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SYNTHESIS, ANALYSIS ADME-TOX PARAMETERS AND ANTI-CANCER ACTIVITY OF *N*-(5-R-BENZYLTHIAZOLE-2-YL)-2-MORPHOLIN-4-YL-2-THIOXOACETAMIDES

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Aim. Study of the synthesis, analysis of ADME-Tox parameters and anti-cancer activity of a series of N-(5-R-benzylthiazole-2-yl)-2-morpholin-4-yl-2-thioxoacetamides.

Methods. Organic synthesis, 1H NMR spectroscopy, analytical method, in silico ADME-Tox analysis and in vitro cytotoxicity assay.

Results. The series of new N-(5-R-benzylthiazole-2-yl)-2-morpholin-4-yl-2-thioxoacetamides was synthesized according to a convenient synthetic method. Their structures were confirmed by 1H NMR spectroscopy and microanalyses. Using the internet resources of SwissADME and pkCSM-pharmacokinetics, the ADME-Tox profiles of the synthesized compounds were calculated. It was determined that the substances were within the optimal limits of bioavailability. All compounds meet the criteria of drug similarity according to the rules of Lipinski, Weber, Egan and Mugge. It is also determined that low toxicity is predicted for these substances.

The synthesized compounds were tested in vitro for their antitumor activity according to the Developmental Therapeutic Program of the National Cancer Institute (NCI) (www.dtp.nci.nih.gov) against 60 cancer lines in the concentration of 10 μM. Human tumor cell lines from nine different cancer types were used: leukemia, melanoma, lung, colon, CNS, ovarian, kidney, prostate and breast cancer. Screening results showed that, in most cases, these compounds are of low activity. An exception is the renal cancer line UO-31, which was moderately sensitive to all synthesized compounds.

Conclusions. A series of 2-aminothiazole hybrids containing morpholine moiety was synthesized and studied in silico ADME-Tox profiles. The ADME-Tox profiles indicated good oral bioavailability and low toxicity. Synthesized compounds were tested in vitro for their anti-cancer activity. They showed moderate antiproliferative activity.

Keywords: Organic synthesis, 2-aminothiazole, morpholine, privileged structure, ADME-Tox, anti-cancer activity.

Introduction

Bemis GW and Murcko MA [1] formulated the concept of privileged frameworks at the end of the last century. They analyzed 5,120 structures of drugs available on the pharmaceutical market and identified 1,179 topological molecular frameworks among them. As it turned out, 32 of them represent more than half of medicines. Molecular framework A (Figure 1) is one of the most widespread medicinal products (the blue color indicates the number according to the gradation of the prevalence of molecular frameworks).

Compounds containing a 2-aminothiazole ring have a special place here. Among them, substances with antitumor, anti-inflammatory, antiviral, antimicrobial, neuroprotective and other types of activity were found [2-5]. Several such compounds are used as medicinal preparations. These are, in particular, well-known drugs such as famotidine, abafungin, cefdinir, and sudoxicam.

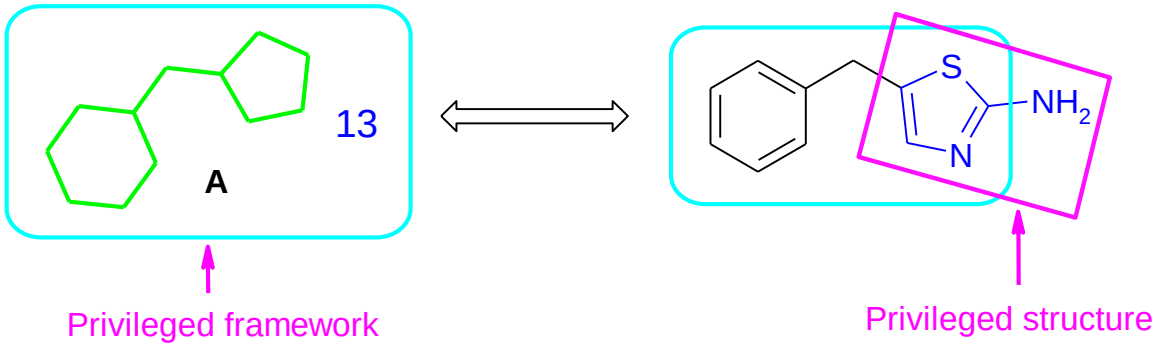


Figure 1. 2-Amino-5-benzylthiazole as privileged structure

Morpholine is a privileged pharmacophore in medicinal chemistry. This scaffold, substituted accordingly, affects a variety of biological activities. Morpholine enhances the molecule's efficacy through molecular interaction with multiple molecular targets and modulates pharmacokinetic properties. Therefore, pharmaceutical chemists have shown scientific interest in synthesizing the morpholine ring efficiently and incorporating this moiety to develop various compounds with diverse therapeutic effects [6–10].

The combination of pharmacological agents 2-aminothiazole and morpholine leads to a synergistic interaction, in which the effectiveness of one compound is increased by the addition of the other (Figure 2). Two pharmacophores, conjugated through a link unit, form a single chemical entity. The obtained "hybrid" may have advantages over combinations of two-component drugs, in particular, increased absorption of one component of the drug due to the physicochemical properties of the other component of the drug, improvement of individual pharmacokinetic parameters, reduction of side effects [11–13].

The morpholine and 2-aminothiazole reagents are commercially available, versatile, readily accessible synthetic building blocks.

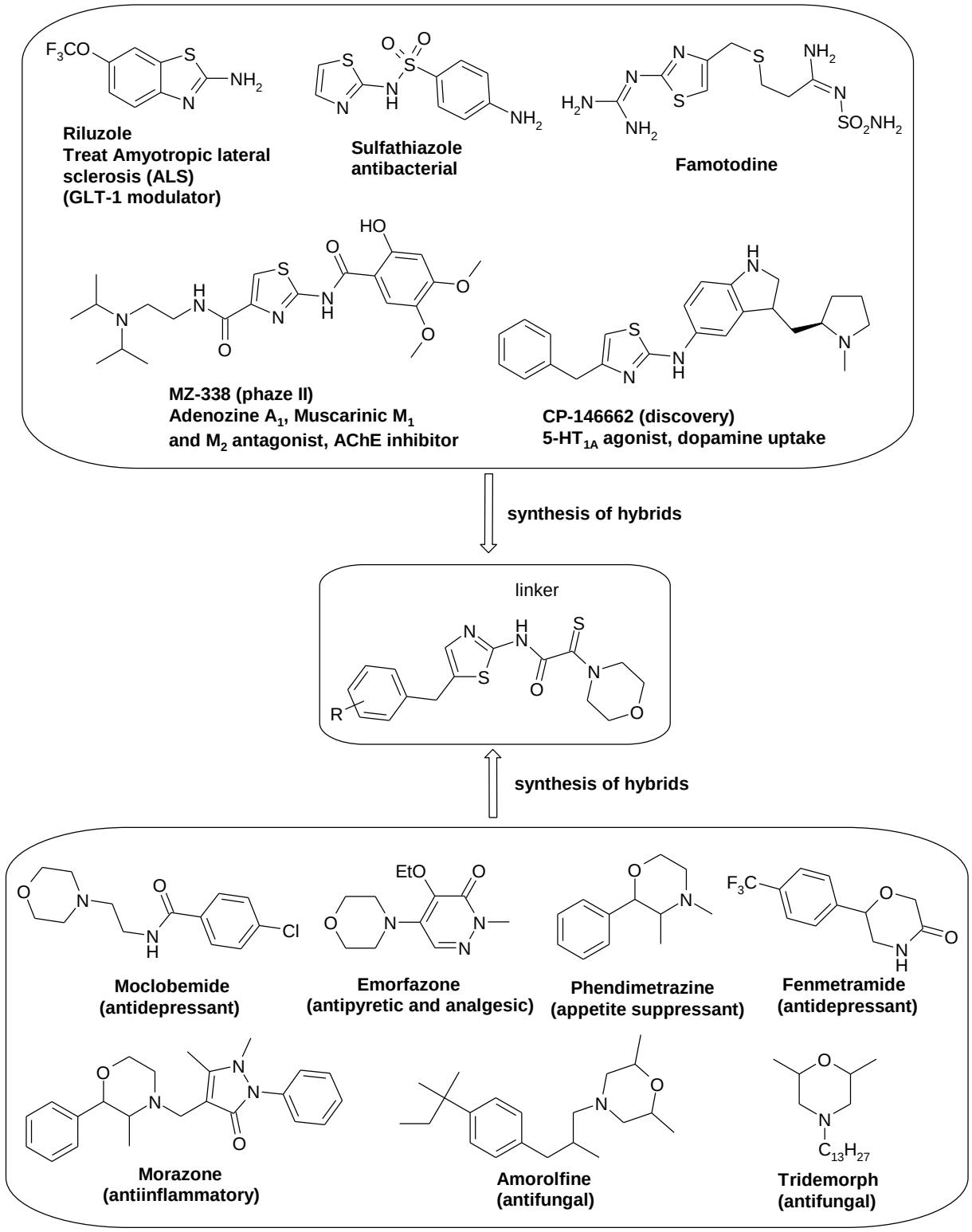


Figure 2. Biologically active derivatives of 2-aminothiazole and morpholine and an approach to the design of *N*-(5-R-benzylthiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamides

Materials and Methods of Synthetic Experiments

Melting points of all substances were determined in an open capillary. ^{1}H NMR spectra of the synthesized compounds in dimethylsulfoxide DMSOd$_6$ solutions were recorded on a Varian Mercury VX-400 spectrometer (Agilent Technologies, USA), 400 MHz at 298 K. Chemical shifts are given as δ, ppm relative to tetramethylsilane as an internal standard. The spin-spin interaction constant *J* is expressed in Hz. The experimental results from elemental analysis for the Carbon, Hydrogen, and Nitrogen percentages fell within ±0.3% of the expected values.

The general method of synthesis of morpholine-4-yl-2-thioxoacetamides ***9a-j***

A mixture containing 0.01 mol (0.32 g) of sulfur powder in 10 ml of morpholine is stirred for 5 minutes. Then, a solution of 0.003 mol of chloroacetamide in 4 ml of *N,N*-Dimethylformamide is gradually added to the resulting cherry-brown solution. The reaction mixture is stirred for 60 minutes before being poured into 100 ml of water and allowed to sit for 24 hours. The resulting precipitate is filtered, washed with water, dried, and subjected to recrystallization from ethanol.

N-(5-Benzylthiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamide ***9a****.* Yield 89%. Mp 188–190°C. ^{1}H NMR, δ, ppm: 12.61 (s, 1H, NH), 7.50–7.16 (m, 6H, C$_6$H$_4$, thiazole), 4.10 (s, 2H, PhCH$_2$), 4.08 (d, *J* = 4.1 Hz, 2H, CH$_2$), 3.73 (d, *J* = 4.0 Hz, 2H, CH$_2$), 3.64 (s, 2H, CH$_2$), 3.58 (s, 2H, CH$_2$). Anal. Calculated for C$_{16}$H$_{17}$N$_3$O$_2$S$_2$, %: C, 55.31; H, 4.93; N, 12.09. Found, %: C, 55.21; H, 4.90; N, 12.00.

N-[5-(4-methylbenzyl)-1,3-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide ***9b****.* Yield 94%. Mp 211–213°C. ^{1}H NMR, δ, ppm: 12.62 (s, 1H, NH), 7.30 (s, 1H, thiazole), 7.15 (d, *J* = 7.7 Hz, 2H, C$_6$H$_4$), 7.12 (d, *J* = 7.7 Hz, 2H, C$_6$H$_4$), 4.11–4.05 (m, 2H, CH$_2$), 4.04 (s, 2H, ArCH$_2$), 3.72 (s, 2H, CH$_2$), 3.64 (s, 2H, CH$_2$), 3.57 (s, 2H, CH$_2$), 2.26 (s, 3H, CH$_3$). Anal. Calculated for C$_{17}$H$_{19}$N$_3$O$_2$S$_2$, %: C, 56.49; H, 5.30; N, 11.62. Found, %: C, 56.39; H, 5.25; N, 11.60.

N-[5-(4-ethylbenzyl)-1,3-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide ***9c****.* Yield 99%. Mp 230–232°C. ^{1}H NMR, δ, ppm: 12.71–12.53 (b.s, 1H, NH), 7.31 (s, 1H, thiazole), 7.17 (d, *J* = 8.0 Hz, 2H, C$_6$H$_4$), 7.14 (d, *J* = 7.9 Hz, 2H, C$_6$H$_4$), 4.14–3.99 (m, 4H, CH$_2$), 3.76–3.70 (m, 2H, CH$_2$), 3.64 (s, 2H, CH$_2$), 3.57 (s, 2H, CH$_2$), 2.56 (кв, *J* = 7.4 Hz, 2H, CH$_2$), 1.15 (т, *J* = 7.6 Hz, 3H, CH$_3$). Anal. Calculated for C$_{18}$H$_{21}$N$_3$O$_2$S$_2$, %: C, 57.57; H, 5.64; N, 11.19. Found, %: C, 57.50; H, 5.65; N, 11.14.

N-[5-(4-fluorobenzyl)-1,3-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide ***9d.*** Yield 98%. Mp 233–235°C. ^{1}H NMR, δ, ppm: 12.69–12.55 (b.s, 1H, NH), 7.34–7.27 (m, 3H, C$_6$H$_4$, thiazole), 7.13 (*дд*, *J* = 7.9, 5.2 Hz, 2H, C$_6$H$_4$), 4.09 (s, 2H, CH$_2$), 4.07 (s, 2H, CH$_2$), 3.73 (d, *J* = 3.6 Hz, 2H, CH$_2$), 3.65 (s, 2H, CH$_2$), 3.58 (s, 2H, CH$_2$). Anal. Calculated for C$_{16}$H$_{16}$FN$_3$O$_2$S$_2$, %: C, 52.59; H, 4.41; N, 11.50. Found, %: C, 52.24; H, 4.44; N, 11.41.

N-[5-(2-chlorobenzyl)-1,3-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide ***9e.*** Yield 95%. Mp 191–193°C. ^{1}H NMR, δ, ppm: 12.67 (s, 1H, NH), 7.59–7.39 (m, 2H, C$_6$H$_4$), 7.37–7.17 (m, 3H, C$_6$H$_4$, thiazole), 4.21 (s, 2H, ArCH$_2$), 4.14–3.93 (m, 2H, CH$_2$), 3.72 (s, 2H, CH$_2$), 3.63 (d, *J* = 4.1 Hz, 2H, CH$_2$), 3.57 (s, 2H, CH$_2$). Anal. Calculated for C$_{16}$H$_{16}$ClN$_3$O$_2$S$_2$, %: C, 50.32; H, 4.22; N, 11.00. Found, %: C, 50.30; H, 4.18; N, 10.95.

N-[5-(4-chlorobenzyl)thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide ***9g.*** Yield 93%. Mp 238–240°C. ^{1}H NMR, δ, ppm: 12.65 (s, 1H, NH), 7.36 (d, *J* = 8.2 Hz, 2H, C$_6$H$_4$), 7.34–7.26 (m, 3H, thiazole, C$_6$H$_4$), 4.10 (s, 2H, ArCH$_2$), 4.07 (s, 2H, CH$_2$), 3.73 (s, 2H, CH$_2$), 3.64 (s, 2H, CH$_2$), 3.58 (s, 2H, CH$_2$). Anal. Calculated for C$_{16}$H$_{16}$ClN$_3$O$_2$S$_2$, %: C, 50.32; H, 4.22; N, 11.00. Found, %: C, 50.35; H, 4.26; N, 10.96.

N-[5-(4-bromobenzyl)-1,3-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide ***9h.*** Yield 95%. Mp 231–233°C. ^{1}H NMR, δ, ppm: 12.78–12.52 (b.s, 1H, NH), 7.57–7.46 (d, *J* = 8.4 Hz, 2H, C$_6$H$_4$), 7.33 (s, 1H, thiazole), 7.27–7.17 (d, *J* = 8.3 Hz, 2H, C$_6$H$_4$), 4.09 (s, 2H, CH$_2$), 4.08 (s, 2H, CH$_2$), 3.73 (s, 2H, CH$_2$), 3.64 (d, *J* = 3.2 Hz, 2H, CH$_2$), 3.57 (s, 2H, CH$_2$). Anal. Calculated for C$_{16}$H$_{16}$BrN$_3$O$_2$S$_2$, %: C, 45.07; H, 3.78; N, 9.86. Found, %: C, 45.01; H, 3.73; N, 9.90.

2-Morpholin-4-yl-2-thioxo-N-[5-(3-trifluorobenzyl)-thiazol-2-yl]-thioxoacetamide ***9i.*** Yield 81%. Mp 187–189°C. ^{1}H NMR, δ, ppm: 12.73–12.64 (b.s, 1H, NH), 7.67 (s, 1H, C$_6$H$_4$), 7.63–7.54 (b.s, 3H, C$_6$H$_4$), 7.38 (s, 1H, thiazole), 4.23 (s, 2H, ArCH$_2$), 4.11–4.03 (m, 2H, CH$_2$), 3.72 (s, 2H, CH$_2$), 3.64 (s, 2H, CH$_2$), 3.61–3.53 (m, 2H, CH$_2$). Anal. Calculated for C$_{17}$H$_{16}$F$_3$N$_3$O$_2$S$_2$, %: C, 49.15; H, 3.88; N, 10.11. Found, %: C, 49.20; H, 4.00; N, 10.08.

N-[5-(2-chloro-5-trifluoromethylbenzyl)-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide ***9j.*** Yield 77%. Mp 207–209°C. ^{1}H NMR, δ, ppm: 12.67 (s, 1H, NH), 7.89 (s, 1H, C$_6$H$_3$), 7.71 (d, *J* = 7.3 Hz, 1H, C$_6$H$_3$), 7.66 (d, *J* = 7.0 Hz, 1H, C$_6$H$_3$), 7.36 (s, 1H, thiazole), 4.32 (s, 2H, ArCH$_2$), 4.08 (s, 2H, CH$_2$), 3.73 (d, *J* = 2.5 Hz, 2H, CH$_2$), 3.64 (s, 2H, CH$_2$), 3.59 (s, 2H, CH$_2$). Anal. Calculated for C$_{17}$H$_{15}$ClF$_3$N$_3$O$_2$S$_2$, %: C, 45.39; H, 3.36; N, 9.34. Found, %: C, 45.36; H, 3.41; N, 9.30.

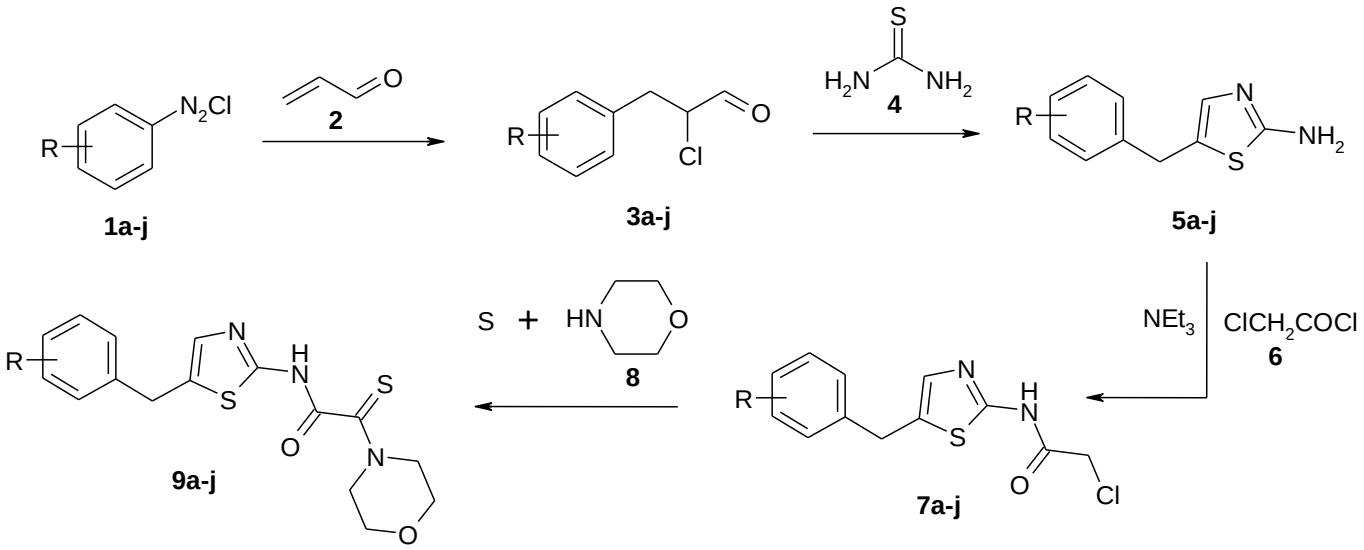
Results and discussion

This work continues our research aimed at developing effective antitumor drugs using a hybrid pharmacophore approach involving morpholine and 2-aminothiazole scaffolds (Figure 2).

Synthesis of N-(5-R-benzylthiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamides

We have developed a method for synthesizing new thioxoacetamides of the thiazole series. 5-R-benzyl-1,3-thiazol-2-amines **5a-j** were synthesized using diazonium salts **1a-j** as starting reagents. Diazonium salts **1a-j** react with acrolein **2** to form 3-aryl-2-chloropropanals **3a-j**, which interact with thiourea to form 2-amino-5-R-benzyl thiazoles **5a-j** [14], which were transformed into chloroacetamides **7a-j** upon interaction with chloroacetyl chloride **6** [14].

It was established that chloroacetamides **7a-j** react with sulfur and morpholine to form morpholin-4-yl-2-thioxoacetamides **9a-j**. A previously obtained sulfur solution in morpholine should be used for a successful synthesis. To do this, the specified reagents were kept under stirring for 5 minutes. This time is necessary for a sufficient amount of polysulfides to form in the reaction mixture (Figure 3).



1a-j, 3a-j, 5a-j, 7a-j, 9a-j: R = H(**a**), 4-CH_3(**b**), 4-C_2H_5(**c**), 4-F(**d**), 2-Cl(**e**), 3-Cl(**f**), 4-Cl(**g**), 4-Br(**h**), 3-CF_3(**i**), 2-Cl-5-CF_3(**j**)

Figure 3. Synthesis of *N*-(5-R-benzylthiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamides **9a-j**

The obtained thioxoacetamides **9a-j** are yellow powders, soluble in cold DMF when heated in alcohols, acetic acid, and dioxane, and insoluble in benzene, hexane, and petroleum ether.

The ^{1}H NMR spectra of 2-morpholin-4-yl-2-thioxoacetamides **9a-j** contain signals of all protons. In the strong magnetic field, we observe signals of aliphatic protons CH_2NCH_2 of the morpholine cycle group as two singlets. In our opinion, this is explained by the difficult rotation around the C(S)-N bond.

Prediction ADME-Tox parameters of synthesized compounds

To evaluate the prospects of synthesized compounds as biologically active substances, ADME-Tox (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis was performed, and the properties of drugs were examined (Table 1). Drug-likeness descriptors were selected using the Lipinski [15], Ghose [16], Veber [17], Egan [18], and Muegge [19] rules. Leadlikeness was determined by the method [20]. Water solubility was calculated according to [21].

Table 1

Drug-, lead likeness and water solubility of N-(5-R-benzylthiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamides 9a-j [22]

Compound	Druglikeness (Lipinski, Ghose, Veber, Egan, Muegge rules)	Leadlikeness	Water Solubility (Log S (Ali))
9a	Yes	Yes	-4.60 Moderately soluble
9b	Yes	No; MW>350	-4.97 Moderately soluble
9c	Yes	No; MW>350	-5.43 Moderately soluble
9d	Yes	No; MW>350	-4.70 Moderately soluble
9e	Yes	No; MW>350	-5.25 Moderately soluble
9f	Yes	No; MW>350	-5.25 Moderately soluble
9g	Yes	No; MW>350	-5.25 Moderately soluble
9h	Yes	No; MW>350	-5.31 Moderately soluble
9i	Yes	No; MW>350	-5.51 Moderately soluble
9j	Yes	No; MW>350, XLOGP3>3.5	-6.16 Poorly soluble

After analyzing the results, it was found that no criteria for these rules were violated for the newly synthesized compounds. They are also predicted to be well absorbed or penetrated, suggesting that they are suitable drug candidates. Percentage absorption value ABS 90.21% (calculated by the formula % ABS = 109(0.345xTPSA)) [23].

We also analyzed the synthesized compounds using the Pan Assay Interference Compounds (PAINS) [24] and Brenk [25] methods for problematic fragments of molecules that have high reactivity with any protein and can cause a false positive result in further studies or worsen pharmacokinetics, having high toxicity and chemical reactivity, as well as metabolically unstable. According to the concept of PAINS [24], the analysis of the manifestation of toxicity is not foreseen. There is a danger at that time due to the presence of a highly reactive thioamide group, according to the Brenk concept [25].

For each tested compound and reference preparates (gefitinib and acortatarin A), bioavailability radars (Figure 4) were created. The pink area represents the optimal range for such properties as lipophilicity (XLOGP3 -0.7 – +5.0), size (molecular weight 150 – 500 g/mol), polarity (TPSA 20 – 130 Å^2), solubility (log S ≤ 6), saturation (fraction of carbon in sp^3 hybridization should be at least 0.25) and flexibility (rotary bonds ≤ 9) [26]. All compounds **9a-j** are in the optimal range on the bioavailability radar. Regarding the drug similarity score, our compounds had a good bioavailability score of 0.55 [27].

Figure 4. Bioavailability radars of *N*-(5-R-benzylthiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamides **9a-j** and reference preparations

To predict gastrointestinal absorption and blood-brain barrier (BBB) penetration, we used the BOILED-Egg model using an electronic resource [22] (Figure 5). Both parameters are crucial in the process of drug design. According to the visualization method proposed by the authors [28], in the BOILED-Egg plot, the white area indicates a high probability of passive absorption in the gastrointestinal tract. In contrast, the yellow area represents a high probability of crossing the blood-brain barrier. The blue color of the indicator of the molecule shows that the compound is actively secreted by P-glycoprotein (P-gp), denoted as (PGP+), while the red indicator signifies the non-substrate P-gp (PGP-) [29].

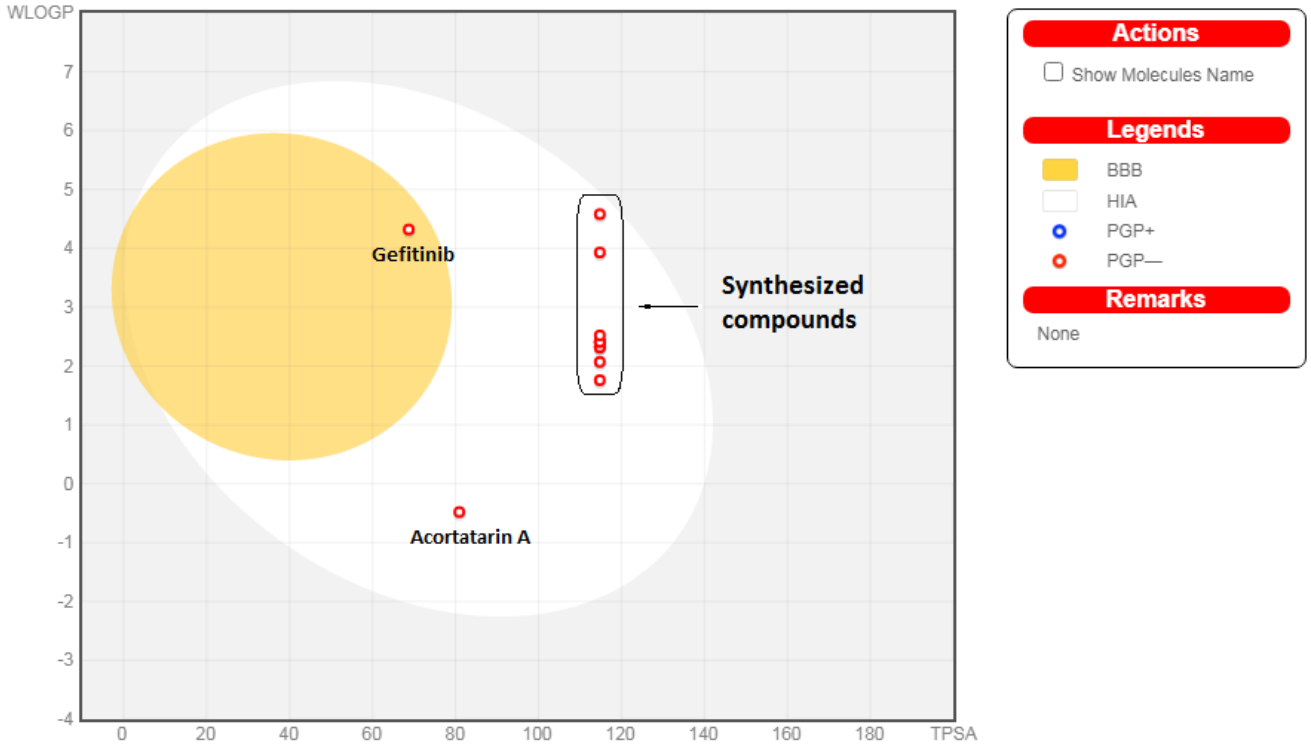


Figure 5. The BOILED-Egg plot of *N*-(5-R-benzylthiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamides **9a-j** and reference preparations

The BOILED-Egg diagram (Figure 5) showed that our compounds **9a-j** are in the graph's white area; therefore, they will be absorbed in the gastrointestinal tract and not penetrate the blood-brain barrier. In addition, all compounds **9a-j** are predicted not to be substrates for P-glycoprotein PGP-, which means that their bioavailability will not be reduced.

Prediction of pharmacokinetic parameters and toxicity of synthesized compounds using the pkCSM method

Absorption and distribution are important processes that determine the effectiveness of drugs. Absorption is the process by which a drug moves from the administration site into the systemic bloodstream. During distribution, drug molecules move from systemic circulation to extravascular sites.

We predicted a number of parameters that have a decisive influence on absorption and distribution using the pkCSM web tool [30] (Table 2).

Table 2

Prediction of absorption and distribution of synthesized compounds 9a-j

Absorption and distribution	Numbers of tested synthesized compounds									
	9a	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**9i**	**9j**
Caco2 permeability (log Papp in 10^{-6} cm/s)	1.311	1.119	1.115	1.117	1.098	1.113	1.117	1.114	1.123	1.125
Skin permeability (log Kp) (sm/h)	-3.14	-3.266	-3.222	-3.269	-2.901	-3.248	-3.221	-3.214	-3.167	-3.051
VDss (human) (log L/kg)	-0.295	0.186	0.28	-0.034	0.245	0.187	0.12	0.134	0.044	0.01
Fraction unbound (human) (Fu)	0.155	0.234	0.196	0.251	0.168	0.216	0.217	0.21	0.198	0.161
BBB permeability (log BB)	0.017	-0.071	-0.129	-0.091	0.002	-0.082	-0.084	-0.085	-0.123	-0.132
CNS permeability (log PS)	-2.427	-2.303	-2.342	-2.416	-2.248	-2.274	-2.262	-2.24	-2.228	-2.105

For predicting permeability of the absorption of orally administered drugs we used the Caco-2 monolayer of cells. The value of Caco-2 permeability (log Papp) of the synthesized 2-morpholin-4-yl-2-thioxoacetamides **9a-j**, calculated using the pkCSM web tool, ranges from 1.098 to 1.311 cm/s (Table 2). Therefore, all compounds **9a–j** are predicted to have high Caco-2 permeability.

Assessment of skin permeability is important in developing effective drugs for transdermal drug delivery. If the value of log Kp is greater than -2.5 cm/h, then it is considered that the drug almost does not penetrate through the skin. The skin permeability (log Kp) of synthesized 2-morpholin-4-yl-2-thioxoacetamides **9a-j** ranges from -3.269 to -2.901 cm/h (Table 2). This implies a good permeability of our compounds through the skin.

The volume of distribution (VD) represents the value of the drug that will be distributed at an equal blood plasma level. According to Pires et al. VDss > 2.81 L/kg or log VDss > 0.45 is considered high, and VDss < 0.71 L/kg or log VDss < -0.15 is considered low. An increase in the VDss value indicates a higher probability of drug distribution in tissues rather than in plasma. The value of VDss, log L/kg of the synthesized compounds **9a-j** is from -0.295 to 0.28 (Table 2). However, for compound **9a** (R = H), log VDss < -0.15, so it can be predicted that compound **9a** can be distributed in blood plasma rather than tissue. The tendency to diffuse into the plasma is also observed for the bioisosteric derivative **9d** (R = 4-F). All other compounds **9b**, **c**, **e-j** will be distributed evenly.

An important parameter to consider when evaluating the pharmacokinetic parameters of a compound is its ability to penetrate the blood-brain barrier (BBB). This is especially relevant in the development of biologically active substances for drugs, the therapeutic effect of which is expected in the brain. The pkCSM web tool uses a predictive model built using a database of compounds whose logBB was experimentally measured. Compounds can cross the BBB easily when log BB > 0.3, and compounds with log BB < -1 have poor brain penetration. The value of log BB of the synthesized 2-morpholin-4-yl-2-thioxoacetamides **9a-j** ranges from -0.132 to 0.017 (Table 2), so it can be assumed that all compounds **9a-j** are capable of moderately penetrating the blood-brain barrier.

Also, using the pkCSM web tool, the probability of penetration of the synthesized compounds into the central nervous system (CNS) was estimated. If the logPS is greater than -2, then the compounds penetrate the CNS. If the logPS is less than -3, they are considered unable to penetrate the CNS. The value of log PS for the synthesized compounds 9a-j is -2.427 – -2.105 (Table 2), which suggests a moderate penetration into the CNS.

Metabolism of xenobiotics is the process of transformation of drugs in the organs and tissues of the body, during which their pharmacological activity changes. This biotransformation plays an important role in the process of elimination of pharmaceutical substances.

The enzyme cytochrome P450 (CYP450) plays an important role in drug metabolism. CYP450, a key enzyme in detoxification, actively oxidizes xenobiotics, promoting their elimination from the body. CYP450 inhibitors can significantly affect the pharmacokinetics of these drugs, so it should be predicted whether the test compound can act as a CYP450 substrate. The major drug-metabolizing isoforms include cytochrome P2D6 (CYP2D6) and cytochrome P3A4 (CYP3A4). The inhibition of these enzymes could result in the buildup of xenobiotics, necessitating metabolic processing [31]. The pkCSM web tool allowed us to assess that the synthesized compounds **9b**, **d**, **f**, and **g** do not affect CYP2D6 and CYP3A4 enzymes and do not inhibit them (Table 3).

Table 3

Prediction of the metabolism of synthesized compounds 9a-j

Metabolism	Numbers of tested synthesized compounds									
	9a	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**9i**	**9j**
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate	Yes	No	Yes	No	Yes	No	No	Yes	Yes	No
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No
CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No	No	Yes

One of the essential factors leading to the withdrawal of in vivo tested compounds is the unsatisfactory toxicological profile of the substance [32]. Therefore, in silico prediction of the toxicity of organic compounds plays an important role in drug design. The results of potential toxicity are performed in Table 4.

Table 4

Prediction of the toxicity of synthesized compounds 9a-j

Toxicity	Numbers of tested synthesized compounds									
	9a	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**9i**	**9j**
Ames toxicity	No	No	No	No	No	No	No	No	No	No
Max. tolerated dose (human) (log mg/kg/day)	-0.159	-0.008	0.002	0.027	-0.092	-0.018	0.01	0	-0.014	0.157
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Oral rat acute toxicity (LD_{50}) log(1/(mol/kg))	2.801	2.411	2.462	2.361	2.569	2.487	2.48	2.484	2.561	2.745
Oral rat chronic toxicity (LOAEL) (log mg/kg_bw/day)	1.642	1.333	1.333	1.461	1.533	1.22	1.38	1.363	1.153	1.544
Hepatotoxicity	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Skin sensitization	No	No	No	No	No	No	No	No	No	No
Tetrahymena pyriformis toxicity (log ug/L)	1.286	0.989	1.05	0.897	0.911	0.976	0.98	0.982	0.85	0.761
Minnow toxicity (log mM)	2.207	1.572	1.302	1.687	1.896	1.19	1.354	1.208	1.411	1.74

As found, no Ames toxicity is predicted for all compounds **9a-j**, which means that these ligands are unlikely to be mutagenic and, consequently, carcinogenic. Also, all analyzed compounds **9a-j** will not affect skin sensitization. It is assumed that compounds **9e** and f are non-hepatotoxic, while hepatotoxicity is possible for the rest of the studied substances. All compounds **9a-j** are considered hERG II inhibitors, which may cause cardiotoxicity. The MRTD value for compounds **9a-j** ranges from -0.159 to 0.157 log(mg/kg/day), so they are characterized by moderate tolerability. All compounds are predicted to exhibit significant acute toxicity to Tetrahymena pyriformis, but no toxicity to Minnow. According to the predicted value of Oral rat acute toxicity (LD50), which is in the range of 2.361 – 2.801 log(1/(mol/kg)). The above toxicity risks should be considered when optimizing the specified class of compounds.

Anti-cancer activity

The biological activity of thiooxamides was hardly studied (studied sporadically). In particular, Ramkumar K et al. [33] showed that compound A exhibit HIV-1 Integrase Inhibitors and indicated the importance of the thioxoamide linker in the molecule (Figure 6).

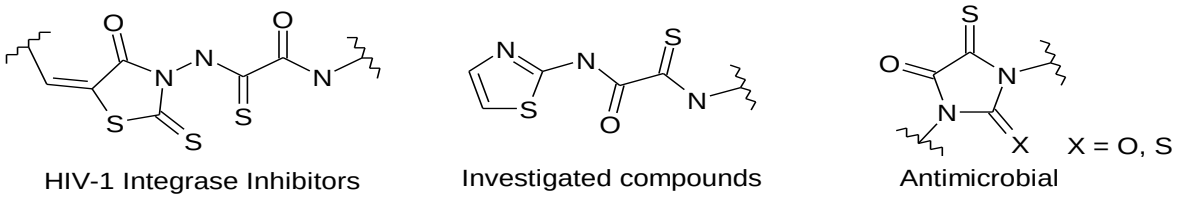


Figure 6. Biological activity of compounds with a thiooxamide fragment

Antimicrobial [34–36] and antitumor effects were observed for heterocyclic analogues [36, 37]. Given the lack of information on the biological activity of thiooxamides, we conducted a study of the antitumor activity of the synthesized compounds per the Developmental Therapeutic Program of the National Cancer Institute (NCI, USA). Anti-cancer screening was performed according to the NCI protocol [39-42]. The results of in vitro cell line screening for the study of their antitumor activity are shown in Table 5.

Table 5

Cytotoxicity of compounds 9a-j at a concentration of 10-5 M on 60 cancer cell lines

Compound	Mitotic activity against 60 lines, GP, %		Most sensitive lines (cancer type), GP, %
	Average growth	Range of growth	
9a	100.84	78.99 – 115.40	UO-31 (Renal Cancer) 78.99
9b	102.04	81.97 – 115.32	UO-31 (Renal Cancer) 81.97
9c	102.92	82.41 – 113.23	UO-31 (Renal Cancer) 82.41
9d	102.21	76.56 – 115.28	UO-31 (Renal Cancer) 76.56
9e	99.72	69.30 – 110.27	CCRF-CEM (Leukemia) 69.30 CAKI-1 (Renal Cancer) 82.07 UO-31 (Renal Cancer) 72.21
9f	102.36	77.10 – 122.25	UO-31 (Renal Cancer) 77.10
9g	103.91	80.33 – 123.38	UO-31 (Renal Cancer) 80.33
9i	100.00	74.53 – 118.71	UACC-62 (Melanoma) 74.77 CAKI-1 (Renal Cancer) 79.31 UO-31 (Renal Cancer) 74.53
9j	90.13	67.14 – 114.39	HOP-92 (Non-Small Cell Lung Cancer) 70.37 UACC-62 (Melanoma) 72.57 CAKI-1 (Renal Cancer) 67.14 UO-31 (Renal Cancer) 69.52

It was established that, in most cases, these compounds are of low activity. An exception is the kidney cancer line UO-31, which was moderately sensitive to all compounds **9a-j**.

In conclusions: A series of 2-aminothiazole hybrids containing morpholine moiety was synthesized, and in silico ADME-Tox profiles were studied. The ADME-Tox profiles indicated good oral bioavailability and low toxicity. Synthesized compounds were in vitro tested for their anti-cancer activity. They showed moderate antiproliferative activity.

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