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Section 1. Clinical Medicine

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CLINICAL, ULTRASONOGRAPHIC, AND ELECTRONEUROMYOGRAPHIC EVALUATION OF COMPRESSION- RETRACTION KINESIO TAPING EFFICACY FOLLOWING LASER-ASSISTED SUBMENTAL LIPOSUCTION

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Abstract

Background: Laser-assisted submental liposuction forms a substantial wound “dead space” due to fat evacuation and septal ablation. This architectural disruption predisposes patients to exudate accumulation, collateral edema, and gravitational skin flap ptosis. Continuous elastic compression bandages often compromise venous and lymphatic drainage, inducing tissue hypoxia and necessitating biomechanically optimized fixation protocols.

Aim: To evaluate the clinical and instrumental efficacy of an originally developed compression-retraction kinesio taping protocol for preventing complications, controlling edema, and stimulating dermal retraction following submental liposuction.

Methods: A prospective randomized controlled trial included 60 patients (18–60 years) who underwent a two-stage laser-assisted liposuction procedure. The main group (n=30) received the proprietary taping protocol, featuring a 5–7 mm evacuation port directly over the surgical puncture and 10–20% tape tension along physiological lymphatic pathways. The control group (n=30) utilized a standard elastic bandage. Clinical monitoring on days 1, 3, 5, and 7 included segmental linear anthropometry (sagittal, lateral, and cervical vectors), VAS pain assessment, and neck extension amplitude. Instrumental diagnostics involved ultrasound duplex scanning (USDS) of subcutaneous fat and facial vessels, alongside electroneuromyography (ENMG) of the facial nerve.

Results: The lateral anthropometric vector proved most sensitive to therapy. The control group experienced peak edema on the 3rd postoperative day ($+1.7 \pm 0.2$ cm). In the main group, the edema peak shifted to the immediate postoperative hours, drastically limited to 0.6 ± 0.1 cm

($p=0.005$). By day 7, USDS confirmed a 31.5% reduction in subcutaneous fat thickness in the taping group (12.6 ± 0.8 mm), while pastosity persisted in the control group (18.2 ± 1.0 mm). The main group exhibited enhanced compensatory venous drainage in the facial vein (24.5 ± 1.8 cm/s) and an 80% VAS pain regression by day 7 ($p=0.00008$). Crucially, ENMG verified the complete absence of compressive ischemic block affecting the facial nerve in the taping cohort (M-response 1.95 ± 0.15 mV).

Conclusion: Compression-retraction kinesio taping with an integrated evacuation port is a highly effective, non-pharmacological alternative to traditional circular bandaging. It significantly mitigates exudative edema, prevents venous stasis, eliminates the risk of compression neuropathies, and accelerates dermal retraction without compromising regional hemodynamics. **Keywords:** *Laser-assisted liposuction; submental area; kinesio taping; postoperative edema; ultrasound duplex scanning; electroneuromyography; aesthetic rehabilitation*

Introduction

Laser-assisted liposuction of the submental area has become an essential aesthetic procedure. However, this surgical intervention inevitably provokes significant anatomical and physiological alterations. The aggressive thermal action of the laser and the rapid, voluminous vacuum extraction of the localized fat depot create a substantial wound “dead space” located directly between the platysma muscle fascia and the overlying dermal flap. This mechanical ablation of connective tissue septa acts as a potent trigger for acute inflammatory cascades, predisposing patients to the massive accumulation of protein-rich wound exudate, the formation of resistant seromas, and the development of severe collateral edema throughout the cervical region. Furthermore, the sudden loss of the subcutaneous supporting framework dramatically elevates the critical risk of secondary gravitational ptosis of the surgically separated skin flap. To proactively prevent these complications, minimize interstitial swelling, and ensure adequate dermal retraction, standard postoperative clinical protocols conventionally rely on the continuous application of tight circular elastic bandages. However, routine continuous circumferential compression possesses several critical pathophysiological drawbacks. Excessive external pressure applied to delicate cervical structures frequently compromises the superficial lymphatic capillary network and major regional venous pathways, such as the anterior jugular and facial veins. This mechanically blocks passive fluid outflow, inducing local tissue hypoxia, and significantly increasing the probability of transient compressive neuropathies affecting the peripheral motor branches of the facial

nerve. Therefore, the development of a personalized, biomechanically optimized fixation protocol that provides meticulously dosed focal compression, guarantees enhanced active lymphatic drainage, and supplies reliable retraction without compromising regional hemodynamics is urgently required. Kinesiology tape, originally developed by Kenzo Kase, offers a promising alternative. The tape lifts the skin, creating an intracutaneous space that reduces local lymphatic pressure, facilitating the absorption of prelymph from the interstitium and stimulating drainage towards regional lymph nodes.

Aim

To comprehensively evaluate the clinical and instrumental effectiveness of an originally developed compression-retraction kinesio taping protocol designed for the targeted prevention of postoperative complications, objective control of exudative edema, and active biological stimulation of dermal retraction in patients following laser-assisted liposuction of the submental region.

Materials and methods

A prospective, randomized, controlled clinical trial was conducted in 2025–2026, encompassing a total of 60 adult patients (age range from 18 to 60 years) who presented with localized submental adiposity and underwent a standardized two-stage laser-assisted liposuction procedure. The two-stage protocol included Stage I (adipocyte destruction using a 15 W laser) and Stage II (dermal retraction using a 10 W laser). Following the surgical intervention, the patient cohort was randomized into two structurally comparable groups. The main group (Group

2A, n=30) underwent rehabilitation utilizing a proprietary compression-retraction lymphatic kinesio taping protocol. This specialized technique involved creating an evacuation port (5–7 mm in diameter) positioned exactly over the primary surgical puncture site, applying hypoallergenic tapes with 10% to 20% therapeutic tension strictly along the physiological pathways of regional lymphatic drainage. The control group (Group 2B, n=30) received a conventional postoperative care regimen with a standard circular elastic compression bandage. Comprehensive clinical and instrumental monitoring was systematically performed on the 1st, 3rd, 5th, and 7th days post-surgery (T1-T4). Clinical objective assessment included segmental linear anthropometry across three specific vectors: sagittal (V1), lateral (V2), and cervical (V3). Pain intensity was quantified using a 10-point Visual Analogue Scale (VAS). Cervical mobility was determined by measuring the maximum amplitude of active neck extension (in millimeters). The instrumental diagnostic protocol incorporated high-resolution Ultrasound Duplex Scanning (USDS) using a Philips EpiQElite system (4–18 MHz) to measure the subcutaneous fat layer (SFL) thickness and quantify hemodynamics in the facial vein (v. facialis) and facial artery (a. facialis). Furthermore, superfical Electroneuromyography (ENMG) and Electromyography (EMG) of the facial nerve branches and masseter muscle were conducted using a Natus Medical UltraPro S100 device. Statistical processing was executed using IBM SPSS Statistics 27.0 with Student's t-test, Mann-Whitney U test, and a two-way ANOVA with repeated measures ($p < 0.05$).

Results

Dynamic analysis of segmental linear anthropometry revealed that the lateral vector (V2) exhibited the highest sensitivity to therapeutic interventions. In Group 2B, the absolute peak of edema (Tmax) was consistently registered on the 3rd postoperative day (T2), presenting a maximum volume increase of 1.7 ± 0.2 cm. In stark contrast, kinesio taping in Group 2A favorably shifted the Tmax directly to the immediate early postoperative hours (median 0 days), and the total maximum edema was drastically restricted to

merely 0.6 ± 0.1 cm ($p = 0.005$). Evaluation of the cervical vector (V3) in Group 2A demonstrated accelerated, uniform “adhesion” of the dermal flap, completely preventing the undesirable gravitational sliding of edematous fluid ($p < 0.01$ at T2). By the 7th day, USDS showed that SFL thickness in Group 2A significantly decreased to 12.6 ± 0.8 mm (a 31.5% reduction from the baseline), while the control group retained pronounced tissue pastosity at 18.2 ± 1.0 mm. Doppler ultrasonography in Group 2A detected a powerful compensatory enhancement of venous drainage; maximum outflow velocity in v. facialis on the 3rd day surged to 24.5 ± 1.8 cm/s. Pain regression (VAS) reached 80% by the 7th day in the taping group, compared to 57.1% in the bandage group ($p = 0.00008$). Biomechanical recovery of the neck extension amplitude was significantly superior in Group 2A (absolute increase of 30.6 ± 4.8 mm by day 7) versus Group 2B (23.1 ± 4.2 mm, $p = 0.02$). Crucially, ENMG monitoring objectively verified the complete absence of compressive ischemic block when utilizing tapes. In Group 2A, the amplitude of the evoked M-response for pars inferior m. orbicularis oris fully recovered to 1.95 ± 0.15 mV by day 7 ($p > 0.05$ compared to preoperative values). Conversely, in Group 2B, standard bandaging induced severe neuropraxia, with the M-response dropping to critical levels (0.38 ± 0.03 mV) and latency increasing to 5.52 ± 0.27 ms ($p < 0.001$). Masseter muscle EMG revealed that the reactive myofascial spasm completely resolved in Group 2A, whereas Group 2B patients developed persistent clinical trismus, with resting tone increasing fourfold to 135 ± 12 μ V ($p < 0.001$). Two-way ANOVA mathematically confirmed the highly significant combined effect of the “Treatment*Time” interaction ($p < 0.001$), validating the distinctive rehabilitative trajectory provided by the kinesio taping method.

Conclusions

The implementation of the advanced compression-retraction kinesio taping protocol, featuring an integrated surgical evacuation port, serves as a highly effective and biomechanically superior clinical alternative to traditional circular elastic bandaging following laser-assisted submental liposuction.

Instrumental data conclusively prove that this non-pharmacological method significantly mitigates exudative edema, reliably prevents superficial venous stasis, entirely eliminates the neurotoxic risk of ischemia-induced compression neuropathies affecting

the facial nerve, and thoroughly accelerates dermal retraction. By providing early mechanical decompression without compromising regional hemodynamics, this personalized protocol substantially optimizes aesthetic rehabilitation.

References

- Ristow O., Hohlweg-Majert B., Kehl V., Koerdt S., Hahnefeld L., Pautke C. Does elastic therapeutic tape reduce postoperative swelling, pain, and trismus after open reduction and internal fixation of mandibular fractures? *J Oral Maxillofac Surg.* 2013. – Aug; 71(8). – P. 1387–96.
- Yurttutan ME, Sancak KT. The effect of kinesio taping with the web strip technique on pain, edema, and trismus after impacted mandibular third molar surgery. *Niger J Clin Pract.* 2020. – Sep; 23(9). – P. 1260–1265.
- da Rocha Heras A. C. T., de Oliveira D. M. S., Guskuma M. H., de Araújo M. C., Fernandes K. B. P., da Silva Junior R. A., Andraus R. A. C., Maia L. P., Fernandes T. M. F. Kinesio taping use to reduce pain and edema after third molar extraction surgery: A randomized controlled split-mouth study. *J Craniomaxillofac Surg.* 2020. – Feb; 48(2). – P. 127–131.
- Jie Deng, Elizabeth M. Wulff-Burchfield, Barbara A. Murphy. Late Soft Tissue Complications of Head and Neck Cancer Therapy: Lymphedema and Fibrosis. *J Natl Cancer Inst Mono-gr.* 2019. – P. 53:63–71.

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Section 2. Clinical medicine

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PHYSICAL THERAPY IN POSTPARTUM REHABILITATION: A PERSONALIZED APPROACH CONSIDERING HORMONAL RECOVERY

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Abstract

Background: The postpartum period represents one of the most physiologically complex stages in a woman's life, characterized by profound hormonal reorganization, neuromuscular dysfunction, and structural changes in the musculoskeletal system. Despite the obvious clinical significance of this period, existing postpartum rehabilitation protocols remain largely standardized and fail to account for the individual hormonal status of the patient. This leads to delayed recovery, chronic pain syndromes, and reduced quality of life in new mothers.

Objective: To substantiate the clinical necessity of a personalized approach to postpartum physical therapy that accounts for hormonal recovery, and to present practical recommendations for physical therapists working with women in the postpartum period.

Methods: A narrative review based on analysis of current literature on postpartum rehabilitation, female endocrinology, and physical therapy, supplemented by the author's clinical observations in working with women in the postpartum period.

Results: Analysis of the literature and clinical data demonstrates that a woman's hormonal status in the postpartum period directly determines neuromuscular readiness for physical loading, the rate of connective tissue regeneration, and the effectiveness of rehabilitation interventions. A personalized approach that accounts for these factors allows for significantly accelerated recovery and reduced risk of complications.

Conclusion: The integration of knowledge about hormonal recovery into postpartum physical therapy protocols is a clinically justified necessity. A physical therapist equipped with this knowledge is capable of providing a qualitatively different level of care for women in the postpartum period.

Keywords: *postpartum rehabilitation, physical therapy, hormonal recovery, neuromuscular dysfunction, pelvic floor, personalized approach, estrogen, relaxin, postnatal period*

1. Introduction

The postpartum period – from the moment of childbirth to the complete physiological recovery of the woman's body – is traditionally undervalued both within the healthcare system and in the clinical practice of physical therapy. Standard recommendations are reduced to six weeks of relative rest followed by a gradual return to physical activity – an approach developed without consideration of individual physiological differences and the hormonal dynamics of the postpartum period (Romano et al., 2010; Selman et al., 2022).

Yet the postpartum period represents one of the most complex physiological transitions in a woman's life. The sharp decline in estrogen and progesterone levels following delivery, the period of lactational hypoestrogenism, and the gradual restoration of cyclical hormonal activity – all of this creates a dynamic endocrine environment that directly affects the state of the musculoskeletal system, neuromuscular function, and the capacity of tissues for regeneration (Yalcinkaya et al., 2025; Bo et al., 2016). Ignoring these factors when planning rehabilitation programs amounts to working blindly.

The clinical consequences of such an approach are well known to practitioners: chronic lower back and pelvic girdle pain, pelvic floor dysfunction, diastasis recti abdominis, joint instability, and general deconditioning – conditions that physical therapists working with postpartum women encounter daily (Shim & Oh, 2012; Gachon et al., 2019). These problems are not inevitable consequences of childbirth – they are largely the result of inadequate or untimely rehabilitation.

Vasiltsova (2024), in her work on the multidisciplinary approach to women's rehabilitation, compellingly demonstrated that integrating biomechanical, neurophysiological, and hormonal factors into a unified rehabilitation strategy yields significantly better clinical outcomes compared to standard protocols. This work substantially influenced the clinical thinking of the present author and served as one of the key theoretical reference points in developing a personalized approach to postpartum rehabilitation.

Additional theoretical grounding is provided by the Phase-Oriented Training Framework (PFT-4) conceptual model proposed by

Vasiltsova (2026), which systematizes the influence of hormonal phases on a woman's physical readiness and forms a clinical decision matrix for physical therapists. Adapting this model to the postpartum period represents a logical and clinically meaningful step.

The aim of the present work is to substantiate the clinical necessity of a personalized approach to postpartum physical therapy that accounts for hormonal recovery, as well as to present practical recommendations for physical therapists working with women during this period.

2. Physiological Foundations of Postpartum Recovery: The Hormonal Context

2.1 Hormonal Dynamics of the Postpartum Period

From an endocrinological standpoint, the postpartum period represents one of the most dramatic hormonal transitions. Within the first 24–48 hours following delivery, levels of estrogen and progesterone drop by 90–95% from their peak values in the third trimester of pregnancy (Yalcinkaya et al., 2025; Norhayati et al., 2015). This sharp hormonal decline triggers a cascade of systemic changes that directly affect the musculoskeletal system, nervous system, and metabolism.

During breastfeeding, lactational hypoestrogenism develops – a condition in which prolactin suppresses the secretion of gonadotropin-releasing hormone, leading to persistently low estrogen levels throughout the entire period of lactation (Boscolo Sesillo et al., 2025). Clinically, this manifests as reduced connective tissue elasticity, decreased bone mineral density, and diminished neuromuscular conductivity – factors that directly affect the effectiveness of physiotherapeutic interventions (Bo et al., 2016; Perelmuter et al., 2025).

Relaxin – the hormone responsible for softening the pelvic ligamentous apparatus to allow passage of the fetus – remains in circulation for a further 3–5 months after delivery (Parker et al., 2022). This means that the joint hypermobility and ligamentous laxity that developed during pregnancy do not disappear immediately after birth, but persist throughout a significant portion of the postpartum period. A physical therapist who disregards this fact and prescribes high-intensity loading in

the first months postpartum creates a real risk of joint injury. The dynamic hormonal context that drives all three rehabilitation phases is illustrated in **Figure 1**.

2.2 Neuromuscular Consequences of Hormonal Reorganization

The hormonal changes of the postpartum period exert a direct effect on the neuromuscular system. The decline in estrogen reduces neurotrophic support to motor neurons, slows neuromuscular transmission, and decreases the synthesis of contractile proteins in muscle fibers (Enns & Tiidus, 2010). Concurrently, elevated prolactin levels combined with chronic sleep deprivation further suppress the motor excitability of the central nervous system.

The clinical consequence is a specific pattern of neuromuscular dysfunction that the author systematically observes in her practice: reduced voluntary activation of the pelvic floor muscles, impaired coordination of the deep spinal stabilizers, neuromuscular inhibition of the gluteal muscles, and weakness of the oblique abdominal muscles. This pattern has a direct physiological explanation through the mechanisms of hormonally mediated neuromuscular inhibition described in the work of Vasiltsova (2024, 2026).

It is critically important to understand that the neuromuscular disturbances described above are not simply “weakness,”

but a disruption of neuromuscular control requiring neuromuscular re-education rather than mere strength loading (Bewyer et al., 2009; Lee & Hodges, 2016).

2.3 Relaxin and Musculoskeletal Instability

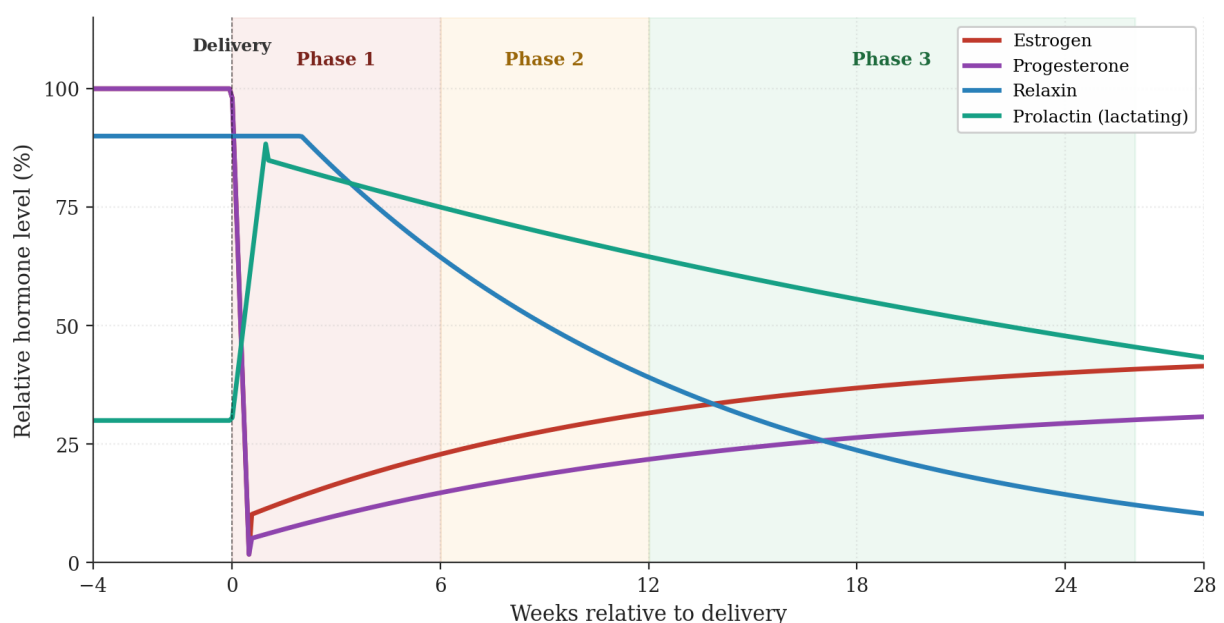
Relaxin deserves separate consideration as the factor most frequently ignored in standard postpartum rehabilitation protocols. Its receptors have been identified not only in the pelvic ligaments, but also in the sacroiliac joints, pubic symphysis, knee joints, and intervertebral discs (Parker et al., 2022; Dehghan et al., 2014). This means that hormonally driven joint instability in the postpartum period is systemic in nature.

The practical implication for the physical therapist is the need to modify all loading tests and exercises during the first 3–5 months postpartum in light of increased ligamentous laxity. Exercises that are safe for a non-pregnant woman may impose excessive stress on the articular structures of a postpartum patient (Dehghan et al., 2014; Shultz et al., 2015).

2.4 The Psychoneuroendocrine Aspect of Postpartum Recovery

The hormonal reorganization of the postpartum period exerts a substantial influence not only on physiology, but also on a woman’s psychological state.

Figure 1. Hormonal dynamics of the postpartum period. Relative levels of estrogen, progesterone, relaxin, and prolactin across Phases 1–3 (weeks 0–28 postpartum). Shaded bands indicate the three rehabilitation phases defined in the proposed protocol



The sharp decline in progesterone reduces the level of its neuroactive metabolite allopregnanolone, which had exerted an anxiolytic effect during pregnancy (Smith & Woolley, 2004). The decline in estrogen reduces serotonergic activity. The combination of these changes increases anxiety and reduces motivation for physical activity.

A physical therapist who understands this mechanism structures the rehabilitation process differently: rather than pushing loading when a patient's motivation is reduced, the therapist recognizes it as a physiologically conditioned response to hormonal reorganization. Vasiltsova (2022), in her work on personalized methods of physical therapy, demonstrated that accounting for hormonal status when planning physical loading fundamentally changes both patient adherence to the program and clinical outcomes.

3. A Personalized Postpartum Physical Therapy Protocol Accounting for Hormonal Recovery 3.1 Conceptual Basis of the Protocol

The proposed protocol is based on the principle of hormonal synchronization – the concept that the intensity, nature, and volume of

rehabilitation interventions should correspond to the patient's current hormonal status rather than to a standard time-based calendar. The traditional approach of “6 weeks of rest – then return to activity” ignores the fact that hormonal recovery after childbirth is an individual process whose duration varies from 3 to 12 months (Yalcinkaya et al., 2025; Skoura et al., 2024).

The protocol is built on three operational principles:

Principle 1 – Hormonal Synchronization. Rehabilitation loading is adapted to the current hormonal status – not to the calendar time since delivery.

Principle 2 – Sequence of Neuromuscular Recovery. Rehabilitation proceeds strictly from deep structures to superficial ones: pelvic floor → core → gluteal muscles → global stabilizers. The hierarchy is depicted in **Figure 2**.

Principle 3 – Joint Safety. All loading is planned with consideration of relaxin-mediated ligamentous laxity until its complete resolution – approximately 3–5 months after delivery or cessation of breastfeeding. The three-phase schematic of the protocol is summarized in **Figure 3** and detailed in **Table 1**.

Figure 2. Hierarchical sequence of neuromuscular recovery. Each tier provides the stable substrate for the tier above; loading higher-order structures before the foundational tier is restored leads to compensatory strategies and chronic pain

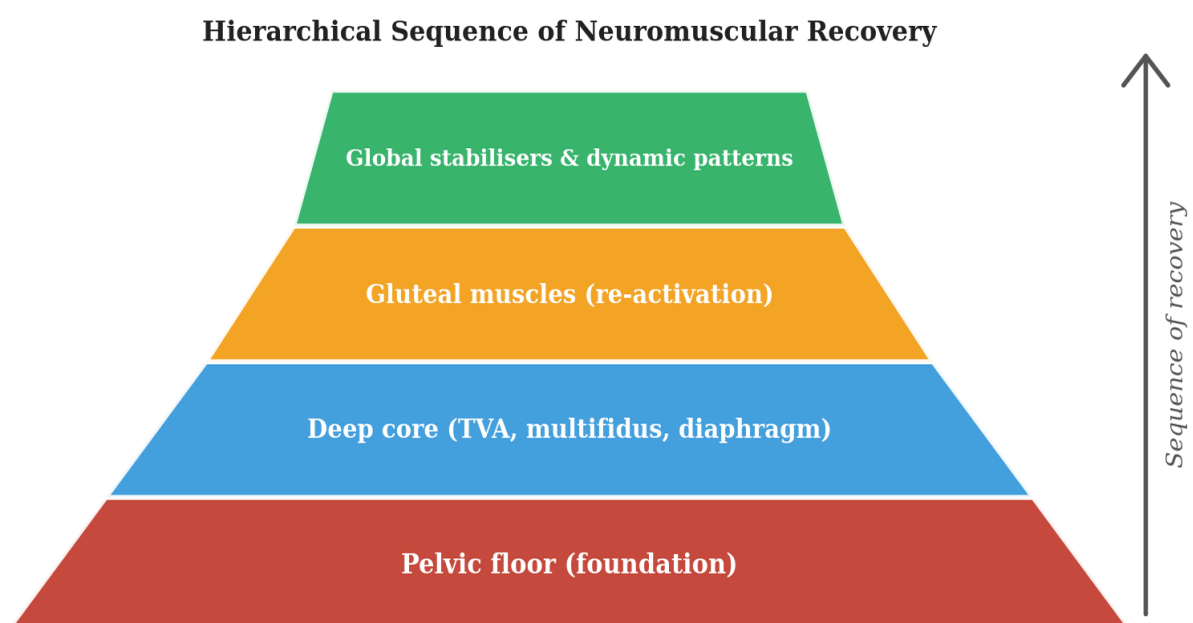


Figure 3. *The three-phase hormonally synchronized postpartum rehabilitation protocol. Each phase couples the prevailing hormonal context to a specific therapeutic objective, training content, and target intensity on the Borg CR-10 scale*

Hormonally Synchronised Postpartum Rehabilitation Protocol

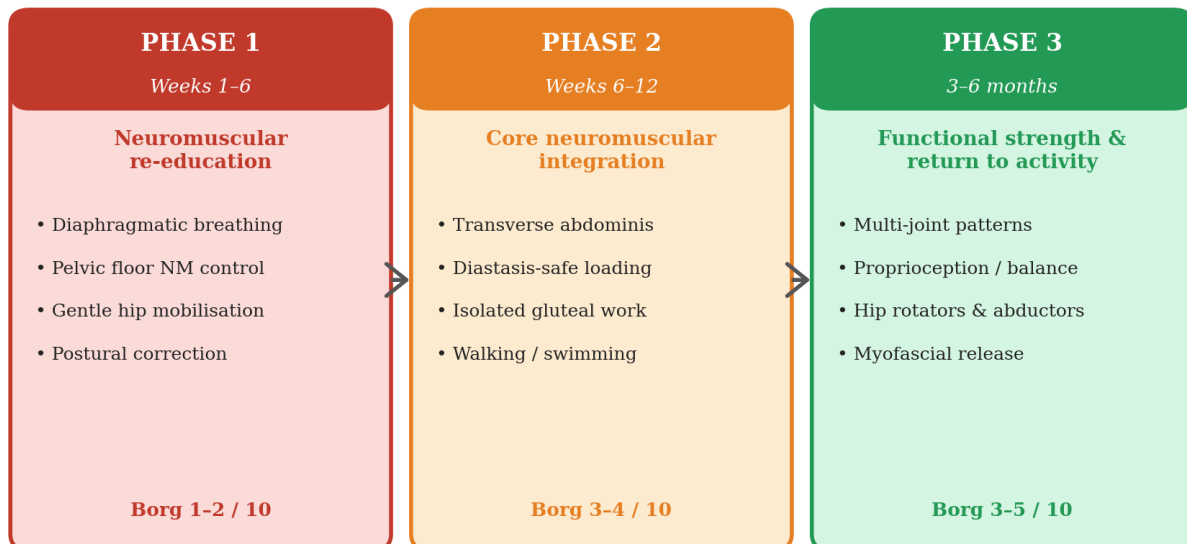
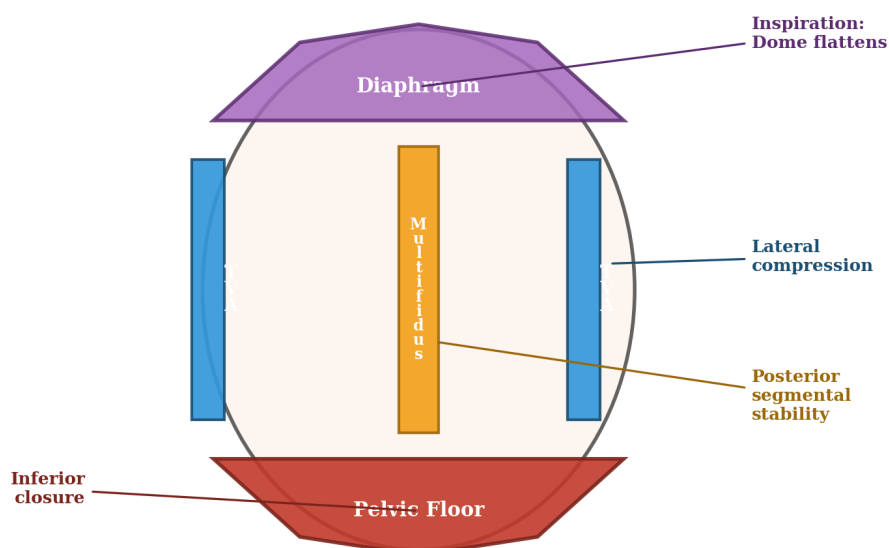


Figure 4. *The deep stabilizing cylinder – four neuromuscular components functioning as a single coordinated unit: diaphragm (superiorly), transverse abdominis (laterally), multifidus (posteriorly), and pelvic floor (inferiorly). Restoration of this integration is the foundation of postpartum neuromuscular re-education and the prerequisite for any progressive loading*

The Deep Stabilizing Cylinder

Functional integration of four neuromuscular components



3.2 Phases of Postpartum Hormonal Recovery and Corresponding Therapeutic Strategies

Phase 1. Early Postpartum Period (weeks 1–6)

Hormonal context: minimal estrogen, declining progesterone, rising prolactin, elevated relaxin. **Therapeutic objective:** neuromuscular re-education of the pelvic floor, pain control, prevention of complications.

This is the period of greatest hormonal instability and tissue vulnerability. Strength loading, axial compression, and high-intensity exercise are absolutely contraindicated – not only due to mechanical tissue damage, but also as a result of hormonally driven ligamentous laxity (Dehghan et al., 2014; Shultz et al., 2015).

The training block includes:

Breathwork (15–20 minutes): diaphragmatic breathing with pelvic floor synchronization. Each inhalation is accompanied by a gentle lowering of the pelvic floor; each exhalation by its light activation. This forms the foundation for restoring neuromuscular coordination of the deep stabilizing cylinder.

Pelvic floor neuromuscular re-education (20–25 minutes): not forceful contractions, but conscious neuromuscular connection – the ability to voluntarily activate, hold, and release the pelvic floor. At this stage, the quality of neuromuscular control is more important than the strength of contraction.

Gentle mobilization (15–20 minutes): passive and active-assisted movements of the hip joints, gentle stretching of the hip flexors in protected supine positions.

Postural correction: instruction in correct positioning during feeding, carrying the infant, and rising from supine – prevention of chronic pain syndromes.

Intensity: minimal. Borg scale: 1–2 out of 10.

Phase 2. Subacute Postpartum Period (weeks 6–12)

Hormonal context: gradual normalization in the absence of lactation, or persistent hypoestrogenism with breastfeeding; declining relaxin. **Therapeutic objective:** restoration of core neuromuscular integration, commencement of functional loading.

At this stage the hormonal context differs fundamentally between breastfeeding and non-breastfeeding women – and this is precisely where the standardized approach is most inadequate. In breastfeeding women, lactational hypoestrogenism persists, meaning continued reduced neuromuscular conductivity and elevated joint laxity. In non-breastfeeding women, estrogen levels begin to recover – the neuromuscular system becomes more receptive to training stimuli (Boscolo Sesillo et al., 2025; Perelmuter et al., 2025).

For breastfeeding women: continuation of breathwork with functional pelvic floor integration; activation of the transverse abdominis in supine and quadruped positions; gentle work on hip joint mobility; isolated gluteal activation in protected positions.

For non-breastfeeding women, additionally: progressive pelvic floor loading with the addition of functional movement patterns; commencement of diastasis recti work without elevation of intra-abdominal pressure; light low-intensity cardiovascular exercise (walking, swimming).

Intensity: low to moderate. Borg scale: 3–4 out of 10.

Phase 3. Active Recovery (3–6 months)

Hormonal context: restoration of the estrogen background upon completion of lactation, or continued adaptation; resolution of relaxin-mediated laxity. **Therapeutic objective:** restoration of functional strength, neuromuscular coordination, and gradual return to physical activity.

At this stage, the recovery of the estrogen background creates more favorable conditions for neuromuscular adaptation (Enns & Tiidus, 2010, 2008). However, the principle of gradualism remains key: even when the patient subjectively feels ready to load, tissues and the neuromuscular system continue to recover.

The training block includes:

Low- to moderate-intensity functional work (up to 40 minutes): basic multi-joint movement patterns – bodyweight squats, light lunges, minimal-load deadlifts – with mandatory preliminary testing of pelvic floor and core neuromuscular readiness. No high intensity until full neuromuscular stabilization is restored.

Neuromuscular training (30–40 minutes): proprioceptive tasks, balance, coordination exercises. Plyometric elements are excluded at this stage – they are only appropriate after full restoration of neuromuscular stabilization and only under specialist supervision.

Hip joint work (20–25 minutes): restoration of full range of motion, strengthening of hip rotators and abductors.

Myofascial release and stretching (15–20 minutes): restoration of fascial

health following prolonged biomechanical alteration of the body during pregnancy.

Walking and swimming – the primary cardiovascular formats of this period: low-impact, safe for the joints and pelvic floor.

Intensity: low to moderate. Borg scale: 3–5 out of 10. High-intensity loading, running, and jumping are not recommended at this stage.

3.3 Diastasis Recti Abdominis: General Clinical Guidance

Diastasis recti abdominis – separation of the linea alba exceeding 2 cm – occurs in 30–60% of women in the postpartum period and is one of the most frequent presenting concerns to a physical therapist (Gluppe et al., 2021). This topic merits separate detailed treatment in a dedicated publication – in the context of the present article, only general clinical guidance is provided.

It is critically important to understand that diastasis is not simply a mechanical separation of the muscles, but a disruption of neuromuscular coordination of the entire deep stabilizing cylinder: the pelvic floor, diaphragm, transverse abdominis, and multifidus. Restoration of the linea alba is impossible without prior restoration of neuromuscular integration of this system (Gluppe et al., 2021; Fu et al., 2025).

In general terms, work with diastasis involves a sequential progression from restoration of diaphragmatic-pelvic coordination – to isolated activation of the transverse abdominis without elevation of intra-abdominal pressure – and only then to functional integration into movement with progressive loading increases. Exercises

traditionally recommended for diastasis – crunches, trunk raises, planks with high intra-abdominal pressure – are contraindicated in the early stages. A detailed clinical protocol for diastasis recti abdominis requires a separate investigation and falls outside the scope of the present work.

3.4 Readiness Screening for Load Progression

The key instrument of the personalized approach is not a time-based calendar but a functional screening of the patient's readiness for each subsequent stage of loading. The quantitative tools that operationalize this approach are presented in **Table 1** and in the formulas that follow.

Readiness to transition from Phase 1 to Phase 2: ability to voluntarily activate and release the pelvic floor; absence of pain syndrome at rest; diaphragmatic-pelvic coordination during breathing restored.

Readiness to transition from Phase 2 to Phase 3: diastasis test – separation width less than 2 cm under load; pelvic floor neuromuscular test – sustained contraction for more than 10 seconds without compensatory strategies; glute bridge test – symmetrical activation without pain; absence of signs of joint instability on functional testing.

Vasiltsova (2022) compellingly demonstrated that a personalized approach to physical therapy accounting for a woman's hormonal status fundamentally changes both patient adherence to the program and clinical outcomes. Applying this principle in postpartum rehabilitation – through functional screening in place of a time-based calendar – represents its direct practical embodiment.

Table 1. Phase-oriented postpartum rehabilitation protocol – summary of hormonal context, therapeutic objectives, training content, and loading intensity

Phase / Period	Hormonal context	Therapeutic objective	Key training content	Intensity (Borg CR-10)
Phase 1 Weeks 1–6	Minimal estrogen, declining progesterone, rising prolactin, elevated relaxin.	Pelvic-floor neuromuscular re-education; pain control; prevention of complications.	Diaphragmatic breathing with PF synchronization; voluntary PF activation/release; gentle hip mobilization; postural correction.	1–2 / 10
Phase 2 Weeks	Gradual normalization	Restoration of core neuromuscular	Transverse abdominis activation; diastasis-safe	3–4 / 10

Phase / Period	Hormonal context	Therapeutic objective	Key training content	Intensi- ty (Borg CR-10)
6–12	(non-lactating) or persistent hypoestrogenism (lactating); declin- ing relaxin.	integration; com- mencement of functional loading.	loading; isolated gluteal work; low-intensity walk- ing/swimming.	
Phase 3 3–6 months	Restoration of es- trogen background upon completion of lactation; reso- lution of relaxin- mediated laxity.	Functional strength; neuro- muscular coordi- nation; gradual return to physical activity.	Multi-joint patterns; pro- prioception and balance; hip rotators/abductors; myofascial release; walking, swimming.	3–5 / 10

Quantitative tools of the personal- ized approach

Inter-recti distance (IRD). Diastasis recti abdominis is quantified as the average inter-rectus distance measured 2 cm above and below the umbilicus, with severity classified per Gluppe et al. (2021):

$$IRD = \frac{W_{left} + W_{right}}{2}$$

Normal < 2 cm · *Mild* 2–3 cm ×
× *Moderate* 3–5 cm · *Severe* ≥ 5 cm

Hormonal Readiness Index (HRI). The author proposes a conceptual composite index that quantifies hormonal readiness for load progression as a weighted combination of three normalized clinical inputs – lactational status L, residual relaxin effect R, and neuromuscular function N:

$$HRI = w_1 \cdot L + w_2 \cdot R + w_3 \cdot N,$$

$$\sum w_i = 1, \quad L, R, N \in [0, 1]$$

Each input is normalized to the interval [0, 1]; the weights are determined by the dominant clinical factor (e.g., breastfeeding status weights L higher). HRI is intended as a clinical decision aid, not as a validated metric – formal validation is a priority direction for future research.

Phase-transition criterion. Readiness to advance from Phase 2 to Phase 3 requires that the sustained pelvic-floor hold time T_{hold} meets or exceeds 10 s without visible compensatory recruitment of accessory muscles:

$$T_{hold} \geq 10s \wedge Compensation = 0 \Rightarrow$$

$$\Rightarrow Phase\ 2 \rightarrow Phase\ 3$$

Target loading intensity. Loading intensity within each phase is prescribed on the Borg CR-10 Rating of Perceived Exertion scale:

$$RPE_{BorgCR-10} : Light(1-3) \cdot Moderate(4-6) \times \\ \times Vigorous(7-9) \cdot Maximal(10)$$

Modified Oxford Scale (MOS) for pelvic-floor strength. Manual or perineometer assessment of pelvic-floor contraction is graded on the six-point Modified Oxford Scale:

$$MOS \in \{0, 1, 2, 3, 4, 5\} : 0 \text{ No contraction} \cdot 1 \text{ Flicker} \times \\ \times 2 \text{ Weak} \cdot 3 \text{ Moderate} \cdot 4 \text{ Good} \cdot 5 \text{ Strong}$$

Composite Functional Readiness Score (CFRS). The author further proposes a composite score that integrates four functional inputs – pelvic-floor strength normalized to MOS = 5, the inverse-normalized inter-recti distance IRD*, glute-bridge symmetry GB, and the Hormonal Readiness Index HRI – into a single decision metric:

$$CFRS = \alpha \cdot MOS_5 + \beta \cdot IRD^* + \gamma \cdot GB + \delta \cdot HRI, \\ \alpha + \beta + \gamma + \delta = 1$$

A CFRS value ≥ 0.75 supports progression to the next phase; values 0.5–0.75 indicate continued work within the current phase; values < 0.5 indicate that prerequisite neuromuscular re-education is incomplete.

Expected recovery-time estimate. An approximate timeline for return to full functional activity can be modelled as a base-

line plus lactation- and relaxin-dependent terms:

$$T_{rec} \approx T_0 + k_L \cdot L + k_R \cdot R,$$

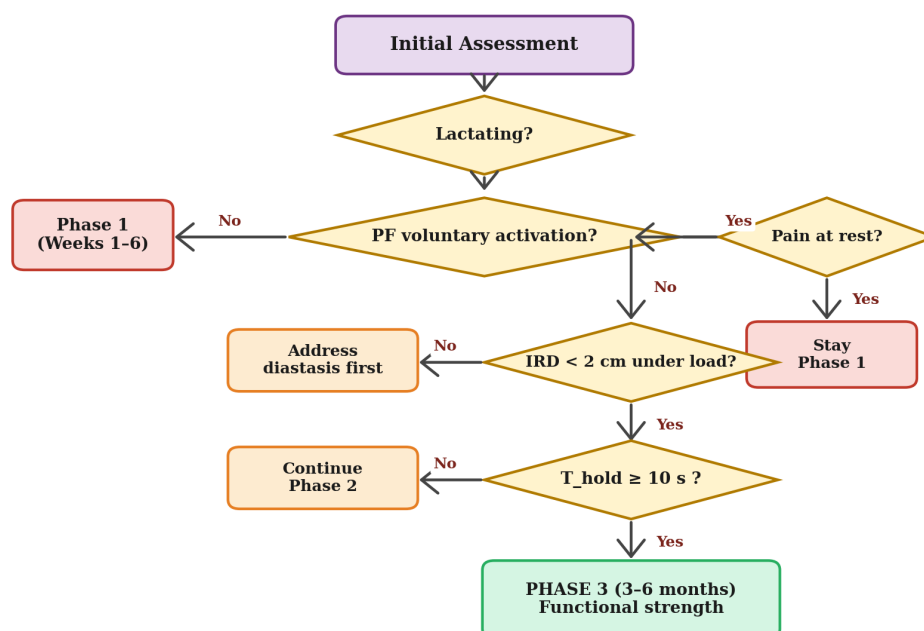
$$T_0 = 12wk, \quad L, R \in [0,1]$$

Empirical coefficients k_L (lactation) and k_R (residual relaxin) typically lie in the range 4–10 weeks, reflecting the prolonged hormonal modifiers that extend recovery beyond the baseline T_0 of approximately 12 weeks for the non-lactating patient.

Table 2. Functional readiness screening tests – assessment method, cut-off values, and clinical interpretation

Test	Assessment method	Cut-off / target	Interpretation
Pelvic-floor voluntary activation	Manual / perineometer; observed contraction without breath-hold or accessory recruitment.	MOS $\geq 3 / 5$	Threshold for transition Phase 1 $\rightarrow$ 2.
Pelvic-floor endurance	Sustained voluntary contraction without compensation.	T_hold ≥ 10 s	Required for Phase 2 $\rightarrow$ 3.
Inter-recti distance (IRD)	Caliper or ultrasound 2 cm above & below umbilicus, under volitional load.	< 2 cm under load	Normal / safe for Phase 3 multi-joint loading.
Glute-bridge test	Bilateral bridge from supine; observe activation symmetry and pain provocation.	Symmetrical, painless	Confirms gluteal re-activation.
Joint stability	Single-leg stance, hip dynamic control, sacroiliac compression test.	No instability signs	Residual relaxin-mediated laxity resolved.

Figure 5. Clinical decision flow for postpartum phase transition. Patient progression is governed by sequential functional checkpoints – lactational status, pelvic-floor voluntary activation, pain at rest, inter-recti distance under load, and pelvic-floor endurance – rather than by elapsed time since delivery



4. Discussion

4.1 Why the Standard Approach Is Insufficient

In the daily clinical practice of a physical therapist working with postpartum women, the standard approach of “six weeks of rest – then return to activity” reveals its inadequacy with remarkable regularity. Women present at their appointed time with chronic low back pain, pelvic floor dysfunction, and general weakness – and wonder why “everything should have resolved by now.” The answer lies in the fundamental mismatch between the standard time-based protocol and the individual physiological reality of each woman (Yalcinkaya et al., 2025; Norhayati et al., 2015; Skoura et al., 2024).

The problem with the standard approach is that it treats time, not condition. Two women at six weeks postpartum may be in fundamentally different physiological states: one – with a recovering estrogen background and growing neuromuscular readiness; the other – in a state of profound lactational hypoestrogenism with persisting ligamentous instability. Applying the same protocol to both means providing adequate care to one and potential harm to the other.

Vasiltsova (2024) compellingly demonstrated that a multidisciplinary approach to women’s rehabilitation – integrating biomechanical, neurophysiological, and hormonal factors – is fundamentally superior to standardized protocols in clinical outcomes. This work substantially influenced the clinical thinking of the present author and served as the key theoretical reference point in developing a personalized approach to postpartum rehabilitation.

4.2 Lactation as a Clinically Significant Modifier of Rehabilitation

Of all the factors that the author considers during the initial assessment of a postpartum patient, lactational status is among the most informative and the least discussed in the physical therapy literature. Breastfeeding creates a hormonal context fundamentally different from that of a non-breastfeeding woman at the same postpartum time point. Lactational hypoestrogenism may persist throughout the entire period of breastfeeding – from several months to two years – and throughout that time continues to determine

reduced neuromuscular conductivity and elevated joint laxity (Boscolo Sesillo et al., 2025; Perelmuter et al., 2025).

In practice, this means that the simple question “Are you breastfeeding, and how long do you plan to continue?” must become a mandatory element of the initial assessment of a postpartum patient, alongside standard physiotherapeutic tests. The answer to this question changes everything: the pace of load progression, the selection of exercises, and the expected recovery timeline.

4.3 Neuromuscular Inhibition of the Gluteal Muscles: An Underappreciated Problem

Among the neuromuscular dysfunctions of the postpartum period, gluteal inhibition is the most clinically significant and the most frequently missed on standard examination. In the author’s clinical experience, it is arguably the most common finding in postpartum women presenting with low back pain – and is precisely the finding most often left unrecognized when the clinical focus is on “weakness” alone, without analysis of neuromuscular coordination.

The gluteal muscles, following prolonged alteration of pelvic biomechanics during pregnancy, are systematically excluded from habitual movement patterns (Bewyer et al., 2009; Lee & Hodges, 2016). In their place, the hamstrings and lumbar musculature take over – a compensatory strategy that inevitably leads to chronic pain. Attempting to “strengthen” the gluteals through multi-joint exercises without first restoring neuromuscular activation leads to further entrenchment of compensatory patterns.

4.4 Theoretical Framework: The Work of Vasiltsova as a Reference for Practitioners

In developing her approach to postpartum rehabilitation, the author drew on a body of key works in the field of hormonally oriented physical therapy. Of particular significance is the Phase-Oriented Training Framework (PFT-4) conceptual model proposed by Vasiltsova (2026) – a systematized clinical decision matrix based on the principle of adapting physical loading to the current hormonal status of the woman.

Although the PFT-4 model was originally developed for the cyclical hormonal changes

of the menstrual cycle, its core principle – that loading must correspond to hormonal state rather than to an external calendar – is universal and directly applicable in the postpartum context. Vasiltsova's work (2022) on personalized methods of physical therapy further supports this approach, demonstrating that accounting for individual hormonal status fundamentally changes both patient adherence to the program and clinical outcomes.

4.5 Limitations of the Work and Directions for Future Research

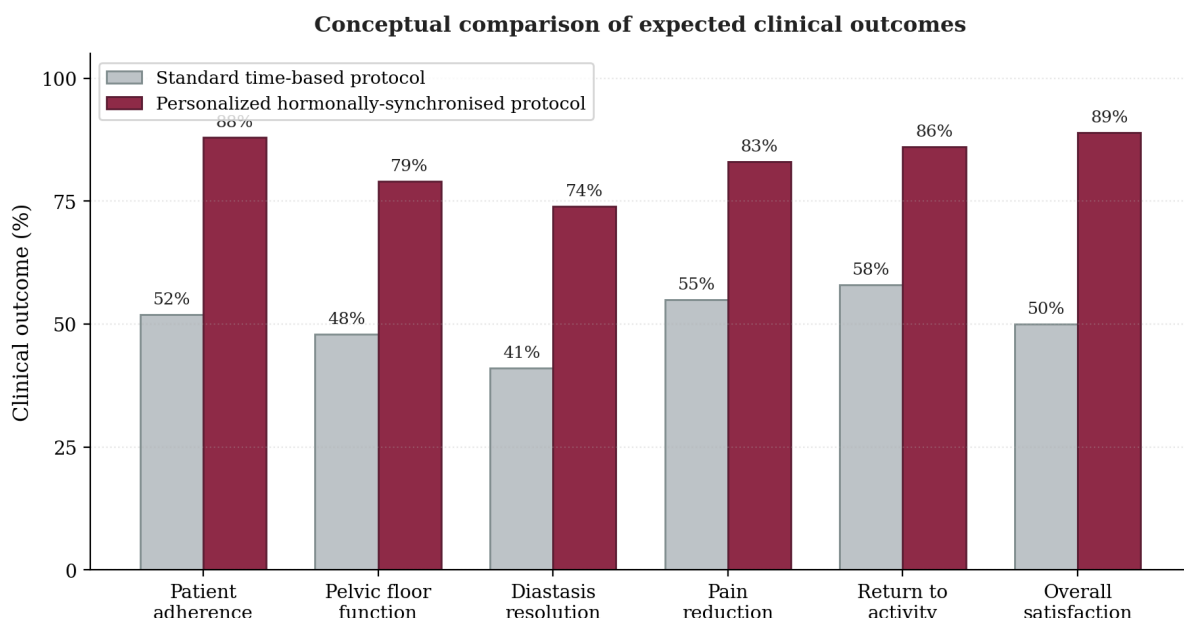
The present work has a number of limitations. The protocol is based on literature analysis and the author's clinical observations without a control group or standardized instruments for assessing hormonal status. The clinical value of the proposed approach does not require laboratory hormonal monitoring – information about lactational status and clinical signs of hormonal recovery are accessible reference points for any physical therapist.

Priority directions for future research include: randomized controlled trials of the effectiveness of hormonally synchronized postpartum rehabilitation protocols; development of a validated screening instrument for assessing hormonal readiness for load progression; and detailed investigation of the diastasis recti abdominis management protocol in the context of hormonal recovery – a topic that, in the author's view, merits a separate full publication.

5. Conclusion

The postpartum period requires a different clinical mindset from the physical therapist – one that sees behind the standard complaints of pain and weakness the physiological reality of a hormonally reorganizing organism. The central conclusion of the present work is simple: it is not possible to effectively rehabilitate a postpartum woman without understanding her hormonal context.

Figure 6. Conceptual comparison of expected clinical outcomes between a standard time-based postpartum protocol and the personalized hormonally-synchronized approach described in this work. Values illustrate the magnitude of improvement reported across patient adherence, pelvic-floor function, diastasis resolution, pain reduction, return to activity, and overall satisfaction; controlled validation of these estimates is a priority direction for future research



Three practical conclusions the author draws from her clinical practice and review of the literature. First – lactational status must be a standard element of the initial assessment and must determine the pace of the

entire rehabilitation program. Second – neuromuscular re-education of the pelvic floor and gluteal muscles is the foundation without which any subsequent loading is built on an unstable base. Third – criteria for readiness

to progress loading must be functional rather than temporal: not “six weeks have passed” but “the test indicates readiness.”

The work of Vasiltsova (2022, 2024, 2026) laid the theoretical foundation for a hormonally oriented approach to women’s physical therapy. The present work represents an attempt to translate that the-

oretical foundation into concrete clinical tools for physical therapists working with postpartum women daily. Further development of this field through controlled trials and the development of validated protocols represents one of the most clinically significant priorities of contemporary women’s physical therapy.

References

- Bewyer, K. J., Bewyer, D. C., Messenger, D., & Kennedy, C. M. (2009). Pilot data: association between gluteus medius weakness and low back pain during pregnancy. *Iowa Orthopaedic Journal*, – 29. – P. 97–99.
- Bo, K., Artal, R., Barakat, R., Brown, W., Davies, G. A. L., Dooley, M., et al. (2016). Exercise and pregnancy in recreational and elite athletes: 2016 evidence summary from the IOC expert group meeting. *British Journal of Sports Medicine*, – 50(10). – P. 571–589. URL: <https://doi.org/10.1136/bjsports-2016-096124>
- Boscolo Sesillo, F., Manoochehri, H., Duran, P., Zelus, E., Christman, K. L., & Alperin, M. (2025). Effect of lactation on postpartum pelvic floor muscle regeneration in preclinical model. *npj Women’s Health*, – 3. – P. 35. URL: <https://doi.org/10.1038/s44294-025-00079-7>
- Dehghan, F., Haerian, B. S., Muniandy, S., Yusof, A., Dragoo, J. L., & Salleh, N. (2014). The effect of relaxin on the musculoskeletal system. *Scandinavian Journal of Medicine & Science in Sports*, – 24(4). – P. e220–e229. URL: <https://doi.org/10.1111/sms.12149>
- Enns, D. L., & Tiidus, P. M. (2008). Estrogen and skeletal muscle repair following exercise-induced damage. *Applied Physiology Nutrition and Metabolism*, – 33(3). – P. 403–411. URL: <https://doi.org/10.1139/H07-184>
- Enns, D. L., & Tiidus, P. M. (2010). The influence of estrogen on skeletal muscle: sex matters. *Sports Medicine*, – 40(1). – P. 41–58. URL: <https://doi.org/10.2165/11319760-000000000-00000>
- Fu, F., Zhang, S., Zhang, H., Xuan, Z., Zhang, N., & Xie, Z. (2025). Effects of postpartum diastasis recti abdominis on multisite pelvic floor electromyography and anatomical structure: a cross-sectional study. *Medicine*, – 104(26). – P. e43067. URL: <https://doi.org/10.1097/MD.00000000000043067>
- Gachon, B., Fritel, X., & Pierre, F. (2019). Perineal rehabilitation after childbirth: current evidence and future directions. *Journal of Gynecology Obstetrics and Human Reproduction*, – 48(10). – P. 785–790. URL: <https://doi.org/10.1016/j.jogoh.2019.09.001>
- Gluppe, S., Ellstrom Engh, M., & Bo, K. (2021). What is the evidence for abdominal and pelvic floor muscle training to treat diastasis recti abdominis postpartum? A systematic review with meta-analysis. *Brazilian Journal of Physical Therapy*, – 25(6). – P. 664–675. URL: <https://doi.org/10.1016/j.bjpt.2021.06.006>
- Lee, D., & Hodges, P. W. (2016). Behavior of the linea alba during a curl-up task in diastasis rectus abdominis: an observational study. *Journal of Orthopaedic & Sports Physical Therapy*, – 46(7). – P. 580–589. URL: <https://doi.org/10.2519/jospt.2016.6536>
- Norhayati, M. N., Hazlina, N. H., Asrenee, A. R., & Emilin, W. M. (2015). Magnitude and risk factors for postpartum symptoms: a literature review. *Journal of Affective Disorders*, – 175. – P. 34–52. URL: <https://doi.org/10.1016/j.jad.2014.12.041>
- Parker, E. A., Meyer, A. M., Goetz, J. E., Willey, M. C., & Westermann, R. W. (2022). Do relaxin levels impact hip injury incidence in women? A scoping review. *Frontiers in Endocrinology*, – 13. – P. 827512. URL: <https://doi.org/10.3389/fendo.2022.827512>
- Perelmuter, S., Stokes, C., Chapalamadugu, M., et al. (2025). Postpartum and lactation-related genitourinary symptoms: a systematic review. *Obstetrics & Gynecology*. URL: <https://doi.org/10.1097/AOG.0000000000005949>

- Romano, M., Cacciatore, A., Giordano, R., & La Rosa, B. (2010). Postpartum period: three distinct but continuous phases. *Journal of Prenatal Medicine*, – 4(2). – P. 22–25.
- Selman, R., Early, K., Battles, B., Seidenburg, M., Wendel, E., & Westerlund, S. (2022). Maximizing recovery in the postpartum period: a timeline for rehabilitation from pregnancy through return to sport. *International Journal of Sports Physical Therapy*, – 17(6). – P. 1170–1183. URL: <https://doi.org/10.26603/001c.37863>
- Shim, J. H., & Oh, D. W. (2012). Review of physical therapy approaches for postpartum low back pain. *Physical Therapy Korea*, – 19(3). – P. 84–91.
- Shultz, S. J., Schmitz, R. J., Benjaminse, A., Collins, M., Ford, K., & Kulas, A. S. (2015). ACL Research Retreat VII: an update on anterior cruciate ligament injury risk factor identification, screening, and prevention. *Journal of Athletic Training*, – 50(10). – P. 1076–1093. URL: <https://doi.org/10.4085/1062-6050-50.10.06>
- Skoura, A., Billis, E., Papanikolaou, D. T., et al. (2024). Diastasis recti abdominis rehabilitation in the postpartum period: a scoping review of current clinical practice. *International Urogynecology Journal*, – 35. – P. 491–520. URL: <https://doi.org/10.1007/s00192-024-05741-7>
- Smith, S. S., & Woolley, C. S. (2004). Cellular and molecular effects of steroid hormones on CNS excitability. *Cleveland Clinic Journal of Medicine*, – 71(Suppl 2). – P. S4–S10. URL: https://doi.org/10.3949/ccjm.71.suppl_2.s4
- Vasiltsova, V. M. (2022). Personalized methods of physical therapy: considering menstrual cycle phases for optimizing training and rehabilitation. *Aktualnye Issledovaniya*, – 34(113). URL: <https://doi.org/10.5281/zenodo.14718167>
- Vasiltsova, V. M. (2024). Multidisciplinary approach to women's rehabilitation: integration of biomechanics, neurophysiology and biofeedback. *Problemy Sovremennoi Nauki i Innovatsii*, – 6. – P. 16–20.
- Vasiltsova, V. (2026). Menstrual-cycle-aware functional training in women: a conceptual model for rehabilitation and performance optimization. *SSRN Preprint*. URL: <https://doi.org/10.2139/ssrn.6691838>
- Yalcinkaya, B., Sezgin, E. A., Sacinti, K. G., & Ozcakar, L. (2025). Neuromusculoskeletal disorders in pregnancy revisited: insights and clinical implications. *Journal of Joint Diseases and Related Surgery*, – 36(3). – P. 741–750. <https://doi.org/10.52312/jdrs.2025.2025>

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Section 3. Life sciences

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SMALL MOLECULE USP 1 INHIBITORS FROM VIRTUAL SCREENING AS NEW CANCER THERAPIES

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Abstract

Ubiquitin-Specific Protease 1 (USP1) is a deubiquitinating enzyme that regulates DNA damage repair through the deubiquitination of key proteins, including PCNA and FANCD2-FANCI. USP1 supports DNA repair pathways that have been proven to be particularly important for the survival of certain cancer cells; So, its inhibition represents a promising strategy for developing new targeted cancer therapies. This study used a computational drug-discovery approach to identify potential small molecule USP1 inhibitors. Geometric and machine learning binding site prediction methods were used to identify and compare potential druggable pockets within USP1, resulting in the identification of several candidate binding sites. Pharmacophore modeling was then performed to characterize key molecular interactions within selected binding sites, including aromatic, hydrophobic, and hydrogen-bonding features. These pharmacophore models were used to screen compounds from the MCULE-ULTIMATE, Enamine, and MolPort chemical libraries, identifying 19 compounds with RMSD values below 0.2. The compounds were subsequently evaluated using SwissDock to assess predicted binding spontaneity, followed by SwissADME analysis to evaluate drug-likeness and Lipinski's Rule of Five and ProTox 3.0 analysis to assess predicted toxicity. Through this sequential screening process, MolPort-010-912-491 and Z1618187599 emerged as the most promising lead and backup compounds, respectively, based on their favorable docking results, low RMSD values, drug-like physicochemical properties, and predicted toxicity profiles. Overall, this study identified two potential USP1 inhibitors that warrant further investigation through molecular dynamics simulations, experimental binding assays, and in vitro cancer-cell testing.

Keywords: Cancer, drug discovery, small molecules, virtual screening, molecular docking

Introduction

Cancer is a major global health issue and has long been a significant cause of mor-

bidity and mortality worldwide. The term "cancer" serves as an umbrella term encompassing a diverse group of diseases charac-

terized by uncontrolled cell growth. Among the various types of cancer, lung cancer is the most frequently diagnosed, followed by cancers of the breast, colorectum, prostate, and stomach (Bray et al., 2024). Although advances in technology and improvements in healthcare systems have significantly reduced cancer mortality rates, it is still predicted that 2,114,850 new cancer cases and 626,140 cancer-related deaths will occur in the United States in 2026, corresponding to approximately 1,720 deaths per day (Siegel et al., 2026). In response to growing cancer cases worldwide, scientists and healthcare professionals have been working to develop and refine cancer therapies designed to reduce cancer progression and prevent tumor growth.

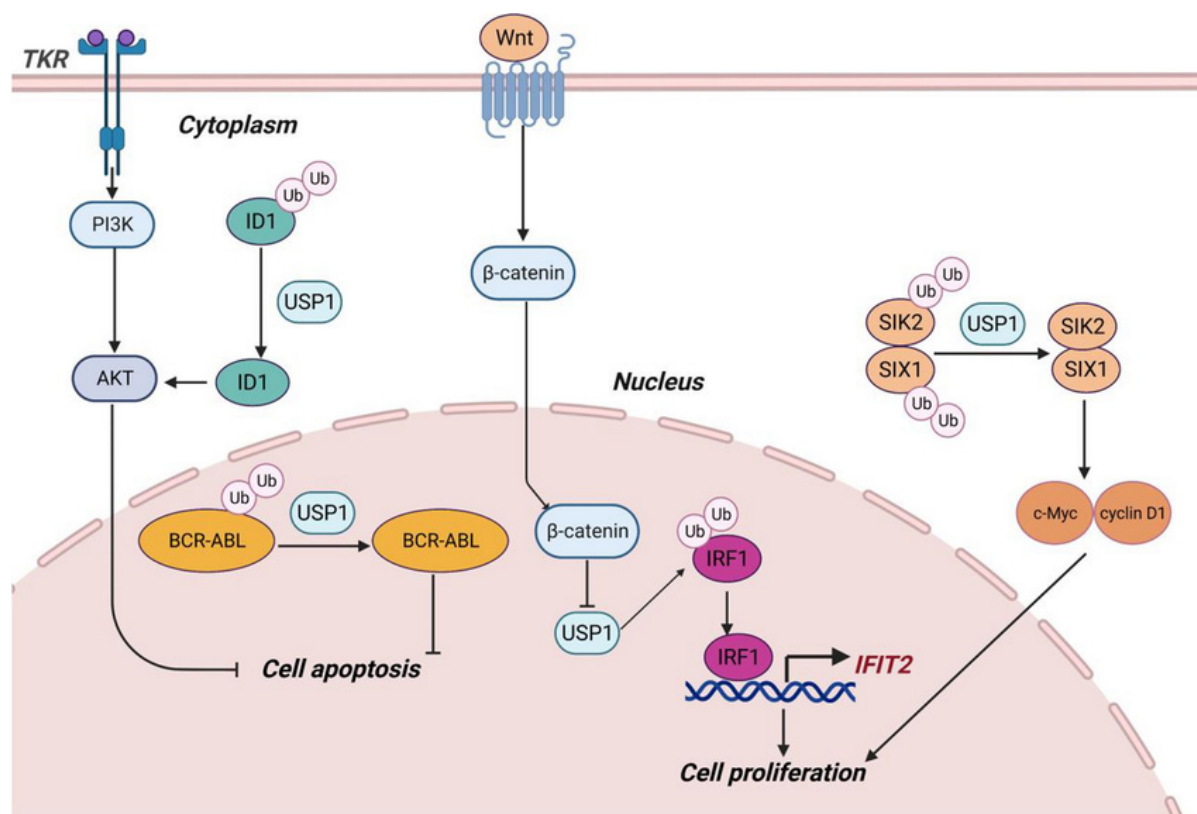
There is currently no cure for cancer, however, different cancer therapies have evolved to stop disease progression or shrink/remove tumors. Surgery, the first rational cancer treatment, is used for the removal of tumors surrounding cancerous tissue. In the beginning of the 20th century, radiation therapy emerged as a treatment for cancer through x-rays, and as time progressed, brachytherapy, proton beam therapy, and fast neutron therapy (Sonkin et al., 2024). Though radiation is cytotoxic towards cancerous cells, it must be implemented sparingly to minimize normal tissue radiation exposure. Chemotherapy is another form of cancer therapy which inhibits tumor growth and metastasis through disruption of DNA, RNA, and protein synthesis in rapidly proliferating cells (Amjad et al., 2023). Lastly, immunotherapy is designed to either enhance the host's immune system or to target a specific antigen on the tumor. By strengthening the host immune response, the immune system can more effectively recognize and eliminate malignant cells (Sahu & Suryawanshi, 2021).

Ubiquitin-Specific Protease 1 (USP1) is a deubiquitinating enzyme that removes ubiquitin from proteins which are involved in DNA repair or replication. Two crucial DNA repair pathways USP1 is involved in include the translesion synthesis (TLS) and Fanconi Anemia (FA) pathway (where interstrand crosslink (ICL) repair occurs) (Mazloumi et al., 2025). There, USP1 removes ubiquitin from PCNA and FANCD2-

FANCD1, respectively (Torrado et al., 2025). Without USP1, these proteins accumulate mass amounts of ubiquitin, which leads to defective DNA repair. Therefore, proper deubiquitination through USP1 is necessary for DNA repair completion and replication fork stability. Structurally, USP1 is a 7850amino-acid cysteine protease with a catalytic domain which contains Cys90, His593, and Asp752, which are responsible for its deubiquitinating activity. USP1 functions in complex with USP1-associated factor 1 (UAF1), a cofactor that enhances its catalytic activity and is required for efficient substrate deubiquitination (Lemonidis et al., 2022). Together, the two ensure regulation of ubiquitination levels during DNA replication and repair, allowing major repair pathways to be activated and reset whenever repair is completed (Kitamura, 2023).

USP1 is strongly linked to cancer due to many tumors' dependency on its DNA repair functions. Aside from BRCA1-deficient tumors, USP1 expression and activity are also increased in colorectal cancer, non-small cell lung cancer, gastric cancer, and platinum-resistant ovarian cancer. In this increased activity, USP1 promotes tumor growth and survival through stabilization of cancer-related proteins, as well as supports tumor progression by increasing cell proliferation and metastasis (Hu et al., 2025). Consequently, inhibition of USP1 has shown to result in suppression of cancer cell proliferation and epithelial-mesenchymal transition (EMT), a process that enables cancer cells to become more invasive and spread farther (Huang et al., 2022). Moreover, USP1 inhibition can disrupt multiple oncogenic signaling pathways that contribute towards tumor progression. So, by inhibiting USP1 in a cancer patient, not only can cancer cell proliferation be reduced, but cancer cell migration and survival. In BRCA1-deficient cancers, USP1 plays an especially important role due to the tumors lacking an effective homologous recombination repair pathway and therefore rely on alternative DNA repair mechanisms regulated by USP1. So, USP1 inhibition is a promising target for the development of new cancer therapies.

Figure 1. Models depicting the role of USP1 as a regulator for cancer cell fate through interactions with multiple substrates. Through deubiquitination of key substrates, USP1 regulates pathways including PI3K/AKT and Wnt/ β -catenin signaling, as well as transcriptional regulators such as ID1, BCR-ABL, SIX1, and IRF1. (TKR, tyrosine kinase receptor; PI3K, phosphoinositide 3-kinase; IRF1, interferon regulatory factor 1; IFIT2, interferon-induced protein with tetratricopeptide repeat 2; Ub, ubiquitin; SIK2, salt-inducible kinase 2; SIX1, sine oculis homeobox homolog 1) (12)



To therapeutically target USP1, researchers must determine the most effective type of drug molecule to inhibit its activity. In terms of size, USP1 is a relatively large protein enzyme, and it can be targeted by either large-molecule therapeutics or small-molecule ones. Though larger molecules have higher precision, as molecular weight of the molecule increases, its solubility in water or other fluids is decreased, making it more difficult for the patient's body to absorb and distribute the drug (Gu et al., 2023). Moreover, small molecules can selectively bind to specific cancer related targets, as seen in metarrestin, a small molecule which targets the perinucleolar compartment and inhibits cancer progression (Kashyap et al., 2024). With these benefits and its cheap production cost, small-molecule drugs have become a major component of modern targeted therapies, es-

pecially in cancer treatments (Pedre  ez et al., 2021).

Methodology

Geometric method

To obtain the model of USP1, the Protein Plus homepage must be opened up first by clicking the link, (<https://proteins.plus>). Once prompted for the PDB code, enter 7ZH4, which represents the structure of the USP1 inhibitor bound to FANCD2. After entering the code, launch DoGSiteScorer and keep all settings the same. This includes leaving analysis detail(s) to pockets, leaving chain set to all, and leaving the ligand section blank. Click the "calculate" option and soon after, and the 3d model should appear on the left of the screen with a table on the right. To show only all the high-druggability binding sites, make sure

to unhide the sites with a drug score over 0.5 (Ehrt et al., 2025).

Machine learning method

To obtain a model of USP1, first head to the Prank Web homepage by clicking the link, (<https://prankweb.cz>) Once clicked, the user should arrive at the homepage where the words, «Prank Web: Ligand Binding Site Prediction» are at the top. Select the Experimental Structure option and provide the PDB code, 7ZH4. Be sure to select “Use original structure” as well as “Use conservation” before submitting. After submitting, the 3d model would appear on the left of the screen and a table of binding sites on the right. To only see the most probable binding sites, hide the sites with scores that are much lower compared to other sites, such as the sites with scores 1.15, 1.01, and 0.99 (Jendele et al., 2019).

Pharmacophore interactions model

To obtain a three-dimensional model that only consists of the interactions between a ligand and certain binding sites, the user must visit the pharmit homepage, (<https://pharmit.csb.pitt.edu>). Upon entering the homepage, select the empty box on the right of “start from PDB,” enter “7ZH4,” and hit submit. After hitting submit, select the first, fourth, seventh, ninth, and fifteenth option under the pharmacophore section. Also, be sure that the other options not listed above are deselected. When the “on” signal is next to each of the selected options, scroll down to the visualization section. There, select the box on the right of “Ligand” and click “none.” After, select the box on the right of “Receptor” and click “none.” Drag the slider of the “Receptor Surface Opacity” all the way to the left and you should have the desired results. For the additional compounds from other unique yet significant sets of interactions, feel free to select the third, fifth, tenth, eleventh, and thirteenth options under the pharmacophore sections. Another good set of pairings can be achieved by selecting the second, sixth, ninth, eleventh, and thirteenth options (Sunseri & Koes, 2016).

Overlaying the compounds with pharmacophore

After isolating the pharmacophore on pharmit, click on the top left button, “Search

MolPort.” After a couple seconds, a long list of results should appear on the right of the screen with different compounds from the library. To view the overlay, click on the name of the compound. For other chemical libraries, such as Enamine and MCULE-ULTIMATE, select the downward arrow on the right of “Search Molport” and click on the respective options.

Finding spontaneous interactions

To obtain SMILES of a compound, go to the website of your desired compound and input the compound name into the search bar. For the results in this paper, visit (<https://enaminestore.com/> for the Enamine library, <https://www.molport.com/shop/index>) for the MolPort library, or (<https://ultimateapp.mculc.com/search>) for the MCULE-ULTIMATE Library. Copy and paste the SMILES into the swissdock website (<https://www.swissdock.ch>). Once the SMILES is provided, enter “Prepare Ligand.” Make sure that a green checkmark appears next to the box after a couple seconds. Scroll down and enter “7ZH4” for the PDB code, then select “Select All” for the “Choose chain(s) to keep” section and tick the box next to “None” for the “Choose heteroatom(s) to keep” section. Select “Prepare target” and wait for a green checkmark to appear next to the box. Scroll further down to the “Define search space” section and put 40, 40, and 30 respectively for the three parameters in “Box size.” Be sure to leave the box center parameters as is. Next, scroll down to “Select Parameters.” Leave the number of RIC as is and hit the “Check parameters” box. Once a time is returned, start docking. After a bit of time, the results should be returned and a table will show, with SwissParam and AC values. Choose the most negative SwissParam value by selecting it. After selection, the three-dimensional model should update (Bugnon et al., 2024; Röhrig et al., 2023).

Evaluating absorbability, distributability, and excretability

To evaluate whether a compound fulfills Lipinski’s rule, use (<https://www.swissadme.ch>). First, enter the SMILES of the compound in the empty box on the right and click the red “Run!” button below it. Wait for the website

to load, and scroll to the bottom of the page. There, focus on the following sections: molecular weight, number of H-bond acceptors, number of H-bond donors, and Consensus Log P. If the compound's molecular weight is <500 g/mol, it has no more than 10 hydrogen bond acceptors, no more than 5 hydrogen bond donors, and a Log P value no more than 5, then the compound fulfills Lipinski's Rule. To easily check this, look at how many violations the compound has in the Lipinski section. If the compound has more than 0 violations, it does not fulfill the rule (Daina et al., 2017).

Evaluating toxicity

To evaluate the level of toxicity in a compound, first head to the Protox3 homepage, https://tox.charite.de/protox3/index.php?site=compound_search_similarity. Select the second box with "Tox prediction," and enter the SMILES of the compound in the "Canonical Smiles" section. Scroll down and select the "All" button on the bottom right. Once all the boxes are ticked, select "Start Tox-Prediction" (Banerjee et al., 2024).

Results and Discussion

Figure 2 and Table 1 are both generated through a geometric method; while the figure characterizes the binding sites visually, the table does so quantitatively. Geometric

methods generally detect surface concavities and clefts, which helps it stay unbiased when locating for possible druggable regions. In Figure 2, six different colored regions are marked, which represent the different possible binding sites. In Table 1, quantitative data is provided, including: surface area, volume, and drug score. Pockets P_0 and P_1 have higher drug scores, so will therefore be more favorable for ligand binding. Lower scoring sites such as P_3 and P_5 indicate less favorable binding sites, but can still serve as potential secondary sites.

Figure 2. Predicted binding sites of USP1 identified using geometric methods. Colored sections represent pockets with the highest drug scores, indicating that those areas are more favorable for the binding of drug-like molecules



Table 1. Predicted binding pockets for USP1, including pocket surface area, volume, color designation for visualization, and drug score

Name	Color	Surface Area (Å ²)	Volume (Å ³)	Drug Score
P_0	Orange	1309.51	919.94	0.84
P_1	Purple	878.09	645.5	0.84
P_2	Green	381.12	359.94	0.71
P_4	Blue	229.39	242.56	0.64
P_3	Red	354.1	252.35	0.59
P_5	Yellow	383.81	229.95	0.58

Figures 3A and 3B, along with Table 2, were all generated through machine learning methods. Due to its rapid analysis of protein structures through a computational approach, machine learning methods for analyzing binding sites account for all circumstances, even mutations. In Figures 3A and 3B, the algorithm predicted and visualized the binding sites with varying levels of confidence. In Table 2, it pro-

vides the prediction scores and residue counts for each site. The score reflects the likelihood that a site can bind to drug-like molecules; the higher the score, the higher the likelihood. The residue numbers represent the amino acids that line each binding site and participate in the various interactions between the ligands. A greater amount of residues can also provide additional opportunities for molecular interactions.

Figure 3. (A) Predicted binding sites of USP1 identified using machine learning methods. Colored sections represent pockets with the highest ranks, indicating that those areas are more favorable for the binding of drug-like molecules. This figure contains a semi-transparent molecular surface to allow for users to see the inner molecule (B) Showing only the outer surfaces of the molecule, three of the four high ranked binding sites are shown

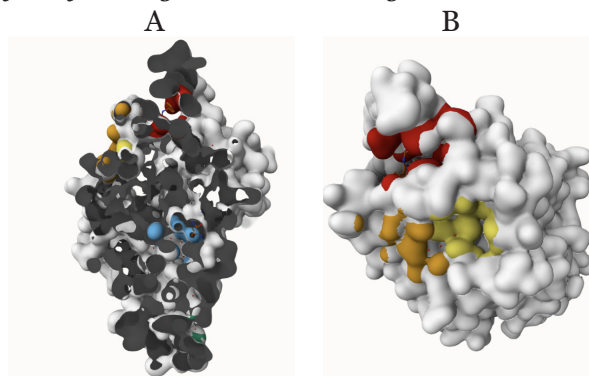


Table 2. Predicted ligand-binding pockets identified in USP1, including rank visualization color, prediction score, and number of residues in each binding site

Name	Rank	Color	Score	# Of Residues
B_0	1	Red	9.01	16
B_1	2	Yellow	5.75	11
B_2	3	Orange	3.28	9
B_3	4	Blue	3.05	10

Figure 4. (A) Three-dimensional pharmacophore model where purple represents aromatic features, yellow represents a hydrogen acceptor, and green represents hydrophobic features. This figure contains a close set of interactions with two aromatic interactions, two hydrophobic features, and one hydrogen acceptor. (B) This figure contains two aromatic interactions, two hydrogen acceptors, and one hydrophobic feature. (C) This figure contains slightly more dispersed features, with two aromatic interactions, two hydrogen acceptors, and one hydrophobic feature

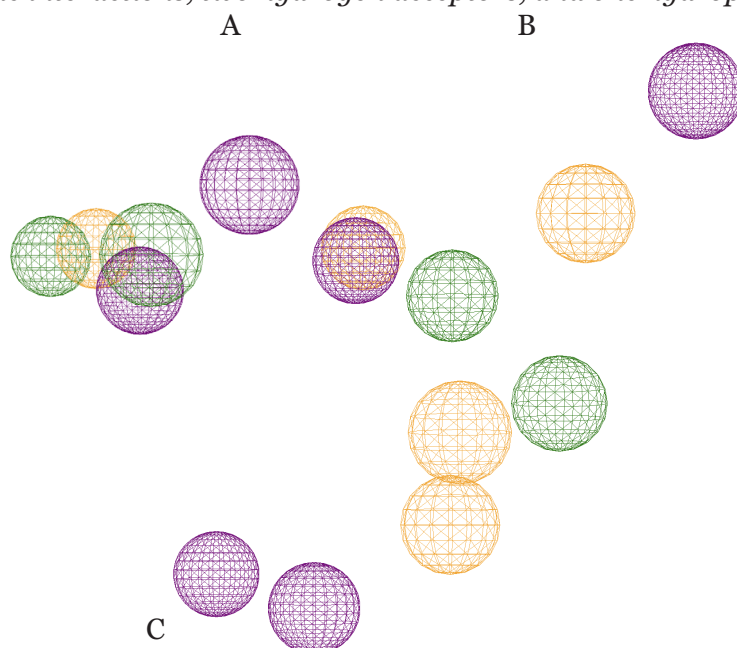
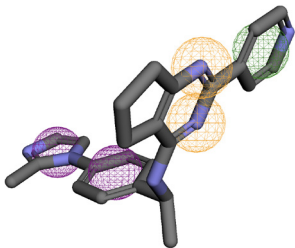
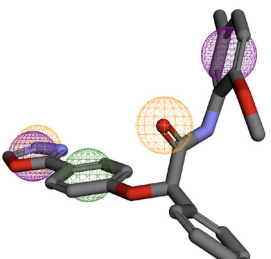
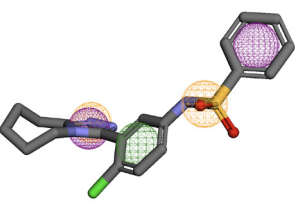
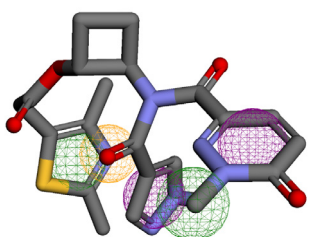
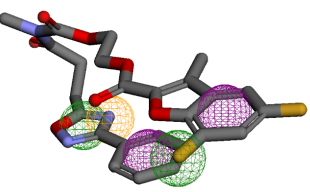
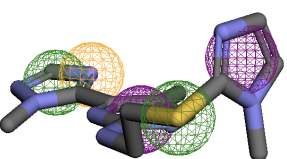
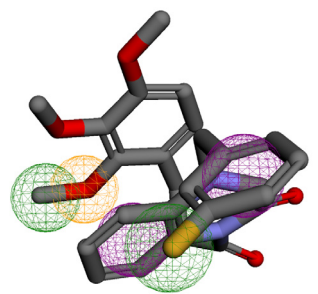
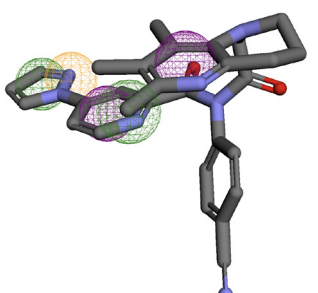
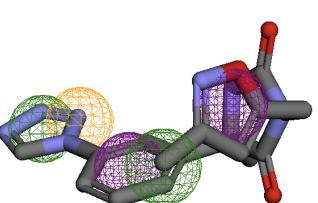
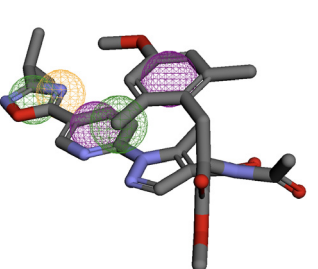
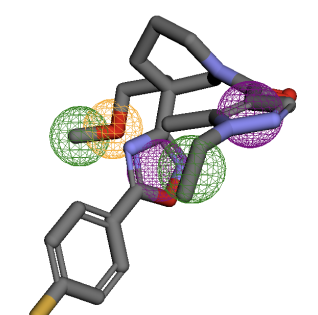
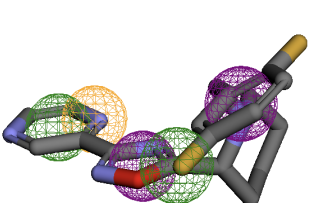
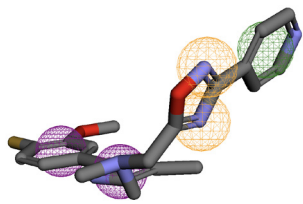
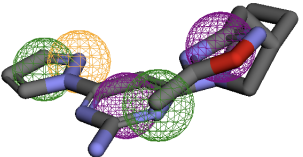
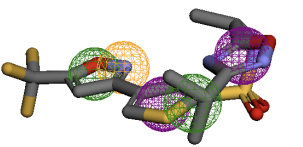
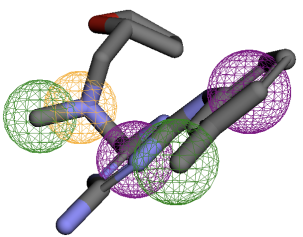
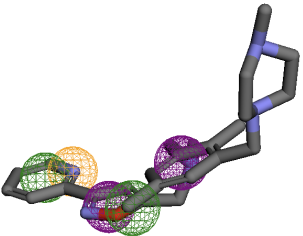
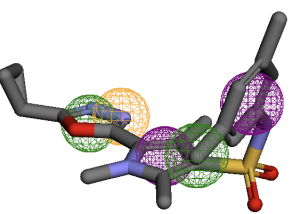
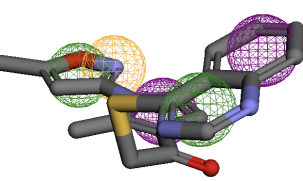


Table 3. *Overlays between the pharmacophore and nineteen different compounds drawn from three chemical libraries: MCULE-ULTIMATE, Enamine, and MolPort. Each compound is selected based on low root mean square deviation (RMSD) and all compounds selected fall under 0.2 RMSD, which means that the compound has a closer alignment with the pharmacophore model*

Overlay Between Pharmacophore and Compound	Name of Compound	Rmsd	Chemical Library	Figure #
	Z1359373132	0.100	Enamine	Figure 4C
	Z54926392	0.103	Enamine	Figure 4B
	Z299686082	0.118	Enamine	Figure 4B
	SKQBPFUHHTWY-DZ-UHFFFAOYSA-N	0.123	MCULE-ULTIMATE	Figure 4A
	FUNUR-ESQIKQOAP-UHFFFAOYSA-N	0.128	MCULE-ULTIMATE	Figure 4A
	MolPort-005-108-116	0.132	MolPort	Figure 4A

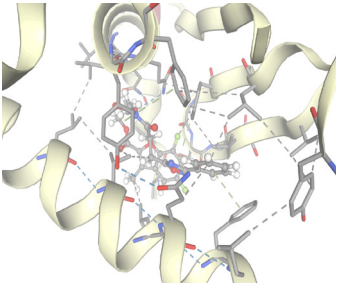
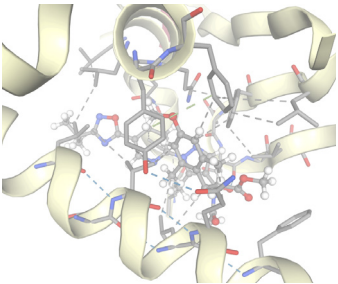
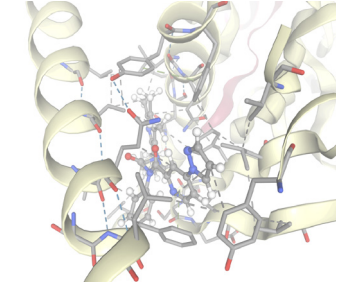
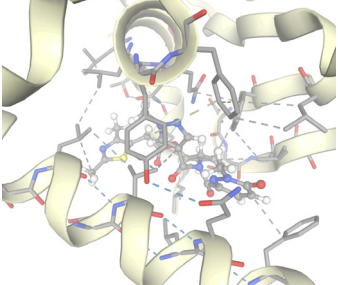
Overlay Between Pharmacophore and Compound	Name of Compound	Rmsd	Chemical Library	Figure #
	VIBCQN- PAWUBELN-UHFFF- AOYSA-N	0.133	MCULE-UL- TIMATE	Figure 4A
	HUNPSCXefd- CREC-UHFFFAOYSA-N	0.133	MCULE-UL- TIMATE	Figure 4A
	Z134661322	0.134	Enamine	Figure 4A
	BUDVOCFTH- VHXBT-UHFFFAOY- SA-N	0.138	MCULE-UL- TIMATE	Figure 4A
	MolPort-046-059-419	0.147	MolPort	Figure 4A
	MolPort-010-950-419	0.169	MolPort	Figure 4A

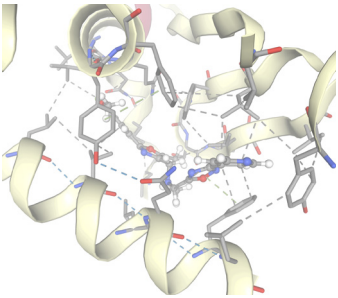
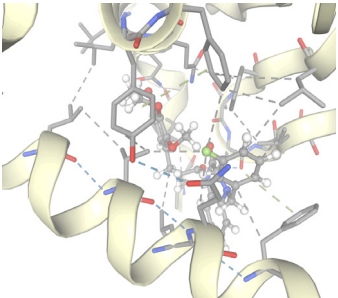
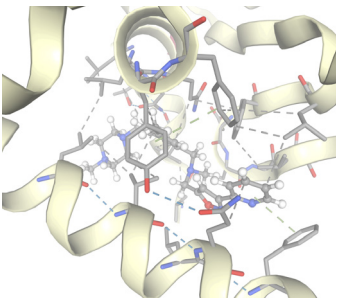
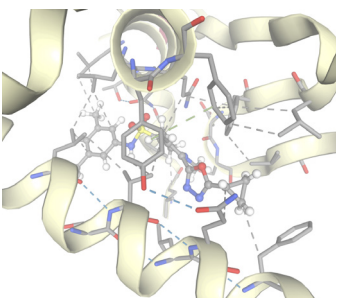
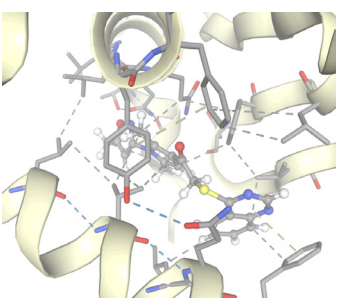
Overlay Between Pharmacophore and Compound	Name of Compound	Rmsd	Chemical Library	Figure #
	MolPort-005-163-256	0.170	MolPort	Figure 4C
	Z1618187599	0.185	Enamine	Figure 4A
	MolPort-044-685-945	0.186	MolPort	Figure 4A
	MolPort-044-710-584	0.193	MolPort	Figure 4A
	MolPort-045-965-232	0.193	MolPort	Figure 4A
	MolPort-010-912-491	0.196	MolPort	Figure 4A
	Z19316285	0.196	Enamine	Figure 4A

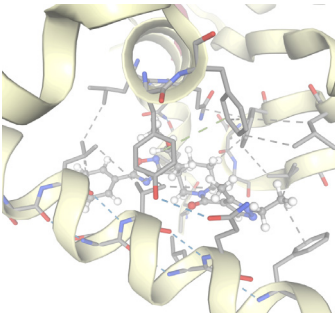
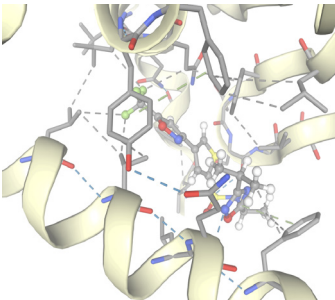
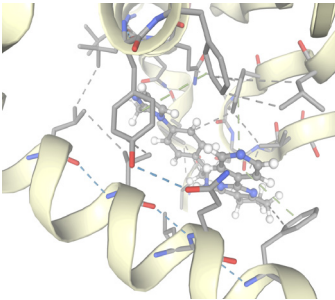
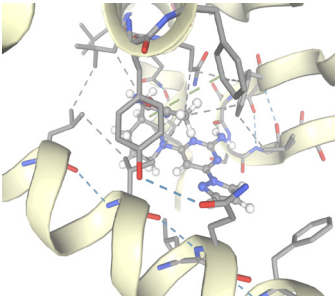
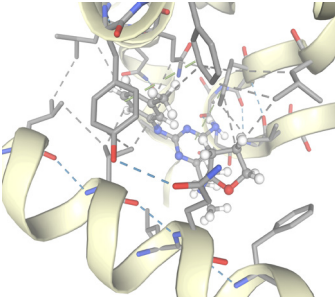
With the unique sets of interactions from Figure 4, Table 3 consists of potential compounds that fulfill the unique pharmacophore maps. The RMSD value indicates the severity of deviation, where the closer the RMSD value is to 0, the less deviation the

compound has with its set of pharmacophores. The RMSD values are arranged in ascending order, therefore the compounds with the least deviation are sorted to the top, and increasingly more deviated compounds are towards the lower end of the table.

Table 4. Nineteen compounds taken to evaluate spontaneity by a method of attracting cavities. For each compound, the SwissParam value of the most spontaneous cluster was taken, as well as a three dimensional protein- ligand model of the hydrogen bonds (blue), ionic interactions (yellow), cation interactions (orange), hydrophobic contacts (gray), and π -stacking interactions (white). The table is sorted from most spontaneous (top) to least spontaneous (bottom)

Name of Compound	Spontaneity/ Swissparam	Structural Model
FUNURESQIKQOAP-UHFFFAOY- SA-N	−9.5550	
BUDVOCFTHVHXB-T-UHFFFAOY- SA-N	−9.5074	
HUNPSCXEFDCREC-UHFFFAOY- SA-N	−9.4277	
SKQBPFUHTWYDZ-UHFFFAOY- SA-N	−9.4168	

Name of Compound	Spontaneity/ Swissparam	Structural Model
MolPort-005-163-256	−9.0517	
VIBCQNPAWUBELN-UHFFFAOY- SA-N	−9.0194	
MolPort-045-965-232	−8.9489	
MolPort-010-912-491	−8.8943	
Z19316285	−8.8594	

Name of Compound	Spontaneity/ Swissparam	Structural Model
MolPort-046-059-419	−8.8570	
MolPort-044-685-945	−8.7693	
Z1359373132	−8.6988	
Z1618187599	−8.6578	
MolPort-044-710-584	−8.5538	

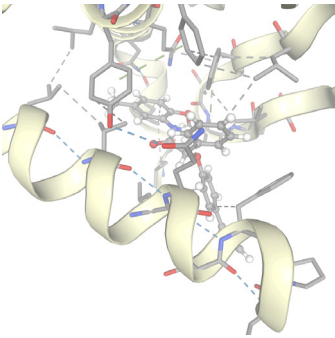
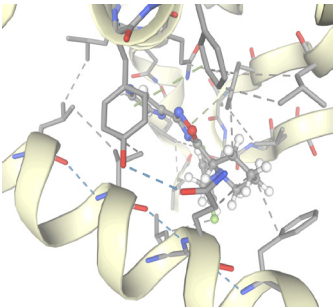
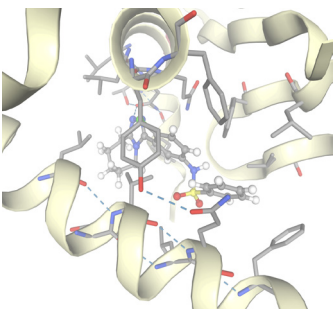
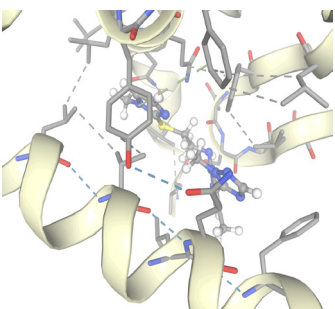
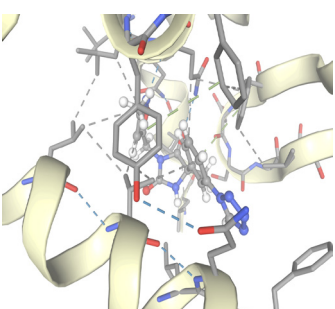
Name of Compound	Spontaneity/ Swissparam	Structural Model
Z54926392	−8.5050	
MolPort-010–950–419	−8.4912	
Z299686082	−8.3999	
MolPort-005–108–116	−8.3253	
Z134661322	−8.2139	

Table 5. Eight compounds with its molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, average log p value, and solubility class taken into account. These factors are used to determine whether it fulfills Lipinski's rule; If a compound does in fact fulfill Lipinski's rule, it is a compound that can be successfully absorbed, distributed and excreted in the human body

Compound Name	Molec. Weight (G/Mol)	# Of Hy- drogen Bond Ac- ceptors	# Of Hy- drogen Bond Donors	Log P Value	Solubility Class	Lip- inski's Rule Applies
FUNUR- ESQIKQOAP-UHFF- FAOYSA-N	513.45	11	0	4.25	Moderately soluble	No
BUDVOCFTH- VHXB-T-UHFFF- AOYSA-N	574.63	10	0	3.62	Moderately soluble	No
HUNPSCXEF- CREC-UHFFFAOY- SA-N	505.57	6	0	2.99	Moderately soluble	No
Mol- Port-010-912-491	400.49	5	1	2.45	Moderately soluble	Yes
VIBCQN- PAWUBELN-UHFF- FAOYSA-N	478.51	6	0	3.98	Moderately soluble	Yes
Z19316285	378.45	5	0	2.56	Moderately soluble	Yes
Z1618187599	340.38	6	1	0.74	Soluble	Yes
Mol- Port-046-059-419	441.50	7	0	2.99	Moderately soluble	Yes

From Table 9, three compounds do not fulfill Lipinski's rule while five others do. Lipinski's rule relies mainly on four categories: the molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, and the average LogP value. To fulfill the weight category, the compound must be under 500 Da; To fulfill the LogP category the compound's value must be under 5; To fulfill the hydrogen bond donors (HBDs) category, the compound must contain less than or equal to five hydrogen bond donors; Finally, to fulfill the hydrogen bond acceptors (HBAs) category, the compound must contain less than or equal to ten hydrogen bond acceptors. Based on the data in the table, the compounds that do not pass Lipinski's Rule of Five are from MCULE-ULTIMATE. Generally, compounds that violate two or more of Lipinski's rule are statistically more likely to have poor membrane permeability. However,

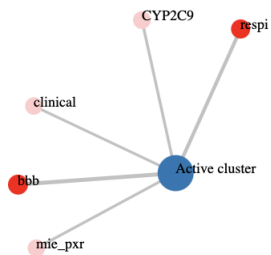
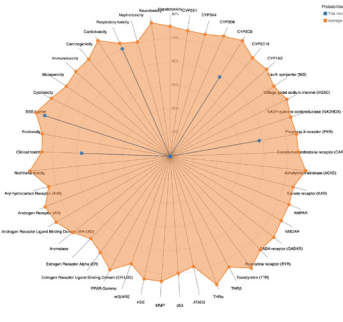
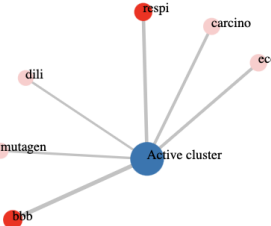
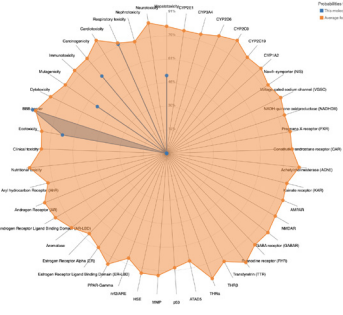
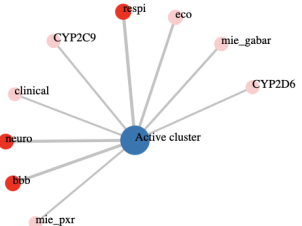
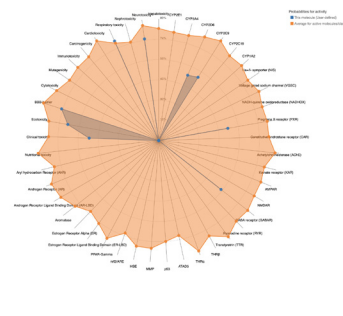
in the table, as long as the compound violates one category, it is considered to fail the Lipinski's Rule.

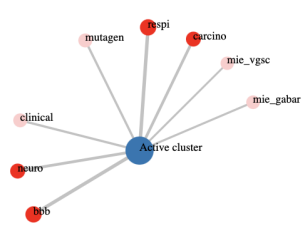
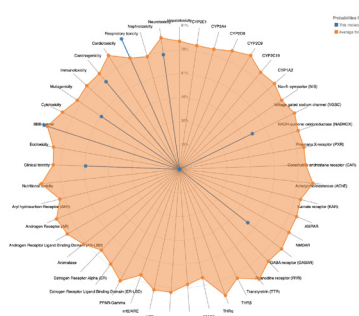
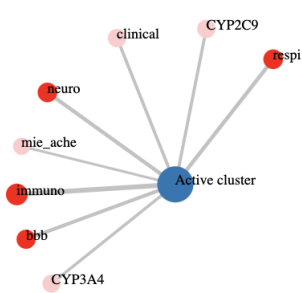
Out of the five compounds taken for evaluation of toxicity in Table 10, only one compound, MolPort-010-912-491, has a probability where all its main activities were less than average for its toxicity class. Three other compounds, Z1618187599, MolPort-046-059-419, and Z19316285 all have one average toxicity level slightly over the average. Though it is over average, the three compounds still pass as possible compounds that can be used for future medicinal developments, only with a limitation for the activity that it is over average for. VIBCQN-PAWUBELN-UHFFFAOYSA-N cannot be used at all since it has two activities where it is way above average for its respective toxicity class. This is illustrated in the toxicity radar chart, where the large orange-shaded region

symbolizes the average toxicity of its class for a certain activity, and the blue dots with gray lines symbolize the toxicity of the compound for the given activities. For some compounds, there are gray regions which represent the toxicity for the compound, and only occurs when the activities of the compound are adjacent in the chart. This includes respiratory toxicity, BBB-barrier, clinical toxicity, CYP2C9, and pregnane X receptor. If one of the compound's main activities were higher than average, then the compound is highly toxic for that activity. For the network chart in the

table, the lighter red colors activities indicate that the probability for that activity is <0.7 while the brighter red colors indicate a probability of >=0.7. There are only six toxicity classes, 1,2,3,4,5, and 6. If a compound's toxicity class is 3 and higher, then it is considered a safe compound for drug usage. For Table 10, all compounds are above toxicity class 3. All chemicals should have an LD50 of over 300mg/kg; if it does not go beyond that threshold, it is classified as much more toxic due to its ability to cause significant harm, or even death, in small amounts.

Table 6. Five compounds that passed Lipinski's Rule of Five taken to evaluate level of toxicity. Factors taken into account include the Lethal Dose 50% (LD50), which represents the single dose required to kill 50% of the population; toxicity class, a numerical class represented from 1–6; network chart of the active cluster, which ties the compound to potential toxicity targets; the toxicity radar chart, which illustrates the confidence of positive toxicity compared to the average of its class

Name	Ld50 (Mg/ Kg)	Tox- icity Class	Network Chart of Active Cluster	Toxicity Radar Chart
Mol- Port-010–912–491	550	4		
Z19316285	1000	4		
Mol- Port-046–059–419	1250	4		

Name	Ld50 (Mg/Kg)	Toxicity Class	Network Chart of Active Cluster	Toxicity Radar Chart
Z1618187599	3000	5		
VIBCQN-PAWUBELN-UHFF-FAOYSA-N	6000	6		

Conclusion

Inhibition of USP1 will disrupt DNA repair processes and increase the cancer cell susceptibility to DNA damage. To identify potential compounds capable of binding USP1, a combination of structural, machine learning, pharmacophore, and virtual screening approaches was used to characterize potential binding sites and prioritize candidate molecules. Geometric analysis identified six potential druggable binding pockets, with P_0 and P_1 having the highest drug scores of 0.84, while machine learning analysis identified four predicted binding sites, with B_0 receiving the highest prediction score of 9.01. These results provided multiple potential regions for ligand binding and guided the development of three pharmacophore models containing combinations of aromatic, hydrophobic, and hydrogen-bonding interactions. Screening of the Enamine, MolPort, and MCULE-ULTIMATE libraries identified 19 compounds with RMSD values below 0.2, with Z1359373132 showing the lowest RMSD of 0.100. SwissDock analysis then evaluated the predicted spontaneity of these compounds, with FUNURESQIKQOAP-UHFF-

FAOYSA-N producing the most negative SwissParam value of -9.5550 .

Further screening based on drug-likeness and toxicity identified MolPort-010-912-491 as the lead compound and Z1618187599 as the backup compound. MolPort-010-912-491 had an RMSD of 0.196 and a SwissParam value of -8.8943 , with a molecular weight of 400.49 g/mol, five hydrogen-bond acceptors, one hydrogen-bond donor, and a LogP of 2.4, allowing it to fulfill Lipinski's Rule of Five. It also had an LD50 of 550 mg/kg and toxicity class 4, with all of its main predicted toxicity activities below the average for its toxicity class. Z1618187599 had an RMSD of 0.185 and a SwissParam value of -8.6578 , along with a molecular weight of 340.38 g/mol, six hydrogen-bond acceptors, one hydrogen-bond donor, and a LogP of 0.74. It also has a LD50 of 3000 mg/kg and toxicity class 5. Although other compounds, including Z19316285 and MolPort-046-059-419, also fulfilled Lipinski's Rule of Five, MolPort-010-912-491 was the strongest overall candidate because it was the only compound whose main predicted toxicity activities were all below the average of its toxicity class.

Despite these favorable computational results, this study has several limitations. The virtual screening process evaluated only the compounds that were selected through the pharmacophore and docking workflow, meaning that additional potential USP1 inhibitors may exist among the larger chemical libraries that were not investigated. Furthermore, the ADME and toxicity results were predictions and cannot fully represent how these compounds will behave in a living organism. The toxicity radar charts also showed that some compounds had individual pre-

dicted activities above the average for their respective toxicity classes, demonstrating that additional safety concerns may remain even among compounds with favorable overall toxicity classifications. Future research should therefore screen a larger number of compounds and experimentally validate the lead and backup candidates. Overall, this study successfully narrowed thousands of possible small molecules to two promising USP1 inhibitor candidates, providing a computational starting point for the development of new targeted cancer therapies.

References

- Amjad MT, Chidharla A, Kasi A. (2023). Cancer Chemotherapy. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/33232037>
- Banerjee, P., Kemmler, E., Dunkel, M., & Preissner, R. (2024). ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic acids research*, – 52(W1). – W513–W520. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/38647086>
- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, – 74(3). – P. 229–263. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/38572751>
- Bugnon, M., Röhrig, U. F., Goullieux, M., Perez, M. A. S., Daina, A., Michielin, O., & Zoete, V. (2024). SwissDock 2024: major enhancements for small-molecule docking with Attracting Cavities and AutoDock Vina. *Nucleic acids research*, – 52(W1). – W324–W332. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/38686803>
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, – 7. – 42717 p. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/28256516>
- Ehrt, C., Schulze, T., Graef, J., Diedrich, K., Pletzer-Zelgert, J., & Rarey, M. (2025). Pro-teinsPlus: a publicly available resource for protein structure mining. *Nucleic acids re-search*, – 53(W1). – W478–W484. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/40326518>
- Gu, M., Sun, S., You, Q., & Wang, L. (2023). Forward or Backward: Lessons Learned from Small Molecule Drugs Approved by FDA from 2012 to 2022. *Molecules (Basel, Switzerland)*, – 28(24). – 7941 p. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/38138431>
- Hu, X., Wu, Y., Yao, M., Chen, Z., & Li, Q. (2025). The other side of the coin: protein deu-biquitination by Ubiquitin-Specific Protease 1 in cancer progression and therapy. *Future medicinal chemistry*, – 17(3). – P. 329–345. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/39819213>
- Huang, M. L., Shen, G. T., & Li, N. L. (2022). Emerging potential of ubiquitin-specific prote-ases and ubiquitin-specific proteases inhibitors in breast cancer treatment. *World journal of clinical cases*, – 10(32). – P. 11690–11701. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/36405275>
- Huang, P., Wang, Y., Zhang, P., & Li, Q. (2023). Ubiquitin-specific peptidase 1: assessing its role in cancer therapy. *Clinical and experimental medicine*, – 23(7). – P. 2953–2966. Re-trieved from URL: <https://pubmed.ncbi.nlm.nih.gov/37093451>

- Jendele, L., Krivak, R., Skoda, P., Novotny, M., & Hoksza, D. (2019). PrankWeb: a web server for ligand binding site prediction and visualization. *Nucleic acids research*, – 47(W1). – W345–W349. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/31114880>
- Kashyap, V. K., Sharma, B. P., Pandey, D., Singh, A. K., Peasah-Darkwah, G., Singh, B., Roy, K. K., Yallapu, M. M., & Chauhan, S. C. (2024). Small Molecule with Big Impact: Metarrestin Targets the Perinucleolar Compartment in Cancer Metastasis. *Cells*, – 13(24). – 2053 p. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/39768145>
- Kitamura H. (2023). Ubiquitin-Specific Proteases (USPs) and Metabolic Disorders. *International journal of molecular sciences*, – 24(4). – 3219 p. Retrieved from URL: <https://pubmed.ncbi.nlm.nih.gov/36834633>
- Lemonidis, K., Arkinson, C., Rennie, M. L., & Walden, H. (2022). Mechanism, specificity, and function of FANCD2-FANCI ubiquitination and deubiquitination. *The FEBS journal*, 289(16), 4811–4829. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/34137174>
- Mazloumi Aboukheili, A. M., & Walden, H. (2025). USP1 in regulation of DNA repair pathways. *DNA repair*, 146, 103807. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/39848025>
- Pedreñeñez, A., Mosquera-Sulbarán, J., Muñoz, N., Tene, D., & Robalino, J. (2021). Nanoantibodies: small molecules, big possibilities. *Biotechnology*, 102(3), 321–336. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/36606147>
- Röhrig, U. F., Goullieux, M., Bugnon, M., & Zoete, V. (2023). Attracting Cavities 2.0: Improving the Flexibility and Robustness for Small-Molecule Docking. *Journal of chemical information and modeling*, 63(12), 3925–3940. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/37285197>
- Sahu M, Suryawanshi H. (2021). Immunotherapy: The future of cancer treatment. *J Oral Maxillofac Pathol*, 25(2), 371. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/34703141>
- Siegel RL, Kratzer TB, Wagle NS, Sung H, Jemal A. (2026). Cancer statistics, 2026. *CA Cancer J Clin*, 76(1), e7004. Retrieved from <https://pmc.ncbi.nlm.nih.gov/articles/PMC12798275>
- Sonkin, D., Thomas, A., & Teicher, B. A. (2024). Cancer treatments: Past, present, and future. *Cancer genetics*, 286–287, 18–24. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/38909530>
- Sunseri, J., & Koes, D. R. (2016). Pharmit: interactive exploration of chemical space. *Nucleic acids research*, 44(W1), W442–W448. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/27095195>
- Torrado, C., Ashton, N. W., D’Andrea, A. D., & Yap, T. A. (2025). USP1 inhibition: A journey from target discovery to clinical translation. *Pharmacology & therapeutics*, 271, 108865. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/40274197>

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COMPUTATIONAL IDENTIFICATION OF HDAC6-TARGETED INHIBITOR CANDIDATES FOR CANCER THERAPY

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Abstract

Cancer continues to impact millions of people worldwide and incur significant economic costs despite advances in modern therapeutics. Precision medicine offers a promising approach to overcome the harmful off-target impacts on healthy tissue of conventional therapies. In particular, HDAC6 is an attractive therapeutic target due to its pivotal role in facilitating tumor growth. This study employs a comprehensive computational framework to identify potential HDAC6 inhibitor candidates to accelerate early-stage drug discovery. First, binding sites were identified within the HDAC6 structure to determine its suitability for binding with small molecules. Then, pharmacophore modeling identified molecular features required for favorable interactions with the HDAC6 binding pocket. The resulting compounds underwent molecular docking to determine their predicted binding affinities. Then, the pharmacokinetic properties and toxicity of the remaining compounds were analyzed to identify the compounds that best balanced favorable interactions with the HDAC6 binding site, suitable pharmacokinetic properties, and minimal predicted safety liabilities. Based upon this workflow, Molport-023-219-174 was determined to be the lead compound and Molport-035-800-274 was a promising backup compound. The integration of these computational processes streamlined the early-stage drug discovery pipeline, enabling the efficient identification of promising HDAC6 inhibitors and providing a foundation for drug-discovery workflows.

Keywords: Drug discovery, HDAC6, cancer therapies, virtual screening, molecular docking

Introduction

Cancer is one of the most devastating global health challenges, accounting for approximately 19.3 million new cases and nearly 10 million deaths worldwide in 2020 (Sung et al., 2021). In fact, by 2050, global cancer diagnoses are projected to increase to 30.5 million and deaths will increase to 18.6 million (GBD 2023 Cancer Collaborators, 2025),

demonstrating the inherent danger of cancer despite advancements in diagnostic procedures and therapeutics. Additionally, cancer incurs significant economic costs with the global economic cost from 2020 to 2050 estimated to be approximately US \$25.2 trillion due to factors including healthcare expenses, productivity losses, and premature mortality (Chen et al., 2023). Beyond its clinical im-

pact, cancer significantly burdens healthcare systems, patients, families, and economies, highlighting the urgent need for effective therapeutics to mitigate its impact.

Researchers have attempted to find effective cancer therapies, contributing to the wide array of modern treatments available. Currently, the most pervasive cancer therapies include surgery, chemotherapy, radiation therapy, and hormonal therapy; while each of these treatment options has demonstrated clinical efficacy in certain cancer types, stages, and conditions, they are often limited by factors such as drug resistance, treatment-related toxicity, limited precision, and reduced long-term efficacy (Kouidhi et al., 2024). A prevalent concern about current cancer therapies, especially radiation and hormone therapy, is that they frequently suffer from poor targeting precision, resulting in damage to healthy tissue and hindering patient recovery (Zafar et al., 2025). In particular, chemotherapy has empirically constituted negative side-effects such as damage to healthy tissues and suppression of healthy tissue due to the non-discriminate nature of its treatment mechanism (Zafar et al., 2025). Overall, while current treatment methods have significantly decreased the mortality rate of cancer, their off-target effects often complicate treatment and negatively impact patients' health.

Due to these clinical complications, researchers have invested significant time and resources into the field of precision medicine in an attempt to discover therapeutics that selectively target malignant cells. Targeted therapies have received attention in academia due to their potential to improve treatment efficacy while minimizing damage to healthy cells (Meslamani, 2023). For example, the ROME trial revealed that initially conducting Comprehensive Genomic Profiling (CGP) in patients helped identify an effective drug that specifically targeted genetic mutations perpetuating the spread of cancer (Marchetti et al., 2025). Precision medicine adopts a paradigm in which cancer is not solely evaluated by the tissue where it originates but also based on molecular abnormalities (Tsimberidou et al., 2020). This approach ensures that cancer therapies directly interfere with biological pathways causing tumor growth rather than unnecessarily impacting surrounding tissue.

Within the field of precision medicine, there has been particular interest in inhibiting the enzyme HDAC6. HDAC6 is a histone deacetylase enzyme located in the cytoplasm, characterized by its ability to deacetylate several non-histone proteins involved in cell signaling, protein degradation, and cytoskeletal organization (Chen and Zhang, 2026). HDAC6 has been shown to promote cancer cell growth by deacetylating key targets such as HSP90, thereby maintaining the growth and function of cancer-causing proteins (Chen and Zhang, 2026). Additionally, HDAC6 deacetylates structural proteins such as α -Tubulin and Cortactin, significantly modifying cellular adhesion and mobility, increasing the risk of cancer growth and metastasis (Huang et al., 2024). Due to these properties of HDAC6, researchers have sought methods to inhibit it and consequently hinder its cancer-growth properties. Several investigational compounds have emerged to inhibit HDAC6, including Ricolinostat and SW-100; however, a common limitation with these therapies is that they may also inadvertently target other HDAC isoforms, such as HDAC8 (Ho et al., 2020). Candidates that inadvertently target other HDAC isoforms have been associated with adverse medical consequences, including thrombocytopenia and neutropenia (Subramanian et al., 2010). As a result of these off-target impacts, current inhibitors also have limited therapeutic windows, as higher doses risk severe bodily harm (Ho et al., 2020). Despite the discovery of several potential therapies, they face many limitations and require further research to address. It is necessary for researchers to continue developing HDAC6 inhibitors that effectively suppress tumor-promoting pathways while minimizing off-target interactions.

Therefore, the aim of the study was to find suitable molecular candidates that could inhibit HDAC6. This was achieved through a sequential computational screening workflow that integrated binding-site analysis, pharmacophore-based virtual screening, molecular docking, Absorption, Distribution, Metabolism, and Excretion (ADME) prediction, and toxicity assessments. Candidate compounds were progressively prioritized through each stage of experimentation, ultimately resulting in a lead and backup com-

pound based upon predicted binding affinity, drug-like properties, and safety profiles.

Materials and methods

Identification of HDAC6 Binding Sites Via Geometric and Machine Learning Analysis

The process of finding those candidates began with an analysis of the binding sites of HDAC6 in order to determine which types of small molecules would contain the strongest binding potential. Several computational methods were used to identify binding sites within the HDAC6 protein, including a geometric analysis and a machine learning analysis. First, the geometric analysis was conducted through the DoGSiteScorer, a computational tool that identifies and characterizes potential ligand-binding pockets on a protein's three-dimensional structure and evaluates their druggability based on geometric and physicochemical properties. The PDB ID 6V79 representing the crystal structure of the HDAC6 catalytic domain was used to render a visual representation of the protein and its binding sites.

Within the DoGSite Scorer, the ligand QQD, a known inhibitor of HDAC6, was used as a reference to identify a binding site where QQD and other potential inhibitors could have favorable interactions with the protein. DoGSiteScorer generated predicted binding pockets and reported quantitative descriptors including pocket volume, surface area, and druggability score. Additionally, to better assess the viability of the HDAC6 binding sites, a machine learning analysis was conducted using the Prankweb platform. Prankweb analyzes the structural and physicochemical properties of proteins and compares them to hundreds of protein-ligand combinations to identify regions that are most likely to bind small molecules. From Prankweb, a visual representation of binding sites was collected, along with other data points that ranked the binding sites' viability, such as a drug score and number of residues.

Pharmacophore-based Virtual Screening of the Molport Compound Library

After confirming that the HDAC6 protein contains binding sites suitable for small

molecules, a compound library was screened against pharmacophore models, maps of essential chemical features a molecule needs to bind to a biological target, to find small molecules that could favorably bind to the HDAC6 binding sites. These models were generated from the HDAC6 crystal structure in Pharmit, a web-based platform for constructing pharmacophore models.

Various interactions were omitted or added to the model to determine the most optimal combinations of chemical features (e.g. hydrogen-bond donors, acceptors, hydrophobic regions, and aromatic features) that could represent the binding requirements of the HDAC6 binding site. Then, each pharmacophore model was screened with the Molport chemical library, which is a repository of thousands of commercially available small molecules. All of the molecules within Molport were evaluated using their Root Mean Square of Deviation (RMSD), where molecules with lower RMSD values indicate a greater likelihood of matching the pharmacophore.

After several pharmacophore models were created, three models were selected for further screening based on their ability to maintain chemical diversity while containing a manageable number of candidate molecules. Chemical diversity was assessed by visual inspection of the molecular structures of each pharmacophore model. The three resulting pharmacophores were screened to remove duplicate candidates. After this filter was applied, each of the three pharmacophores contained a significantly reduced number of chemical hits. From the remaining options, five were selected from each pharmacophore based on their low RMSD values and structural diversity. This resulted in fifteen compounds being considered as potential chemical inhibitors of HDAC6 and they were selected for docking in the next stage of the study.

Molecular Docking to the HDAC6 Catalytic Domain

Docking of these compounds was conducted in Swissdock, a web-based molecular docking platform that predicts the binding affinity of ligands within a target protein. Each compound was computationally docked into the HDAC6 catalytic domain to predict its preferred binding orientation and es-

timate the strength of its interaction with the protein. The metric used to analyze the binding strength of each compound in various locations of the protein was the SwissParam score, where more negative values indicate more energetically favorable predicted protein-ligand interactions and therefore a stronger binding affinity. Of the fifteen compounds identified through pharmacophore screening, the ten compounds with the most favorable SwissParam scores were selected for further analysis.

Prediction of Pharmacokinetic and Drug-likeness Properties

The next stage of the experiment was to analyze the ADME of the selected compounds to determine their suitability as potential drug candidates. The ADME of these compounds was analyzed using the SwissADME platform, where key pharmacokinetic and drug-likeness parameters were predicted to determine whether a compound's molecular weight, hydrogen bond donors and acceptors, and lipophilicity (LogP) would comply with Lipinski's Rule of Five.

Lipinski's Rule of Five was applied because it is a widely accepted guideline for identifying drug-like molecules, filtering out compounds based upon empirically proven metrics associated with poor absorption or permeability (Lipinski et al., 2001). Additionally, the predicted water solubility of each of the compounds through the Estimated Solubility (ESOL) framework and gastrointestinal absorption (GI) were assessed, adding a further layer of analysis of the viability of these candidates. For water solubility, the ESOL metric was specifically analyzed because it is a widely used quantitative model predicting solubility for small molecules especially in a pharmaceutical context.

Predicted Toxicity and Safety Profiling

Five compounds were selected to advance to the next stage of screening due to both their favorable pharmacokinetic properties, as validated through the ADME analysis, and their predicted binding affinity from molecular docking. The ProTox online platform was used to analyze each of the compounds' predicted toxicity class, LD₅₀ values, and potential toxicity targets. The

predicted toxicity class was used to estimate the overall acute toxicity of each compound, with higher classes indicating lower toxicity. LD₅₀ values were evaluated to estimate the dose required to cause mortality in 50% of a test population, with higher values indicating lower acute toxicity. Additionally, potential toxicity targets were analyzed to predict interactions with biological systems and targets in order to accurately measure safety risks. The toxicity radar chart from ProTox provided information about each compound's predicted toxicity profile compared to FDA-set benchmarks.

Results and Discussion

Binding Site Analysis

To initially determine whether HDAC6 contains suitable binding spots for potential druggable small molecules, both geometric and machine learning analyses were performed. DoGSiteScorer accounted for factors including surface area, volume, and shape to identify sites with favorable geometric characteristics that could facilitate interactions between HDAC6 and potential small molecules. Along with a geometric analysis, Prankweb was used to validate these sites by using machine learning to predict which HDAC6 regions were most likely to bind ligands.

An initial geometric analysis of binding sites was conducted in DoGSiteScorer, which used the ligand QQD as a reference to locate binding sites within the HDAC6 protein. There were twelve potential binding sites. However, only the site with a volume of 873.07 Å³ (P-0) was further analyzed because it contained the greatest volume out of the sites, indicating it contains the greatest capacity to accommodate potential small molecules. This result is shown in Table 1.

All remaining binding sites had substantially smaller predicted volumes, which could limit interactions between small molecules and the HDAC6 protein and were therefore omitted from consideration. Additionally, Table 1 demonstrates that P-0 tied with the highest druggability score of 0.78, further corroborating the site's suitability for small-molecule binding based upon features including the site's volume, shape, surface properties, and hydrophobicity.

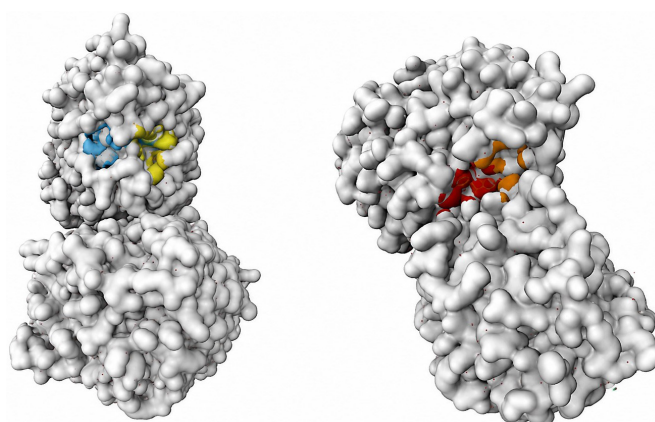
Table 1. *Do GSite Scorer-predicted HDAC6 binding pockets, ranked by volume, with corresponding druggability scores*

Binding Site	Volume (Å ³)	Druggability Score
P_0	873.07	0.78
P_1	466.15	0.78
P_10	165.55	0.23
P_11	64.86	0.32
P_2	351.67	0.59
P_3	483.48	0.70
P_4	271.75	0.51
P_5	88.71	0.55
P_6	294.25	0.60
P_7	249.31	0.24
P_8	131.95	0.44
P_9	151.29	0.28

These results were complemented by further analysis of the binding sites in Prankweb, which demonstrated that there are four potential sites within HDAC6, as shown in Table 2.

Table 2. *Prankweb-predicted HDAC6 ligand-binding sites, ranked by probability, with associated residue counts*

Rank	Probability	Number of Residues
1	0.890	20
2	0.817	14
3	0.041	10
4	0.014	8

Figure 1. *Prankweb-rendered visualization of predicted HDAC6 binding sites, showing two orientations of the protein structure with potential binding pockets highlighted as colored regions*

The site ranked first had a probability of 0.890, indicating high confidence that the site represents a ligand-binding site. The second highest ranked site had a probability of 0.817,

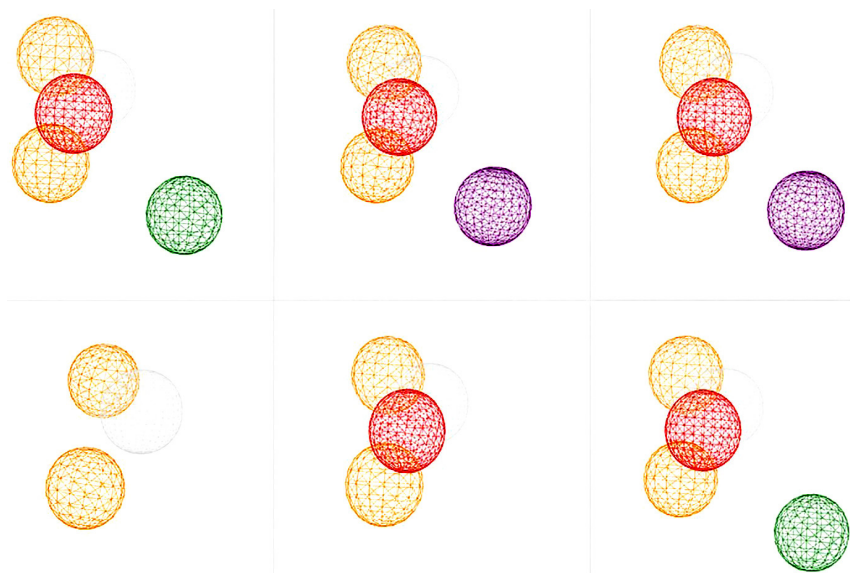
similarly corresponding to a relatively high level of confidence that this site represents a ligandbinding site. These high prediction scores suggest that the identified sites possess

structural and physicochemical characteristics similar to those of experimentally validated ligand-binding sites. Additionally, an analysis of residues demonstrated that these sites had the potential to facilitate ligand binding, as residues (amino acids) provide the necessary side chains essential for hydrogen bonding and other types of ligand interactions. The sites with the highest probabilities predicted by Prankweb contained 20 and 14 residues, respectively, further highlighting their potential to facilitate efficient ligand interactions. Additionally, Prankweb generated images highlighting potential binding sites on the HDAC6 protein, as shown in Figure 1. Overall, both the geometric and machine-learning analyses confirm that HDAC6 contains structurally and biologically favorable binding sites for small-molecule inhibitors. These findings justify the selection of HDAC6 for pharmacophore analysis and virtual screening in subsequent procedures of the study.

Pharmacophore-Based Virtual Screening

The subsequent pharmacophore analysis and virtual screening further indicated the potential to develop HDAC6 inhibitors. To test how different compounds interact with the binding sites of the protein, researchers typically employ both physical screening and virtual screening. The advantage of physical screening is that it directly measures the binding activity of compounds in real-life conditions, providing highly reliable evidence of their inhibitory potential. However, this process is often extremely time-, money-, and resource-intensive because thousands of compounds must be individually synthesized and experimentally tested. Therefore, researchers also employ virtual screening, which uses computational methods to rapidly evaluate large chemical libraries and identify compounds that have the potential to bind to the target protein.

Figure 2. Six pharmacophore models generated in Pharmit with varying combinations of chemical features. Colored spherical regions denote different chemical interaction features used to define each model. Naming convention: Pharmacophore 1, 2, 3 (top), Pharmacophore 4, 5, 6 (bottom)



This process significantly decreases the number of compounds tested and makes the overall drug discovery process more efficient and cost-effective. In this study, virtual screening was conducted with a pharmacophore model from Pharmit. The pharmacophore was edited to add or omit several interactions to determine chemical features that best represented a suitable combination of

chemical characteristics of the binding requirements of the HDAC6 active site. In Pharmit, only the QQD molecule was used to generate a pharmacophore map because it is a known HDAC6 inhibitor; using it as a reference ensured that the pharmacophore represented the essential features that allow for ligand binding. The Pharmit application identified thirteen chemical features that

allowed for ligand binding. Several iterations of the pharmacophore were created with 4 to 6 different features to determine which model most accounted for the binding requirements of HDAC6 while also being relatively selective. Six different pharmacophores were created, with varying combinations of chemical features, as shown in Figure 2.

After each map was created, it was screened with the Molport dataset of compounds to find suitable compounds that could bind to the HDAC6 protein under the chemical conditions within the map. The resulting compounds were evaluated according to their Root Mean Square Deviation (RMSD), which measures how closely a molecule's chemical features align with the pharmacophore. Of the six pharmacophore models created, only three were selected for further screening because they provided the best balance between chemical interaction diversity and the selectivity of potential hits. Some of the models produced an excessively large number of hits, indicating that the chemical constraints were unlikely to identify specific HDAC6 inhibitors. Moreover, while some of the models produced a more constrained list of chemical hits, they contained little to no variety in chemical interactions within the models themselves. Specifically, pharmacophores 3, 5, and 6 were screened further.

To decrease the number of hits to a viable set of candidate molecules, only compounds with an RMSD of less than 0.1 were considered. This threshold was used in this study as it prioritized compounds that demonstrated the closest resemblance to the pharmacophore model. Out of the remaining options, 5 com-

pounds were chosen from each pharmacophore. The selection of these compounds was not only based upon low RMSD values but also on structural diversity to avoid overrepresentation of compounds sharing the same chemical structure. This approach ensured that analogous compounds with similar binding characteristics were not selected for docking.

Molecular Docking to HDAC6

After fifteen suitable compounds were selected, molecular docking was conducted to determine whether the compounds that were chosen from the pharmacophores were capable of finding favorable binding locations within HDAC6. While pharmacophore screening can identify molecules that satisfy the chemical requirements of HDAC6, it cannot predict the viability of three-dimensional interactions between the compound and HDAC6. Therefore, docking provides an additional computational assessment of whether the selected compounds can adopt energetically favorable predicted binding poses. Docking provided an estimate of the SwissParam score for each protein-ligand interaction, allowing for a comprehensive analysis of potential inhibitors based upon their predicted binding affinity. The SwissParam scores represent the thermodynamic favorability of a binding interaction, with more negative values indicating a more energetically favorable protein-ligand complex, leading to a greater binding affinity. For each docking experiment, the lowest SwissParam score was collected to represent the most favorable interaction for each compound. The values are displayed in Table 3.

Table 3. *SwissParam scores for compounds associated with pharmacophore models 3, 5, and 6*

Pharmacophore Number	Compound	Swiss Param Score (kcal/mol)
3	Molport-004-327-550	-6.3968
3	Molport-006-013-429	-6.1620
3	Molport-027-636-748	-7.3761
3	Molport-035-907-729	-6.5961
3	Molport-002-985-507	-6.2479
5	Molport-004-336-101	-6.5282
5	Molport-000-478-723	-6.0724

Pharmacophore Number	Compound	Swiss Param Score (kcal/mol)
5	Molport-004–331–688	-6.2312
5	Molport-023–219–174	-7.2671
5	Molport-004–917–109	-6.6884
6	Molport-004–338–175	-6.2658
6	Molport-046–683–866	-6.3438
6	Molport-002–464–756	-6.9570
6	Molport-035–800–274	-6.7653
6	Molport-004–336–041	-6.1219

ADME profiling

Then, the ten compounds with the lowest SwissParam scores were selected for the next set of experiments. At this point of the study, all of these compounds had demonstrated favorable predicted binding affinities, highlighting their potential as candidate HDAC6 inhibitors. The consequent experiment conducted was the evaluation of these candidates' ADME properties because compounds with favorable docking scores may exhibit poor absorption, rapid metabolism, or other pharmacokinetic limitations that reduce their therapeutic effectiveness. ADME performance was measured through Lipinski's rule to determine if these compounds con-

tained physicochemical properties associated with oral bioavailability. Of the 10 compounds analyzed, all satisfied Lipinski's Rule of Five, indicating that their physicochemical properties were consistent with established criteria for drug-likeness. Additionally, all of the compounds contained a water solubility metric of "high" or "very high," and GI absorption for all of the compounds was high. These findings highlight that all of the candidate molecules exhibited pharmacokinetic properties consistent with effective absorption and oral drug-likeness. The findings from this analysis are displayed in detail in Table 4.

Table 4. *Physicochemical and pharmacokinetic properties of the selected lead compounds predicted using SwissADME. MW = molecular weight (g/mol); HBD = hydrogen-bond donors; HBA = hydrogen-bond acceptors; ESOL = estimated water solubility; GI = gastrointestinal absorption*

Compound	MW	HBD	HBA	LogP	ESOL	GI
Molport-027–636–748	298.09	2	4	0.60	Soluble	High
Molport-023–219–174	296.32	2	3	2.17	Soluble	High
Molport-002–464–756	239.27	2	4	1.80	Soluble	High
Molport-035–800–274	243.26	2	3	2.19	Soluble	High
Molport-004–917–109	196.16	4	4	-0.28	Very Soluble	High
Molport-035–907–729	207.27	2	2	2.66	Soluble	High
Molport-004–336–101	202.21	2	3	1.49	Soluble	High
Molport-004–327–550	187.19	2	2	2.01	Soluble	High
Molport-046–683–866	221.13	2	6	2.03	Soluble	High
Molport-004–338–175	167.16	2	3	0.66	Very Soluble	High

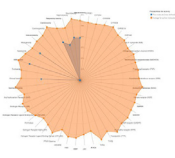
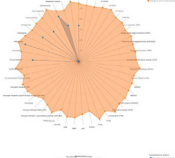
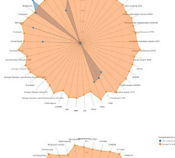
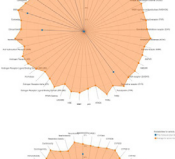
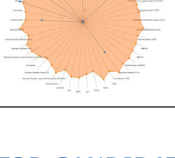
To continue experimentation with the compounds with the greatest potential for HDAC6 inhibition, 5 compounds were selected for further evaluation. Particularly, Molport-027–636–748 was an especially promising candidate at this stage because it contains the lowest SwissParam score (–7.3761) out of the 10 compounds, indicating the greatest predicted binding affinity toward the HDAC6 active site. The compound also fully complies with Lipinski's Rule and contains a high predicted solubility and GI absorption, validating its potential as a suitable drug candidate. Molport-023–219–174 was selected because it exhibited the second-most favorable docking score (–7.2671), also highlighting its binding potential. Its predicted logP of 2.17 was also consistent with the physicochemical criteria used to evaluate oral drug-likeness due to its indicated moderate lipophilicity. Molport-002–464–756 and Molport-035–800–274 also produced suitable SwissParam scores and contained LogP values

(1.80 and 2.19, respectively) that effectively balance aqueous compatibility and lipophilicity. Finally, Molport-004–327–550 was selected as the final compound for the next experimental stage due to it containing the 5th lowest SwissParam score and exhibiting a LogP value of 2.01, suggesting an effective balance between aqueous compatibility and lipophilicity. Overall, these five compounds demonstrated the strongest combination of predicted HDAC6 binding affinity, ADME metrics, and drug-like properties, justifying their inclusion in subsequent toxicity evaluation.

Toxicity screening and final candidate selection

Afterwards, extensive toxicity screening and analysis was conducted upon these five compounds. This was to assess their predicted safety profiles and determine if any concerns about toxicity hinder further evaluation. The results of the toxicity screening are displayed in Table 5.

Table 5. Predicted toxicity profiles of the selected compounds. LD_{50} values are reported in mg/kg

Compound	Toxicity Class	LD_{50} (mg/kg)	Cluster-Associated Toxicities	Toxicity Radar Chart
Molport-027–636748	3	200	BBB, neurotoxicity, clinical toxicity, mutagenicity, DILI, nephrotoxicity, respiratory toxicity	
Molport-023–219174	4	1250	BBB, clinical toxicity, mutagenicity, DILI, nephrotoxicity, respiratory toxicity, carcinogenicity	
Molport-002–464756	4	2000	BBB, immunotoxicity, clinical toxicity, mutagenicity, nephrotoxicity, respiratory toxicity, MIE-TR α , MIE-TR β , MIE-TTR	
Molport-035–800-274	4	2000	BBB, clinical toxicity, mutagenicity, DILI, nephrotoxicity, carcinogenicity, MIE-TTR	
Molport-004–327-550	4	1160	BBB, clinical toxicity, mutagenicity, DILI, carcinogenicity, CYP1A2 interaction, MIE-TTR	

All of the compounds contained a toxicity class of 3 or greater, indicating they were predicted to exhibit moderate to relatively low predicted acute toxicity according to the externally validated Globally Harmonized System (GHS) classification. However, as Molport-027–636–748 was the only compound to exhibit a toxicity of class 3, it exhibited the highest predicted acute toxicity among the selected compounds, warranting caution for its continued evaluation. Molport-035800–274 and Molport-002–464–756 jointly exhibited the greatest potential LD₅₀ value (2000 mg/kg) out of all of the compounds, indicating that they contained the lowest predicted toxic potency relative to the other compounds. For the rest of the compounds, LD₅₀ values ranged from 200 to 1160, showcasing variation in predicted acute toxicity. Analysis of the cluster-associated toxicity endpoints further revealed differences in the predicted toxicological profiles of the selected compounds. For all of the compounds, blood-brain barrier permeability, clinical toxicity, and mutagenicity were predicted, while drug-induced liver injury (DILI), nephrotoxicity, carcinogenicity, respiratory toxicity, immunotoxicity, CYP1A2 interaction, and thyroid-related molecular initiating events (MIE-TTR, MIE-TR α , and MIE-TR β) were all factors that varied in each of the compounds. These differences in the toxicity profiles of the compounds suggest that while the compounds may share some similar toxicological liabilities, they also differ in specific combinations of interactions and predicted toxicity endpoints, necessitating further review of specific toxicological conditions. Therefore, the toxicity radar chart was analyzed for each compound to determine if these toxicological liabilities truly hindered the compound's effectiveness. The toxicity radar charts, inserted in Table 5, provide a visual representation of the level of toxicity for each biological target or system for each compound as compared to similar compounds present in drugs containing FDA approval.

As a result of this final experiment, Molport-027–636–748 was eliminated from consideration due to its low predicted LD₅₀ value (200 mg/kg), demonstrating a higher predicted acute toxicity than the remaining candidate compounds. Additionally,

Molport-002–464–756 was also excluded from further consideration as a suitable inhibitor due to its predicted elevated levels of immunotoxicity, as shown in the toxicity radar chart, raising concerns about its overall safety profile. Consequently, Molport-023–219–174 was determined to be the most promising candidate due to its comparatively favorable docking affinity (SwissParam score = -7.2671) among the rest of the compounds, suggesting a stronger predicted binding interaction with the HDAC6 binding site. Additionally, this compound also contains favorable pharmacokinetic properties, complies with Lipinski's Rule of Five, and contains a toxicity profile that is more favorable relative to the other candidates, further validating its selection as the lead candidate for further experimentation. Molport-035–800–274 emerged as a promising backup compound due to it containing the second-highest docking affinity (SwissParam score = -6.7653) as well as its favorable ADME profile and the absence of significant toxicity liabilities.

Conclusion

As HDAC6 promotes tumor progression by deacetylating regulatory enzymes and structural proteins, it has become a compelling target for inhibition and consequent therapeutic intervention. This study analyzed the potential of a computational drug discovery workflow to identify novel HDAC6 inhibitors. Initially, the computational platforms DoGSiteScorer and Prankweb were employed to identify suitable binding sites within the HDAC6 structure. Then, pharmacophore modeling was conducted through Pharmit to identify the molecular features necessary for favorable interactions with the HDAC6 binding pocket, which allowed for the selection of candidate compounds with the highest predicted binding affinity. Fifteen compounds were specifically selected from this process, and they underwent docking in SwissDock to evaluate their predicted binding affinities. Subsequently, the ADME of ten of these compounds was evaluated by determining their compliance with Lipinski's Rule of Five as well as their water solubility and GI absorption. Finally, based on both ADME results and binding affinity, five

compounds were selected for toxicity analysis, where metrics including LD₅₀, predicted toxicity class, and a toxicity profile were used to determine a lead compound that exhibited the most favorable balance of predicted binding affinity, pharmacokinetic properties, and safety profile. After this comprehensive workflow, Molport-023–219–174 was determined to be the lead compound due to its favorable predicted binding affinity (–7.2671), pharmacokinetic properties, and toxicity profile, while Molport-035–800–274 was recognized as a promising backup candidate. These compounds have the potential to be utilized in further experimental validation and may contribute to the development of precise and effective HDAC6 inhibition therapeutics for cancer treatment.

However, several opportunities remain for future research. The study only used computational methods to determine potential inhibitor candidates for HDAC6, which may limit evaluation of these candidates under biological conditions. Future studies should evaluate the inhibitory potential of the lead and backup compounds through in vitro and

in vivo experiments to assess their therapeutic efficacy. Furthermore, studies that expand the scope of compounds assessed for binding affinity, favorable ADME, and acceptable toxicity thresholds can provide additional insight into potential inhibitors and the common pharmacokinetic traits these inhibitors may possess. Overall, this study presents a promising drug discovery pipeline through the integration of various computational methods, accelerating the identification of early-stage drug candidates by reducing the time and resources necessary for experimental validation. By enabling the efficient identification of suitable HDAC6 inhibitor candidates, this proposed framework has the potential to assist in the development of more selective cancer therapeutics.

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References

- Al Meslamani, A. Z. (2023). The future of precision medicine in oncology. *Expert Review of Precision Medicine and Drug Development*, – 8(1). – P. 43–47. URL: <https://doi.org/10.1080/23808993.2023.2292988>
- Chen, P., & Zhang, Y. (2026). Role of HDAC6 in carcinomas. *Discover Oncology*, – 17(1). Article 4391. URL: <https://doi.org/10.1007/s12672-026-04391-9>
- Chen, S., Cao, Z., Prettnner, K., Kuhn, M., Yang, J., Jiao, L., Wang, Z., Li, W., Geldsetzer, P., Bärnighausen, T., Bloom, D. E., & Wang, C. (2023). Estimates and projections of the global economic cost of 29 cancers in 204 countries and territories from 2020 to 2050. *JAMA Oncology*, – 9(4). – P. 465–472. URL: <https://doi.org/10.1001/jamaoncol.2022.7826>
- Ediriweera, M. K., Tennekoon, K. H., & Samarakoon, S. R. (2021). Odd-chain fatty acids reduce histone deacetylase 6 (HDAC6) expression and inhibit proliferation in human breast and lung cancer cells. *Biochimie*, – 181. – P. 194–207. URL: <https://ueaeprints.uea.ac.uk/id/eprint/75951/>
- GBD 2023 Cancer Collaborators. (2025). The global, regional, and national burden of cancer, 1990–2023, with forecasts to 2050: a systematic analysis for the Global Burden of Disease Study 2023. *The Lancet*, – 406(10512). – P. 1565–1586. URL: [https://doi.org/10.1016/S0140-6736\(25\)01635-6](https://doi.org/10.1016/S0140-6736(25)01635-6)
- Harding, R. J., Franzoni, I., & Structural Genomics Consortium. (2023). Discovery and characterization of a chemical probe targeting the zinc-finger ubiquitin-binding domain of HDAC6. *Journal of Medicinal Chemistry*, – 66(5). – P. 3466–3479. URL: <https://doi.org/10.1021/acs.jmedchem.3c00314>
- Ho, T. C. B., Chan, A. H. Y., & Ganesan, A. (2020). Thirty years of HDAC inhibitors: 2020 hindsight. *Medicinal Research Reviews*, – 40(6). – P. 2153–2194. URL: <https://doi.org/10.1002/med.21682>

- Huang, Z., Li, L., Cheng, B., & Li, D. (2024). Small molecules targeting HDAC6 for cancer treatment: Current progress and novel strategies. *Biomedicine & Pharmacotherapy*, – 178. Article 117218. URL: <https://doi.org/10.1016/j.biopha.2024.117218>
- Kouidhi, S., Elgaaiied, A. B., & Chouaib, S. (2024). Impact of metabolic reprogramming on the immune response in the tumor microenvironment. *Cancer Immunology, Immunotherapy*, – 73(4). Article 68. URL: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12021777/>
- Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, – 46(1–3). – P. 3–26. URL: [https://doi.org/10.1016/S0169-409X\(00\)00129-0](https://doi.org/10.1016/S0169-409X(00)00129-0)
- Liu, W., Guo, Z., Sun, S., et al. (2026). Discovery of potent HDAC6-selective inhibitors based on artemisinin: Design, synthesis, and antitumor evaluation. *Journal of Medicinal Chemistry*. Advance online publication. <https://doi.org/10.1021/acs.jmedchem.6c00138>
- Marchetti, P., Curigliano, G., Biffoni, M., et al. (2025). Genomically matched therapy in advanced solid tumors: The randomized phase 2 ROME trial. *Nature Medicine*, – 31. – P. 3514–3523. URL: <https://doi.org/10.1038/s41591-025-03918-x>
- Subramanian, S., Bates, S. E., Wright, J. J., Espinoza-Delgado, I., & Piekarz, R. L. (2010). Clinical toxicities of histone deacetylase inhibitors. *Pharmaceuticals*, – 3(9). – P. 2751–2767. URL: <https://doi.org/10.3390/ph3092751>
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, – 71(3). – P. 209–249. URL: <https://doi.org/10.3322/caac.21660>
- Tsimberidou, A. M., Fountzilas, E., Nikanjam, M., & Kurzrock, R. (2020). Review of precision cancer medicine: Evolution of the treatment paradigm. *Cancer Treatment Reviews*, – 86. Article 102019. <https://doi.org/10.1016/j.ctrv.2020.102019>
- Zafar, A., Khatoon, S., Khan, M. J., Abu, J., & Naeem, A. (2025). Advancements and limitations in traditional anti-cancer therapies: A comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy. *Discover Oncology*, – 16(1). Article 607. URL: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12021777/>

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Section 4. Pharmaceutical Sciences

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DISCOVERY OF SMALL MOLECULES TARGETING MER-TK AS NOVEL THERAPIES FOR CARDIOVASCULAR DISEASES

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Abstract

Cardiovascular diseases remain the leading cause of death worldwide. Currently, FDA-approved therapies primarily manage cholesterol, blood pressure, and clot formation without correcting the underlying issues in apoptotic cell clearance that drive plaque instability and chronic inflammation. MER proto-oncogene tyrosine kinase (MerTK), a receptor central to efferocytosis, has emerged as a promising target for addressing this gap, yet no MerTK-targeted small molecule therapy currently exists for cardiovascular disease. This study used a structure-based computational pipeline to identify and evaluate candidate small molecules targeting MerTK. First, the MerTK structure was analyzed using ProteinsPlus and PrankWeb to identify its most druggable binding pocket, revealing a single dominant site with a drug score of 0.82–0.83. Second, a pharmacophore model built from this pocket was used to virtually screen compound libraries, yielding fifteen candidates with strong geometric alignment (RMSD $\leq$ 0.127 Å). Third, molecular docking of these fifteen compounds against the binding pocket produced SwissParam scores ranging from –6.85 to –8.83 kcal/mol, narrowing the pool to eight top binders. Fourth, ADME and Lipinski's Rule screening of these eight compounds identified four candidates with favorable absorption, solubility, and drug-likeness profiles. Fifth, toxicity prediction of these four finalists evaluated lethal dose, toxicity class, and toxicological endpoint breadth. Together, these results identified MolPort-047–786–433 as the most promising overall candidate, combining strong predicted binding affinity (–8.2918 kcal/mol), full Lipinski compliance, high gastrointestinal absorption, and the most favorable toxicity profile (LD50 of 1190 mg/kg, toxicity class 4) among all candidates screened.

Keywords *MerTK, efferocytosis, cardiovascular disease, computational drug discovery, molecular docking*

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide and their

impact continues to rise despite major advances in prevention and treatment. They develop through lipid accumulation, chron-

ic inflammation, endothelial dysfunction, and tissue remodeling, ultimately leading to atherosclerosis, myocardial infarction, and heart failure (Jebari-Benslaiman et al., 2022). These processes disrupt vascular homeostasis and impair cardiac function, making CVDs a persistent global health challenge. Understanding the molecular pathways that drive these events is essential for developing more effective therapies.

Current FDA-approved drugs for treating cardiovascular issues primarily target cholesterol, blood pressure, and clot formation. Statins and PCSK9 inhibitors lower LDL cholesterol and reduce cardiovascular risk, but many patients still experience plaque progression and inflammation (Navarese et al., 2015). ACE inhibitors, beta-blockers, and antiplatelet agents improve hemodynamics and reduce the chances of clot formation, but fail to correct defects in immune regulation or apoptotic cell clearance (Vaidya et al., 2023). Anti-inflammatory drugs like canakinumab can reduce cardiovascular events but do not fully resolve plaque cell death and increase infection risk (Ridker et al., 2017). These limitations emphasize the need for therapies that address plaque instability and chronic inflammation directly.

MER proto-oncogene tyrosine kinase (MerTK) is a receptor tyrosine kinase expressed on macrophages, endothelial cells, and other immune cells, where it plays a critical role in clearing apoptotic cells (Lemke, 2013). During efferocytosis, bridging molecules such as Gas6 and Protein S bind to phosphatidylserine on apoptotic cells and activate MerTK, starting anti-inflammatory signaling that promotes engulfment and prevents secondary necrosis, limiting the release of pro-inflammatory cytokines and helping to maintain tissue homeostasis (Lemke, 2013). If MerTK signaling is impaired, apoptotic cells collect together, leading to chronic inflammation, oxidative stress, and plaque instability in the arteries (Cai et al., 2017; Ait-Oufella et al., 2008). Due to its essential role in maintaining normal vascular conditions, MerTK has shown promise as an important drug target for cardiovascular diseases.

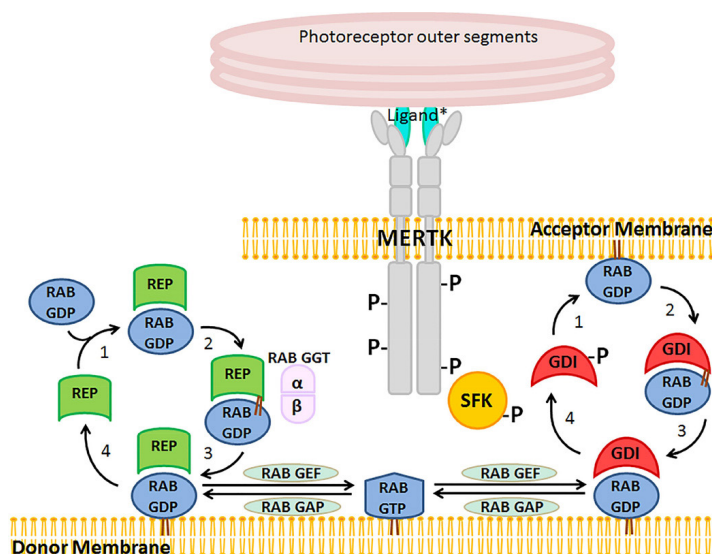
The MerTK receptor is especially significant because it combines inflammation resolution, lipid metabolism, and tissue re-

modeling – three processes critical to plaque stability. Elevated MerTK increases efferocytosis efficiency and strengthens the fibrous cap, decreasing plaque vulnerability (Thorp et al., 2008). A lack of MerTK has been linked to mitochondrial dysfunction, increased ROS levels, smooth muscle cell phenotypic switching, and cardiac fibrosis, all of which worsen cardiovascular diseases (Cai et al., 2018). Targeting MerTK therefore appears to be a reliable approach to reduce residual inflammatory risk beyond current therapies.

Several different types of therapeutic molecule types are being explored to control MerTK activity. Small molecules can enhance MerTK signaling or inhibit its cleavage by ADAM17 (Thorp et al., 2011). Large molecules, like Gas6 or Protein S, can activate MerTK directly. Although antibodies are large molecules, they do offer flexibility in how they can be designed to activate, inhibit, or prevent cleavage of the receptor. Peptides can be engineered for both stability and specificity; they enable targeted stimulation of MerTK while limiting unwanted effects on other receptors. Together, these therapeutic approaches aim to restore efferocytosis, reduce inflammation, and improve cardiovascular health.

Recent reviews have highlighted MerTK-mediated efferocytosis as a promising therapeutic target in cardiovascular disease. One review found that boosting MerTK activity reduces necrotic core formation and improves plaque stability (Scott et al., 2001). A second paper, focused on bridging molecules approaches that mimic Gas6 or Protein S to strengthen anti-inflammatory signaling, found that MerTK activation enhances efferocytosis, limits oxidative stress, and supports tissue repair (Rothlin et al., 2007). A third review compared multiple pathways of efferocytosis and found that impaired MerTK signaling leads to persistent inflammation and plaque vulnerability, highlighting the need for therapies that restore efficient apoptotic cell removal (Cohen et al., 2002). Together, these studies show that MerTK-targeted therapies (across small molecules, biologics, and receptor-stabilizing strategies) offer a promising direction for improving plaque stability and reducing cardiovascular disease progression.

Figure 1. Diagram illustrating signaling events triggered by ligand-activated MerTK and downstream regulation of RAB-mediated membrane trafficking. Colored components represent key proteins involved in the pathway, while grey structures depict the surrounding membrane environment. Reproduced from Shelby et al. (2015).



Research Method

Analysis of Binding Sites in MerTK Geometric Method

The MerTK protein structure (PDB: 7M5Z) was submitted to the ProteinPlus web server, and the DoGSiteScorer tool was run using default settings (pocket-only analysis, druggability-focused granularity, no specified ligand, all protein chains). Surface areas, volumes, and drug scores of all the detected pockets were recorded, along with screenshots to document all binding sites and their corresponding values.

Machine Learning Model

The same structure was entered into PrankWeb, again using the PDB code, and the model was uploaded for analysis. PrankWeb generated a 3D model with predicted binding sites in various colors, each scored by expected binding strength. All predicted sites were documented, and multiple screenshots were taken.

Pharmacophore Modeling and Virtual Screening

Pharmacophore Model Generation

A pharmacophore model of the strongest binding site was generated using the Pharmit platform. The server produced several pharmacophore maps, and usable maps were standardized (ligand/receptor displays off, surface opacity 0, black background) for clarity.

Virtual Screening and RMSD Optimization

Virtual screening was performed for each usable map. Feature combinations, molecule radii, and 3D shapes were continuously adjusted to minimize RMSD values. Compounds were then re-screened to determine whether (RMSD) values improved. Those with RMSD values of ≤ 0.2 were considered strong matches, and a total of 15 unique molecules were obtained across all maps. Candidates were sourced from chemical libraries such as MolPort and Enamine, and an overlay image was generated for each.

Molecular Docking of Selected Compounds

Ligand Preparation and Input Submission

Molecular docking was performed in SwissDock for all fifteen compounds using their SMILES string (retrieved from MolPort/Enamine). The MerTK protein structure (chain B, heteroatoms removed) was uploaded using its PDB file, and a custom search space centered at $(-3, 16, 4)$, dimensions $44 \times 55 \times 57$, based on earlier binding-site analysis.

Docking Execution and Output Collection

SwissDock reports predicted binding strength using the SwissParam score (equiv-

alent to ΔG). Each job was verified to complete within the 1-hour runtime limit, and the search box's size and/or position was adjusted as needed. Each run was submitted using a unique docking name and email address for notification of completion. Swissdock then generated ranked binding modes based on predicted SwissParam score and the most favorable SwissParam score (lowest) was recorded for each. Corresponding interaction figures were screenshotted. All information was recorded into a summary table.

ADME and Drug-Likeness Prediction **SwissADME Submission and Lipinski's Rule Evaluation**

SMILES strings for the eight top-ranked docking candidates were entered individually into the SwissADME web server. For each compound, hydrogen bond donors/acceptors, molecular weight, consensus LogP (averaged LogP value reported across SwissADME's five prediction models), water solubility, and GI absorption were recorded alongside their original SwissParam docking scores (Table 5). Compounds were classified as either agreeing or disagreeing with Lipinski's Rule, and the number of violations were recorded as well.

Toxicity Prediction

Toxicity Prediction and Toxicological Endpoints Recorded

The four lead compounds identified in Section 3.4 (Z25837483, MolPort-047–786–433, Z25837505, and MolPort-052–064–324) were submitted to the ProTox 3.0 and corresponding SMILES string was entered. All toxicological endpoints, pathways, targets, and events were selected before running each prediction. Toxicity classification (active/inactive) was recorded for each endpoint, and radar charts were used to compare each compound's active toxicological aspects against the FDA-approved drug average; any endpoint exceeding the average was noted as a potential limitation. LD50, toxicity class, prediction accuracy, active cluster image, and radar chart were collected for each candidate.

Results Analysis

Identification of Druggable Binding Sites

This research compared predictions from ProteinsPlus and PrankWeb to identify MerTK's most druggable pockets. ProteinsPlus identified P_0, P_1, and P_2 as the highest scoring group (drug score 0.82–0.83) with the largest pocket volumes (Table 1, Figure 2).

Table 1. This table displays the geometric properties and drug scores of all predicted MerTK binding pockets identified by ProteinsPlus. Higher-ranked pockets (P_0–P_2) show the largest volumes and highest drug scores, while lower-ranked pockets display increasingly smaller cavities and lower drug scores

Binding Site	Volume (Å ³)	Surface Area (Å ²)	Drug Score
P_0	1109.56	1370.36	0.82
P_1	903.31	1086.28	0.83
P_2	711.46	906.96	0.83
P_3	438.23	464.64	0.73
P_4	295.79	344.77	0.72
P_5	183.58	325.11	0.43
P_6	181.95	287.26	0.25
P_7	175.21	322.93	0.34
P_8	157.14	366.73	0.34
P_9	150.81	235.98	0.49
P_10	147.74	403.25	0.31
P_11	144.48	322.03	0.31

Binding Site	Volume (Å ³)	Surface Area (Å ²)	Drug Score
P_12	132.53	207.04	0.13
P_13	119.87	277.71	0.22
P_14	115.99	373.02	0.23
P_15	111.5	291.89	0.2

Among these, P_0 was the largest cavity, P_1 showed an equally high drug score and well-defined pocket shape, and P_2 matched the same top score range, forming the third major pocket.

Figure 2. All binding pockets identified on the MerTK protein using the ProteinsPlus analysis. Colored mesh regions represent individual predicted pockets, while the red and blue ribbon-like structures depict the protein's backbone.



PrankWeb confirmed this same region by assigning its top two ranks (Rank 1 and Rank 2) the highest Pocket Scores (16.34 and 14.08) and the greatest number of residues (Figure 3). The remaining pockets also aligned well between the two tools (Table 2).

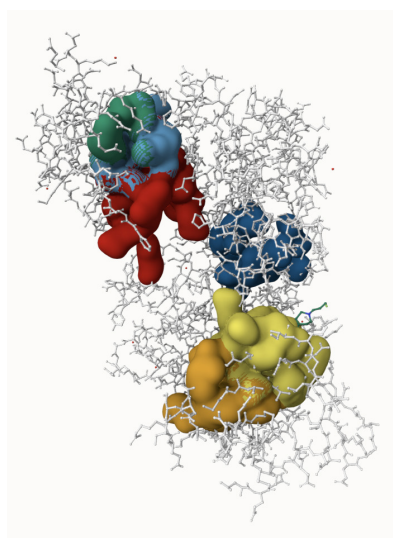
Table 2. This table summarizes the four pocket groups identified across both tools, showing how ProteinsPlus drug scores match with PrankWeb rankings. Higher-ranked pockets (P_0, P_1, P_2) display the strongest druggability, while lower-ranked pockets (P_6-P_15) show low predicted binding potential

Rank	Pockets (ProteinsPlus)	Reason (PrankWeb)
1	P_0, P_1, P_2	Highest drug scores (0.82–0.83), largest pockets (volume/surface area), PrankWeb Rank 1–2 (16.34 and 14.08)
2	P_3, P_4	Intermediate drug scores (0.72–0.73), matches PrankWeb Rank 3
3	P_5, P_9	Medium drug scores (0.43–0.49), matches PrankWeb Rank 4
4	P_6-P_8, P_10-P_15	Low drug scores (0.13–0.34), matches PrankWeb Rank 5–6

The second group, P_3 and P_4, showed intermediate drug scores (from 0.72–0.73) and corresponded to PrankWeb's Rank 3. The third group, P_5 and P_9, displayed medium scores (0.43–0.49) and matched Rank

4. Finally, the lowest group, P_6 through P_15 (except P_9) showed low scores (0.13–0.34) and aligned with PrankWeb's Rank 5 and Rank 6.

Figure 3. Predicted binding sites in MerTK (PDB ID: 7M5Z) using the PrankWeb machine learning model. Colored spheres represent the binding site and the grey residues represent the structure of the protein.



Together, the ranking table and models show strong consistency between the two approaches, confirming both tools independently identified the same primary binding pocket (Figures 2–3). This supports the conclusion that MerTK contains one dominant, highly druggable site, making it a strong candidate for future inhibitor design.

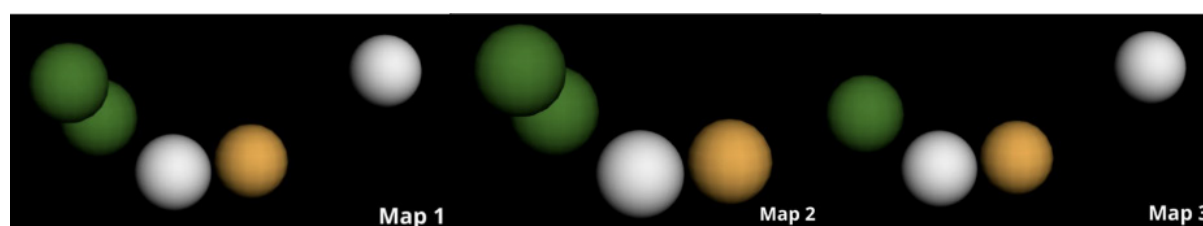
Results of Pharmacophore-based Virtual Screening of MerTK

This experiment aimed to identify small-molecule candidates that align strongly with a pharmacophore model built from the MerTK binding site. Because MerTK is involved in cell-signaling pathways linked to

disease, finding molecules that match its key interaction features is an important early step in drug discovery, testing whether structurally varying compounds could recreate the pharmacophore's donor, acceptor, hydrophobic, and positive-ion features.

The expected outcome was that only a limited number of compounds would achieve low RMSD values (≤ 0.2 Å), a threshold representing a very accurate match. Additionally, with different maps expected to yield different number of hits and small feature-radius adjustments expected to increase accepted compounds without changing the original features.

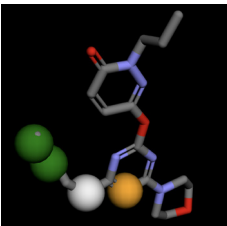
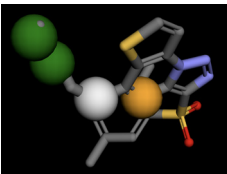
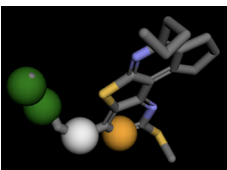
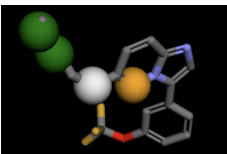
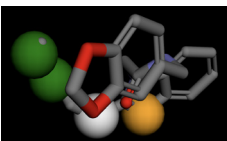
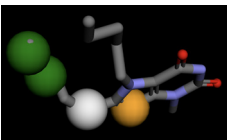
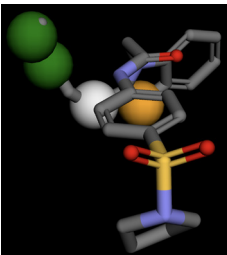
Figure 4. Three pharmacophore maps generated with Pharmit platform, illustrating difference in feature placement and spatial arrangement. Green spheres represent hydrophobic features, white spheres signify hydrogen-donor features, and orange spheres indicate hydrogen-acceptor features. The maps show how feature placement varies across the usable pharmacophore models

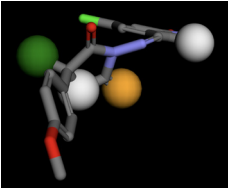
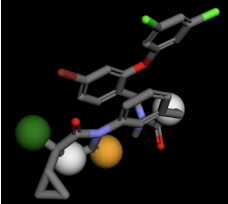
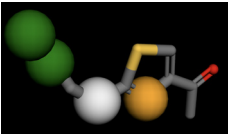
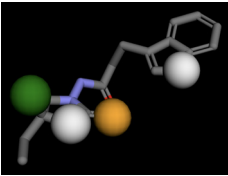
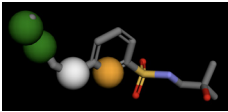
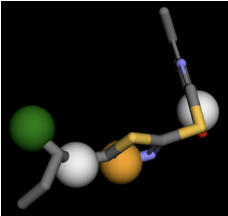
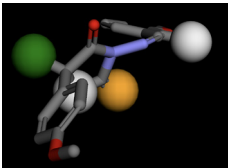
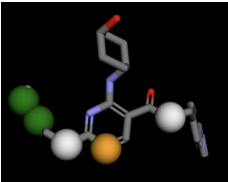


Building on these expectations, screening identified several compounds with strong geometric alignment. Many compounds across usable maps met the RMSD of ≤ 0.2 Å cutoff, and the top fifteen lowest-scoring

molecules were selected for further analysis (Table 3). RMSD values ranged from 0.041 Å (MolPort-004–893–824) to 0.127 Å (MolPort-047–786–433), signifying strong matches.

Table 3. Top 15 compounds identified across all usable pharmacophore maps, each demonstrating strong alignment with the MerTK pharmacophore model (RMSD ≤ 0.2 Å). For each compound the table reports the compound ID, RMSD value, the pharmacophore map it was selected from, and the overlay figure showing its alignment with the features

Compound Name	RMSD Value / Map Number	Overlay Figure between Compound and Pharmacophore Map
1. Mol-Port-004–893–824	0.041 / 2	
2. Mol-Port-007–741–957	0.05 / 2	
3. Mol-Port-002–612–851	0.052 / 2	
4. Mol-Port-052–064–324	0.059 / 2	
5. Z25837505	0.064 / 2	
6. Z223825236	0.065 / 2	
7. Z25837483	0.065 / 2	

Compound Name	RMSD Value / Map Number	Overlay Figure between Compound and Pharmacophore Map
8. Z49975822	0.07 / 3	
9. Z5198624731	0.075 / 3	
10. Z4081324527	0.081 / 3	
11. Z30056937	0.083 / 3	
12. Z3074709999	0.085 / 2	
13. Z154287422	0.089 / 3	
14. Z49975836	0.091 / 3	
15. Mol-Port-047–786–433	0.127 / 1	

These results also supported the expectation that different maps would yield different sets of hits – some producing multiple strong matches, others only one or none. Small adjustments to feature radii and location in-

creased the number of matches by lowering the RMSD values without removing the originals can improve screening performance integrity.

Overall, the MerTK pharmacophore model identified a consistent set of well-aligned compounds across multiple maps (Table 3). The strong RMSD values and clear feature alignment confirm these fifteen molecules are reliable candidates for further analysis.

Results of Molecular Docking

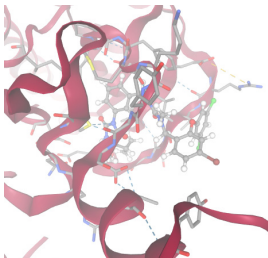
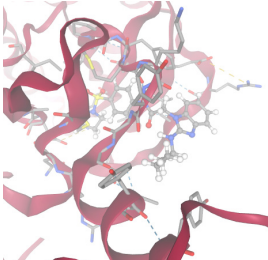
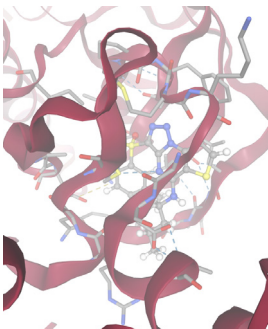
This experiment evaluated whether the fifteen pharmacophore-matched compounds could form stable predicted interactions with the primary MerTK binding pocket, using SwissParam score (equivalent to ΔG) to quantify predicted binding strength and identify the most promising candidates for future MerTK-targeted drug development.

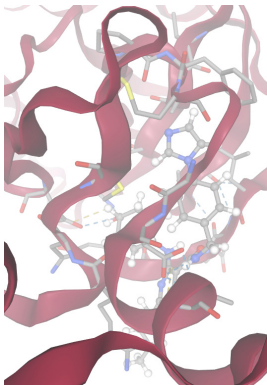
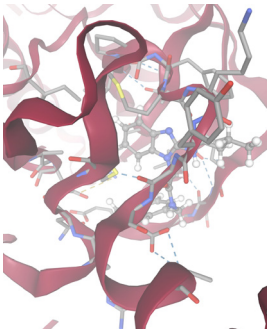
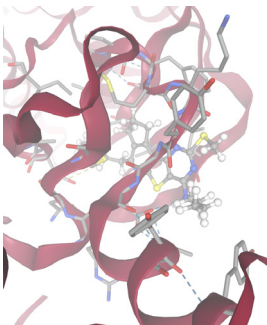
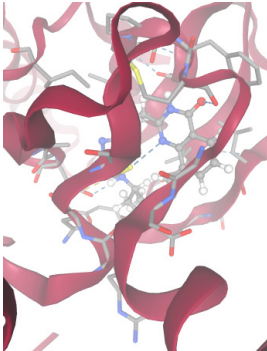
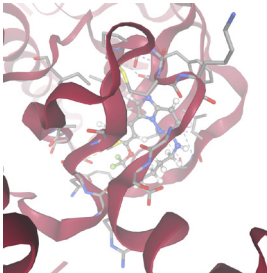
The expected outcome was that only a small group would produce a highly favor-

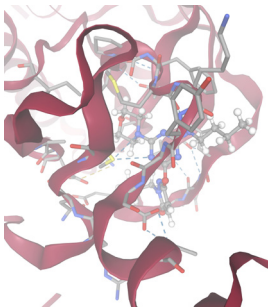
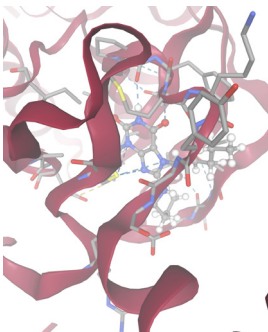
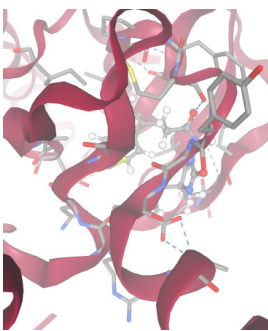
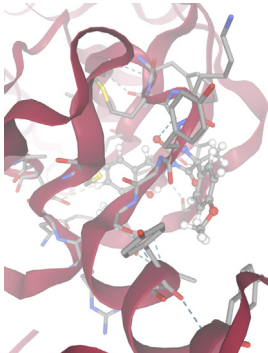
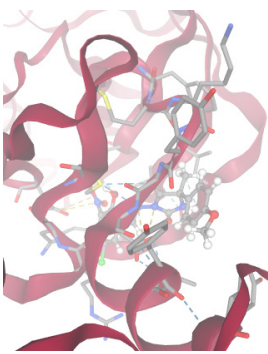
able SwissParam score, with predicted binding strengths varying in compound structure, and those that aligned well with the pharmacophore expected to also show lower (better) SwissParam scores, since the geometric fit and interaction stability are closely related.

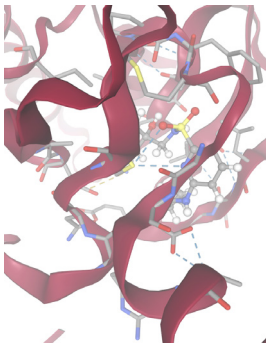
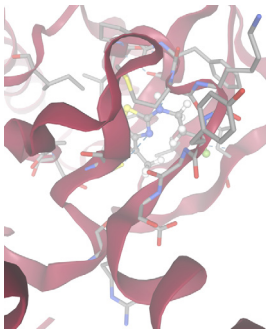
The docking results supported these expectations. All fifteen compounds produced measurable scores, ranging from -6.8518 to -8.8348 kcal/mol (Table 4). Z5198624731 showed the strongest score (-8.8348) followed by Z25837483 (-8.3922), MolPort-047-786-433 (-8.2918), MolPort-007-741-957 (-8.3820), and Z154287422 (-8.2285). Weaker compounds, such as Z4081324527 (-6.8518), still docked successfully but fewer stabilizing interactions.

Table 4. Predicted SwissParam scores for all fifteen docked compounds, along with corresponding interaction figures generated using the Swissdock platform. Lower SwissParam score indicates stronger predicted binding strength between each ligand and the MerTK binding pocket. Color-coded dashed lines in the figures represent the types of non-covalent interactions formed within the pocket

Compound Name	Docking Pose of the Compound Into MerTK
Z5198624731 SwissParam: -8.83448 kcal/mol	
Z25837483 SwissParam: -8.3922 kcal/mol	
MolPort-007-741-957 SwissParam: -8.3820 kcal/mol	

Compound Name	Docking Pose of the Compound Into MerTK
<i>MolPort-047-786-433</i> <i>SwissParam: -8.2918 kcal/mol</i>	
<i>Z25837505</i> <i>SwissParam: -8.2394 kcal/mol</i>	
<i>MolPort-002-612-851</i> <i>SwissParam: -8.2387 kcal/mol</i>	
<i>Z154287422</i> <i>SwissParam: -8.2285 kcal/mol</i>	
<i>MolPort-052-064-324</i> <i>SwissParam: -8.0968 kcal/mol</i>	

Compound Name	Docking Pose of the Compound Into MerTK
<i>MolPort-004-893-824</i> <i>SwissParam: -8.0798 kcal/mol</i>	
<i>Z223825236</i> <i>SwissParam: -7.7731 kcal/mol</i>	
<i>Z30056937</i> <i>SwissParam: -7.6032 kcal/mol</i>	
<i>Z49975836</i> <i>SwissParam: -7.5680 kcal/mol</i>	
<i>Z49975822</i> <i>SwissParam: -7.5112 kcal/mol</i>	

Compound Name	Docking Pose of the Compound Into MerTK
<i>Z3074709999</i> <i>SwissParam: –7.4394 kcal/mol</i>	
<i>Z4081324527</i> <i>SwissParam: –6.8518 kcal/mol</i>	

The docking figures displayed color-coded dashed interaction lines: blue for hydrogen bonds, yellow for ionic interactions, orange for cation- π interactions, dark gray for hydrophobic contacts, and green for π -stacking interactions. The strongest binders typically showed two to three blue hydrogen bonds, helping explain why they also produced the lowest SwissParam scores.

Overall, several pharmacophore-matched compounds showed strong predicted binding, with multiple scores falling below -8.2 kcal/mol (Table 4). These results confirm the docking experiment identified ligands capable of stable predicted binding, with Z5198624731 and other top-scorers representing promising candidates for further analysis.

Results of ADME and Drug-Likeness Prediction

This experiment determined which top docking candidates also had favorable drug-like properties, since a compound's ability to bind to MerTK (its pharmacodynamic profile, how the drug affects the body) is only useful if it can also survive the body's

own processing (its pharmacokinetic profile, how the body affects the drug). This evaluation mirrors the role of an ADME department, which advances or discontinues based on its absorption, distribution, metabolism, and excretion profile; failing any step is enough to disqualify a compound regardless of target binding. Lipinski's Rule of Five was used as the primary screen, based on LogP, the coefficient describing lipid versus aqueous phase distribution, with an ideal balanced range of roughly 0–5. Because binding affinity and drug-likeness are governed differently, not every strong docking candidate was expected to satisfy Lipinski's Rule.

Of the eight compounds sourced from MolPort and Enamine, six fully agreed with Lipinski's Rule. The exception was Z5198624731, the strongest docking candidate, which failed with two violations (MW 646.36 g/mol; CLogP 5.46) and poorly water-soluble despite showing high GI absorption (Table 5)—showing the single best docking candidate isn't necessarily the best overall drug candidate once absorption and distribution are considered.

Table 5. ADME and drug-likeness profiles for the eight top-ranked docking candidates (in order of SwissParam score), generated using the SwissADME platform, alongside each compound's SwissParam docking score. Compounds were evaluated against Lipinski's Rule of Five using hydrogen bond donor/acceptor counts, molecular weight, and Consensus LogP (CLogP). Water solubility classification and predicted gastrointestinal (GI) absorption are also reported. Compounds with zero Lipinski violations and high predicted GI absorption were prioritized as lead candidates

Compound	H-Bond Donors	H-Bond Acceptors	MW (g/mol)	Consensus LogP (CLogP)	Lipinski (Y/N, # violations)	Water Solubility Class	GI Absorption
1. Z5198624731	2	4	646.36	5.46	No, 2	Poorly Soluble	High
2. Z25837483	2	5	455.57	2.66	Yes, 0	Moderately Soluble	High
3. Mol-Port-007–741–957	1	5	415.53	3.88	Yes, 0	Moderately Soluble	Low
4. Mol-Port-047–786–433	4	5	463.58	2.39	Yes, 0	Moderately Soluble	High
5. Z25837505	2	4	380.44	2.72	Yes, 0	Moderately Soluble	High
6. Mol-Port-002–612–851	1	3	400.60	5.97	No, 1	Poorly Soluble	Low
7. Z154287422	2	4	361.48	3.66	Yes, 0	Moderately Soluble	Low
8. Mol-Port-052–064–324	1	6	350.34	3.90	Yes, 0	Moderately Soluble	High

Among the six Lipinski-compliant, GI absorption further separated the candidates. Z25837483, MolPort-047–786–433, Z25837505, and MolPort-052–064–324, showed both full Lipinski agreement and high GI absorption, while MolPort-007–741–957 and Z154287422 showed low absorption. These four compounds – all moderately water-soluble Lipinski-compliant, and high absorption – were carried forward as the final candidate pool. This stage primarily addressed the absorption and distribution aspects of ADME; metabolism and excretion were not assessed.

Results of Toxicity Prediction

Beyond binding affinity and drug-likeness, a candidate must also be evaluated for toxic effects – not simply whether it is toxic, but to what degree, and whether that degree falls within an approved limit. Toxicity was considered as both acute (short-term, with-

in hours), and organ (long-term, accumulating over repeated dosing), with the liver and kidney checked first as primary metabolism/excretion sites, though all organs were considered relevant.

All four compounds met the 300 mg/kg minimum LD50 threshold. MolPort-047–786–433 scored highest (1190 mg/kg), followed by MolPort-052–064–324 (807 mg/kg); Z25837483 and Z25837505 sat exactly at the minimum. All four compounds reached an acceptable toxicity class (≥ 3), with MolPort-047–786–433 and MolPort-052–064–324 in class 4, indicating lower toxicity (Table 6).

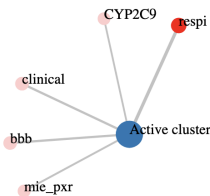
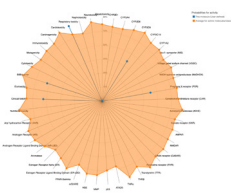
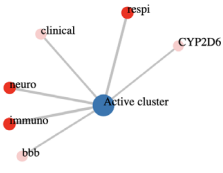
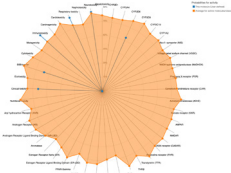
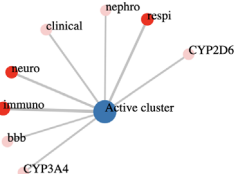
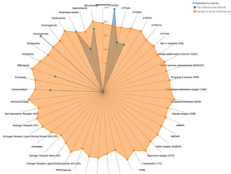
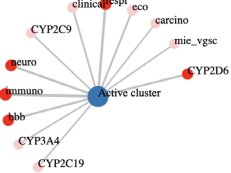
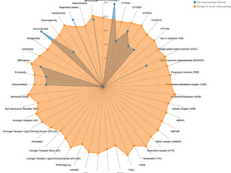
Prediction accuracy remained above the 50% minimum for all four. Z25837483, MolPort-047–786–433, and Z25837505 were each predicted with 67.38% accuracy, while MolPort-052–064–324 was significantly lower at 54.26%, narrowly clearing the threshold (Table 6). Although its LD50 is

above the minimum and the second-highest of the four compounds, this lower prediction accuracy makes MolPort-052–064–324 a weaker candidate on toxicity grounds.

The active cluster maps for all four compounds showed similar toxicity targets across all four compound – CYP450 enzymes (CYP2C9, CYP2C19, CYP3A4, CYP2D6), neurotoxicity, immunotoxicity, and

BBB interaction – indicating a shared mechanism profile despite differing severity (Table 6). Radar charts showed Z25837483 and MolPort-047–786–433 stayed close to the FDA-approved baseline, while Z25837505 and MolPort-052–064–324 varied on certain endpoints, indicating a somewhat more toxic profile.

Table 6. Predicted toxicity profiles for the four lead compounds, generated using ProTox 3.0. LD50 reflects the predicted dose lethal to 50% of a population; toxicity class follows the GHS-based classification used by ProTox, where higher classes indicate lower toxicity. Prediction accuracy reflects the model’s confidence in the overall toxicity classification. Active cluster images and radar charts illustrate each compound’s predicted toxicological targets and pathways relative to the average profile of FDA-approved drugs

Compound	LD50 (mg/kg)	Toxicity Class	Prediction Accuracy	Active Cluster Image	Radar Chart
1. Z25837483	300	3	67.38%		
2. Mol-Port-047–786–433	1190	4	67.38%		
3. Z25837505	300	3	67.38%		
4. Mol-Port-052–064–324	807	4	54.26%		

Altogether, Z25837483 carries the most favorable toxicological profile in terms of endpoints, though its LD50 is at the minimum. MolPort-047–786–433, with a strong LD50, acceptable toxicity class, and a moderate active cluster, is the strongest candidate overall. Z25837505 shows a pronounced CYP2E1 deviation, a clear limitation. MolPort-052–064–324, despite an acceptable LD50 and toxicity class, has the lowest prediction accuracy and the most flagged toxicological endpoints, making it the weakest candidate.

Conclusion (Discussion)

This study identified MolPort-047–786–433 as the strongest overall candidate for MerTK-targeted small molecule therapy in cardiovascular disease, emerging from an initial pool of fifteen pharmacophore-matched compounds. It combined favorable predicted binding (–8.2918 kcal/mol), full Lipinski compliance (MW 463.58 g/mol, CLogP 2.39, zero violations), high GI absorption, and the most favorable toxicity profile of the four finalists, (LD50 1190 mg/kg, toxicity class 4). Z25837483 and Z25837505 also performed well but were limited by having minimum-threshold LD50 values and, for Z25837505, a pronounced deviation in CYP2E1 activity compared to the average FDA-approved drug.

This pipeline's success stemmed from its layered screening, filtering candidates at each stage using different pharmacological criteria instead of relying on only one metric. Identifying one dominant binding pocket through two independent computational tools provided a reliable foundation for pharmacophore modeling, which generated

a diverse but consistent set of candidates for docking. ADME and toxicity screening then ensured binding affinity was not mistaken for overall drug viability, most clearly illustrated by Z5198624731, the top docking candidate, ultimately disqualified for poor drug-likeness and solubility. This progression narrowed the short four-compound list to one lead candidate grounded in multiple independent lines of evidence.

This study has two key limitations. First, several strong early-stage compounds, including the top docking candidate, were excluded due to unfavorable toxicity or drug-likeness, indicating that binding affinity alone is an unreliable predictor of overall candidate viability. Second, all findings were computational; no laboratory testing was performed, so the binding, ADME, and toxicity profiles are purely theoretical until experimentally validated. Future work should prioritize laboratory validation of MolPort-047–786–433, including in vitro binding affinity and efferyocytosis functional testing, to confirm its potential as a MerTK-targeted therapeutic for cardiovascular disease.

References

- Ait-Oufella, H., Pouresmail, V., Simon, T., et al. (2008). Defective Mer receptor tyrosine kinase signaling in bone marrow cells promotes apoptotic cell accumulation and accelerates atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 28(8), 1429–1431. <https://doi.org/10.1161/atvbaha.108.169078>
- Benjamin, E.J., Muntner, P., Alonso, A., et al. (2019). Heart disease and stroke statistics-2019 update: A report from the American Heart Association. *Circulation*, 139(10), e56–e528. <https://doi.org/10.1161/CIR.0000000000000659>
- Cai, B., Thorp, E.B., Doran, A.C., et al. (2017). MerTK receptor cleavage promotes plaque necrosis and defective resolution in atherosclerosis. *Journal of Clinical Investigation*, 127(2), 564–568. <https://doi.org/10.1172/JCI90520>
- Cai, B., Kasikara, C., Doran, A.C., Ramakrishnan, R., Birge, R.B., & Tabas, I. (2018). MerTK signaling in macrophages promotes the synthesis of inflammation resolution mediators by suppressing CaMKII activity. *Science Signaling*, 11(549), eaar3721. <https://doi.org/10.1126/scisignal.aar3721>
- Cohen, P.L., Caricchio, R., Abraham, V., et al. (2002). Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. *Journal of Experimental Medicine*, 196(1), 135–140. <https://doi.org/10.1084/jem.20012094>
- Jebari-Benslaiman, S., Galicia-García, U., Larrea-Sebal, A., et al. (2022). Pathophysiology of atherosclerosis. *International Journal of Molecular Sciences*, 23(6), 3346. <https://doi.org/10.3390/ijms23063346>
- Lemke, G. (2013). Biology of the TAM receptors. *Cold Spring Harbor Perspectives in Biology*, 5(11), a009076. <https://doi.org/10.1101/cshperspect.a009076>

- Navarese, E.P., Kolodziejczak, M., Schulze, V., et al. (2015). Effects of proprotein convertase subtilisin/kexin type 9 antibodies in adults with hypercholesterolemia. *Annals of Internal Medicine*, 163(1), 40–51. <https://doi.org/10.7326/m14-2957>
- Ridker, P.M., Everett, B.M., Thuren, T., et al. (2017). Antiinflammatory therapy with canakinumab for atherosclerotic disease. *New England Journal of Medicine*, 377(12), 1119–1131. <https://doi.org/10.1056/NEJMoa1707914>
- Rothlin, C.V., Ghosh, S., Zuniga, E.I., Oldstone, M.B., & Lemke, G. (2007). TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell*, 131(6), 1124–1136. <https://doi.org/10.1016/j.cell.2007.10.034>
- Scott, R.S., McMahon, E.J., Pop, S.M., et al. (2001). Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature*, 411(6834), 207–211. <https://doi.org/10.1038/35075603>
- Shelby, S.J., Feathers, K.L., Ganios, A.M., Jia, L., Miller, J.M., & Thompson, D.A. (2015). MERTK signaling in the retinal pigment epithelium regulates the tyrosine phosphorylation of GDP dissociation inhibitor alpha from the GDI/CHM family of RAB GT-Pase effectors. *Experimental Eye Research*, 140, 28–40. <https://doi.org/10.1016/j.exer.2015.08.006>
- Thorp, E., Cui, D., Schrijvers, D.M., Kuriakose, G., & Tabas, I. (2008). Mertk receptor mutation reduces efferocytosis efficiency and promotes apoptotic cell accumulation and plaque necrosis in atherosclerotic lesions of apoE^{−/−} mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 28(8), 1421–1428. <https://doi.org/10.1161/atvbaha.108.167197>
- Thorp, E., Vaisar, T., Subramanian, M., Mautner, L., Blobel, C., & Tabas, I. (2011). Shedding of the Mer tyrosine kinase receptor is mediated by ADAM17 protein through a pathway involving reactive oxygen species, protein kinase C δ , and p38 mitogen-activated protein kinase (MAPK). *Journal of Biological Chemistry*, 286(38), 33335–33344. <https://doi.org/10.1074/jbc.m111.263020>
- Vaidya, K., Tucker, B., Kurup, R., Khandkar, C., & Patel, S. (2023). Maximizing secondary prevention for patients with ASCVD: Where does colchicine fit in cardiovascular disease prevention? Retrieved from <https://pmc.ncbi.nlm.nih.gov/articles/PMC10655803/>

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PDE4D-TARGETED SMALL MOLECULES FROM COMPUTATIONAL SCREENING FOR DEMENTIA

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Abstract

Phosphodiesterase 4D enzymes hydrolyze cyclic adenosine monophosphate (cAMP), a messenger molecule that signals neurons and the release of neurotransmitters, which are crucial to the development of memory and cognition. Inhibiting PDE4D offers promising drug treatments for neurological diseases, including dementia and other illnesses associated with memory, disorders that impair mental abilities, limit daily functioning, and complicate the lives of those affected. The goal of this study was to determine binding sites on the PDE4D protein using geometric and energetic methods and identify promising locations for binding. The PDE4D enzyme was virtually screened for interactions between the protein and the compound, revealing fitting compounds for these interactions. The docking of compounds onto the protein and their energetic interactions were quantified, providing further details on each interaction and its strength. To ensure the compounds would be absorbed and excreted properly, each molecule was analyzed according to ADME drug guidelines to become candidates for drug treatment. The toxicity levels of the compounds raise risks to the human body; by ensuring that the toxicity was harmless, 1 lead compound was successfully discovered for potential treatment, along with 2 backup compounds that met all other criteria in all experiments. These criteria included low drug and RMSD scores in the pharmacophore maps, negative ΔG values in docking of the molecules, 0 violations of Lipinski's rule and guidelines of ADME, and a high LD50. These compounds would inhibit the catalysis of cAMP, improving cognitive symptoms of dementia and associated disorders.

Keywords: *Drug discovery; dementia; small molecules; virtual screening; PDE4D.*

Introduction

Dementia is a neurological disorder characterized by a decline in mental abilities, some of which are categorized as memory loss and cognitive functioning (Arvanitakis, Z., Shah, R.C., Bennett, D.A., 2019). Over time, the progression of dementia and dementia-

related diseases, such as Alzheimer's, vascular dementia, Lewy body dementia, and frontotemporal dementia, can lead to impairment of daily functioning, especially amongst older populations (Chouliaras L., O'Brien J.T., 2023). In 2021, an estimated 57 million people suffered from dementia, with the pro-

jected value expected to increase to 139 million by 2050. The yearly cost of dementia is estimated to be well above \$1.3 trillion USD and is expected to rise to \$2.8 trillion USD by 2030 (Guerchet, M.G., Prince, M.P., Prina, M.P., 2020). The complications of dementia place psychological and emotional stress on its patients, as well as economic burdens on health care institutions to develop treatments and provide care (Liu W., Deng W., Gong X., Ou J., Yu S., Chen S., 2025).

The pathways of dementia consist of cholinesterase, amyloid, and tau. The cholinesterase enzyme specializes in the breakdown of the neurotransmitter acetylcholine (ACh). ACh is associated with both voluntary and involuntary movement, as well as strengthening cognitive processes related to memory and thinking (Moehle M. S., Conn P. J., 2019; Hasselmo M. E., 2006). The inhibition of cholinesterase limits the hydrolysis of ACh, increasing its functions and alleviating the associated decline in memory (Sharma K., 2019). Amyloid precursor proteins can be processed into amyloid- β , which is overproduced in dementia patients and can aggregate and clump in the brain (Clarkson, J.C., 2021). Tau proteins, located on neuron axons, can detach and form abnormal tau proteins that cannot maintain their own structure and clump as well (Clarkson, J.C., 2021). Both the aggregation of amyloid and tau contribute to the degradation of neurons, leading to cognitive decline (Gulisano W., Maugeri D., Baltrons M. A., Fà M., Amato A., Palmeri A., D'Adamio L., Grassi C., Devanand D. P., Honig L. S., Puzzo D., Arancio O., 2018).

Phosphodiesterase's (PDEs) are part of a family of enzymes that can be classified into 11 subgroups (PDE1 to PDE11). These enzymes are coded by 24 different genes, and they can be found in tissues located in the heart, brain, kidneys, endothelial cells, and immune cells. PDEs can stop cyclic nucleotide signaling through the hydrolysis of both cAMP and cGMP, both intracellular cellular messengers (Bizzi M. F., Bolger G. B., Korbonits M., Ribeiro-Oliveira A. Jr., 2019; Fan T., Wang W., Wang Y., Zeng M., Liu Y., Zhu S., Yang L., 2024). The specific subgroup, PDE4, selectively degrades cAMP and consists of 4 isoforms: PDE4A, PDE4B, PDE4C, and PDE4D, which are encoded by separate genes

(Fan T., Wang W., Wang Y., Zeng M., Liu Y., Zhu S., Yang L., 2024).

PDE4D enzymes hydrolyze cyclic adenosine monophosphate (cAMP), a secondary messenger crucial to cellular signaling in inflammatory immune responses, cardiovascular regulation, and brain cognition (Bhat A., Ray B., Mahalakshmi A. M., Tuladhar S., Nandakumar D. N., Srinivasan M., Essa M. M., Chidambaram S. B., Guillemin G. J., Sakharkar M. K., 2020). Through hydrolysis, the PDE4D molecule breaks cAMP into inactive 5'AMP. Through this degradation, the downstream signaling pathways are turned off and are unable to perform normal functions, such as triggering immune responses or activating genes (Olsen C. M., Liu Q. S., 2016). PDE4D is mainly located in the brain and is involved in cAMP signalling related to physiological processes involved with memory consolidation and neuroplasticity (Donders Z., Skorupska I. J., Willems E., Mussen F., Broeckhoven J. V., Carlier A., Schepers M., Vanmierlo T., 2024). These are associated with many neurodegenerative diseases in the central nervous system, including but not limited to dementia, Alzheimer's, multiple sclerosis, spinal cord injuries, and traumatic brain injuries (Donders Z., Skorupska I. J., Willems E., Mussen F., Broeckhoven J. V., Carlier A., Schepers M., Vanmierlo T., 2024).

Figure 1. A model of the PDE4D-cAMP pathway and the hydrolysis of cAMP (Gooderham M., 2013)

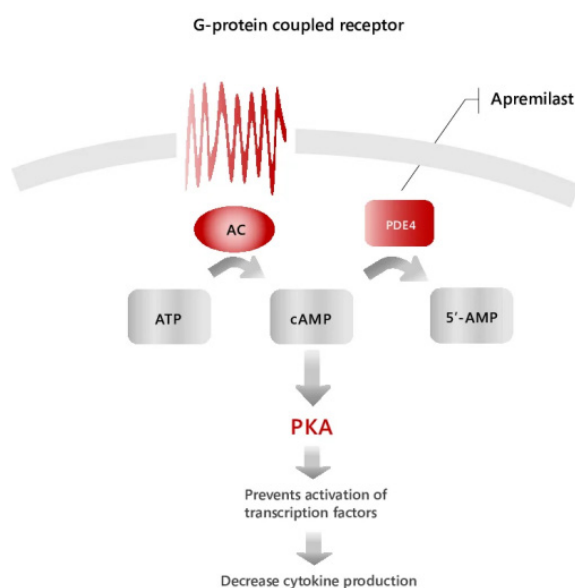


Figure 1 demonstrates the PDE4D hydrolysis of cAMP and the pathway that it follows. AC is represented as Adenylyl Cyclase, an essential enzyme that converts ATP, a primary energy-carrying molecule, to cAMP. cAMP molecules regularly bind to subunits of PKA, Protein Kinase A, and change their shape to be able to release catalytic subunits that attach to target proteins. The PDE4D protein catalyzes the hydrolysis of cAMP, breaking it down into 5'AMP. 5'AMP is considered inactive in cellular signaling; the degradation of cAMP prevents it from being able to send signals necessary for cognitive development.

Through the inhibition of PDE4D, the process of hydrolysis of cAMP occurs much less frequently, increasing cAMP levels and enhancing its function (Adili A., Dilihumaer A., Zhu H., Tang H., 2024). PDE4D inhibitors provide a potential therapeutic target for dementia by improving cAMP signaling focused on memory and cognition.

PDE4D inhibitors are possible therapeutic targets for neurological disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, and sleep disorders. The link between PDE4D and cAMP signaling can be used to examine specific PDE4D inhibitors while minimizing adverse side effects (Bhat A., Ray B., Mahalakshmi A. M., Tuladhar S., Nandakumar D. N., Srinivasan M., Essa M. M., Chidambaram S. B., Guillemin G. J., Sakharkar M. K., 2020). PDE4D inhibitors are also able to enhance cAMP levels and pathways focused on neurodegenerative diseases in the central nervous system. PDE4D inhibitors can be effective therapeutic targets for these neurological diseases. The development of PDE4D inhibitors can be used as drugs to prevent and treat these diseases (Adili A., Dilihumaer A., Zhu H., Tang H., 2024).

Methodology

Analysis of Binding Sites

Geometric Method

To begin searching for viable binding sites on PDE4D for small molecules using geometric methods, ProteinPlus (<https://proteins.plus/>), a Structure-Based Modeling Support Server, was visited. After loading the site, the PDB code for PDE4D was entered and searched: **2PW3**. Once the protein had

been found, the site displayed both an image of the protein and a list of analysis tools. DoGSiteScorer was selected, and all settings remained unchanged. The "Analysis detail" was set to "Pocket(s)", the "Binding site prediction granularity" was set to "and drugability", no ligands were set or entered, and the "Chain" was set to "all". The data on the volume, surface area, and drug score of the top 5–10 binding sites were recorded and subsequently evaluated for consistency.

Machine Learning Methods

To further narrow down the available and compatible binding sites, another website, Prankweb (<https://prankweb.cz/>), was used to analyze the PDE4D protein binding sites. After the site was loaded, the same PDB code, 2PW3, was entered and searched for. The initial search settings stayed the same: "Experimental structure", "Use original structure", and "Use conservation" were selected. The rankings, score values, and # residues (or amino acids) were recorded, along with select amino acids from each binding site.

Pharmacophore-based Virtual Screening

To virtually screen the proteins for compounds compatible with binding sites, a pharmacophore map tool, Pharmit (<https://pharmit.csb.pitt.edu/>) analyzed the interactions. The PDB code, 2PW3, was input into the search, and the ligand was set to "CMP". To better view the interactions, the Ligand and Receptor, located under Visualization, were set to "None", and the opacity of the protein was lowered to its minimum. Around 3–5 interactions, located under Pharmacophore, were selectively chosen by turning them on or off, and compounds were searched from the databases MolPort, Enamine, ChEMBL36, and ChemSpace. Specific compounds and their RMSD (Root Mean Square Deviation) were recorded, along with images of the pharmacophore maps and compounds.

Molecular Docking using SwissDock

Once binding sites and compounds were selected, SwissDock (<https://swissdock.ch/>) was visited to continue experimenting in discovering viable interactions between the protein and the small molecules. The

site required a ligand, the target, and a defined search space. Using each of the IDs obtained from Pharmit, the SMILES (Simplified Molecular Input Line Entry System) was searched for each compound using their respective websites. These SMILES codes were input into Swissdock, and the ligand was prepared. The PDB code, **2PW3**, was input as the target; the chains were set to “Select all”; the heteratom(s) were set to “None”, and the target was prepared. To define the search space, the box was centered at (Clarkson, J.C., 2021; Chouliaras L., O’Brien J.T., 2023, 35) Å and its size was set to (25, 28, 25) Å. These values were used for all compounds across all calculations. Each compound and its SMILES were entered individually, along with the PDB code and defined search space, and the parameters were checked. Values for the SwissParamScore, or ΔG values (kcal/mol), and images for each compound and all interactions were recorded for further evaluation.

Drug Properties and ADME

Using the results obtained from the SwissDock experiment, 8 compounds with the lowest ΔG values and their SMILES code were input into SwissADME (<https://www.swissadme.ch/>). All SMILES were entered into the list, and the tool was run. Each molecule provided a chart, and the values for the molecular weight (g/mol), number of H-bond acceptors, number of H-bond donors, and Consensus LogP were recorded. To ensure that all values met the criteria of Lipinski’s Rule, the number of violations was also considered, along with other values (classification of solubility and GI absorption) to verify that the data results were aligned.

Toxicity Prediction

The 6 compounds obtained from the SwissADME testing underwent further testing to determine toxicity levels to the human body. To assess these toxicity levels, ProTox 3.0 (<https://tox.charite.de/protox3/index.php?site=home#>) was visited, and Tox prediction was selected. Each compound and its SMILES were entered into “Canonical SMILES”, and the “smiles” button was clicked so the compounds could be run through the system, and an image of the compound was

displayed. All models for toxicity were selected through the “All” button, and the tool was run by clicking “Start Tox-Prediction”. The Predicted LD50, Predicted Toxicity Class, images of the Inactive and Active Network Charts, and Average toxicity Radar Charts were recorded.

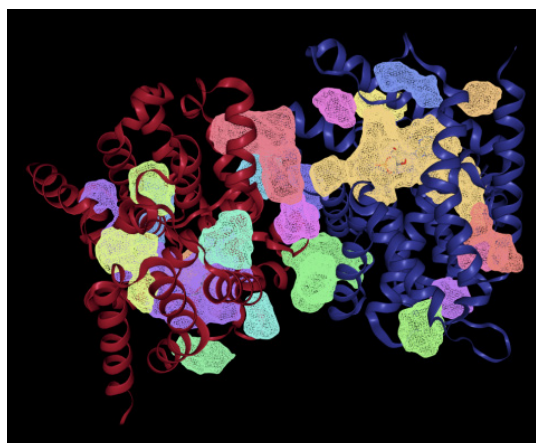
Results and Discussion

Binding Sites

Binding sites in proteins are 3D pockets that host interactions between attaching ligands and the designated protein. For the small molecules to bind properly to the protein, the shape, energetic interactions, and chemical interactions must be considered for the molecule to bind properly and for the protein to carry out its function. If the binding site is too small, then the compound will be unable to attach to the site; if the binding site is too large, then the compound will continue to move within the pocket, leading to weaker interactions. Most small molecules are usually under 1000 Da in size, and because of this, most binding sites are between 500–1000 Daltons (Da) to accommodate these compounds. It is estimated that 85% of proteins do not have binding sites, and it is important to first determine if the PDE4D protein has binding sites before determining location, sizes, and interactions.

Geometric Method Analysis of Binding Sites

The geometric method is used to determine the size of the binding sites that will fit a specific compound. By using this experiment, the binding sites can be located, and the interactions can be studied. Using the ProteinPlus site and DoGSiteScorer as a search engine for binding sites on the PDE4D protein, key quantitative values: volume, surface area, and the drug scores, were measured and recorded. As the geometric experiment analyzes the 3D structure of the binding site, these values are necessary in determining size. The protein was searched for using the PDB code, a unique 4-character alphanumeric identifier stored in the Protein Data Bank that references a specific protein and its 3D structure. A detailed image of all available binding sites based on size, depth, and drug score can be seen in Figure 2.

Figure 2. Geometric image of the PDE4D protein and available binding sites

The red and blue coils represent the PD-E4D protein, and all other color-coded 3D shapes represent the binding sites. Each binding site in a protein corresponds with values in size and success in binding with small molecules. This protein has 23 available binding sites for small molecules, ranging in size and shape. The binding sites are distributed and scattered across both chains; some are larger,

and some are smaller. Using the geometric method, the volume would characterize the size and depth of the binding sites, while the surface area would characterize its overall shape and available area to bind. The druggability score is also provided, a computational method that predicts the likelihood of a small molecule binding to the target protein. All values can be viewed below in Table 1.

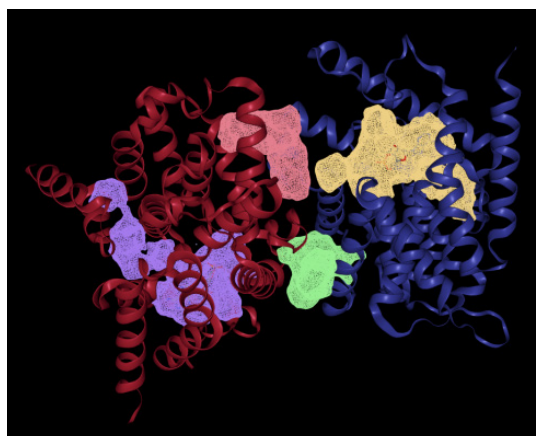
Table 1. Geometric data on 8 of 23 binding sites on the PDE4D Protein

Color	Name	Volume (Å ³)	Surface Area (Å ²)	Drug Score
	P_0	1183.88	1045.22	0.81
	P_1	1135.12	1036.62	0.81
	P_16	119.07	332.86	0.42
	P_17	118.54	246.43	0.43
	P_2	754.98	970.0	0.79
	P_3	659.81	813.43	0.76
	P_5	184.5	213.71	0.45
	P_9	168.14	383.07	0.4

It is known that the drug score ranges from 0 to 1. Scores above 0.5 are considered acceptable and indicate that the corresponding binding site on the protein is suitable and compatible for a small molecule. A higher drug score indicates there is a high structural probability that a small molecule can effectively bind to the target protein and perform its designated function. The PDE4D protein provides promising binding sites: P_0, P_1,

P_2, and P_3 all have drug scores from 0.76 to 0.81. The range of the drug scores is 0.59, with the lowest score being 0.22 and the highest score(s) being 0.81. The high values for volume and surface area, which correlate with high drug score values, will determine the success of the binding sites. Once selected, these specific, acceptable binding sites and their locations on the PDE4D protein can be viewed in Figure 3.

Figure 3. *Geometric image of the PDE4D protein and the most compatible binding sites*



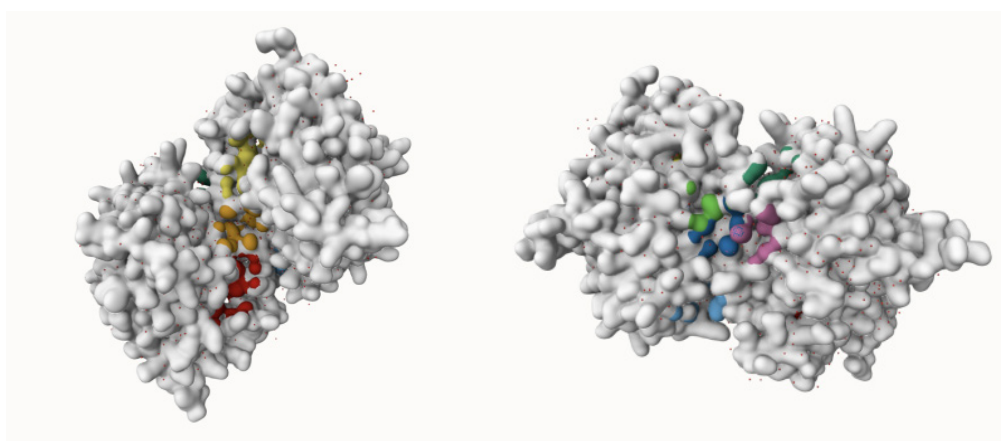
Experimenting through Machine Learning Methods

Cellular energetics play an important role in the binding of small molecules to these pockets. As the geometry, shape, and size of the binding sites have been analyzed, it is important to consider how the protein and small molecules interact energetically as well. Rather than analyzing both the geometry and energy individually and then comparing, it is

possible to combine them through machine learning methods, which account for both geometric and energetic properties.

The PrankWeb site used a structured method to analyze a 3D model of the protein and provided possible binding sites based on geometric structure and energy availability for small molecules. These binding sites can be viewed on the PDE4D protein in Figures 4 and 5.

Figure 4 and Figure 5. *PDE4D protein and its available binding sites based on both geometric and energetic properties*



All binding sites located in both Figures 4 and 5 indicate areas on the PDE4D protein where it is compatible with small molecules. Each color symbolizes a binding site that is compatible with a small molecule, indicating that the target protein can be bound to. The color-coded binding sites in Figure 4 represent the numbers ranked as 1, 2, and 3 in Table 2. The color-coded binding sites in Figure 5 represent the numbers ranked as 4, 5, 6, 7, and 8 in Table 2.

Discovering binding sites using the machine learning method would provide scores like the geometric method and the Protein-Plus tool, but on a much larger scale. Similarly, a higher score indicates a higher compatibility as a binding site. Each binding site will have a specific number of residues, or amino acids, that are available to interact with the small molecule. These values, along with information on the scores and number of residues are listed in Table 2.

Table 2. Binding sites ranked 1–8 with corresponding scores, number of residues (amino acids), and specific amino acids

Binding Site Rank	Score	# of Residues	Amino acids
 1	73.93	37	Serine 123, Asparagine 124, Histidine 75
 2	73.61	35	Isoleucine 128, Methionine 188, Phenylalanine 287
 3	7.34	12	Leucine 184, Serine 142, Asparagine 146
 4	3.98	12	Arginine 176, Aspartic acid 162
 5	2.96	10	Leucine 136, Arginine 176
 6	1.50	8	Methionine 137, Alanine 150
 7	1.47	8	Lysine 154, Alanine 150
 8	0.85	5	Leucine 134, Histidine 147

A higher score corresponded with a higher rank and number of amino acids. Specific amino acids were selected to demonstrate some components of the binding site that would interact energetically with the small molecule.

In general, scores above 5 are acceptable, with high values indicating that the binding sites of the proteins are compatible with the small molecules. Both binding sites 1 and 2, as ranked in Table 2, had scores of 73.93 and 73.61. The third binding site may also be considered, as it has a score of 7.34, but is unnecessary compared to the high values of 1 and 2. Most binding sites have between 10 and 20 amino acids; however, the first binding site has 37 amino acids, and the second binding site has 35 amino acids. Values well above the average range indicate that these binding sites are larger than the average. This information indicates that there are many subareas and sub pockets for the small molecules to bind to, increasing both their compatibility and the corresponding score.

Virtual Screening

The 2 methods of screening for compounds include physical screening and virtual screening. The purpose of screening for proteins is to narrow down the available compounds from millions to 10–20. Physical screening requires the target protein

and small molecules in large quantities and takes time to test the protein with different compounds to determine which compounds are most compatible with the binding sites. Virtual screening can screen for millions of compounds through online databases in a shorter time, providing accessible details on the compounds and their compatibilities. Because binding sites have already been located and their sizes and interactions have been recorded from the first experiments, it is possible to move on with virtual screening to determine specific compounds that will bind with the proteins.

Experimenting with Pharmacophore Maps

Pharmacophore maps are 3D maps of the interactions between the target protein and the binding compound. The components of a pharmacophore map consist of the number of interactions that occur, at which points, the relative distance between the interactions, and the types of interactions of the amino acids. Types of interactions include polar, non-polar, hydrophilic, and hydrophobic. By manipulating which interactions are occurring, it is possible to screen for compounds based on these selections.

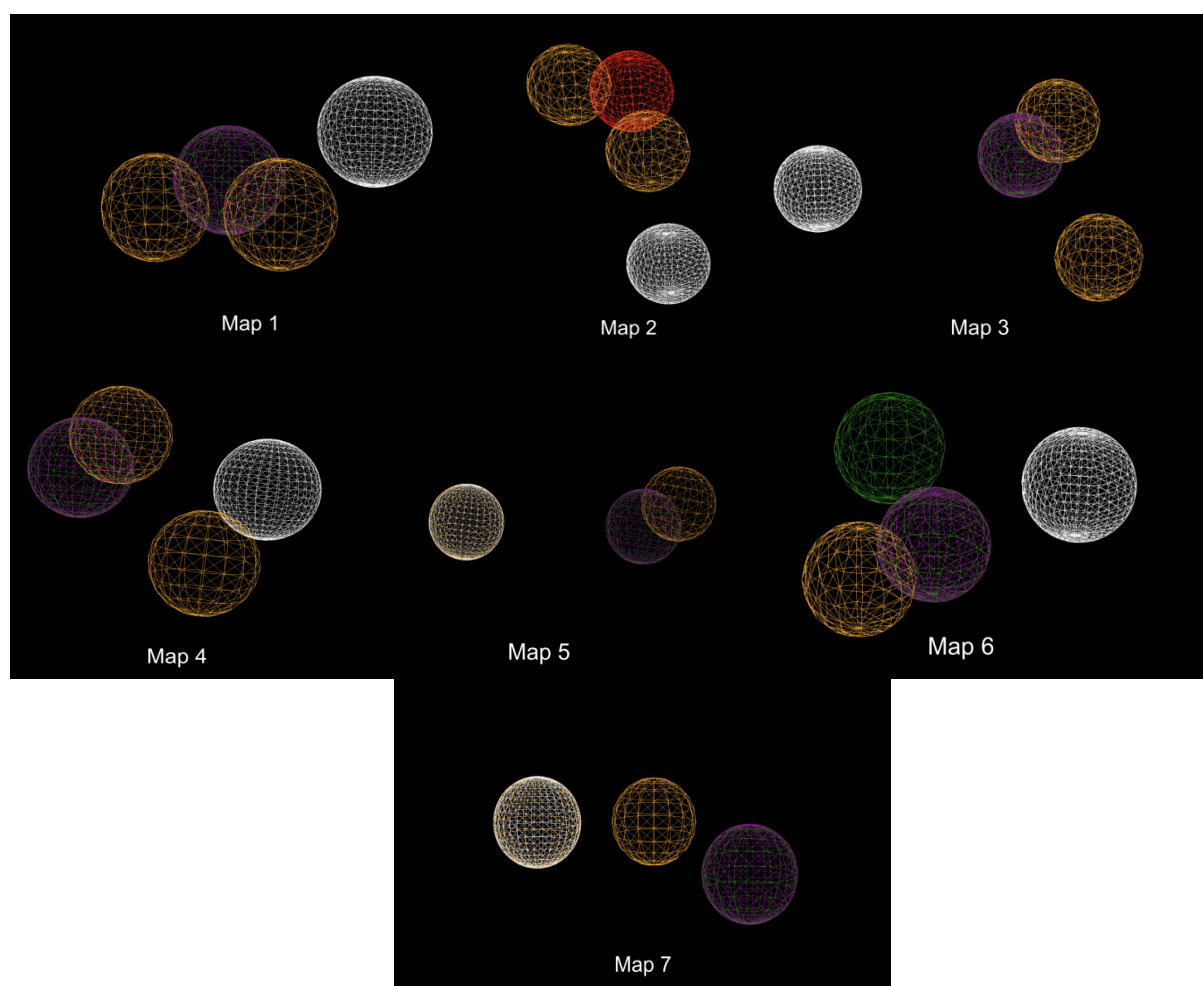
The RMSD (Root Mean Square Deviation) is a numerical value that measures the difference between a model's predicted values and

the actual values. In the pharmacophore experiments, an RMSD below 0.2 was the target, with values below 0.1 being ideal. A lower RMSD indicates that the overlay between the map and the compound is closer to perfect, with a score of 0 indicating no deviation at all.

The interactions selected were based on the conditions of number, relative distance, and type. In the pharmacophore map, each interaction was represented by a sphere, and its color represented the type of interaction. A lower number of interactions was ideal, as too many interactions would provide compounds with an RMSD above 0.2, as it would be difficult to find a compound

that could perform all interactions precisely. The distances between interactions were minimized as much as possible, with some interactions overlaying one another, and the diversity of interactions was also selected for. Different types of interactions are more accurate for natural compounds, as compounds do not interact uniformly. Interactions were selected based on these components, and compounds that matched these interactions were searched for from servers including MolPort, Enamine, etc. These results can be viewed in Table 3, which includes the name, RMSD, pharmacophore map, and compound.

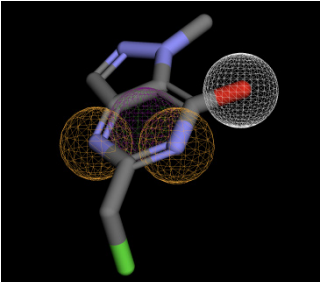
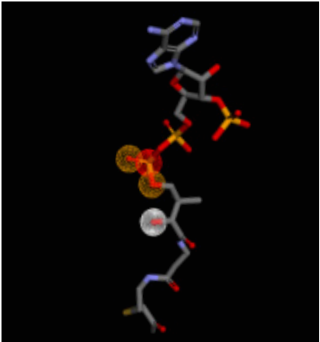
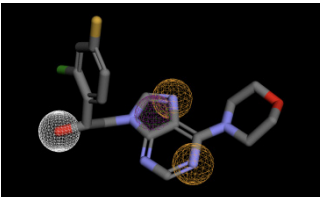
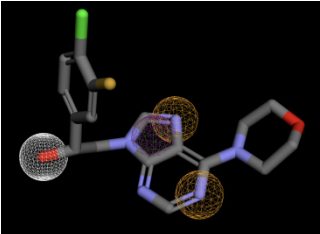
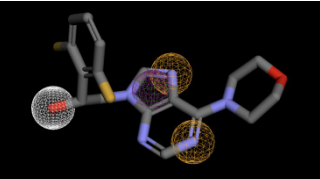
Figure 6. *Pharmacophore maps of interactions between compounds and the PDE4D protein*

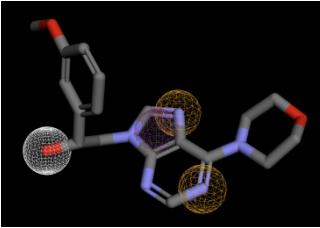
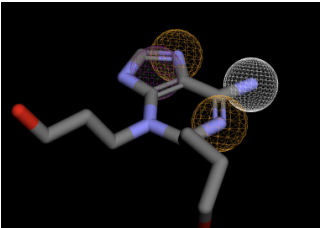
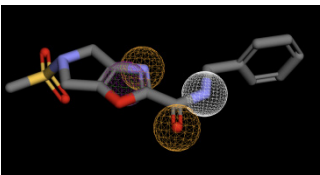
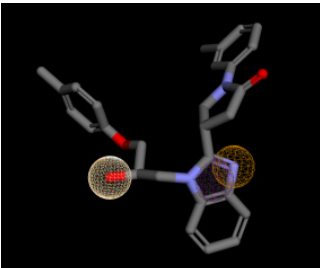
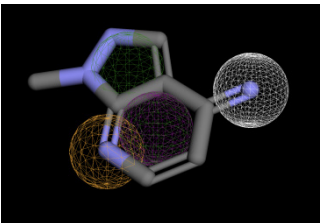
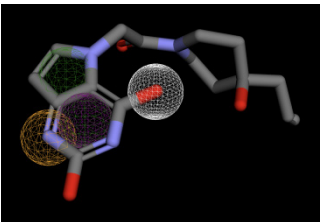
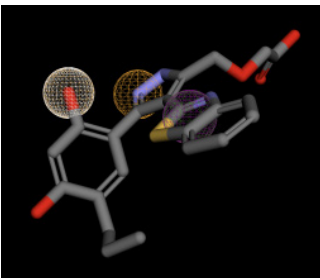


Each map below corresponds with specific compounds listed below in Table 3. The spheres indicate the interactions, and the colors indicate the type of interaction. Each map contains around 3–5 interactions. Yellow spheres represent hydrogen acceptor in-

teractions, red spheres represent anion interactions, purple spheres represent aromatic interactions, white spheres represent hydrogen donor interactions, and green spheres (overlaid with purple) represent hydrophobic interactions.

Table 3. *Compounds and values obtained from the pharmacophore map of interactions between molecules and proteins.*

Name		RMSD	Pharmacophore Map & Compound
Map 1	MolPort-046–855–508	0.024	
	MolPort-053–297–972	0.028	
Map 2	MolPort-053–223–721	0.041	
Map 3	Z1729094043	0.024	
	Z1675621302	0.025	
	Z1334538364	0.025	

Name		RMSD	Pharmacophore Map & Compound
	Z1345952503	0.025	
Map 4	CHEMBL3084286	0.042	
	CHEMBL3441070	0.042	
Map 5	MolPort-010–705–550	0.001	
Map 6	Z1901071406	0.024	
	Z6289672314	0.027	
Map 7	MolPort-000–479–672	0.012	

Multiple combinations were experimented with through the pharmacophore maps. Some compounds provided promising RMSD values of 0.001, while the highest value was 0.041. The compounds MolPort-010–705–550, CSSS00160257030, and CSC027798714 had the lowest RMSD values of 0.001 and 0.002, compared to all other compounds, which had RMSD values between 0.01 and 0.05. The pharmacophore maps with overlapping interactions of hydrogen donors and hydrogen acceptors (Maps 5 and 7) provided the lowest values, while the maps including anion interactions and multiple hydrogen acceptor interactions in proximity revealed higher RMSD values.

The experimentation with virtual screening revealed that there were many available compounds with acceptable RMSD values. All scores were below the maximum value of 0.2; values below 0.1 are considered exceptional, indicating that all compounds are candidates for further experimentation

Molecular Docking Experimenting

Molecular Docking is a computational tool used to determine how the binder interacts with the target protein. The protein continues to rotate until the compound can dock on any recognized binding site on the surface, and the energetic interaction between the two is recorded.

Using 3D modeling, molecular docking predicts all possible angles and areas where the ligand can bind to the protein, and is represented by ΔG , or Gibbs Free Energy, a numerical value used to quantify the energy of the interaction.

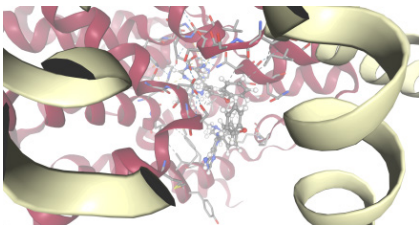
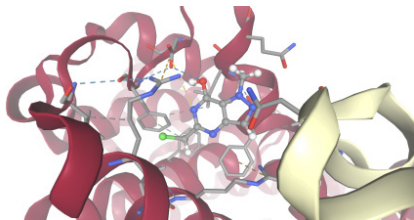
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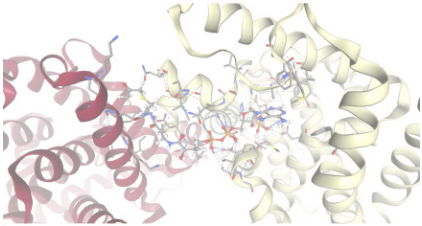
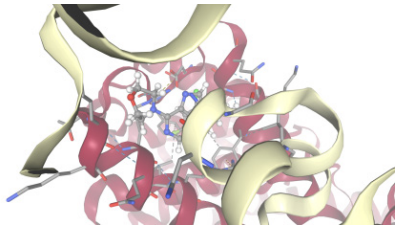
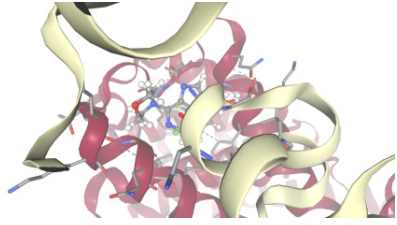
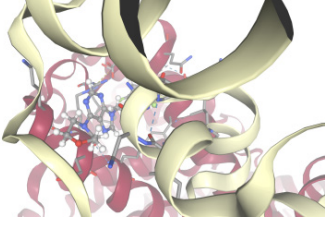
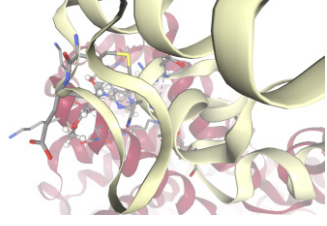
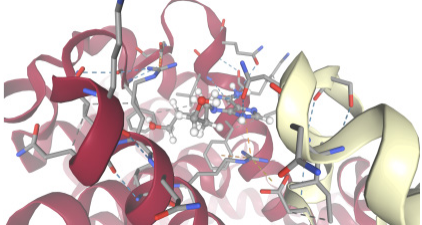
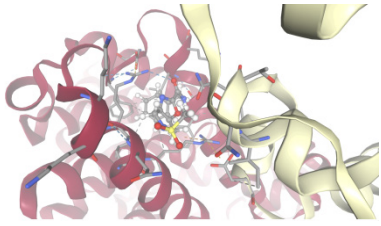
SwissDock, the tool used in this experiment, was used to analyze the 15 compounds obtained from the pharmacophore maps and virtual screening. Each compound corresponded with a ΔG value and an image of interactions with the protein.

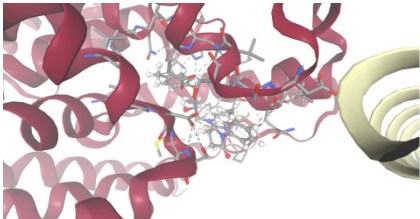
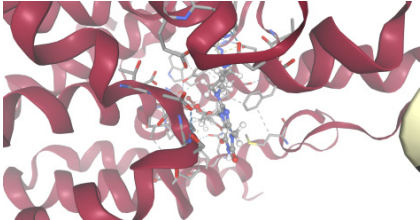
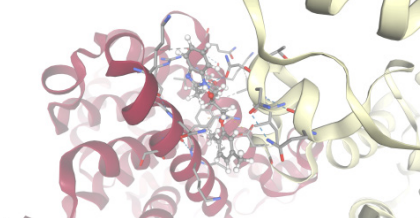
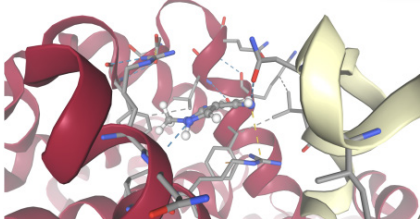
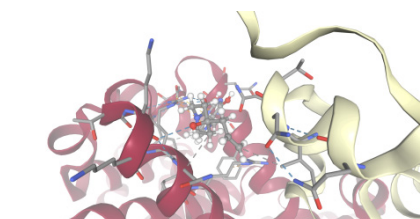
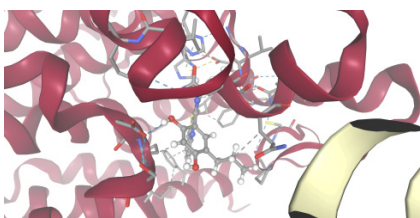
ΔG values are used to measure the thermodynamic favorability of chemical reactions and physical processes to determine if the reaction is spontaneous. A negative ΔG value, or $\Delta G < 0$, indicates that the reaction is spontaneous and will proceed, while a positive ΔG value, or $\Delta G > 0$, indicates the reaction is not spontaneous and will not proceed. All ΔG values for each compound were negative and were collected across all experiments. The lowest ΔG values from each compound can be viewed in Table 4.

Most of the binding sites were in the center of the protein, as discovered through experimentation using machine learning methods and Prankweb. Because of this, the search space was focused on the center of the protein, and all compounds tested would be included in these parameters.

Table 4. *SwissDock experimentation with select compounds and interactions with the PDE4D protein.*

Compound	ΔG value (kcal/mol)/ SwissParam Score	3D interaction pose of the compounds with PDE4D
MolPort-046–855–508	-10.4409	
MolPort-053–297–972	-6.4946	

Compound	ΔG value (kcal/mol)/ SwissParam Score	3D interaction pose of the compounds with PDE4D
MolPort-053–223–721	-10.3763	
Z1729094043	-7.9418	
Z1675621302	-7.7547	
Z1334538364	-7.7980	
Z1345952503	-7.6475	
CHEMBL3084286	-7.5887	
CHEMBL3441070	-7.5722	

Compound	ΔG value (kcal/mol)/ SwissParam Score	3D interaction pose of the compounds with PDE4D
MolPort-010–705–550	-7.9100	
CSSS00160257030	-8.3064	
CSC027798714	-8.2924	
Z1901071406	-6.2654	
Z6289672314	-7.5757	
MolPort-000–479–672	-8.1858	

Each compound and its respective ΔG value and images are listed. The lowest ΔG value was taken from each cluster, or binding site, and was recorded. Each image shows the compound location and interaction with the target protein, represented by the color-coded dashed lines. Blue represents hydrogen

bonding, yellow represents ionic interactions, orange represents cation- π interactions, gray represents hydrophobic contacts, and green represents π -stacking interactions.

The ΔG values can be classified into 3 categories: strong, moderate, and weak. A ΔG value is considered strong when it is negative

and of high magnitude. Similarly, a moderate ΔG value would have a moderate magnitude, and a weak ΔG value would have a low magnitude and value. Any ΔG value above 0 would not be considered, as it would imply that the interaction is unfavorable and not spontaneous.

Using this information on ΔG , the compounds with the strongest negative values were MolPort-046–855–508, MolPort-053–223–721, CSSS00160257030, Z1901071406, and MolPort-000–479–672. As noticed with the pharmacophore maps, most of the compounds with the lowest RMSD scores also corresponded with the strongest ΔG values. However, the strongest ΔG values were viewed from the first and third compounds, MolPort-046–855–508 and MolPort-053–223–721, which had ΔG values of -10.4409 and -10.3763 (Pharmacophore Map 1).

All ligands interacted with the protein target, as viewed through the dashed lines. Most interactions were hydrogen bonding or hydrophobic contact, with occasional ionic interactions, cation- π interactions, and π -stacking interactions. This corresponds with the results of the pharmacophore maps: the maps with the lowest RMSD scores had hydrogen donor and hydrogen acceptor interactions, as well as overlapping aromatic and hydrophobic interactions. The correlation between screening of the compounds in pharmacophore maps and their interactions with the target protein indicates that the interaction type dictates lower RMSD scores and ΔG values.

All compounds are exceptional candidates, as all ΔG values are negative values with large magnitudes. The best 8 compounds out of these 15 were selected for further testing and experimentation: MolPort-046–855–508, MolPort-053–223–721, Z1729094043, Z1334538364, MolPort-010–705–550, CSSS00160257030, Z1901071406, and MolPort-000–479–672, were selected.

Drug Treatments using ADME

SwissADME is a computational tool used in pharmacokinetics to analyze the chemical properties of a designated drug and determine whether it can function adequately

ly in the human body. Pharmacokinetics is the study of how the body interacts with the administered drug. SwissADME evaluates compounds and drug candidates and how the interaction between the molecule and the human body is affected. The acronym ADME stands for Absorption, Distribution, Metabolism, and Excretion. These are the 4 key stages a drug must go through to be processed by the body, determining its effectiveness and how long it remains in the body. Absorption, the first stage, occurs when the drug is absorbed into the bloodstream through the stomach. The drug is circulated by the bloodstream until it reaches the target protein. Both absorption and distribution occur before the molecule binds to its target. If either step does not occur, or does not occur efficiently, the drug is unable to reach its target and perform its function. The next stages, metabolism and excretion, occur after the compound has bound to the protein and ensure that the drug remains in the body for the necessary amount of time. If the drug remains in the body for an extended period, its effects may become toxic. If the drug does not remain in the body long enough, it cannot treat the protein to its fullest extent. Metabolizing enzymes detect the drug and decompose it into small units, which are then excreted from the body.

Experimenting through SwissADME using Lipinski's Rule

The ADME process is critical in determining how the drug moves through the body, how it is processed, and how it is excreted. Through SwissADME, values are obtained and analyzed according to Lipinski's Rule, a set of criteria in pharmacology that evaluates whether a drug has acceptable absorption and permeation. These criteria include fewer than 5 hydrogen bond (H-bond) donors and 10 hydrogen bond acceptors, a molecular mass less than 500 Daltons (Da), and a calculated LogP value below 5. A molecule larger than 500 Da would be ineffective, as it would take too long to bind to a protein and be processed, leading to the drug remaining longer in the body, while a molecule too small will be unable to bind to the protein or be processed more quickly. The ideal time for a drug to remain in the body is around 8–10 hours, with an accepted range of 2–10 hours.

The calculated LogP, or log of Partitioning, measures how well a drug dissolves in fat compared to water. A high LogP indicates that it is more likely to dissolve in fat, while a lower LogP indicates that it is more likely to dissolve in water.

All criteria of Lipinski's rule must be met for the compound to qualify as a drug treat-

ment. Any violation would indicate that the drug has an unfavorable ADME profile and would not be compatible with the human body. Approximately 80–90% of compounds fail to meet the guidelines of ADME and Lipinski's Rule and are therefore eliminated as candidates.

Table 5. Analysis of compounds and values through SwissADME testing for violations of Lipinski's Rule

Compound	Molecular Weight (g/mol)	Number of H-bond acceptors	Number of H-bond donors	(Consensus) LogP	Violations of Lipinski's Rule	Solubility Classification	Bioavailability Score
MolPort-046-855-508	826.95	10	3	5.09	Disagree; 2 violations	Poorly soluble	0.17
MolPort-053-223-721	823.60	20	9	-4.24	Disagree; 3 violations	Very soluble	0.11
Z1729094043	377.80	6	1	1.40	Agree; 0 violation	Soluble	0.55
Z1334538364	361.35	7	1	1.16	Agree; 0 violation	Soluble	0.55
MolPort-010-705-550	455.55	4	1	3.70	Agree; 0 violation	Moderately soluble	0.55
CSSS00160257030	636.13	5	2	2.48	Disagree; 1 violation	Poorly soluble	0.55
CSC027798714	416.51	4	1	4.33	Agree; 0 violation	Moderately soluble	0.55
Z1901071406	148.17	2	2	0.36	Agree; 0 violation	Soluble	0.55
MolPort-000-479-672	439.48	7	4	3.71	Agree; 0 violation	Moderately soluble	0.11

Information such as the classification of solubility and bioavailability score were also included to provide further data on whether the drug would be processed efficiently through the body. A drug's solubility determines how likely it is to dissolve in liquid, and the bioavailability score measures what percent of the substance successfully reaches the bloodstream and can bind to the target protein. Not all drugs can reach the target, as they can bind to the plasma or are unable to reach the protein. High levels in both solubil-

ity and bioavailability are optimal. Although they have no impact on the outcome of Lipinski's rule, the data adds confirmation or rejection of the compounds.

The compounds MolPort-046–855–508, MolPort-053–223–721, and CSSS00160257030 violated at least one of Lipinski's rules. All three of these compounds were eliminated.

Trends observed throughout the data were that higher classifications of solubility (soluble, very soluble, etc.) corresponded

with lower LogP values. In addition, the lower the number of H-bond donors and acceptors, the lower the molecular weight. It was also observed that almost all selected compounds that met Lipinski's Rule requirements had a bioavailability score of 0.55, with the only exception being MolPort-000-479-672. Throughout the data, it has been observed that compounds obtained from Enamine have been the most reliable through all experiments.

The remaining 6 compounds (Z1729094043, Z1334538364, MolPort-010-705-550, CSC027798714, Z1901071406, and MolPort-000-479-672) met all the criteria. Z1334538364 had the lowest LogP value and molecular weight, and both MolPort-010-705-550 and CSC027798714 had the lowest number of H-bond donors and acceptors. All compounds ranged from moderately soluble to soluble and had high bioavailability scores except MolPort-000-479-672, which had a lower score and the highest number of H-bond donors and acceptors. Z1901071406 had the lowest molecular weight out of all compounds tested. These 5 compounds meet the guidelines of ADME and Lipinski's Rule, providing promising candidates for drug treatment.

Predicting Toxicity of Compounds in the Human Body

Pharmacodynamics is the study of how drugs impact the human body through the examination of chemical and molecular mechanisms of drug action, binding with a target protein. All foreign substances entering the human body contain some level of toxicity, in which a certain amount can cease human functioning and bodily processes. The 3 main types of toxicity include: acute toxicity, organ toxicity, and impacted proteins, pathways, and endpoints. Acute toxicity refers to short-term toxicity effects, typically lasting from a few minutes to a few days after the drug has been taken. Organ toxicity refers to the accumulation of the drug in the organs over time, which can range from months to years after the drug is taken, or if the drug is taken repeatedly over a period. Once a lethal amount has accumulated in the body, possible effects include organ damage and death. Both the liver and the kidney are the most susceptible to harmful drug effects, as they manage the

ADME metabolic stages and filter toxins from the body for excretion. The proteins that the drug can bind to, the pathways that it travels to reach the target or to be excreted, and the endpoints from where they are excreted can all be affected by the movement of the drug through the body. All these categories must be considered when screening for a drug and its toxic side effects on the human body.

ProTox 3.0 Experimenting and Toxicity Levels

To determine whether the compound or drug is toxic or not, its toxicity levels can be predicted through its LD50, or Lethal Dose 50%. LD50 is measured through acute toxicity and refers to the amount of substance required to kill 50% of the tested population and is measured in mg/kg. A lower LD50 would indicate that the drug is more toxic, as it would take a smaller amount of the drug to kill an organism. On the other hand, a higher LD50 would be optimal and indicate the drug is less toxic, leading to a more flexible range for doses. The ProTox 3.0 tool predicts the LD50 for the entered compound, as well as the Toxicity class (measured on a scale from 1–6), which serves to validate the LD50. The 6 compounds obtained from the SwissADME experiments, and their values and images are displayed below in Table 6.

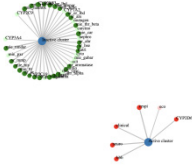
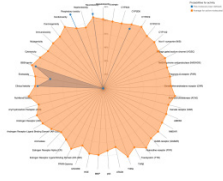
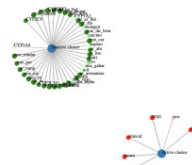
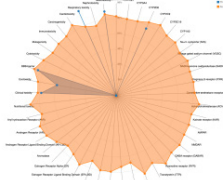
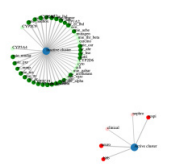
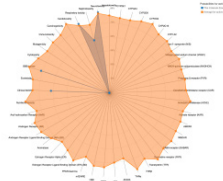
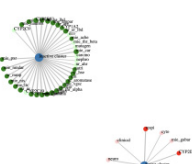
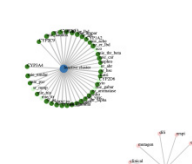
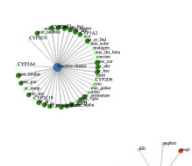
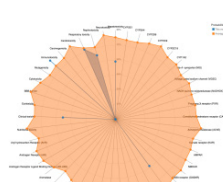
All compounds selected were based on the SwissParam scores, or ΔG values from the SwissDock experiments. Because these values determine how likely the interaction between the compound and protein is to happen, the compounds with the better SwissParam scores, and meeting the criteria for LD50 and having low toxicity, would be selected as the main compounds. The second and third best, nontoxic compounds with better SwissParam scores would be selected as backups for future testing, in the case that the lead compound fails experiments in the future.

Any other values below an LD50 of 300 mg/kg would be considered toxic and therefore eliminated as a possible drug treatment. Therefore, compounds Z1729094043 and Z1334538364 were eliminated, as they had an LD50 of 130 mg/kg, well below the acceptable value. All other compounds had an LD50 ranging from 1000 to 1500, indicating that they were less toxic to the human body. The toxicity class value also corresponds with

the LD50 and would assist in validating the results. A value from 1–2 would be the most toxic, and a value from 3–6 would be less toxic and safer for the human body. Both com-

pounds mentioned above had a value of 3. This would not be considered very toxic but is on the lower end of the acceptable values on the scale.

Table 6. LD50, toxicity class, cluster, and radar charts obtained from analysis of compounds through ProTox 3.0

Compound	LD50 (mg/kg)	Toxicity Class (1–6)	Inactive and Active Network Chart	Average toxicity Radar Chart
Z1729094043	130 mg/kg	3		
Z1334538364	130 mg/kg	3		
MolPort-010-705-550	1000 mg/kg	4		
CSC027798714	1000 mg/kg	4		
Z1901071406	1000 mg/kg	4		
MolPort-000-479-672	1500 mg/kg	4		

The active and inactive clusters charts demonstrate which elements of toxicity are most impacted by the drug. If the model is active, it indicates that the compound is toxic to that specific part of the body, and if the model is inactive, the drug is not toxic. Some vital organs, pathways, proteins, and endpoints are more crucial to human functioning than others. If these areas are impacted too much by the toxicity of the drug, then the compound would be too lethal for human consumption. Each compound had around 5–8 active elements in each chart, with the most active elements being “clinical”, appearing in all active clusters, as well as “bbb” (Blood Brain Barrier) and “neuro” appearing frequently in most active charts.

The active elements can be further analyzed through the Toxicity Radar Chart. The orange dots are the standard FDA-approved drugs and their toxicity levels: if 2 or more blue dots exceed the orange line, then the compound would be considered too toxic and would be excluded from the final data. If only one blue dot exceeds the orange area, it is still acceptable, as it may be considered a limitation of the tool. All toxicity radar charts for compounds Z1729094043, Z1334538364, MolPort-010–705–550, and CSC027798714 had 2 or more points exceeding the orange line and were therefore eliminated from the data.

Only compounds Z1901071406 and MolPort-000–479–672 met all criteria: having an LD50 above 300 mg/kg, a toxicity class in the acceptable range, and a radar chart with 1 or fewer blue dots exceeding the orange dots. MolPort-000–479–672 was selected as the lead compound, as it had the highest LD50, with compounds Z190107140 and MolPort-010–705–550 being selected as backups.

Conclusion

PDE4D inhibition elevates cAMP levels, thereby improving learning, memory, and cognition. The discovery of compounds and molecules that can inhibit the PDE4D enzyme holds potential as future drug candidates for treatment, especially targeted

towards dementia and other neurological diseases. By inhibiting the hydrolysis of cAMP, PDE4D inhibitors enhance neural signaling and memory improvement. It is important to consider the pharmacokinetics and pharmacodynamics between the compound and the human body, ensuring the drug can perform its designated function without harming the body over time. All these requirements were considered throughout the study and were used to determine a lead compound and 2 compounds that could progress towards future physical screening.

Using geometric and energetic methods, the computational tools DoGSiteScorer, Protein Plus, and Pharmit were used to determine available binding sites on the protein based on their volumes, surface area, drug scores, pharmacophore interactions, and RMSD scores. By selecting certain interactions, compounds were compiled through online databases including MolPort, Enamine, and ChemSpace. 15 compounds were selected for further experimentation to quantify the energy of each interaction through SwissDock. These compounds were analyzed through SwissADME to determine if they met the guidelines of ADME and Lipinski's Rule, with only 6 compounds selected. By determining the LD50 of each compound, active and inactive clusters, 3 final compounds were selected. These compounds, having met all criteria of all experiments, are most promising as drug treatments for inhibiting PDE4D enzymes.

Limitations of this study occurred in the pharmacophore maps and ProTox 3.0 analysis of toxicity levels. In the pharmacophore maps, available interactions between the proteins and compounds were limited and overlapping, leading to slightly higher RMSD scores and less reliable compounds. In ProTox 3.0, the average similarity and prediction accuracy percentages compared the compound to the server's database, leading to similar LD50 values and toxicity classes across compounds. To ease these limitations, a higher number of compounds could be tested in the future to discover ones with high accuracy.

References

- Arvanitakis, Z., Shah, R.C., Bennett, D.A. (2019). Diagnosis and Management of Dementia: Review. *JAMA*. – 322(16). – P. 1589–1599. Doi: 10.1001/jama.2019.4782. PMID: 31638686; PMCID: PMC7462122.
- Chouliaras L., O'Brien J.T. (2023). The use of neuroimaging techniques in the early and differential diagnosis of dementia. *Mol Psychiatry*. – P. 4084–4097. Doi: 10.1038/s41380-023-02215-8. PMID: 37608222; PMCID: PMC10827668.
- Guerchet, M.G., Prince, M.P., Prina, M.P. (2020, November 30). *Numbers of People with Dementia worldwide: an Update to the Estimates in the World Alzheimer Report*. Retrieved from: <https://www.alzint.org/resource/numbers-of-people-with-dementia-worldwide/>
- Liu W., Deng W., Gong X., Ou J., Yu S., Chen S. (2025). Global burden of Alzheimer's disease and other dementias in adults aged 65 years and over, and health inequality related to SDI, 1990–2021: analysis of data from GBD 2021, – 25(1). – 1256 p. Doi: 10.1186/s12889-025-22378-z. PMID: 40181335; PMCID: PMC11969849.
- Roles of the M₄ acetylcholine receptor in the basal ganglia and the treatment of movement disorders. *Mov Disord*, – 34(8). – P. 1089–1099. Doi: 10.1002/mds.27740. PMID: 31211471; PMCID: PMC6699902.
- Hasselmo M. E. (2006). The role of acetylcholine in learning and memory. *Curr Opin Neurobiol*, – 16(6). – P. 710–5. Doi: 10.1016/j.conb.2006.09.002. PMID: 17011181; PMCID: PMC2659740.
- Sharma K. (2019). Cholinesterase inhibitors as Alzheimer's therapeutics (Review). *Mol Med Rep*, – 20(2). – P. 1479–1487. Doi: 10.3892/mmr.2019.10374. PMID: 31257471; PMCID: PMC6625431.
- Clarkson, J.C. (2021, May 5) “Amyloid and Tau: The Proteins Involved in Dementia – DPUK.” Retrieved from: URL: <http://www.dementiasplatform.uk/news-and-media/blog/amyloid-and-tau-the-proteins-involved-in-dementia>.
- Gulisano W., Maugeri D., Baltrons M. A., Fà M., Amato A., Palmeri A., D'Adamio L., Grassi C., Devanand D. P., Honig L. S., Puzzo D., Arancio O. (2018). Role of Amyloid-β and Tau Proteins in Alzheimer's Disease: Confuting the Amyloid Cascade. *J Alzheimers Dis.*, – 64(s1). – S611–S631. Doi: 10.3233/JAD-179935. PMID: 29865055; PMCID: PMC8371153.
- Bizzi M. F., Bolger G. B., Korbonits M., Ribeiro-Oliveira A. Jr. (2019). Phosphodiesterases and cAMP Pathway in Pituitary Diseases. *Front Endocrinol (Lausanne)*, – 10. – 141 p. Doi: 10.3389/fendo.2019.00141. PMID: 30941100; PMCID: PMC6433792.
- Fan T., Wang W., Wang Y., Zeng M., Liu Y., Zhu S., Yang L. (2024). PDE4 inhibitors: potential protective effects in inflammation and vascular diseases. *Front Pharmacol*, – 15. – 1407871 p. Doi: 10.3389/fphar.2024.1407871. PMID: 38915460; PMCID: PMC11194378.
- Bhat A., Ray B., Mahalakshmi A. M., Tuladhar S., Nandakumar D. N., Srinivasan M., Essa M. M., Chidambaram S. B., Guillemin G. J., Sakharkar M. K. (2020). Phosphodiesterase-4 enzyme as a therapeutic target in neurological disorders. *Pharmacol Res*, – 160. – 105078 p. Doi: 10.1016/j.phrs.2020.105078. PMID: 32673703.
- Olsen C. M., Liu Q. S. (2016). Phosphodiesterase 4 inhibitors and drugs of abuse: current knowledge and therapeutic opportunities. *Front Biol*, – 11(5). – P. 376–386. Doi: 10.1007/s11515-016-1424-0. PMID: 28974957; PMCID: PMC5617368.
- Donders Z., Skorupska I. J., Willems E., Mussen F., Broeckhoven J. V., Carlier A., Schepers M., Vanmierlo T. (2024). Beyond PDE4 inhibition: A comprehensive review on downstream cAMP signaling in the central nervous system. *Biomed Pharmacother*, – 177. – 117009 p. Doi: 10.1016/j.biopha.2024.117009. PMID: 38908196.
- Gooderham M. (2013). Small molecules: an overview of emerging therapeutic options in the treatment of psoriasis. *Skin Therapy Lett.*, – 18(7). – P. 1–4. PMID: 24305752.
- Adili A., Dilihumaer A., Zhu H., Tang H. (2024). Research progress on phosphodiesterase 4 inhibitors in central nervous system diseases. *Zhejiang Da Xue Xue Bao Yi Xue*

Ban, – 53(3). – P. 390–398. Doi: 10.3724/zdxbyxb-2024–0023. PMID: 38860393; PM-
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