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BIOLOGICAL ACTIVITIES OF THE PRODUCT OBTAINED FROM THE REACTION OF 2-CHLORO-N-(PYRIDIN-2-YL) ACETAMIDE WITH 5-FLUOROURACIL

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Abstract

The chloroacetylation reaction of 2-aminopyridine derivatives was carried out, and the compound synthesized based on the reactions of the obtained chloroacetyl products with 5-fluorouracil was evaluated using the PASS Online program, which predicts the biological activity spectrum of substances based on their structure. When tested against various cancer and normal cell lines, the candidate compound demonstrated high efficacy against leukemia (Kasumi-1) and certain tumors of epithelial origin. At the same time, low activity observed in normal fibroblast cells indicates that the compound possesses selective activity and may potentially exhibit low toxicity. The antibacterial activity of the planned compound was also evaluated, showing the highest activity against Enterococcus faecalis ATCC 29212 and Mycobacterium tuberculosis H37Rv. The drug-like properties and possible adverse effects of the compound were further analyzed using the PASS program.

Keywords: *2-aminopyridine, 5-fluorouracil, PASS Online, cancer cells, tuberculosis bacteria*

Introduction:

Over the past years five- and six-member ring azaheterocyclic compounds have received considerable attention due to their important applications from pharmacological, industrial, and synthetic points of view (Pozharskii A. F., Soldatenkov A. T., Katritzky A. R., 2011). Pharmaceutical industry and modern medicinal science pay a lot of effort in their combat with two aggressive life-threatening diseases: cancer and tuberculosis (TB). Both diseases are leading cause of death worldwide, millions of people dying every year; the incidence of both are continually increasing and the treatment became more and more complicated and sophisticated (Grahman P. L., 2001; Silverman R. B., 1992; World Health Organization Tuberculosis Programme. Available from: Global tuberculosis report 2015/WHO/). The cancer chemotherapy is complex, expensive and often rather inefficient, because of the large variety of neoplasm types, high toxicity levels and non-specificity of drugs and the

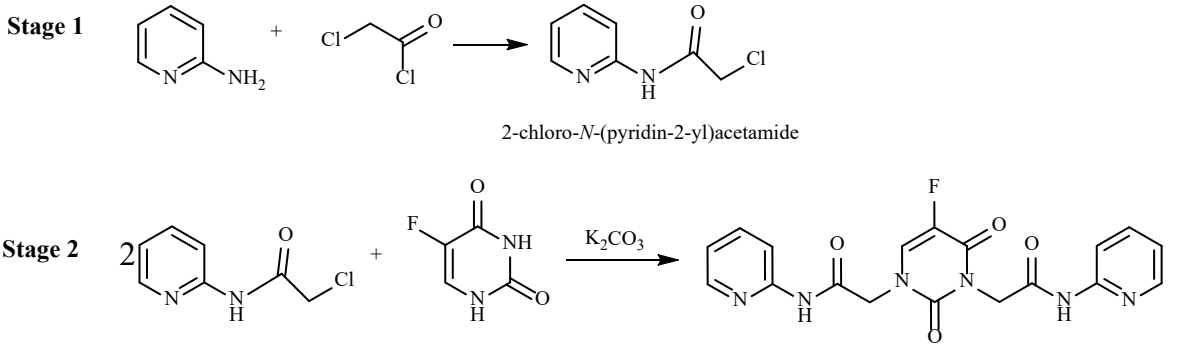
emergence of drug resistance and multidrug-resistant (MDR) (Grahman P. L., 2011; Silverman R. B., 1992). On the other hand, because of the Mycobacterium tuberculosis (Mtb) versatility, the treatment against TB became a challenging and difficult task, and the situation begin to become even worse because of the phenomena of drug resistance, MDR, extensively-drug-resistant (XDR), association of TB with AIDS, etc. (Silverman R. B., 1992; World Health Organization Tuberculosis Programme. Available from: Global tuberculosis report 2015/WHO/).

It is well known from the literature (Pozharskii A. F., Soldatenkov A. T., Katritzky A. R., 2011; Grahman P. L., 2001; Silverman R. B., 1992; World Health Organization Tuberculosis Programme. Available from: Global tuberculosis report 2015/WHO/) that imidazole (and its benzo-derivative benzimidazole) and pyridine (and its benzoderivative quinoline) derivatives are core scaffolds widely present in many classes of drugs (of natural or synthetic origin), displaying a large variety of interesting biological activities (antimicrobials, antifungus, anti-inflammatory, antihypertensive, antineuropathic, antihistaminic, etc.; anticancer (Rescifina A., Zagni C., Varrica M. G., et al., 2014; Xiang P, Zhou T, Wang L, et al.,2012; Wissner A., Mansour T. S., 2008; Zbancioc A. M., Zbancioc G., Tanase C., et al. 2010; Luca M. C., Tura V., 2010) and anti-TB (Koseki Y., Kinjo T., Kobayashi M., Aoki S., 2013; Gising J., Nilsson M. T., Odell L. R., et al., 2012; Villemagne B., Crauste C., Flipo M., et al., 2012; Pandey J., Tiwari V. K., Verma S. S., et al., 2009; Danac R., 2014) also included)).

Results and discussion:

Based on the above considerations, and with the aim of developing new anticancer and anti-tuberculosis agents containing an azaheterocyclic scaffold, it is planned to carry out the chloroacetylation of 2-aminopyridine derivatives, to investigate the reactions of the obtained chloroacetyl products with 5-fluorouracil, and to study the structure of the synthesized compounds as well as their in vitro anticancer and antimycobacterial activity. The reaction is carried out in the following two steps (Bobonazarova S. et al., 2025; Habibullaevna B. S. et al., 2024; Ochilov S. H. et al.).

Figure 1.



2,2'-(5-fluoro-2,4-dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(*N*-(pyridin-2-yl)acetamide)

An important property of chemical compounds is their biological activity, as its presence may serve as a basis for using a substance for therapeutic purposes or, conversely, may limit its practical application due to the manifestation of side effects and toxic effects. The computational evaluation of biological activity spectra makes it possible to identify the most promising directions for studying the pharmacological effects of specific substances and to screen potentially hazardous molecules at the early stages of research.

PASS Online (Prediction of Activity Spectra for Substances) is an in silico program that predicts the biological activity spectrum of compounds based on their structure. The PASS software simultaneously forecasts more than 780 pharmacological effects and biochemical mechanisms using the 2D structural formula of a substance.

The synthesized compound was evaluated for its biological activity against various tumor and normal cell lines using the PASS (Prediction of Activity Spectra for Substanc-

es) online platform. The obtained results were analyzed based on probabilistic parameters, where Pa represents the probability of activity and Pi represents the probability of inactivity. Analysis of tumor cell lines revealed that the highest predicted activity was observed against the Kasumi-1 cell line (Pa = 0.525; Pi = 0.031). This cell line is associated with pediatric acute myeloid leukemia, indicating a pronounced potential effect of the compound, particularly against hematological malignancies. Notable levels of predicted activity were also observed for several other tumor cell lines, including SK-MES-1 (lung squamous cell carcinoma) with Pa = 0.425, SW-620 (colon adenocarcinoma) with Pa = 0.367, PA-1 (ovarian carcinoma) with Pa = 0.356, Hs 683 (brain tumor, oligodendroglioma) with Pa = 0.302, as well as YAPC and CFPAC-1 (pancreatic carcinoma) with Pa values in the range of approximately 0.31–0.30.

In contrast, analysis of normal cell lines demonstrated significantly lower predicted activity: NHDF (normal human dermal fibroblasts) showed Pa = 0.150, WI-38 (embryonic lung fibroblasts) Pa = 0.131, and AG1523 (fibroblasts) Pa = 0.044. These values are substantially lower compared to those observed for tumor cell lines, suggesting a reduced effect of the compound on normal cells.

Overall, the results indicate that the synthesized compound exhibits a broad spectrum of predicted activity against various tumor cell lines, with particularly high potential against leukemia cells (Kasumi-1) and several epithelial-derived cancers. Importantly, the relatively low predicted activity in normal fibroblasts suggests a degree of selectivity and potentially low toxicity. Therefore, the investigated compound can be considered a promising antitumor agent, warranting further comprehensive studies, including detailed in vitro and in vivo evaluations.

The increasing prevalence of antibiotic-resistant bacteria represents a major challenge for global healthcare systems. Therefore, the discovery of new effective antibacterial agents and the early-stage evaluation of their biological activity are of great importance. In this study, the antibacterial potential of a proposed compound was evaluated using the PASS (Prediction of Activity Spectra for Substances) software. The results indicated that the highest probabilities of activity were observed against Enterococcus faecalis ATCC 29212 (Pa = 0.1401) and Mycobacterium tuberculosis H37Rv (resistant strain) (Pa = 0.1176). These findings suggest that the investigated compound may exhibit potential activity against Gram-positive bacteria as well as drug-resistant mycobacteria. In particular, the predicted activity against resistant strains of Mycobacterium tuberculosis, the causative agent of tuberculosis, is of notable scientific interest. According to the obtained data, the probability of activity against the resistant strain Mycobacterium tuberculosis H37Rv (Pa = 0.1176) is relatively low. It is well established that, within the PASS framework, Pa values below 0.5 generally indicate a low probability of pronounced biological activity. However, low Pa values do not necessarily imply the absence of biological effect; rather, they may reflect that the compound belongs to a novel or insufficiently represented chemical class within the training dataset. Importantly, even a low predicted activity against drug-resistant Mycobacterium tuberculosis strains is scientifically significant, as these pathogens exhibit high resistance to existing therapeutics and require compounds with new mechanisms of action. From this perspective, the detected, albeit low, probability of activity may indicate that the studied compound operates via an unconventional or previously unexplored mechanism of action. Furthermore, it should be noted that the PASS algorithm is based on previously characterized compounds. Consequently, molecules with novel or less-studied structural features may receive underestimated activity predictions, which may not fully reflect their true biological potential. Taking these considerations into account, the investigated compound can be regarded as a promising lead structure. Further studies involving structural optimization, as well as comprehensive in vitro and in vivo evaluations, are warranted to enhance and validate its biological activity.

The drug-like properties and potential adverse effects of the investigated compound were analyzed using the PASS program. The highest predicted probability of activity was observed for antineoplastic action (Pa = 0.644; Pi = 0.036), indicating the compound's potential effectiveness against can-

cer cells. Additionally, predicted activities included antieczematic action (Pa = 0.576) and pseudouridylate synthase inhibition (Pa = =0.479), suggesting that the compound may influence multiple biological pathways. The predicted values for kidney function stimulation (Pa = 0.504) and electron-transferring flavoprotein dehydrogenase inhibition (Pa =0.465) indicate a potential impact of the compound on metabolic processes, including renal function and cellular energy metabolism.

The PASS algorithm also predicted possible adverse effects. The highest probabilities were observed for hematemesis (Pa = 0.787), nail discoloration (Pa = 0.691), and gastrointestinal hemorrhage (Pa = 0.682). Cardiovascular effects (Pa = 0.643 – atrial fibrillation) and metabolic effects (Pa = 0.605 – hyperglycemia) were also notable.

These results indicate that the compound possesses high biological activity; however, the presence of high-probability adverse effects necessitates caution in its potential clinical application. In particular, monitoring gastrointestinal and cardiovascular systems is essential, given the high predicted antineoplastic activity of the compound.

Conclusion:

When analyzing the biological activity spectra predicted by the PASS program, it is necessary to take into account the real possibilities of experimental testing. In this case, the general recommendation is to study the various predicted types of biological activity sequentially, from the most probable to the least probable. It should be emphasized that the PASS program cannot predict whether a particular compound will become a drug, as this depends on a number of factors. However, the predictions help determine which biological activities should be tested first and which compounds are most likely to exhibit the desired types of activity.

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