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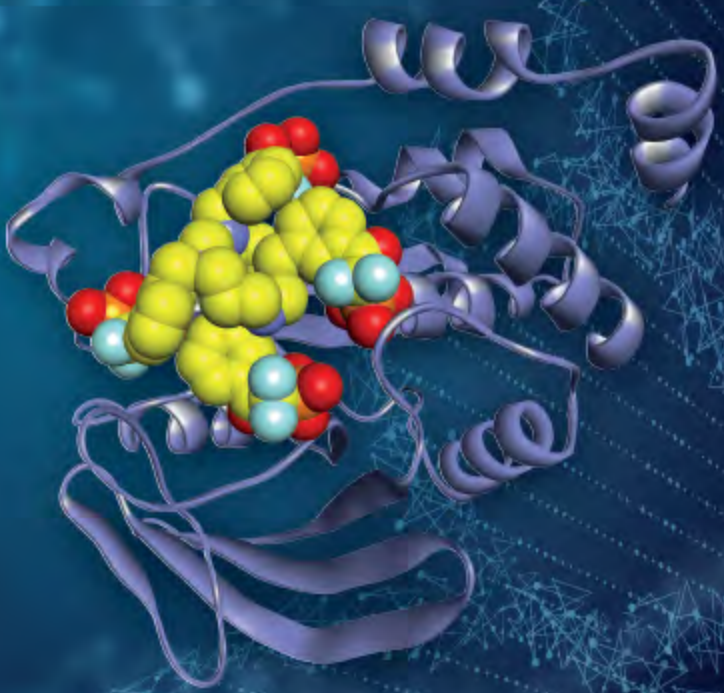

National Academy of Sciences of Ukraine

V.P. KUKHAR INSTITUTE OF BIOORGANIC CHEMISTRY AND PETROCHEMISTRY
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C O N T E N T S

Review article

Research articles

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DIGEST PAPER

Features of the synthesis and biological evaluation of 3-(carboxyphenyl)chromones

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Abstract: Flavonoids and their derivatives have historically been a source of therapeutic agents. Every year, more and more data is published on new flavonoid compounds, both synthetic and isolated from natural sources, and their innumerable physiological and pharmacological activities. This review presents synthetic routes towards 3-(carboxyphenyl)chromones and evaluation of their biological activity as published in both journal and patent literature. We have focused specifically on the 3-(carboxyphenyl)chromones, because while methods of synthesis and biological activity of 2(3)-substituted and 2,3-disubstituted chromones are well studied, literature data on isoflavones containing a carboxyl, ester, or amide group in ring B is scarce and fragmentary. The presented generalization of synthetic strategies and biological activity of 3-(carboxyphenyl)chromone derivatives demonstrates that this class of compounds can be targeted for discovery of new drugs and can be readily prepared owing to recent advances in synthetic organic and medicinal chemistry.

Keywords: chromones, isoflavones, biological activity, synthesis.

Chromones are important structural motifs that serve as useful templates for a design of novel biologically important compounds. The majority of research activity in recent years has been focused on the synthesis of 2(3)-substituted (flavones and isoflavones respectively) and 2,3-disubstituted chromones [1-7]. The synthetic approaches to chromone-pyrazole-fused compounds [8], azachromones, and azachromanones [9] have been summarized. Among numerous currently known isoflavone derivatives, compounds **1** with a carboxyl group in the ring B constitute a small group of compounds (Figure 1).

Isoflavones of natural origin, due to peculiarities of their biogenesis, belong to the category of polyphenols. Any substituents in their structure other than hydroxyl, alkoxyl, and methyl groups are extremely rare [10].

Preparative synthesis of isoflavones mainly focused on making of natural compounds with biological activity as well as the synthesis of analogues and heteroanalogues of isoflavones – 3-hetarylchromones [11]. Methods to synthesize derivatives of pharmaceutically active molecules of 3-(carboxyphenyl)chromones and their synthetic intermediates have also been reported.

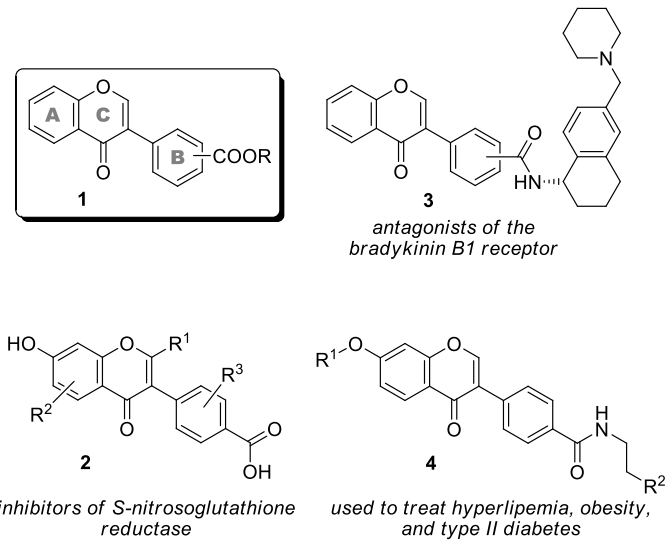

Figure 1. Examples of pharmaceutically active 3-(carboxyphenyl)chromones.

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For example, acids **2** are inhibitors of *S*-nitrosoglutathione reductase and can be used as immunomodulators, anti-inflammatory, and anti-asthma drugs [12]; isoflavone amides of type **3** are antagonists of the bradykinin B1 receptor [13]; and amides **4** are used to prevent or cure hyperlipemia, obesity, and type II diabetes (Figure 1) [14]. Yet, despite the obvious prospect, studies of synthesis of isoflavones containing a carboxyl, ester, or amide group in the B-ring have received little attention. These compounds are usually briefly mentioned in widely scoped publications and rarely feature as individual study objects. In addition, studies of the impact of introducing a highly acidic and reactive carboxyl group or its derivatives, e.g., various amides, on biological activity and approaches to constructing the isoflavone system are isolated.

In this brief review, we present a compilation of the literature data published in the last years, concerning the synthesis and biological activity of the 3-(carboxyphenyl)chromones. Analysis of the data demonstrates that although the introduction of a carboxyl group can complicate the construction of the isoflavone system, these problems are easily overcome. Therefore, we hope that this review will help draw attention to such promising building blocks for medicinal chemistry as 3-(carboxyphenyl)chromones.

Biological activity of 3-(carboxyphenyl)chromones, their esters and amides

The summary of 3-(carboxyphenyl)chromones biological activity presented below illustrates that these compounds can be readily employed as precursors to new drugs with various pharmacological effects.

A series of acids **2** (Figure 2) was reported to inhibit *S*-nitrosoglutathione reductase (GSNOR) [12], which implied that these compounds were promising immunomodulators, anti-inflammatory, and anti-asthma drugs. Similar bioactivity was manifested by corresponding 1-thioisoflavones [15].

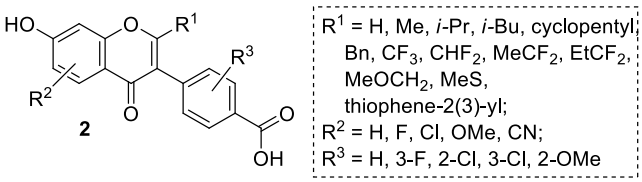
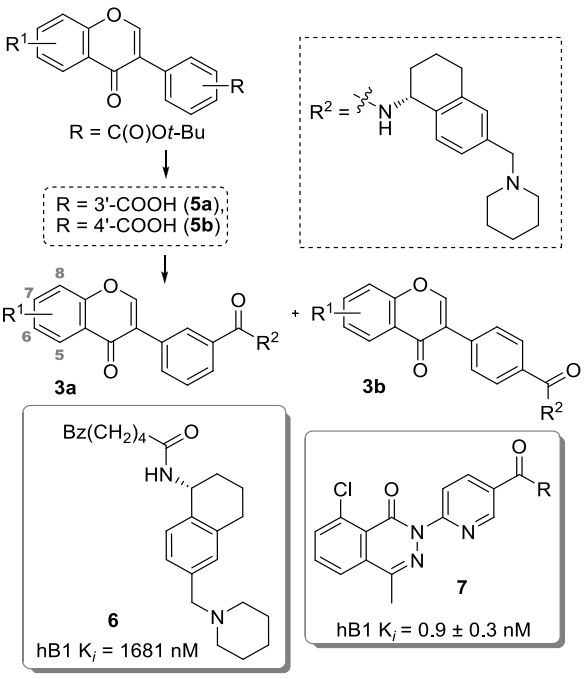


Figure 2. 4-(7-Hydroxy-4-oxo-4*H*-chromen-3-yl)benzoic acids **2** – inhibitors of *S*-nitrosoglutathione reductase.

A number of functionalized isoflavones, including those with carboxyl and carboxamide groups in 4'-position, and their heteroanalogues (at the ring A), featured as possible agents for the treatment of various kinds of mental disorders and addictions due to their ability to inhibit aldehyde dehydrogenase 2 (ALDH-2) [16-17].

Amides **3a,b**, produced from acids **5a,b**, were synthesized in the course of amide **6** modification in order to obtain new antagonists of the bradykinin B1 receptor (hB1) (Scheme 1) [13].

Despite *p*-isomer **3b** being less effective than its analogue with the phthalazinone cycle, it was selected as a promising compound for a more detailed study. It is interesting to note the significantly higher activity of *p*-isomer **3b** in comparison with *m*-isomer **3a**. The same pattern was observed not only for isoflavone derivatives but also for structurally related phthalazinones.



Scheme 1. Isoflavone amides **3** – antagonists of the bradykinin B1 receptor.

Interestingly, subsequent studies of isoflavone amides **3a,b** showed that their inhibitory activity significantly depends on the presence of substituents in the A-ring of isoflavone [18]: *p*-amide **3b** with a F or Cl atom introduced into the 5th position of the chromone ring is quite capable of competing in terms of activity with the most successful inhibitor **7** in a series of phthalazinones from a previous study [13]. Table 1 shows the efficiency of the bradykinin B1 receptor inhibition by the indicated compounds; as a numerical parameter, the inhibitory constant (K_i) is used.

Table 1. The efficiency of inhibition of the hB1 receptor.

Compd	R^1	hB1 K_i (nM)	Compd	R^1	hB1 K_i (nM)
3aa	H	64 ± 11	**3ba**	H	184 ± 32
3ab	5-Cl	29 ± 12	**3bb**	5-Cl	4 ± 2
3ac	6-Cl	767 ± 225	**3bc**	6-Cl	1616 ± 498
3ad	6-F	398 ± 44	**3bd**	7-Cl	42 ± 4
3ae	8-Cl	322 ± 126	**3be**	8-Cl	481 ± 87
			3bf	5-F	17 ± 8
			3bg	5-Me	15 ± 2

A variety of amides **4** of 7-hydroxy-isoflavone 4'-carboxylic acid was presented in a patent development [14] as medicines for preventing or curing hyperlipemia, obesity, or type II diabetes (Figure 3).

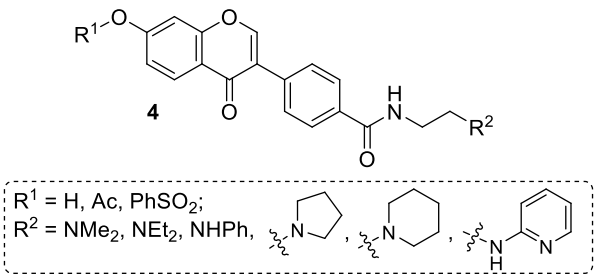


Figure 3. Isoflavone amides **4** – products for the treatment of hyperlipemia, obesity, and type II diabetes.

Of the four synthesized amides (Figure 4), the amide **8d** exhibited transactivation activity and induced the expression of farnesoid X receptor (FXR). The latter is known to regulate a series of target genes, including short heterodimer partner (SHP), bile salt export pump (BSEP) and sterol regulatory element-binding protein 1c (SREBP-1c).

Although the effect of **8d** in modulating FXR gene expression was less pronounced than that of GW4064 – synthetic FXR agonist, **8d** showed less toxicity in HepG2 cells. Overall, results obtained by the authors indicated that **8d** could act as a promising lead compound for the design of novel FXR modulators against dyslipidemia [19]. Moreover, amidation turned out to be a necessary condition for the high level of biological activity as the acid itself and its ester demonstrated much more modest performance. Functionalized isoflavones similar to the depicted ones have been patented for preventing or treating hyperlipidemia, type II diabetes, atherosclerosis, and non-alcoholic fatty hepatitis [20].

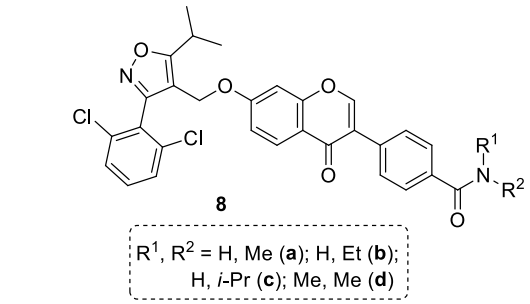


Figure 4. Isoflavone amides **8** – FXR modulators against dyslipidemia.

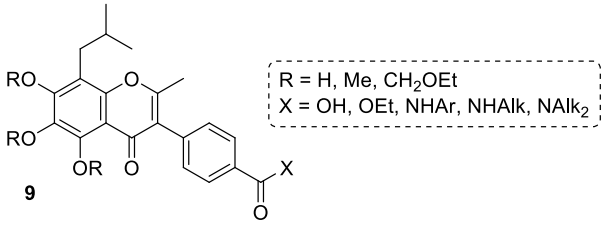


Figure 5. Series of isoflavones **9** – a new class of apoptosis inhibitors.

A large array of isoflavones modified with functional groups, as well as additional aromatic and heterocyclic fragments, were announced to be a new class of apoptosis inhibitors [21]. Compounds with carboxyl and carboxamide groups are represented mainly by structures **9** (Figure 5); several structures with a carboxyl group at the 3'-position of the isoflavone are shown.

Pyranoisoflavones were shown to function as butyrylcholinesterase inhibitor and can therefore be used in the treatment of Alzheimer's disease [22]; one of the studied derivatives was compound **10** with an ester group, the publication focused more on carbamate derivatives of hydroxyisoflavones, in particular, compound **11** – one of the most active substances (Figure 6).

The site docking data given in this work is interesting. Calculations showed that multiple ways of binding pyranoisoflavones to the active site of the enzyme exist depending on the nature and location of the substituents. In one of them, the carbonyl fragment of the chromone ring and π-interaction with the isoflavone ring B plays an important role, which is another argument in favor of the pharmacophoric potential of the isoflavone system. It should also be noted that the removal of the annelated pyran ring led to a noticeable drop in isoflavone activity, while the variation of substituents in ring B was not critical. This confirms the assumption that the B ring substituents can be easily varied (in particular, by obtaining amides of the carboxyl group) in order to introduce required physicochemical properties into a compound, while at the same time preserving the active action of the heterocyclic system itself.

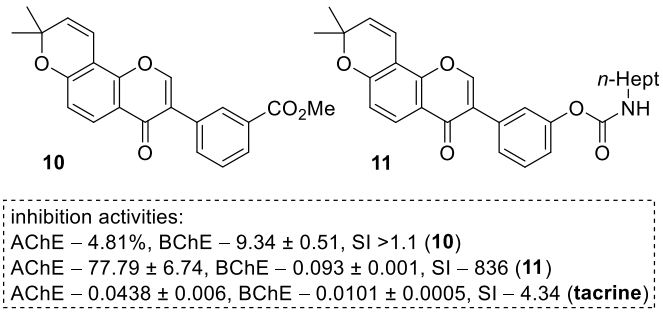
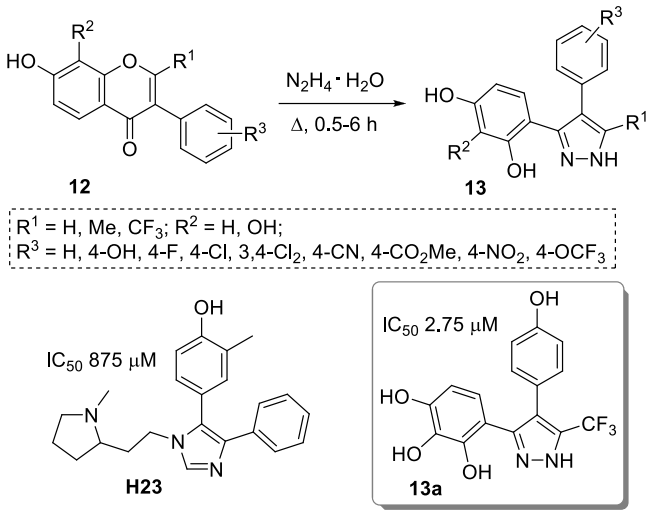


Figure 6. Pyranoisoflavones as butyrylcholinesterase inhibitor. The inhibition activities are expressed as IC_{50} (μM) or as a percentage of inhibition at 10 μM, and the IC_{50} values are the mean of three independent experiments ± SEM; SI represents selectivity index which is determined as ratio AChE IC_{50} / BChE IC_{50}; AChE – Acetylcholinesterase, BChE – butyrylcholinesterase.

Biological activity of 3-(carboxyphenyl)chromone derivatives

Approaches to obtaining biologically active substances from isoflavones can be based not only on the modification of functional groups but also on the recyclization of the labile pyrone fragment. For example, article [23] proposed pyrazoles **13** as an alternative to substance H23 – a previously known inhibitor of glycogen synthase. The pyrazoles **13**, including a compound with an ester group in the ring B (R^1 = OH, R^2 = Me, R^3 = 4-CO_2Me, Scheme 2), were obtained *via* recyclization of 2-substituted (R^1 = H, Me, CF_3) isoflavones **12**. The *p*-hydroxy derivative **13a** exhibited the highest activity (Scheme 2, inhibitory IC_{50} values against human glycogen synthase GS 1 (hGYS1)).



Scheme 2. Isoflavones **12** – precursor inhibitors of glycogen synthase.

Saturated analogs of isoflavones with carboxyl, carboxamide, and ester groups are also mentioned among biologically active substances. Isoflavans **14**, in particular, were investigated as compounds that exhibited valuable pharmacological properties, especially for the treatment of vascular diseases (Figure 7) [24].

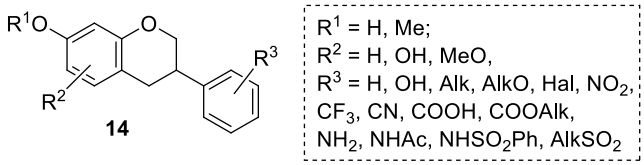
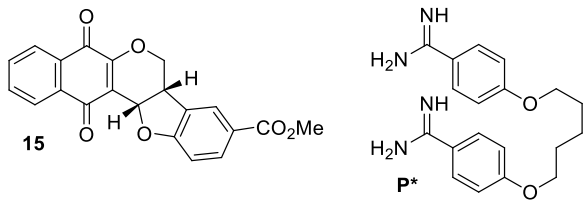


Figure 7. Isoflavans **14** – products for the treatment of vascular diseases.

It was also discovered that the antineoplastic activity on human malignant cell lines and antileishmanial activity on *Leishmania amazonensis* [25] is inherent in quinoid pterocarpans, including substance **15** with an ester group (Table 2).

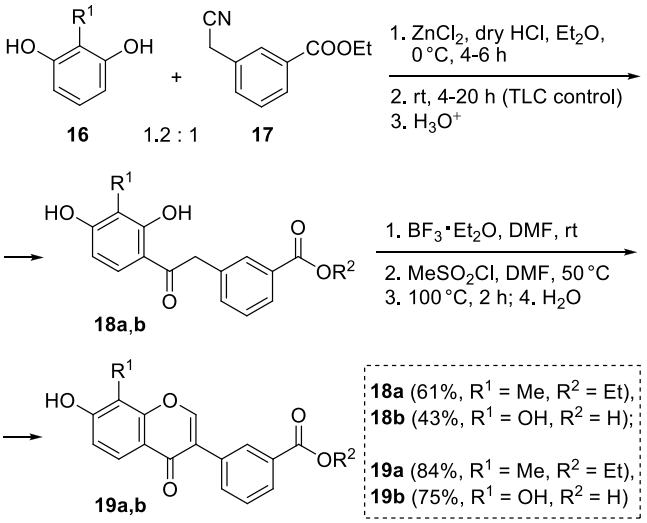
Table 2. Comparison of antileishmanial activity of compound **15** and pentamidine (**P***) on promastigote and amastigote forms of *L. amazonensis* and toxicity for M J774 cells (IC_{50} in μM).



	15	P*
Promastigote	2.8	2.2
Amastigote	0.5	1.5
MJ774	22.5	70
M J774/amastigote	45	46

Synthesis of 3-(carboxyphenyl)chromones, their esters and amides

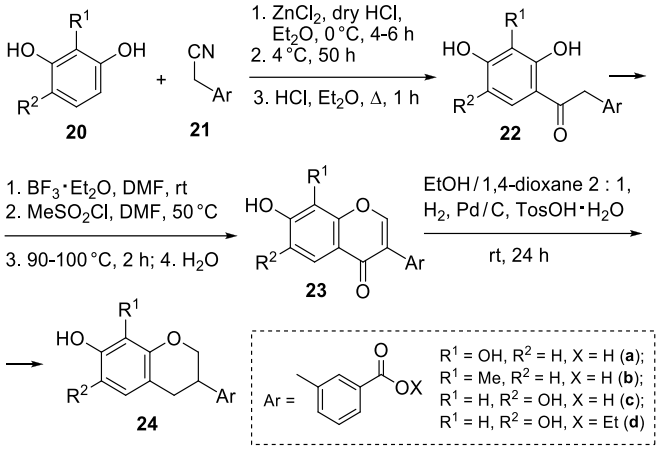
The most obvious "classical" solution to the problem of obtaining isoflavones with a carboxyl group in ring B is the Houben-Hoesch reaction using (carboxyphenyl)acetonitriles as key reagents, followed by formylation and ring-closure of the chromone system. However, the reactivity of phenylacetonitriles with a carboxyl function is questionable and, when ester protection of the carboxyl group is used, the ester fragment stability in both reaction and subsequent isolation conditions is of concern. Paper [26] described the preparation of 2-hydroxyphenylbenzyl ketones **18a,b** (and their analogues with alkoxy, CF_3, and CO_2Et groups) *via* the classical Houben-Hoesch reaction. Substituted phenols **16** and 3-(carboxyphenyl)acetonitrile (**17**) reacted under the action of $ZnCl_2$ in Et_2O, and underwent subsequent ring-closure into the corresponding isoflavones **19a,b** according to the method described by Bass – with $BF_3{\cdot}Et_2O$ followed by formylation with $MeSO_2Cl$-DMF reagent (Scheme 3) [27].



Scheme 3. Synthesis of 3-(carboxyphenyl)chromones **19a,b**.

In two cases when Houben-Hoesch reaction of ethyl 3-(cyanomethyl) benzoate with 2,6-dihydroxytoluene or 1,2,3-trihydroxybenzene was used, the isolation of reaction products was accompanied by partial hydrolysis and the formation of a mixture of ester and acid **18**. Isolation and purification of the main product were based on the acid's solubility in the aqueous solution of $NaHCO_3$. The main product was the ester **18a** in the first case and the acid **18b** in the second. During formylation and heterocyclization, despite treating the reaction mixture with water at a certain stage of the process, hydrolysis of the ester group of derivative **19a** did not occur.

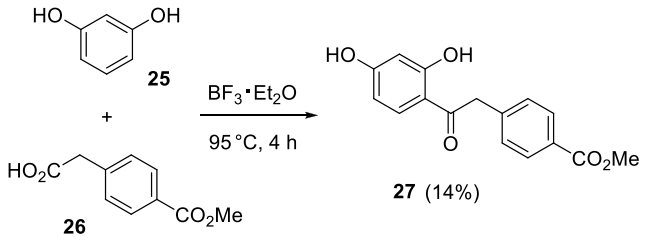
Notably, the conversion of deoxybenzoin to isoflavone is accompanied by partial or complete acylation of free OH-groups, and subsequent addition of water is necessary to remove the acyl residue from the hydroxyl group. Therefore, it can be assumed that the hydrolytic stability of the ester group in ring B is significantly higher; several similar examples are given later in the review.



Scheme 4. Synthesis of isoflavans **24**.

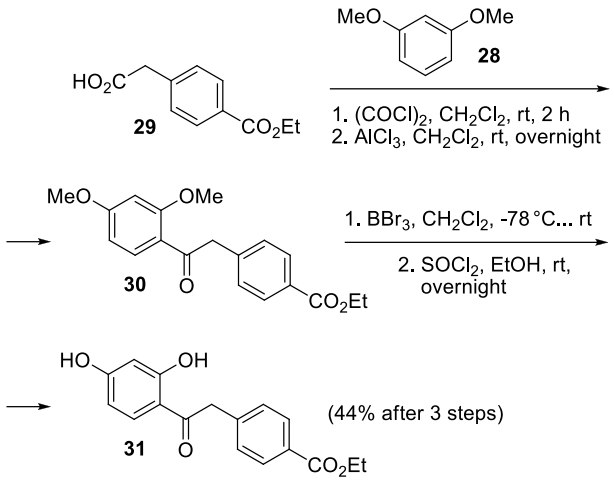
The previously mentioned bioactive isoflavans **14** (Figure 7) were synthesized by a catalytic reduction of corresponding isoflavones [24]. The latter, in turn, were obtained *via* the Houben-Hoesch reaction which was carried out at a rather low temperature. This significantly increased the reaction time but was obviously justified, because the authors successfully obtained a wide series of polyfunctional compounds, including those with a free carboxyl group. The synthetic sequence to these carboxy derivatives is shown in Scheme 4 (on the example of compounds **24** synthesis).

Using a corresponding arylacetic acid **26** instead of a nitrile in the Houben-Hoesch reaction allows to avoid the hydrolysis of the imine fragment and to treat the reaction mixture in milder conditions, which reduces the likelihood of hydrolysis of functional groups present in the main fragment (including ester). If the target product is an isoflavone with a free carboxyl group, the hydrolysis of the ester fragment is expediently carried out after the ring-closure of the pyrone ring. Such sequence of reactions was proposed by the authors of [12], devoted to the synthesis of a large array of bioactive isoflavones with a 4'-carboxyl group, shown in Figure 2. Scheme 5 shows the synthesis of a key compound – methyl 4-(2-(2,4-dihydroxyphenyl)-2-oxoethyl)benzoate (**27**).



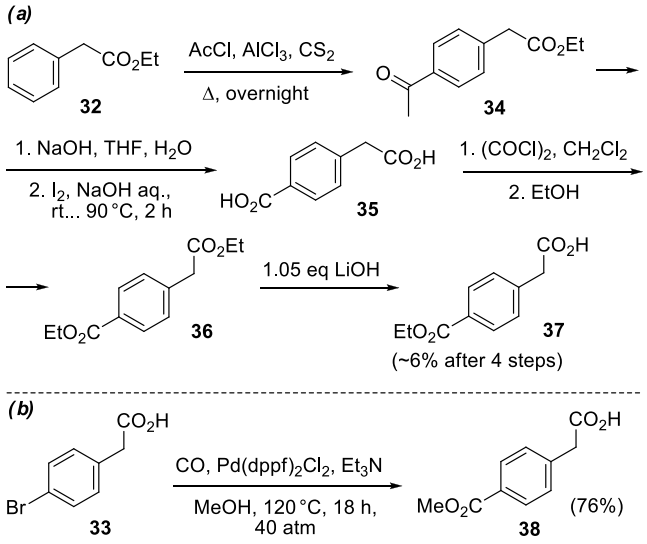
Scheme 5. Synthesis of methyl 4-(2-(2,4-dihydroxyphenyl)-2-oxoethyl)benzoate (**27**).

As an alternative to the Houben-Hoesch reaction between (ethoxycarbonylphenyl)acetic acid **29** and 1,3-dimethoxybenzene **28** in $BF_3 \cdot Et_2O$ medium, the authors of [12] studied in detail the Friedel-Crafts acylation of **28** with (ethoxycarbonylphenyl)acetic acid chlorides in the presence of $AlCl_3$ with subsequent removal of methyl groups of BBr_3. This approach provided higher yields of the target product **31** in comparison to the Houben-Hoesch reaction (Scheme 6).



Scheme 6. Synthesis of ethyl 4-(2-(2,4-dihydroxyphenyl)-2-oxoethyl)benzoate (**31**).

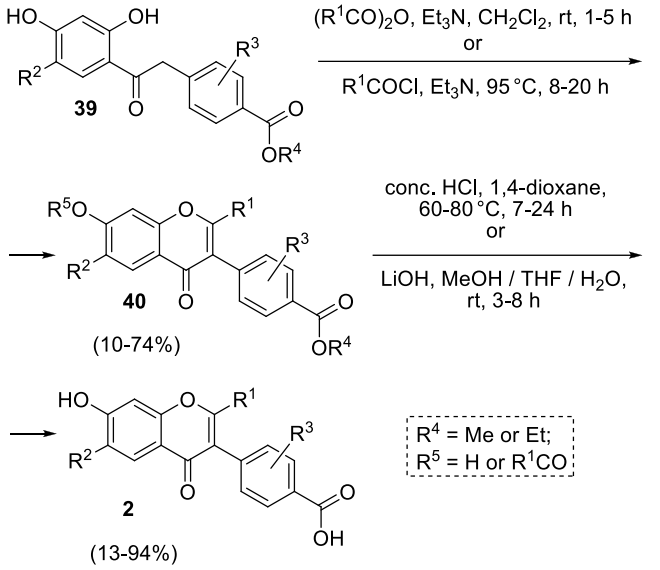
Additionally, two approaches have been successfully used to introduce a carboxyl group in the *p*-position of phenylacetic acid: the Friedel-Crafts acetylation of phenylacetic acid **32** with subsequent oxidation of the acetyl fragment in the iodoform reaction (Scheme **7a**; unfortunately, the total yield of product **37** for four stages was minuscule); or the catalyzed carbonylation of *p*-bromophenylacetic acid **33** with yields of target product **38** of 76% (Scheme **7b**).



Scheme 7. Synthesis of (ethoxycarbonylphenyl)acetic acid **37** and (methoxycarbonylphenyl)acetic acid **38**.

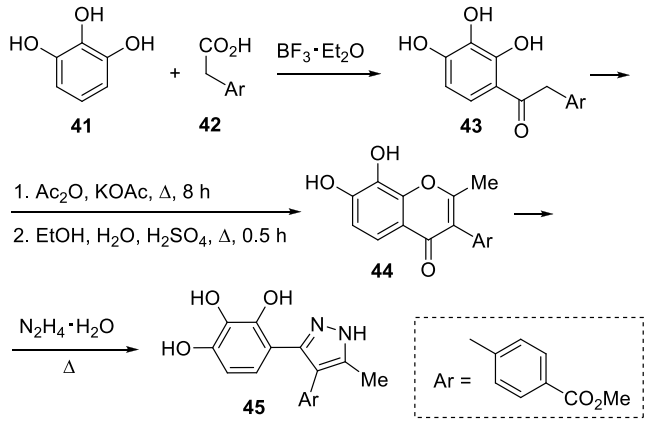
This study is also noteworthy for its use of anhydrides and chloroanhydrides of various carboxylic acids (aliphatic, aromatic, and heteroaromatic, as well as alkoxy and fluorine-substituted) in the cyclization of deoxybenzoins **39** – the products of the Houben-Hoesch reaction. This allowed to obtain isoflavones **40** with various substituents at position 2 of the chromone ring (the list of substituents R^1-R^3 is given in Figure 2 above). Depending on the activity of reagents, the cyclization of deoxybenzoins **39** to flavones **40** took place either with an equivalent amount of Et_3N at

room temperature in a dichloromethane solution or under reflux (Scheme 8). Of course, acylation of the additional hydroxyl group occurred simultaneously with the cyclization. Two methods were also developed for the hydrolysis of the *O*-acyl fragment: heating products **40** in an acidic medium, or treating with LiOH at room temperature, resulting in the formation of corresponding acids **2** with yields of up to 94%.



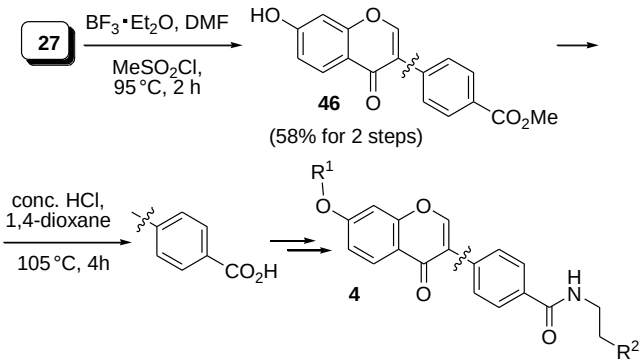
Scheme 8. Synthesis of 4-(7-hydroxy-4-oxo-4*H*-chromen-3-yl)benzoic acids **2**.

Tang et al. demonstrated that the conditions of the Houben-Hoesch reaction should be varied depending on the activity of arylacetic acids or nitriles [23]. Various reagents were used to conduct the cyclization of the chromone ring, allowing for variation of the substituent at position 2 of the system. The data on the synthesis of a derivative with an ester group – compound **44** – is presented in Scheme 9.



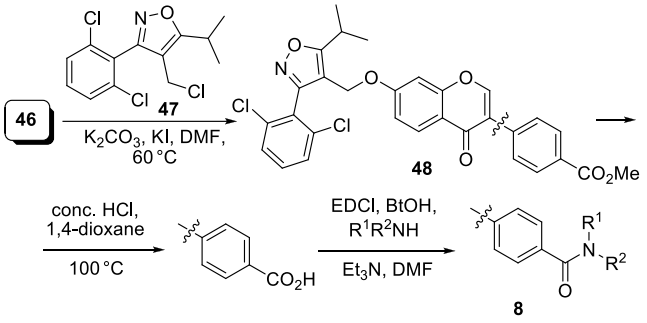
Scheme 9. Synthesis of isoflavone **44** – a precursor of inhibitors of glycogen synthase.

7-Alkoxyisoflavone 4'-carboxylic acid, subsequently converted into various amides **4** (see the list in Figure 3), was synthesized using the Houben-Hoesch reaction as well [14]; the synthesis of 2-(4-(methoxycarbonyl)phenyl)acetic acid required for the reaction was carried out using an approach similar to that shown in Scheme 7a. The authors did not reported a hydrolysis of the ester group neither during the Houben-Hoesch reaction nor during the isolation and subsequent cyclization to isoflavone (Scheme 10).



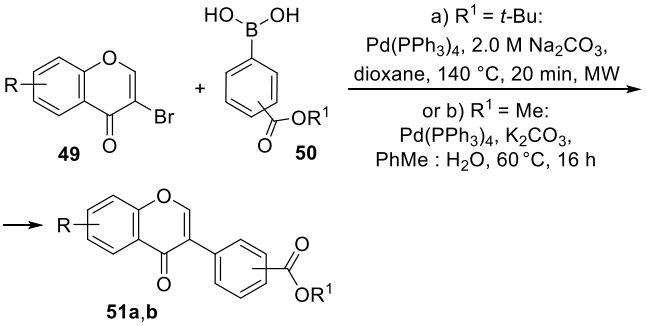
Scheme 10. Synthesis of isoflavone amides **4** – products for the treatment of hyperlipemia, obesity, or type II diabetes.

Isoflavone amides with an isoxazole moiety – compounds **8** – were synthesized in a similar manner; slight differences were related to the temperature regime of the Houben-Hoesch reaction, the conditions of hydrolysis of the ester, and the formation of the amide bond (Scheme 11) [19-20].



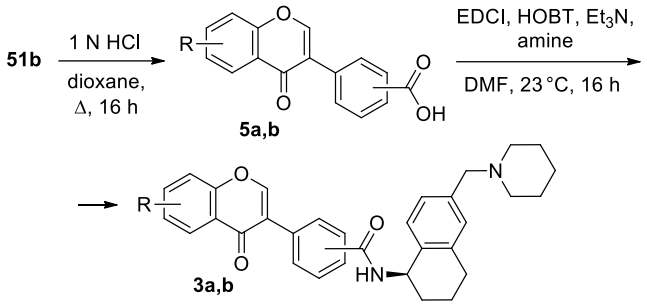
Scheme 11. Synthesis of isoflavone amides **8** – FXR modulators against dyslipidemia.

Another promising modern method for producing isoflavones is the catalytic arylation of 3-halogenochromones. One of the undoubted advantages of this approach is the possibility of using reagents with fragments that are unstable under the conditions of "classical" schemes. Using this method, *tert*-butyl esters **51a** of the acids mentioned above (Scheme 1) were obtained by the Suzuki-Miyaura reaction [13]. It should be noted that the ester fragment remained intact, despite the rather high reaction temperature (Scheme 12).



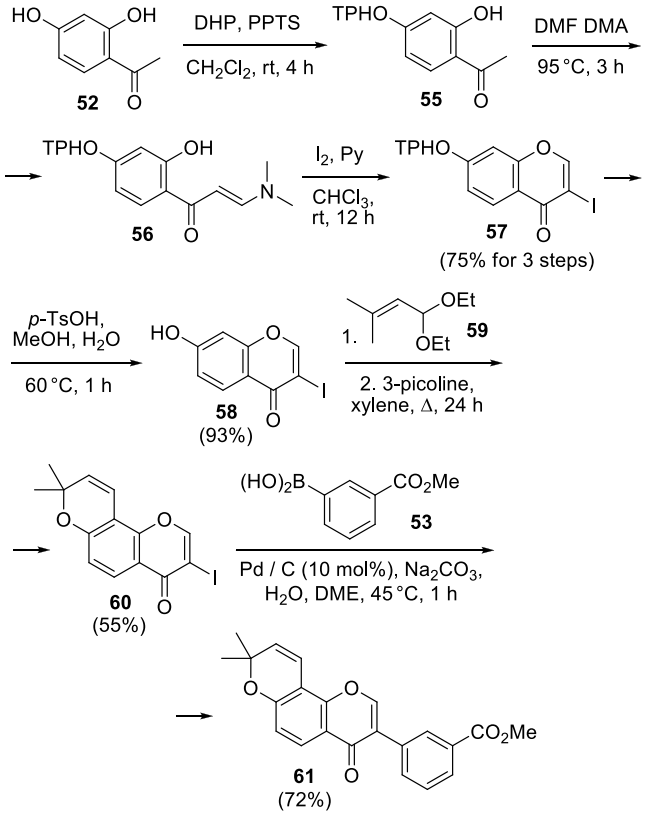
Scheme 12. Synthesis of isoflavones **51a,b** – precursors for antagonists of the bradykinin B1 receptor.

Bioactive isoflavones **3a,b** with amide group (see Table 1 above for a list of substituents) can be easily synthesized from methyl esters **51b** (Scheme 13), as was done in [18].



Scheme 13. Synthesis of amides **3a,b** – antagonists of the bradykinin B1 receptor.

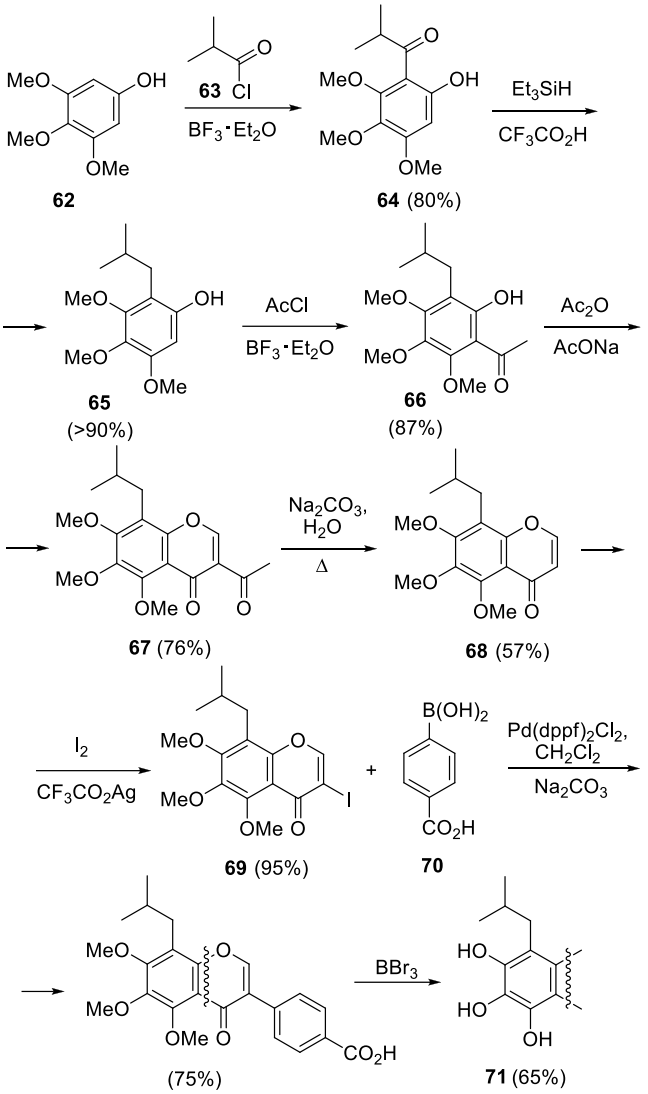
In the preparation of pyranoisoflavones, the pyran ring was first annealed to 7-hydroxy-3-iodochromone **58**, and only then was it combined with boronic acids [22]. At the coupling stage, standard conditions were used – palladium catalyst with triphenylphosphine ligands, aqueous organic medium, sodium carbonate as a base. In this way, an extensive list of 3-substituted pyranoisoflavones was synthesized, mainly with hydroxy and alkoxy groups in the B ring, as well as some 3-pyridylisoflavones. The yield of compound **61** with the ester group is shown in Scheme 14.



Scheme 14. Synthesis of pyranoisoflavone **61**.

The literature contains plenty of examples of the Suzuki reaction's preparative possibilities for the synthesis of isoflavones that are functionalized, among other things, with a carboxyl group. In fact, the main difficulties in this method are associated not so much with the implementation of the combination itself, but with the synthesis of the starting 3-halochromones.

For example, the authors of [28] carried out coupling of 3-iodochromone **69** and arylboronic acid **70** with a free carboxyl group with a yield of 75%, but the synthesis of the starting 3-iodochromone **69** from 3,4,5-trimethoxyphenol **62** required 6 stages (Scheme 15).

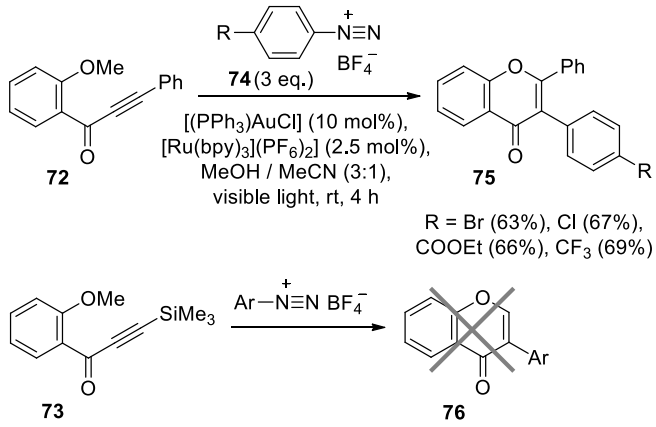


Scheme 15. Synthesis of isoflavone **71** *via* coupling of 3-iodochromone **69** and arylboronic acid **70**.

In the same manner, the same group obtained isoflavone-3'(4')-carboxylic acids with alkyl substituents at positions 2 and 8 and hydroxy groups at positions 5-7, which were used for the synthesis of a large group of amides – potential inhibitors of Bcl-2 (Figure 5) [21].

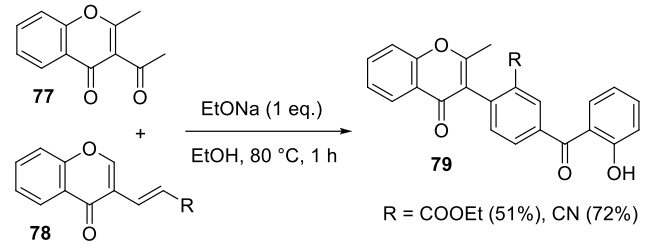
A more recent approach to catalytic heterocyclization of various systems (2*H*-benzo-[*e*][1,2]-thiazine 1,1-dioxides (benzosultams), benzoselenophenes, benzothiophenes, 4*H*-chromen-4-ones (flavones), 3*H*-indoles, 1*H*-isochromen-1-ones (isocoumarins), and 4*H*-thiochromen-4-ones (thioflavones)) was a photopromoted gold-catalyzed arylative heterocyclization of alkynes [29]. This method was tolerant towards many functional groups, including

esters functional groups, and the yield of corresponding 3-aryl-2-phenylchromone **75** with a 4-CO_2Et substituent did not differ significantly from the yield of Cl-, Br-, CF_3-derivatives (Scheme 16). Unfortunately, this technique has so far been developed only for 2,3-diarylchromones, and attempts to obtain 2-unsubstituted isoflavones by introducing silyl derivatives into the reaction have not been successful.



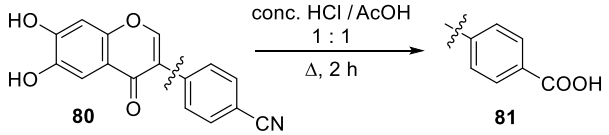
Scheme 16. Synthesis of 3-aryl-2-phenylchromone **75**.

Unusual polycyclic structures – condensed benzo-γ-pyrones – were obtained by a domino reaction between 3-acetyl-2-methylchromone **77** and various 3-(2-*R*-vinyl)-chromones (R = EWG) **78** [30]. In this specific method of constructing isoflavones **79**, the diene fragment of 3-vinylchromone **78** and the acetal group of acethyl-chromone **77** participated in the formation of the benzene ring (Scheme 17).



Scheme 17. Synthesis of isoflavone **79** *via* domino reaction.

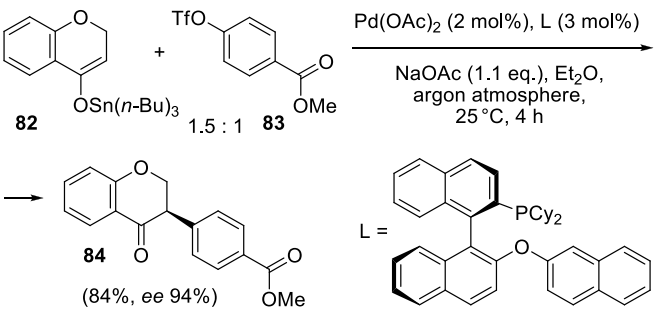
The patent [24] mentioned the possibility of obtaining 4'-carboxyisoflavone **81** by the hydrolysis of the corresponding nitrile in an acidic medium (Scheme 18).



Scheme 18. Synthesis of isoflavone **81**.

Synthesis of 3-(carboxyphenyl)chromone derivatives

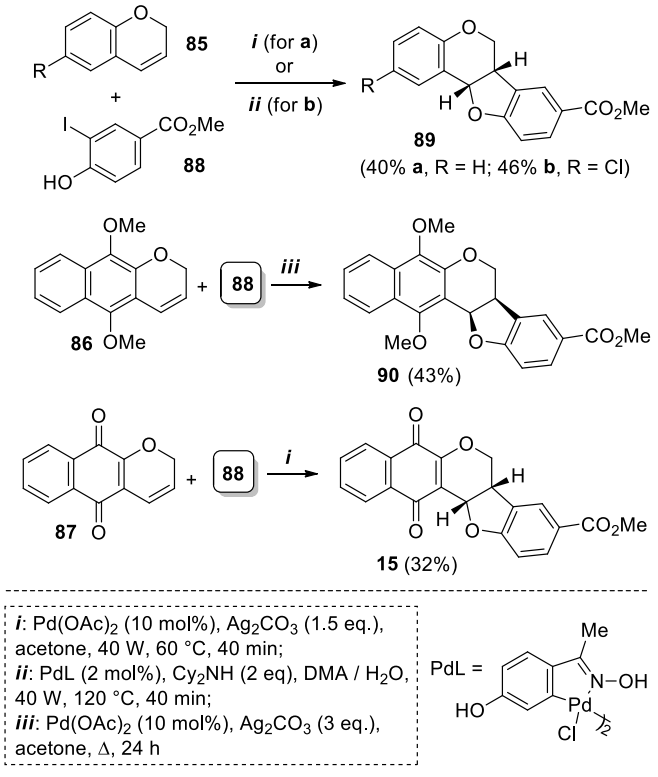
Compound **84** – isoflavanone with an ester group – was obtained by the catalytic arylation of the α-position of chromanone [31]; the high enantioselectivity of this reaction is remarkable (Scheme 19).



Scheme 19. Synthesis of isoflavanone **84**.

As it was mentioned earlier (Figure 7), the adducts that were obtained during a palladium-catalyzed oxyarylation of the multiple bonds of chromanes contained the structural fragment of isoflavane [25, 32-33]. Although such pterocarpans maintain interest as biologically active substances [25], the synthetic method was not very effective because the reaction yield never reached above 65% even under microwave irradiation for 40 min.

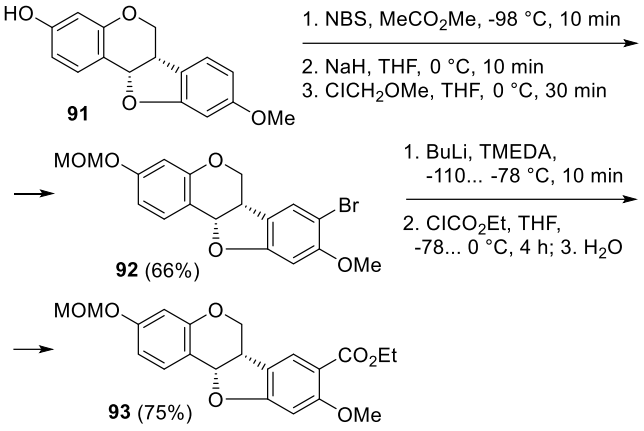
Nevertheless, the ability to use of a wide variety of substituents, as well as the successful use of electron-deficient quinone **87**, are worth mentioning as the reaction's advantages. Not only compounds with ester groups but their analogues with chloro-, nitro- and methoxy- groups were obtained as well (Scheme 20) [33]. The latter reaction led to the formation of biologically active derivatives (Figure 7). The quinone cyclic system also was obtained by the oxidation of dimethoxy derivatives [25].



Scheme 20. Synthesis of pterocarpans **15, 89-90**.

The carboxylation of the heterocyclic system itself as a method for modifying an isoflavonoid with a carboxyl group was carried out only for pterocarpans [34]. The bromination of the (+)-medicarpin **91** under free-radical

conditions with NBS (*N*-bromosuccinimide) in methyl acetate led to the formation of the 8-bromoderivative **92** (Scheme 21). The protected 8-bromo derivative **92** was treated with butyllithium and TMEDA (*N,N,N′,N′*-tetramethylethylenediamine) at low temperature (-110 °C) followed by quenching of the lithio derivative with ethyl chloroformate to give the ethyl ester **93**. This approach, however, is not applicable to isoflavones.



Scheme 21. Synthesis of pterocarpan **93**.

Conclusions

The literature data reveals numerous practical applications for 3-(carboxyphenyl)chromones and their derivatives. A carboxyl group modifications were the main synthetic routs to chromones' derivatives. Although the number of compounds of this class is still relatively small, most of them appeared in many biological studies where they demonstrated a fairly high level of biological activity. The existing data allows us to consider 3-(carboxyphenyl)-chromones as synthetically available compounds. In some cases the presence of the carboxyl group in the molecules necessitated adjustments to the "classical" synthetic protocols. Most of these derivatives were obtained using popular approaches in the synthesis of isoflavones – the Houben-Hoesch reaction or catalytic arylation of 3-halogenochromones. It should be noted that the number of publications on this topic has been steadily increasing since 2000. Moreover, the reports on the preparative synthesis of new 3-(carboxyphenyl)chromones facilitated studies of their biological activity, which undoubtedly will significantly improve the prospects of creating new drugs and other practically useful substances based on this class of compounds.

Notes

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The authors declare no conflict of interest.

Author contributions. The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

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Особливості синтезу та біологічної активності 3-(карбоксифеніл)хромонів

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Резюме: Флавоноїди та їх похідні історично були джерелами терапевтичних засобів. З кожним роком публікується все більше даних про нові флавоноїдні сполуки (як виділені з природних джерел, так і синтезовані) та їх різноманітну фізіологічну та фармакологічну активність. У цьому огляді представлена інформація щодо синтезу 3-(карбоксифеніл)хромонів та їх біологічної активності, опублікована як у періодичних виданнях, так і в патентній літературі. Наш інтерес до 3-(карбоксифеніл)хромонів, було викликано насамперед тим, що є багато літературних даних про методи синтезу та біологічну активність 2(3)-заміщених та 2,3-дизаміщених хромонів, але дані про ізофлавони, що містять карбоксильну, естерну або амідну групу в кільці В обмежені та фрагментарні. Тим не менше, інтенсивність роботи над цією темою в 2000 рр. почала зростати, в першу чергу, завдяки виявленню цікавих біологічних властивостей карбоксиізофлавонів та їх похідних; і це, в свою чергу активізувало розробки оригінальних методів синтезу таких сполук. Представлений огляд літератури дозволяє вважати 3-(карбоксифеніл)хромони та їх похідні синтетично доступними сполуками, хоча в деяких випадках присутність карбоксильної групи в субстраті вимагає певних удосконалень "класичних" методів. Більшість із представлених у огляді 3-(карбоксифеніл)хромонів були отримані з використанням підходів, популярних у синтезі ізофлавонів – реакції Губена-Хеша з наступною циклізацією або каталітичного арилювання 3-галогенохромонів. Структура 3-(карбоксифеніл)хромону має також багато можливостей для модифікації, зокрема: синтез амідів за карбоксильною функцією, анелювання додаткових гетероциклічних фрагментів, одержання частково насичених за кільцем С похідних та продуктів рециклізації хромонової системи. Перелічені перетворення були реалізовані на практиці і привели до створення нових біологічно активних сполук, що беззаперечно підтверджує потенціал подальшого розвитку хімії 3-(карбоксифеніл)хромонів.

Ключові слова: хромони, ізофлавони, біологічна активність, синтез.

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RESEARCH ARTICLE

Synthesis and anticancer activity of 5-sulfonyl derivatives of 1,3-oxazole-4-carboxylates

Stepan G. Pilyo, Olexandr P. Kozachenko, Victor V. Zhirnov, Maryna V. Kachaeva, Oleksandr L. Kobzar, Andriy I. Vovk and Volodymyr S. Brovarets*

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Abstract: A series of new 2-aryl 5-sulfonyl-1,3-oxazole-4-carboxylates for NCI anticancer screening protocol against 60 cancer cell lines were synthesized. Screening was performed *in vitro* on 60 cell lines of lungs, kidneys, CNS, ovaries, prostate, and breast cancer, leukemia, and melanoma. Methyl 5-benzylsulfonyl-2-phenyl-1,3-oxazole-4-carboxylate **15** exhibited potent and broad range of cytotoxic activity against tested human cancer cells with average GI_{50}, TGI, and LC_{50} values of $5.37 \cdot 10^{-6}$, $1.29 \cdot 10^{-5}$ and $3.6 \cdot 10^{-5}$ mol/L respectively. Molecular docking was used to evaluate the possible interaction of compound **15** with tubulin as well as a complex formation with CDK2.

Keywords: 5-sulfonyl-1,3-oxazole-4-carboxylates; synthesis; anticancer activity; selectivity; molecular docking.

Introduction

During the last years, the search for new biologically active compounds, in particular anticancer agents, among 1,3-oxazole-4-carboxylates has stimulated considerable synthetic efforts [1-3]. Oxazoles **I-VIII** containing arylsulfonyl [4] or sulfonamide [5-6] moiety displayed considerable cytotoxicity and selectivity towards diverse cancer subpanels with sub-micromolar GI_{50} values (Figure 1). It has been proposed that possible ways of anticancer influence of sulfonamide derivatives are associated with inhibition of the tubulin polymerization, similar to E7010 [5], DNA damage, BCL6, and NSD2 inhibition [6]. So, previous works [4-6] have focused mostly on 4-cyano-substituted 1,3-oxazoles.

In the present work, we replaced the nitrile with an ester group in oxazole derivatives and synthesized new 5-sulfonyl derivatives of 1,3-oxazole-4-carboxylate, investigated their anticancer screening and elucidated the

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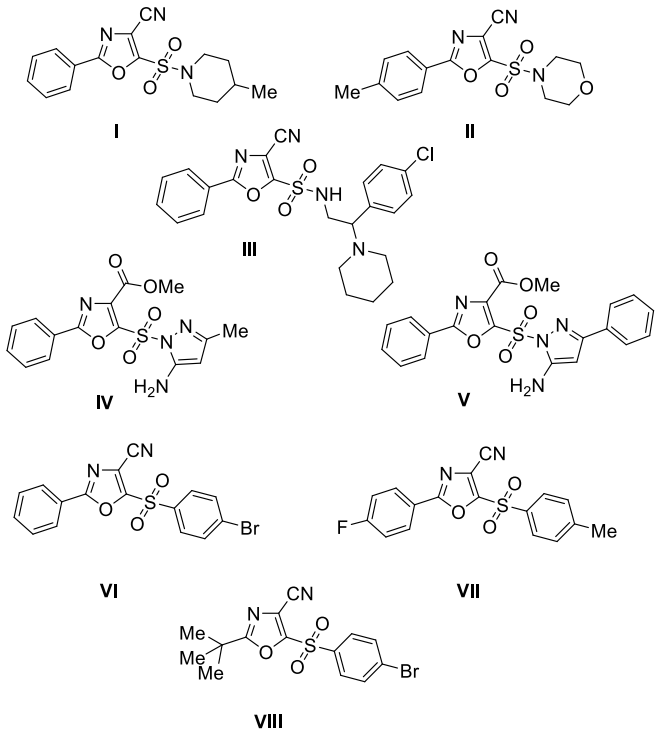


Figure 1. 5-Sulfonyl-substituted 1,3-oxazoles with anticancer activity.

possible mechanism of their action by molecular docking approaches.

Although the nitrile group is quite robust and, in most cases, is not readily metabolized, there are some confirmed facts of its cleavage and modification leading to increased cytotoxicity [7]. Ester group in comparison with nitrile is less cytotoxic for normal cells and it is perspective moiety for the introduction in 1,3-oxazole-5-sulfonamide pharmacophore for the new anticancer drugs search.

Accordingly, in this study, we report the synthesis, characterization and biological activity of novel molecules combined 2-aryl-1,3-oxazole-4-carboxylate and sulfonamide scaffolds.

Results and Discussion

Chemistry

The synthesis of target compounds was accomplished by the reaction sequence illustrated in Scheme 1 and 2. The previously described in the literature [8] dichloroacrylates **1a,b** were chosen as the starting materials.

1,3-Oxazole-5-sulfonamides **3-12** were prepared from methyl 5-chlorosulfonyl-2-phenyloxazole-4-carboxylate (**2**) by refluxing with appropriate amines [9].

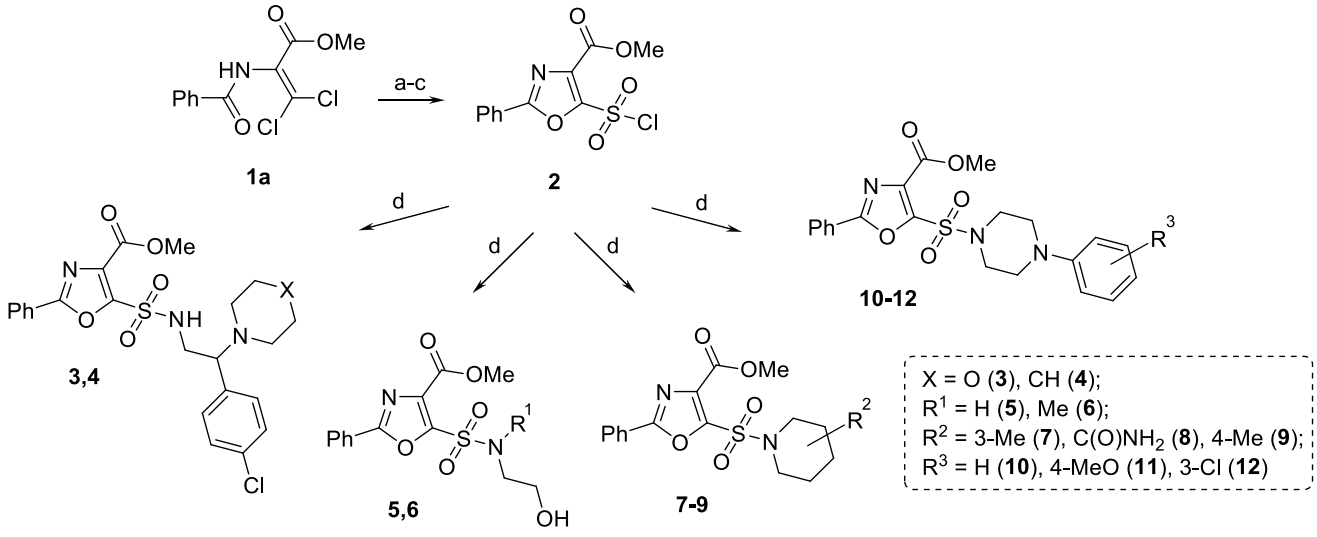
The preparation of arylsulfonyl derivatives **17** and **18** involves the reaction sequences as in Sheme 2. The starting methyl 2-(benzoylamino)-3,3-dichloroacrylate (**1a**) was reacted with sodium sulfide to yield methyl 5-mercapto-2-phenyl-1,3-oxazole-4-carboxylate (**13**), which was converted into methyl 5-(benzylsulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**15**) by alkylation with benzyl chloride and oxidation with hydrogen peroxide [10].

5-((3-Methoxyphenyl)sulfonyl)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile (**18**) was synthesized following the transformation sequence **1b** → **16** → **17** → **18** in Sheme 2. Reaction of methyl 3,3-dichloro-2-((4-methylbenzoyl)-amino)acrylate (**1b**) with benzenemethanethiol in the presence of triethylamine yielded methyl 3,3-bis((3-methoxyphenyl)thio)-2-((4-methylbenzoyl)amino)acrylate (**16**), that was cyclized in the presence of silver carbonate to form methyl 5-((3-methoxyphenyl)thio)-2-(4-methyl-phenyl)-1,3-oxazole-4-carboxylate (**17**). The latter was converted into the corresponding sulfonyl derivative **18** by oxidation with hydrogen peroxide [10].

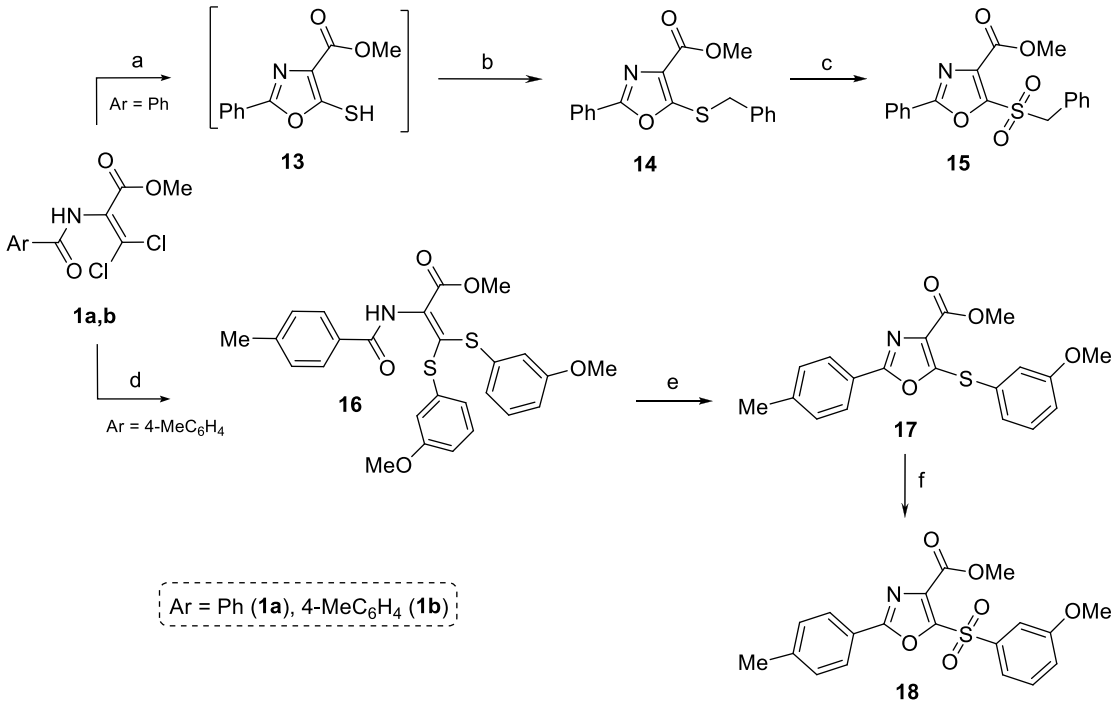
The structure and composition of all obtained compounds **3-12**, **15**, and **18** have been in good agreement with IR, NMR (^{1}H and ^{13}C NMR) spectroscopy, chromato-mass spectrometry (LC-MS), and elemental analysis data. The CH-protons of the methoxy group at the 4th position of the oxazole ring of synthesized compounds appear as a singlet at 3.61-3.93 ppm. All CH_2 and CH-proton signals of sulfamides **3-12** are visible in the ^{1}H NMR spectra. The signal of the OH group of 6 and 7 is located in ^{1}H NMR at 4.80-4.68 ppm. The signal at 8.32 ppm belongs to NH group of compound **3**. The strong absorption bands of SO_2 group appeared at 1152 to 1192 cm^{-1} and 1353 to 1386 cm^{-1} in the IR spectra of all compounds. Also, the broad strong bands at 1731 to 1732 cm^{-1} corresponded to C═O bond of esters.

In vitro evaluation of the anticancer activity

The synthesized 5-sulfonyl-1,3-oxazole-4-carboxylates were screened on human cancer cell lines at the NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI. Results for each compound were reported as a mean graph of the percent growth (GP%) of the treated cells when compared to the untreated control cells and one-dose screening data are summarized in Table 1.



Scheme 1. Synthesis of 5-sulfamide derivatives of 2-phenyl-1,3-oxazole-4-carboxylate **3-12**. Reagents and conditions: (a) 2.5 eq. NaSH; (b) BnCl; (c) Cl_2, AcOH, H_2O; (d) amine, Et_3N.



Scheme 2. Synthesis of 5-aryl-1,3-oxazole-4-carboxylates **15, 18**. Reagents and conditions: (a) 2.5 eq. Na_2S; HCl; (b) $PhCH_2Cl$, Et_3N; (c) H_2O_2, AcOH; (d) 2 eq. $3\text{-}MeOC_6H_4SH$, 2 eq. Et_3N; (e) 2.5 eq. Ag_2CO_3; (f) H_2O_2, AcOH, reflux.

The synthesized compounds showed differential anticancer activity pattern against different types of cancer and cell lines according to the primary one-dose anticancer assay [11-15]. The most active methyl 5-(benzylsulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**15**) had anticancer activity range from -78.70 to 109.63%. Thus, compound **15** revealed high cytotoxic effect on leukemia CCRF-CEM (GP = -6.08%), MOLT-4 (GP = -9.29%), SR (GP = -11.55%), colon cancer COLO 205 (GP = -42.98%), melanoma MALME-3M (GP = -78.70%), renal cancer ACHN (GP = -35.00%) cell lines.

Since compound **15** possessed a significant anticancer effect against several cell lines, it was tested additionally in the fifth-dose assay (10^{-4}-10^{-8} M) [13, 16] (Table 2). Three dose-dependent values were extrapolated from concentration-response curves for each cell line: GI_{50} – the drug concentration of the compound that inhibited 50% net cell growth; TGI – concentration of tested compound with total cell growth inhibition, LC_{50} – concentration of compound leading to 50% net cell death.

Compound **15** exhibited a potent and broad range of cytotoxic activity against tested human cancer cells with average GI_{50}, TGI, and LC_{50} $5.37\cdot10^{-6}$, $1.29\cdot10^{-5}$, and $3.6\cdot10^{-5}$ mol/L respectively. GI_{50} values were ranged from 1.48 μM (renal cancer UO-31 cell line) to 70.2 μM (ovarian cancer SK-OV-3 cell line), TGI – from 3.12 μM (non-small cell lung cancer NOP-92 cell line) to >100 μM (CNS Cancer SF-395 cell line, and Ovarian cancer SK-OV-3 cell line), and LC50 – from 5.45 μM (renal cancer UO-31 cell line) to >100 μM (leukemia panel, non-small cell lung cancer A549/ATCC cell line, ovarian cancer SK-OV-3, breast cancer MCF7, HS 578T, T-47D).

Table 1. Anticancer NCI one-dose screening data (10^{-5} M) for compounds **3-12**, **15**, **18**

Compd NSC	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %
3 802758	65.66	from -21.00 to 120.90	-21.00 (CCRF-CEM/Leukemia) 4.12 (HL-60(TB)/Leukemia) 13.37 (K-562/Leukemia) 7.96 (MOLT-4/ Leukemia) 7.86 (RPMI-8226/ Leukemia) 1.88 (SR/ Leukemia) 23.78 (NCI-H522/ Non-small cell lung cancer) 18.71 (SW-620 / Colon cancer) 31.67 (MCF7/Breast cancer) 29.94 (MDA-MB-468/ Breast cancer)
4 802759	66.95	from -19.22 to 116.57	-19.22 (CCRF-CEM/Leukemia) 17.47 (HL-60(TB)/Leukemia) 9.15 (K-562/Leukemia) 1.79 (MOLT-4/ Leukemia) 20.02 (RPMI-8226/ Leukemia) -3.58 (SR/ Leukemia) 25.60 (NCI-H522/ Non-small cell lung cancer)
5 802760	93.48	from 67.00 to 136.78	67.00 (SR/ Leukemia) 67.05 (MDA-MB-468/ Breast cancer)
6 802761	65.83	from -24.58 to 112.08	16.16 (CCRF-CEM/Leukemia) -24.58 (HL-60(TB)/Leukemia) 22.17 (K-562/Leukemia) 6.31 (MDA-MB-468/ Breast cancer)

Table 1. (*Contd.*)

Compd NSC	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %
7 802781	72.07	from 0.92 to 110.17	1.21 (CCRF-CEM/Leukemia) 14.99 (HL-60(TB)/Leukemia) 17.71 (K-562/Leukemia) 6.09 (MOLT-4/ Leukemia) 0.92 (SR/ Leukemia) 28.58 (NCI-H522/ Non-small cell lung cancer) 29.19 (MDA-MB-468/ Breast cancer)
8 802782	58.77	from -33.25 to 110.68	-3.72 (CCRF-CEM/Leukemia) -33.25 (HL-60(TB)/Leukemia) 14.77 (K-562/Leukemia) 1.95 (MOLT-4/ Leukemia) -3.16 (SR/ Leukemia) 0.81 (NCI-H522/ Non-small cell lung cancer) 20.64 (COLO 205/Colon cancer) 25.38 (SW-620 / Colon cancer) 16.19 (OVCAR-3/Ovarian cancer) 23.15 (TK-10/Renal cancer) -14.59 (MDA-MB-468/ Breast cancer)
9 802797	69.91	from 12.35 to 113.24	12.35 (CCRF-CEM/Leukemia) 22.72 (K-562/Leukemia) 23.59 (MOLT-4/ Leukemia) 17.56 (NCI-H522/ Non-small cell lung cancer) 38.26 (SW-620 / Colon cancer) 16.19 (OVCAR-3/Ovarian cancer)
10 802783	91.60	from 61.48 to 111.90	61.48 (CCRF-CEM/Leukemia) 62.10 (SR/ Leukemia)
11 802784	72.26	from -11.85 to 121.22	-11.85 (CCRF-CEM/Leukemia) 21.81 (HL-60(TB)/Leukemia) 20.41 (K-562/Leukemia) -1.38 (SR/ Leukemia) 22.65 (MDA-MB-468/ Breast cancer)
12 802796	74.57	from 4.91 to 111.22	4.91 (CCRF-CEM/Leukemia)
15 802778	69.01	from -78.70 to 109.63	-6.08 (CCRF-CEM/Leukemia) 2.87 (K-562/Leukemia) -9.29 (MOLT-4/ Leukemia) -11.55 (SR/ Leukemia) -42.98 (COLO 205/Colon cancer) -78.70 (MALME-3M/ Melanoma) -35.00 (ACHN/ Renal cancer) 5.41 (TK-10/Renal cancer)
18 802780	78.38	from -12.30 to 109.75	-9.09 (CCRF-CEM/Leukemia) 11.58 (K-562/Leukemia) -7.44 (MOLT-4/ Leukemia) 0.69 (SR/ Leukemia) -8.96 (COLO 205/Colon cancer) -12.30 (MALME-3M/ Melanoma)

Table 2. Anticancer NCI five-dose-response parameters for compound **15**.

Panel	Cell line	GI_{50}, M	TGI, M	LC_{50}, M
Leukemia	CCRF-CEM	$2.91 \cdot 10^{-6}$	$8.41 \cdot 10^{-6}$	$>1.00 \cdot 10^{-4}$
	HL-60(TB)	$2.30 \cdot 10^{-6}$	$6.22 \cdot 10^{-6}$	$>1.00 \cdot 10^{-4}$
	K-562	$2.07 \cdot 10^{-6}$	$5.29 \cdot 10^{-6}$	$>1.00 \cdot 10^{-4}$
	MOLT-4	$2.11 \cdot 10^{-6}$	$5.57 \cdot 10^{-6}$	$>1.00 \cdot 10^{-4}$
Non-Small Cell Lung Cancer	HOP-92	$1.61 \cdot 10^{-6}$	$3.12 \cdot 10^{-6}$	$6.06 \cdot 10^{-6}$
	NCI-H522	$1.52 \cdot 10^{-6}$	$3.29 \cdot 10^{-6}$	$7.13 \cdot 10^{-6}$
Colon Cancer	COLO 205	$1.84 \cdot 10^{-6}$	$3.66 \cdot 10^{-6}$	$7.30 \cdot 10^{-6}$
	HCT-116	$2.58 \cdot 10^{-6}$	$6.23 \cdot 10^{-6}$	$2.16 \cdot 10^{-5}$
	HCT-15	$1.79 \cdot 10^{-6}$	$3.58 \cdot 10^{-6}$	$7.15 \cdot 10^{-6}$
	SW-620	$2.05 \cdot 10^{-6}$	$4.14 \cdot 10^{-6}$	$8.33 \cdot 10^{-6}$
Melanoma	MALME-3M	$1.73 \cdot 10^{-6}$	$3.44 \cdot 10^{-6}$	$6.85 \cdot 10^{-6}$
Ovarian Cancer	OVCAR-3	$1.94 \cdot 10^{-6}$	$3.52 \cdot 10^{-6}$	$6.40 \cdot 10^{-6}$
	OVCAR-4	$1.82 \cdot 10^{-6}$	$3.40 \cdot 10^{-6}$	$6.32 \cdot 10^{-6}$
Renal Cancer	ACHN	$1.77 \cdot 10^{-6}$	$3.15 \cdot 10^{-6}$	$5.62 \cdot 10^{-6}$
	CAKI-1	$1.84 \cdot 10^{-6}$	$3.42 \cdot 10^{-6}$	$6.37 \cdot 10^{-6}$
	RXF 393	$1.87 \cdot 10^{-6}$	$3.50 \cdot 10^{-6}$	$6.55 \cdot 10^{-6}$
	TK-10	$1.74 \cdot 10^{-6}$	$3.19 \cdot 10^{-6}$	$5.86 \cdot 10^{-6}$
	UO-31	$1.48 \cdot 10^{-6}$	$2.84 \cdot 10^{-6}$	$5.45 \cdot 10^{-6}$
Breast Cancer	MDA-MB-468	$1.85 \cdot 10^{-6}$	$4.02 \cdot 10^{-6}$	$8.75 \cdot 10^{-6}$
Average values		$5.37 \cdot 10^{-6}$	$1.29 \cdot 10^{-5}$	$3.6 \cdot 10^{-5}$

Previously, anticancer activity of 2-substituted 5-arylsulfonyl-4-cyano-1,3-oxazoles was proposed to be related to the inhibition of tubulin polymerization [5]. Given the interest in microtubule-targeting agents [17], as well as to possible inhibitors of oncogenic signaling pathways [18], two series of molecular docking simulations for the 5-sulfonyl-1,3-oxazole-4-carboxylates were carried out in this paper. The compounds were docked into colchicine binding site of tubulin (PDB code 1SA0 [19]) and ATP-binding sites of cyclin-dependent kinases CDK1, CDK2, CDK7 and CDK9 (PDB codes 4Y72 [20], 3QQJ, 1UA2 [21], 4BCF [22], respectively). The highest affinity of compound **15** was observed towards CDK2.

The molecular docking of 5-benzylsulfonyl derivative of 1,3-oxazole-4-carboxylate **15** into colchicine binding site of αβ-tubulin heterodimer structure showed calculated binding affinity of -8.0 kcal/mol. According to the obtained model (Figure 2A), the 5-(benzylsulfonyl)oxazole fragment of ligand provided electrostatic, van der Waals, and hydrophobic interactions with amino acids residues Val238, Leu248, Leu255, Lys352, and Ile378 of β-tubulin subunit (B chain). The complex exhibited hydrogen bond of methyl acetate moiety of ligand with backbone amino group of Ala250 (B chain). The 5-benzylsulfonyl fragment of the

ligand was located near amino acids residue Asn101, Thr179, Ala180, and Val181, which belong to α-tubulin subunit (A chain).

Binding mode of compound **15** into ATP-binding site of cyclin-dependent kinase 2 with binding affinity -9.2 kcal/mol is shown in Figure 2B. In this model, 5-(benzylsulfonyl)-oxazole fragment of ligand was located in hydrophobic pocket and had electrostatic, Wan der Waals, and hydrophobic interaction with amino acids residues Val18, Ile10, Ala31, Val64, Phe80, Leu83, Leu134, and Ala144 as well as showed π-stacking interaction with Phe82. The sulfonyl group of 5-benzylsulfonyl moiety was adjacent to Gln85, Asp86, and Lys89 residues.

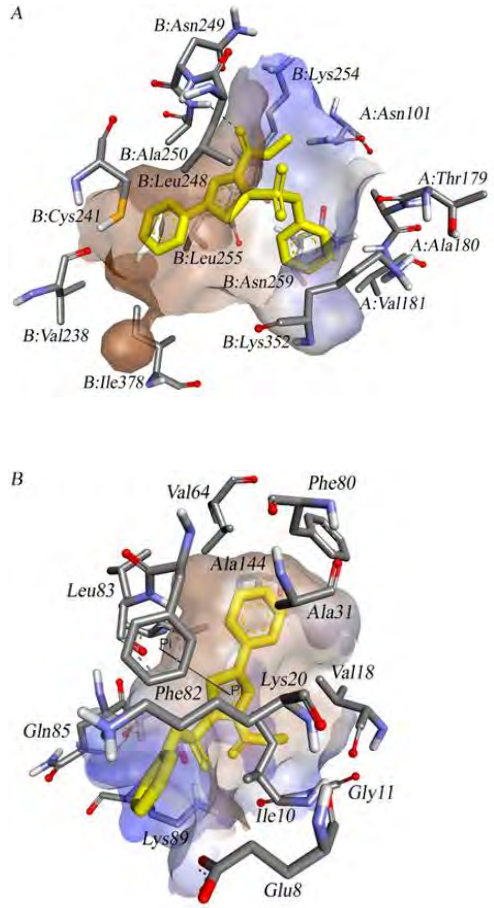


Figure 2. Possible binding modes of 5-benzylsulfonyl derivative of 1,3 oxazole-4-carboxylate **15** into colchicine binding site of tubulin (A) and ATP-binding site of CDK2 (B).

Conclusions

All the reported in this paper substances showed significant inhibitory activity and selectivity over 60 cell lines. Leukemia and non-small cell lung cancer NCI-H522 cell lines were particularly sensitive to all synthesized compounds except **5** and **10**. Colon cancer COLO 205 cell line was sensitive to compounds **15**, **18**, and **8**. Melanoma MALME-3M cell line appeared to be sensitive to compounds **15** and **18**.

The study of the antitumor activity of 5-sulfonyl derivatives of 1,3-oxazole-4-carboxylates towards the NCI 60 human cancer cell lines revealed «leader compound» – methyl 5-benzylsulfonyl-2-phenyl-1,3-oxazole-4-carboxylate (**15**). The Non-Small Cell Lung Cancer, Colon Cancer, Melanoma, Ovarian, Renal and Breast Cancer cell lines showed a significant sensitivity to this compound with sub-micro molar LC_{50} values.

The results of NCI screening make the reported 1,3-oxazole-4-carboxylate derivatives not only interesting for further chemical optimization but also for the elucidation of their mechanism of action. In this regard, molecular docking approaches were used to evaluate the possible interaction between compound **15** and tubulin as well as *in silico* binding to CDK2.

Experimental section

Chemistry

All reagents and solvents used in synthetic procedures were purchased from Aldrich and used as received. Reaction progress was monitored by TLC on Merck Silica gel 60 F_{254} aluminium sheets. Melting points were determined on a Fisher-Johns apparatus. FT-IR (KBr pellet) spectra were performed on a Bruker VERTEX 70 spectrometer and only the most representative frequencies were reported. Absorption bands are reported in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 (500 and 125 MHz, respectively) or Varian Mercury 400 (400 MHz) spectrometers in DMSO-d_6 taking its residual protons signal as a standard. Mass spectra were recorded on an Agilent 1200 LCMSD SL instrument (chemical ionization (APCI), electrospray ionization (ESI)). Combustion elemental analysis was performed in the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry analytical laboratory, their results were found to be in good agreement (±0.4%) with the calculated values. The carbon and hydrogen contents were determined using the Pregl gravimetric method, nitrogen – using the Duma's gasometrical micromethod, sulfur – by the Scheininger titrimetric method, chlorine – by the mercurometric method.

*General procedure for preparation of compounds (**3-12**).*

A mixture of a solution of 0.001 mol of methyl 5-(chlorosulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**2**), 15 ml of anhydrous dioxane, 0.001 mol of the corresponding amine, and 0.001 mol of Et_3N was refluxed for 2 h. Then the mixture was incubated at 20-25 °C during 12 h; the precipitate was filtered off, and the solvent was removed in vacuum. The residue was treated with water, filtered off, dried, and recrystallized.

*Methyl 5-(((2-(4-chlorophenyl)-2-morpholin-4-ylethyl)-amino)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**3**).*

Yield: 0.36 g, 71%; mp 172-174 °C. ^1H NMR (500 MHz, DMSO-d_6) δ 8.32 (s, 1H, N*H*), 8.01-7.98 (m, 2H, Ar), 7.64-7.58 (m, 3H, Ar), 7.29-7.25 (m, 2H, Ar),

7.18-7.15 (m, 3H, Ar), 3.88 (s, 3H, OCH_3), 3.67-3.63 (m, 1H, CH), 3.57-3.55 (m, 1H, CH), 3.43-3.40 (m, 5H, CH, CH_2), 2.52 (br s, 1H, CH), 2.24 (m, 2H, CH_2), 2.16 (m, 2H, CH_2). IR (KBr) ν, 2817, 1718 (C=O), 1552, 1486, 1400, 1342 (SO_2), 1237, 1161, 1113, 1073, 1056, 852, 714, 688. LC/MS (CI) m/z 505.9 $(M+1)^+$. Anal. Calcd. for $C_{23}H_{24}ClN_3O_6S$: C, 54.60; H, 4.78; Cl, 7.01; N, 8.30; S, 6.34. Found: C, 54.58; H, 4.76; Cl, 7.14; N, 8.45; S, 6.52.

*Methyl 5-(((2-(4-chlorophenyl)-2-piperidin-1-ylethyl)-amino)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**4**).*

Yield: 0,37 g, 73%; mp 134-136 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.01-7.98 (m, 2H, Ar), 7.65-7.57 (m, 3H, Ar), 7.27-7.24 (m, 2H, Ar), 7.15-7.11 (m, 2H, Ar), 3.87 (s, 3H, OCH_3), 3.65-3.58 (m, 2H, CH_2), 2.53 (br s, 1H, CH), 2.24 (br s, 2H, CH, CH_2), 2.04 (br s, 2H, CH_2), 1.33 (br s, 4H, CH_2), 1.15 (br s, 2H, CH_2). IR (KBr) ν, 3198 (NH), 2935, 2797, 1718 (C=O), 1558, 1490, 1397, 1340 (SO_2), 1245, 1188, 1158, 1069, 820, 714, 689. LC/MS (CI) m/z 504.0 $(M+1)^+$. Anal. Calcd. for $C_{24}H_{26}ClN_3O_5S$: C, 57.19; H, 5.20; Cl, 7.03; N, 8.34; S, 6.36. Found: C, 57.16; H, 5.18; Cl, 7.19; N, 8.45; S, 6.26.

*Methyl 5-(((2-hydroxyethyl)amino)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**5**).*

Yield: 0,25 g, 76%; mp 108-110 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 (br s, 1H, NH), 8.03-7.99 (m, 2H, Ar), 7.65-7.56 (m, 3H, Ar), 4.71-4.68 (m, 1H, OH), 3.86 (s, 3H, CH_3), 3.43-3.37 (m, 2H, CH_2), 3.14-3.09 (m, 2H, CH_2). IR (KBr) ν, 3538 (OH), 3213 (NH), 2955, 2887, 1726 (C=O), 1556, 1484, 1354, 1338 (SO_2), 1235, 1179, 1153, 1056, 820, 710, 616. LC/MS (CI) m/z 326.3 $(M+1)^+$. Anal. Calcd. for $C_{13}H_{14}N_2O_6S$: C, 47.85; H, 4.32; N, 8.58; S, 9.83. Found: C, 47.82; H, 4.31; N, 8.68; S, 9.93.

*Methyl 5-(((2-hydroxyethyl)(methyl)amino)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**6**).*

Yield: 0,26 g, 75%; mp 119-121 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.02-8.01 (m, 2H, Ar), 7.65-7.57 (m, 3H, Ar), 4.80-4.79 (m, 1H, OH), 3.87 (s, 3H, OCH_3), 3.54-3.53 (m, 2H, CH_2), 3.00 (s, 3H, NCH_3), 2.52 (br s, 2H, CH_2). IR (KBr) ν, 3434 (OH), 2950, 2873, 1742 (C=O), 1557, 1486, 1375 (SO_2), 1297, 1224, 1173, 1082, 1069, 987, 924, 814, 714, 598. LC/MS (CI) m/z 340.4 $(M+1)^+$. Anal. Calcd. for $C_{13}H_{14}N_2O_6S$: C, 49.41; H, 4.74; N, 8.23; S, 9.42. Found: C, 49.38; H, 4.76; N, 8.33; S, 9.52.

*Methyl 5-((3-methylpiperidin-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**7**).*

Yield: 0,27 g, 73%; mp 100-102 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05-8.01 (m, 2H, Ar), 7.69-7.61 (m, 3H, Ar), 3.90 (s, 3H, OCH_3), 3.69 (dd, J 22,1 Hz, J 11,6 Hz, 1H, CH), 2.88 (t, J 12 Hz, 1H, CH), 2.59-2.54 (m, 1H, CH), 1.75-1.61 (m, 3H, CH, CH_2), 1.52-1.43 (m, 1H, CH), 1.06-0.98 (m, 1H, CH), 0.87 (d, J 6,8 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.8, 160.4, 147.0, 134.8, 133.0, 129.9, 127.5, 125.3, 53.3, 52.6, 46.4, 31.5, 30.7, 24.7, 19.0. IR (KBr) ν, 2954, 2929, 1749 (C=O), 1545, 1481, 1374 (SO_2), 1363, 1224, 1179, 1144, 1070, 1053, 1008, 930, 818, 749, 716, 621, 581. LC/MS (CI) m/z 364.4 $(M+1)^+$. Anal. Calcd. for $C_{17}H_{20}N_2O_5S$: C, 56.03; H, 5.53; N, 7.69; S, 8.80. Found: C, 56.00; H, 5.50; N, 7.79; S, 8.89.

*Methyl 5-((4-(aminocarbonyl)piperidin-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**8**).*

Yield: 0,28 g, 77%; mp > 210 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J 7.6 Hz, 2H, Ar), 7.69-7.60 (m, 3H, Ar), 7.17 (s, 1H, C(O)NH_2), 6.70 (s, 1H, C(O)NH_2), 3.91 (s, 3H, OCH_3), 3.83 (d, J 12,4 Hz, 2H, CH_2), 2.98 (t, J 10.8 Hz, 2H, CH_2), 2.26 (t, J 10.8 Hz, 1H, CH), 1.84 (d, J 10.4 Hz, 2H, CH_2), 1.62-1.53 (m, 2H, CH_2). IR (KBr) ν, 3380 (NH_{amide}), 3192, 2943, 1749 (C=O), 1651, 1552, 1449, 1372 (SO_2), 1336, 1299, 1221, 1178, 1148, 1067, 1054, 954, 815, 720, 714, 675, 610, 582. LC/MS (CI) m/z 364.4 $(M+1)^+$. Anal. Calcd. for $C_{17}H_{19}N_3O_6S$: C, 56.03; H, 5.53; N, 7.69; S, 8.80. Found: C, 51.90; H, 4.87; N, 10.68; S, 8.15.

*Methyl 5-((4-methylpiperidin-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**9**).*

Yield: 0,28 g, 78%; mp 102-104 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, J 7.6 Hz, 2H, Ar), 7.67-7.60 (m, 3H, Ar), 3.90 (s, 3H, OCH_3), 3.78 (d, J 12,4 Hz, 2H, CH_2), 2.89 (t, J 12 Hz, 2H, CH_2), 1.71 (d, J 13,2 Hz, 2H, CH_2), 1.48 (br s, 1H, CH), 1.14 (dd, J 23.6 Hz, J 13.2 Hz, 2H, CH_2), 0.88 (d, J 6.4 Hz, 3H, CH_3). IR (KBr) ν, 2942, 1746 (C=O), 1547, 1449, 1371 (SO_2), 1337, 1221, 1157, 1049, 927, 815, 726, 689, 617, 592. LC/MS (CI) m/z 365.2 $(M+1)^+$. Anal. Calcd. for $C_{17}H_{20}N_2O_5S$: C, 56.03; H, 5.53; N, 7.69; S, 8.80. Found: C, 55.90; H, 5.50; N, 7.58; S, 8.19.

*Methyl 2-phenyl-5-((4-phenylpiperazin-1-yl)sulfonyl)-1,3-oxazole-4-carboxylate (**10**).*

Yield: 0,31 g, 73%; mp 119-121 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.01 (d, J 8 Hz, 2H, Ar), 7.66-7.57 (m, 3H, Ar), 7.19 (t, J 7 Hz, 2H, Ar), 6.92 (d, J 7.5 Hz, 2H, Ar), 6.79 (t, J 7.5 Hz, 1H, Ar), 3.90 (s, 3H, OCH_3), 3.48 (br s, 4H, 2CH_2), 3.25 (br s, 4H, 2CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.6, 159.9, 150.4, 146.1, 134.9, 132.5, 129.5, 129.0, 127.1, 125.0, 119.7, 116.2, 52.9, 48.2, 45.6. IR (KBr) ν, 2842, 1737 (C=O), 1598, 1555, 1495, 1451, 1380 (SO_2), 1322, 1225, 1181, 1149, 1069, 1052, 953, 816, 771, 713, 698, 688, 596. LC/MS (CI) m/z 427.5 $(M+1)^+$. Anal. Calcd. for $C_{21}H_{21}N_3O_5S$: C, 59.00; H, 4.95; N, 9.83; S, 7.50. Found: C, 59.03; H, 4.91; N, 9.70; S, 7.62.

*Methyl 5-((4-(4-methoxyphenyl)piperazin-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**11**).*

Yield: 0,34 g, 74%; mp 119-121 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.02 (d, J 7 Hz, 2H, Ar), 7.66-7.58 (m, 3H, Ar), 6.88 (d, J 9 Hz, 2H, Ar), 6.82-6.79 (m, 2H, Ar), 3.90 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.47 (br s, 4H, 2CH_2), 3.11 (br s, 4H, 2CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.6, 160.3, 153.6, 146.0, 144.7, 134.9, 132.6, 129.5, 129.0, 127.1, 125.0, 118.3, 114.4, 55.2, 52.9, 49.6, 45.8. IR (KBr) ν, 2832, 1747 (C=O), 1552, 1513, 1485, 1449, 1374 (SO_2), 1323, 1270, 1249, 1224, 1180, 1151, 1116, 1034, 1052, 951, 818, 732, 716, 688, 614, 601. LC/MS (CI) m/z 457.5 $(M+1)^+$. Anal. Calcd. for $C_{22}H_{23}N_3O_6S$: C, 57.76; H, 5.07; N, 9.18; S, 7.01. Found: C, 57.78; H, 5.04; N, 9.26; S, 7.13.

*Methyl 5-((4-(3-chlorophenyl)piperazin-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**12**).*

Yield: 0,34 g, 75%; mp 119-121 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.00 (d, J 7 Hz, 2H, Ar), 7.66-7.58 (m, 3H, Ar), 6.88-6.79 (m, 4H, Ar), 3.87 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 3.42 (br s, 4H, 2CH_2), 3.15 (br s, 4H, 2CH_2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.0, 160.3, 151.9, 146.4, 135.3, 134.3, 133.0, 130.9, 130.0, 127.5, 125.4, 119.2, 115.7, 114.7, 53.3, 47.9, 45.8. IR (KBr) ν, 2849, 1738 (C=O), 1596, 1556, 1484, 1450, 1380 (SO_2), 1320, 1220, 1181, 1147, 954, 856, 786, 724, 712, 687, 599, 543. LC/MS (CI) m/z 461.9 $(M+1)^+$. Anal. Calcd. for $C_{21}H_{20}ClN_3O_5S$: C, 54.60; H, 4.36; N, 9.10; S, 6.94. Found: C, 54.62; H, 4.38; N, 9.19; S, 6.99.

*Synthesis of methyl 5-(benzylsulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (**15**).*

To a suspension of 0.01 mol of methyl 2-benzoylamino-3,3-dichloroacrylate (**1a**) in 40 ml of methanol 0.025 mol of sodium sulfide was added while stirring, the suspension was mixed for 2-3 h, then kept for 12 h at 20-25 °C. The precipitate was filtered off. The filtrate was diluted with water, acidified with hydrochloric acid to pH < 7, to form red precipitate **13**.

A mixture of methyl 5-mercapto-2-phenyl-1,3-oxazole-4-carboxylate (**13**, 0.01 mol), chloromethylbenzene (0.01 mol) and triethylamine (0.01 mol) was refluxed for 2-3 h. The precipitate was filtered off, the solvent was removed in vacuo, the residue was treated with water, filtered off, and dried to form compound **14**.

A solution of 0.01 mol of methyl 5-benzylthio-2-phenyl-1,3-oxazole-4-carboxylate (**14**) in 40 ml of glacial acetic acid was heated to reflux, then H_2O_2 was added in three portions during 2 h, then the reaction mixture was left for 12 h at room temperature. The mixture was kept for 8 h at 20-25 °C, and the precipitate was filtered off and purified by recrystallization from ethanol to form compound **15**. Yield: 0,26 g, 72%; mp 143-145 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, J 7.6 Hz, 1H, Ar), 7.84 (d, J 6.4 Hz, 1H, Ar), 7.68-7.58 (m, 1H, Ar), 7.41-7.35 (m, 7H, Ar), 5.05 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3). IR (KBr) ν, 2954, 1739 (C=O), 1614, 1545, 1494, 1345 (SO_2), 1318, 1301, 1237, 1180, 1140, 1085, 1062, 825, 776, 736, 696, 652, 640, 540, 522. LC/MS (CI) m/z 357.4 $(M+1)^+$. Anal. Calcd. for $C_{18}H_{15}NO_5S$: C, 60.49; H, 4.23; N, 3.92; S, 8.97. Found: C, 60.46; H, 4.21; N, 3.99; S, 8.85.

*Synthesis of 5-((3-methoxyphenyl)sulfonyl)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile (**18**).*

A mixture of 3,3-dichloro-2-((4-methylbenzoyl)-amino)acrylate (**1b**, 0.01 mol), triethylamine (0.02 mol) and the corresponding 3-methoxybenzenethiol (0.02 mol) in 30 ml of acetonitrile was stirred on a magnetic stirrer at 20-25 °C C for 8 h. The precipitate was filtered off, the solvent was removed in vacuo, the residue was treated with water, filtered and dried. The mixture of formed *N*-(1-cyano-2,2-bis((3-methoxyphenyl)thio)vinyl)-4-methylbenz-amide (**16**, 0.01 mol) and dried silver carbonate (0.025 mol) in 40 ml of acetonitrile was stirred on a magnetic stirrer at reflux for 8-10 h, then left at 20-25 °C for 8 h. The precipitate was filtered off, the solvent was removed in vacuo, the residue was washed with water, filtered off, dried and purified by recrystallization.

The formed 5-((3-methoxyphenyl)thio)-2-(4-methyl-phenyl)-1,3-oxazole-4-carbonitrile (**19**, 0.01 mol) was heated to boiling in glacial acetic acid (20 ml), then 30% H_2O_2 was added in three 2 ml portions over 2 hours. The mixture was left at 20-25 ºC for 8 hours. The precipitate formed was filtered off, dried and recrystallized from ethanol. Yield: 0,27 g, 70%; mp 93-95 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, J 7.6 Hz, 2H, Ar), 7.71-7.61 (m, 3H, Ar), 7.40-7.37 (m, 3H, Ar), 3.90 (s, 3H, CH_3O), 3.86 (s, 3H, CH_3O), 2.39 (s, 4H, 2CH_2). IR (KBr) ν, 3530, 1742 (C=O), 1597, 1496, 1482, 1353 (SO_2), 1333, 1291, 1251, 1227, 1172, 1144, 1036, 821, 734, 702, 677, 642, 618, 534. LC/MS (CI) m/z 387.4 $(M+1)^+$. Anal. Calcd. for $C_{19}H_{17}NO_6S$: C, 58.91; H, 4.42; N, 3.62; S, 8.28. Found: C, 58.93; H, 4.40; N, 3.70; S, 8.39.

In vitro anticancer assay

In vitro screening methodology, screening interpretation information and cancer cell growth calculation method is described in details at the NCI Development Therapeutics Program site [23].

Molecular docking calculation

Molecular docking was carried out by Autodock Vina software [24]. Before the calculation, ligands, water molecules and amino acids conformers were removed from crystal structures of tubulin (PDB code 1SA0 [18]), CDK1, CDK2, CDK7, and CDK9 (PDB codes 4Y7*2* [20], 3QQJ, 1UA2 [21], and 4BCF [22], respectively), which were downloaded from PDB server (https://www.rcsb.org) [25]. The structure of 5-benzylsulfonyl derivative of 1,3 oxazole-4-carboxylate **15** was drawn using MarvinSketch [26] and optimized with the AM1 semi-empirical quantum mechanical method in MOPAC software [27]. Files for docking were prepared by using AutoDockTools (version 1.5.6) [28]. Analysis of models was performed in program Discovery Studio 3.5 Visualizer (Accelrys Inc., San Diego, CA, USA).

Notes

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The authors declare no conflict of interest.

Author contributions. **S. G. P.**: synthesis of compounds, investigation, formal analysis. **O. P. K.**: synthesis of compounds, investigation, formal analysis. **V. V. Z.**: COMPARE analysis. **M. V. K.**: writing most of the manuscript. **O. L. K.**: analysis of literature and molecular docking calculation. **A. I. V.**: conceptualization, writing, review. **V. S. B.**: conceptualization, supervision, writing - review & editing.

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Синтез та протипухлинна активність 5-сульфонільних похідних 1,3-оксазол-4-карбоксилатів

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Резюме: На основі попередніх досліджень протипухлинної активності 5-сульфонілзаміщених 1,3-оксазолів було синтезовано ряд нових 2-арил-5-сульфоніл-1,3-оксазол-4-карбоксилатів для проведення скринінгу щодо 60 ракових клітинних ліній NCI: недрібноклітинного раку легень (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), раку нирок (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), ЦНС (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), яєчників (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), простати (PC-3, DU-145), товстого кишкивніка (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), молочної залози (MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T- 47D, MDA-MB-468), лейкемії (CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, SR) та меланоми (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62). Серед досліджуваних речовин «сполукою-лідером» виявився метил 5-бензилсульфоніл-2-феніл-1,3-оксазол-4-карбоксилат (**15**), який показав значну цитотоксичність на багатьох лініях досліджених ракових клітин людини із середнім значенням GI_{50}, TGI та LC_{50} $5{,}37\cdot10^{-6}$, $1{,}29\cdot10^{-5}$ та $3{,}6\cdot10^{-5}$ моль/л відповідно. Молекулярний докінг було використано для оцінки взаємодій між сполукою **15** і тубуліном, а також для моделювання комплексів сполуки **15** з CDK2, енергія зв'язування в якому становила -9.2 ккал/моль. Серед нових похідних 1,3-оксазолу знайдено перспективні сполуки для подальшого дослідження протиракової активності щодо різних ліній.

Ключові слова: 5-сульфоніл-1,3-оксазол-4-карбоксилати; синтез; протипухлинна активність; селективність; молекулярний докінг.

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RESEARCH ARTICLE

Condition-based switching the multicomponent reactions of 5-amino-3-(methylthio)-1,2,4-triazole, aromatic aldehydes, and pyruvic acid

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Abstract: The multicomponent reactions of 5-amino-3-methylthio-1,2,4-triazole with aromatic aldehydes and pyruvic acid were studied using conventional thermal heating and ultrasonic activation at room temperature. Under conventional heating, dihydrotriazolopyrimidine derivatives were formed in both two- and three-component treatments. In the case of ultrasonic activation, the multicomponent reaction led to the formation of 7-hydroxytetrahydrotriazolopyrimidines.

Keywords: 5-Amino-3-(methylthio)-1,2,4-triazole, multicomponent reaction, ultrasonication, pyruvic acid, heterocyclization.

Introduction

Pyrimidine derivatives and their sulfur analogs have attracted much attention because of their wide range of biological activities involving antibacterial [1], anti-inflammatory [2-3], analgesic [3], antitumour [4-5], anti-microbial [6], anti-infective [7] and antifungal [8-9] activities.

In our previous work, the multicomponent reactions of 3-amino-1,2,4-triazole with aromatic aldehydes and pyruvic acid (arylpyruvic acids) were discussed from the viewpoints of their selectivity and molecular diversity. Different types of heterocycles depending on the structures of the reagents, the solvent, the temperature mode, and the activation method were formed [10-13]. In particular, the multicomponent approaches based on the reaction of 3-amino-1,2,4-triazole, aromatic aldehydes, and pyruvic acid were described for the synthesis of dihydrotriazolopyrimidine-5-carboxylic acids **I**, 7-hydroxy-tetrahydropyrimidine-7-carboxilic acid **II**, benzotriazo-looxadiazocine-5-carboxylic acids **III** and 3-hydroxy-triazolyldihydropyrrolones IV (Scheme 1). The reaction conditions were found for the selective synthesis of each of these compounds (**I-IV**). Application of non-classical activation methods like ultrasonication and microwave irradiation have appeared as convenient tools for tuning the multicomponent treatments.

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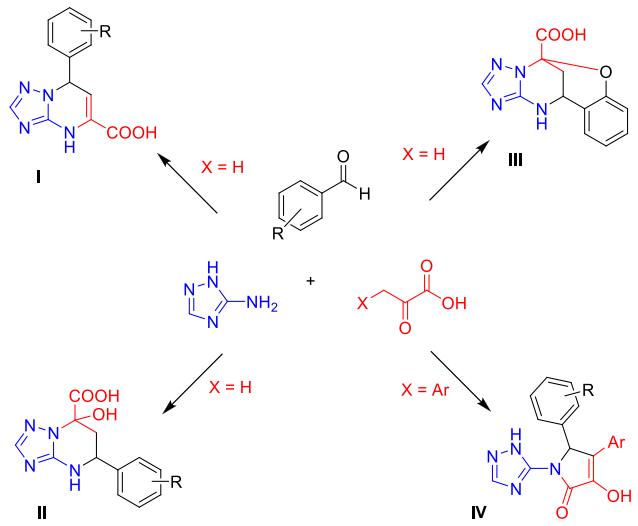
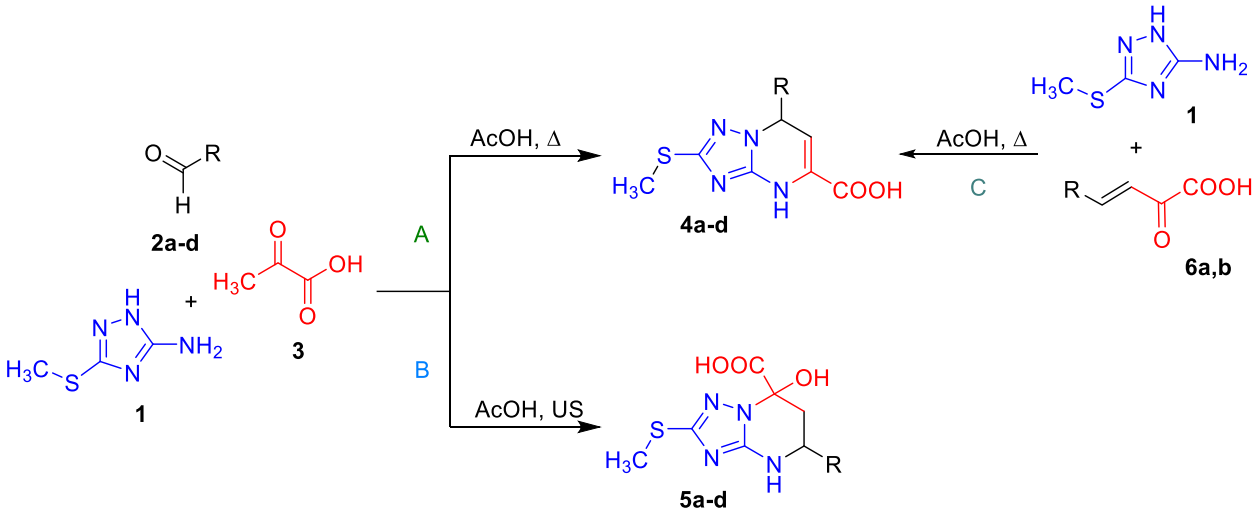


Scheme 1. Diversity of heterocyclizations of 3-amino-1,2,4-triazole with aldehydes and pyruvic acids.



Scheme 2. Switchable heterocyclizations of 3-(methylthio)-1*H*-1,2,4-triazol-5-amine with aldehydes and pyruvic acids.

Some reactions involving sulfur-containing substituents in the 3rd position of 3-amino-1,2,4-triazole were described in the literature as well [14-19].

Results and Discussion

Here, we report the Doebner-type synthesis of undescribed 2-methylthiotetrahydrotriazolo[1,5-*a*]pyrimidine-7-carboxylic acids, and 2-methylthiodihydrotriazolo[1,5-*a*]pyrimidine-5-carboxylic acids. The type of final heterocycle forming in the multicomponent reaction between 3-(methylthio)-1*H*-1,2,4-triazol-5-amine, aromatic aldehydes and pyruvic acid depends on the reaction conditions – conventional heating or ultrasonication.

In particular, it was found that the three-component treatment of an equimolar mixture of 3-(methylthio)-1*H*-1,2,4-triazol-5-amine (**1**), aromatic aldehydes **2a-d**, and pyruvic acid (**3**) under conventional heating at reflux in glacial acetic acid for 4 hours selectively led to the formation of a dihydropyrimidine ring and thus 2-(methylthio)-7-aryl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-5-carboxylic acids **4a-d** were isolated as the only products of the interaction in satisfactory yields (Scheme 2, Method A, and Table 1).

On the other hand, the multicomponent reaction of the same starting materials **1**, **2a-d**, and **3** in glacial acetic acid under ultrasonication at room temperature for 2 hours led to the formation of 7-hydroxy-2-(methylthio)-5-aryl-4,5,6,7-tetrahydro-[1,2,4]triazolo-[1,5-*a*]pyrimidine-7-carboxylic acids **5a-d** (Scheme 2, Method B, and Table 1). Mechanical stirring at room temperature instead of ultrasonication led to the formation of compounds **5a-d** as well. However, this procedure required a longer reaction time (ca. 30 h) and the yield and purity of the products were significantly lower.

It should be noted, that dihydropyrazolopyrimidine carboxylic acids **4** were formed by sequential procedure as well. The linear pathway included two steps: the synthesis of arylidenpyruvic acids **6a,b** starting from appropriate aromatic aldehyde **2** and pyruvic acid **3**, and their further heterocyclization with 3-(methylthio)-1*H*-1,2,4-triazol-5-amine **1** in glacial acetic acid under conventional heating for 30 min gave dihydropyrazolopyrimidine carboxylic acids **4a,b** (Scheme 2, Method C). Thus, the final reaction products **4** were identical to the compounds isolated from the multicomponent reaction (Scheme 2, Method A) in similar yields (Table 1).

The structures of the heterocyclic carboxylic acids **4a-d** and **5a-d** were established by MS, ^{1}H and ^{13}C NMR spectral data. The ^{1}H NMR spectra of 2-(methylthio)-7-aryl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-5-carboxylic acids **4a-d** show the following signals: a broad singlet for NH group (9.82-9.99 ppm), signals of aromatic H-atoms and terminal groups, a doublet for H-atom in position 7 of the heterocycle (6.12-6.30 ppm, J = 3.9 Hz), a doublet for ethylene H-atom at C-5 (5.72-5.77 ppm, J = 3.7 Hz), a singlet for methyl group (2.38-2.39 ppm). The ^{1}H NMR spectra of 7-hydroxy-2-(methylthio)-5-aryl-4,5,6,7-tetrahydro-[1,2,4]-triazolo[1,5-*a*]pyrimidine-7-carboxylic acids **5a-d** show a broad signal for the pyrimidine NH at δ = 7.78-7.94 ppm, a singlet for methyl group (2.42-2.43 ppm), a multiplet for the CH group at C-5 at δ = 4.54-4.80 ppm, a multiplet for the CH_2 group at C-6 at δ = 2.03-2.41 ppm, and peaks for aromatic protons as well as signals for other substituents.

The structure of compounds **4a-d** and **5a-d** was additionally confirmed by the comparison of their ^{1}H NMR spectra with literature data for similar pyrimidines [10-12].

MS and ^{13}C NMR are also in an agreement with the proposed structures.

Table 1. Synthesis of compounds **4a-d** and **5a-d**.

Entry	Starting materials		Reaction time	Pathway	Conditions	Product	Yield
	Compd.	R	(hours)		Δ or US		(%)
1	**2a**	C_6H_5	4	A	Δ	**4a**	37
2	**2b**	4-Cl-C_6H_4	4	A	Δ	**4b**	64
3	**2c**	4-CH_3O-C_6H_4	4	A	Δ	**4c**	44
4	**2d**	4-$COOCH_3$-C_6H_4	4	A	Δ	**4d**	43
5	**2a**	C_6H_5	2	B	US	**5a**	67
6	**2b**	4-Cl-C_6H_4	2	B	US	**5b**	40
7	**2c**	4-CH_3O-C_6H_4	2	B	US	**5c**	69
8	**2d**	4-$COOCH_3$-C_6H_4	2	B	US	**5d**	64
9	**6a**	4-Cl-C_6H_4	0.5	C	Δ	**4b**	40
10	**6b**	4-CH_3O-C_6H_4	0.5	C	Δ	**4c**	43

Conclusions

Thus, we showed that the multicomponent reactions involving 5-amino-3-methylthio-1,2,4-triazole, aromatic aldehydes, and pyruvic acid can be switched between two different pathways using either conventional thermal heating in acetic acid or ultrasonic activation at room temperature in the same solvent. Under conventional heating 2-(methylthio)-7-aryl-4,7-dihydro-[1,2,4]triazolo-[1,5-*a*]pyrimidine-5-carboxylic acids were formed both in the three-component treatment and in sequential two-step reaction *via* preliminary synthesis of arylidenpyruvic acid. In the case of ultrasonic activation the multicomponent reaction led to the formation of 7-hydroxy-2-(methylthio)-5-aryl-4,5,6,7-tetrahydro-[1,2,4]triazolo-[1,5-*a*]pyrimidine-7-carboxylic acids.

Experimental section

Melting points were determined with a Kofler apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 at 400 MHz (100 MHz for ^{13}C NMR) with a Varian MR-400 spectrometer.

The mass spectra were measured Shimadzu GCMS-2020 instrument (70 eV ionizing energy) using the direct inlet (DI) method. Elemental analysis was performed on a Euro Vector EA-3000.

Ultrasound-assisted experiments were carried out using a standard ultrasound bath (SELDI, Ukraine) with a working frequency of 44.2 kHz.

Arylidenpyruvic acids **4a-b** were synthesized according to the literature procedure [20]. 3-(Methylthio)-1*H*-1,2,4-triazol-5-amine (**1**), 2-oxopropa-noic acid **2**, and substituted aldehydes **3a-d** were commercially available and were purchased from Merck.

*General procedure for the preparation of compounds **5a-d**:*

Method A: 100 mg (0.77 mmol) of 3-(methylthio)-1*H*-1,2,4-triazol-5-amine (**1**) were dissolved in glacial AcOH (2 mL), then pyruvic acid (**2;** 0.77 mmol) and the appropriate aldehyde (**2a-2d**; 0.77 mmol) were added. The mixture was refluxed for 4h, then it was cooled and allowed to stand overnight. After that, the resulting precipitate was collected by filtration and dried in air, yielding a colorless solid.

*2-(methylthio)-7-phenyl-4,7-dihydro[1,2,4]triazolo-[1,5-a]pyrimidine-5-carboxylic acid (**4a**):*

Yield: 27 mg, 37%; Colorless solid; mp 259-261 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H, NH), 7.15-7.42 (m, 5H, ArH), 6.18 (d, *J* 3.9 Hz, 1H), 5.77 (d, *J* 3.7 Hz, 1H), 2.39 (s, 3H, SCH_3). Anal. Calcd. for $C_{13}H_{12}N_4O_2S$: C, 54.16; H, 4.20; N, 19.43. Found C, 54.08; H, 4.27; N, 19.51. MS (EI, 70 eV) m/z (%) 288 (M^+, 100), 243 (30), 211 (61), 193 (45), 165 (27), 115 (50), 77 (39).

*7-(4-chlorophenyl)-2-(methylthio)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-5-carboxylic acid (**4b**):*

Yield: 76 mg, 64%; Colorless solid; mp 285-287 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H, NH), 7.20-7.46 (m, 4H, ArH), 6.22 (d, *J* 3.9 Hz, 1H, CH), 5.76 (d, *J* 3.7 Hz, 1H, CH), 2.39 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.7; 159.9; 150.6; 140.6; 133.9; 129.8; 129.7; 128.5; 106.9; 59.6; 14.4. Anal. Calcd. for $C_{13}H_{11}ClN_4O_2S$: C, 48.38; H, 3.44; N, 17.36. Found C, 48.25; H, 3.47; N, 17.39. MS (EI, 70 eV) m/z (%) 322 (M^+, 30), 290 (11), 286 (18), 241 (18), 211 (65), 193 (56), 165 (52), 140 (60).

*7-(4-methoxyphenyl)-2-(methylthio)-4,7-dihydro-[1,2,4] triazolo[1,5-a]pyrimidine-5-carboxylic acid (**4c**):*

Yield: 106 mg, 44%; Colorless solid; mp 273-275 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H, NH), 6.87-

7.18 (m, 4H, ArH), 6.12 (d, *J* 3.9 Hz, 1H, CH), 5.75 (d, *J* 3.7 Hz, 1H, CH), 3.73 (s, 3H, OCH_3), 2.39 (s, 3H, SCH_3). Anal. Calcd. for $C_{14}H_{14}N_4O_3S$: C, 52.82; H, 4.43; N, 17.60. Found C, 52.72; H, 4.51; N, 17.65. MS (EI, 70 eV) m/z (%) 318 (M^+, 89), 271 (65), 211 (34), 193 (40), 158 (100), 145 (72), 128 (50).

*7-(4-(methoxycarbonyl)phenyl)-2-(methylthio)-4,7-dihydro[1,2,4]triazolo[1,5-a]pyrimidine-5-carboxylic acid (**4d**):*

Yield: 120 mg, 43%; Colorless solid; mp 255-257 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H, NH), 7.32-8.02 (m, 4H, ArH), 6.30 (d, *J* 3.9 Hz, 1H, CH), 5.78 (d, *J* 3.7 Hz, 1H, CH), 3.84 (s, 3H, OCH_3), 2.38 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9; 162.7; 159.0; 149.8; 145.6; 129.8; 129.5; 127.6; 127.1; 105.8; 59.1; 52.2; 13.5. Anal. Calcd. for $C_{15}H_{14}N_4O_4S$: C, 52.02; H, 4.07; N, 16.18. Found C, 51.96; H, 4.16; N, 16.25. MS (EI, 70 eV) m/z (%) 346 (M^+, 1), 211 (28), 165 (36), 140 (40), 128 (36), 119 (45), 115 (77).

The same compounds as **4b** and **4c** were obtained via two-component reaction (**Method C**) including appropriate arylidenpyruvic acids **6a-b** and 3-(methylthio)-1*H*-1,2,4-triazol-5-amine (**1**). 100 mg (0.77 mmol) of 3-(methylthio)-1*H*-1,2,4-triazol-5-amine (**1**) were dissolved in glacial AcOH (2 mL), then appropriate 2-oxo-4-arylbut-3-enoic acids were added. The mixture was refluxed for 30 min, then it was cooled and allowed to stand overnight. After that, the resulting precipitate was collected by filtration and dried in air, yielding a colorless solid.

Method B: 100 mg (0.77 mmol) of 3-(methylthio)-1*H*-1,2,4-triazol-5-amine (**1**) were dissolved in glacial AcOH (2 mL), then the appropriate aldehyde (**2a-d;** 0.77 mmol) and pyruvic acid (**3;** 0.77 mmol) were added. The mixture was put into the ultrasound bath for 120 min. Then the mixture was allowed to stand overnight. After that, the resulting precipitate was collected by filtration and dried in air, yielding a colorless solid.

*7-hydroxy-2-(methylthio)-5-phenyl-4,5,6,7-tetrahydro-[1,2,4]triazolo[1,5-a]pyrimidine-7-carboxylic acid (**5a**):*

Yield: 160 mg, 67%; Colorless solid; mp 152-154 °C. ^{1}H NMR (400 MHz, DMSO-d_6); δ 7.84 (s, 1H, NH), 7.26-7.66 (m, 5H, ArH), 4.57-4.70 (m, 1H, CH), 2.42 (s, 3H, SCH_3), 2.04-2.32 (m, 2H, CH_2). Anal. Calcd. for $C_{13}H_{14}N_4O_3S$: C, 50.97; H, 4.61; N, 18.29. Found C, 50.87; H, 4.66; N, 18.32. MS (EI, 70 eV) m/z (%) 288 ($M^{+\cdot}$–H_2O, 8), 243 (15), 211 (18), 130 (100).

*5-(4-chlorophenyl)-7-hydroxy-2-(methylthio)-4,5,6,7-tetrahydro-[1,2,4]triazolo[1,5-a]pyrimidine-7-carboxylic acid (**5b**):*

Yield: 115 mg, 40%; Colorless solid; mp 169-171 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (s, 1H, NH), 6.89-7.51 (m, 4H, ArH), 4.59-4.70 (m, 1H, CH), 3.06 (s, 3H, O-CH_3), 2.42 (s, 3H, SCH_3), 2.04-2.32 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6; 159.4; 155.4; 133.1; 128.2; 114.4; 81.6; 55.6; 50.8; 42.4; 13.9. Anal. Calcd.. for $C_{13}H_{13}ClN_4O_3S$: C, 45.82; H, 3.85; N, 16.44. Found C, 45.76; H, 3.91; N, 16.52. MS (EI, 70 eV) m/z (%) 322 ($M^{+\cdot}$–H_2O, 3), 211 (6), 165 (87), 137 (46), 130 (79).

*7-hydroxy-5-(4-methoxyphenyl)-2-(methylthio)-4,5,6,7-tetrahydro-[1,2,4]triazolo[1,5-a]pyrimidine-7-carboxylic acid (**5c**):*

Yield: 180 mg, 69%; Colorless solid; mp 168-170 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (s, 1H, NH), 6.88-7.41 (m, 4H, Ar), 4.54-4.63 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 2.42 (s, 3H, SCH_3), 2.03-2.31 (m, 2H, CH_2). Anal. Calcd. for $C_{14}H_{16}N_4O_4S$: C, 49.99; H, 4.79; N, 16.66. Found: C, 50.07; H, 4.76; N, 16.71. MS (EI, 70 eV) m/z (%) 318 (M^+ –H_2O, 20), 271 (21), 151 (100), 133 (52), 130 (61).

*7-hydroxy-5-(4-(methoxycarbonyl)phenyl)-2-(methylthio)-4,5,6,7-tetrahydro-[1,2,4]triazolo[1,5-a]pyrimidine-7-carboxylic acid (**5d**):*

Yield: 179 mg, 64%; Colorless solid; mp 159-161 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (s, 1H, NH), 7.46-8.20 (m, 4H, Ar), 4.68-4.80 (m, 1H, CH), 3.90 (s, 3H, OCH_3), 2.43 (s, 3H, SCH_3), 2.13-2.41 (m, 2H, CH_2). Anal. Calcd. for $C_{15}H_{16}N_4O_5S$: C, 49.44; H, 4.43; N, 15.38. Found: C, 49.36; H, 4.51; N, 15.41. MS (EI, 70 eV) m/z (%) 346 ($M^{+\cdot}$–H_2O, 3), 189 (67), 145 (40), 130 (100).

Notes

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Author contributions. **Ya. I. S.**: synthesis of compounds, investigation, formal analysis, writing of the most part of the manuscript, editing. **M. V. M.**: synthesis of compounds, writing experimental section of the manuscript, formal analysis. **N. V. C.**: experimental section. **M. A. K.** and **E. H. S.**: mass experiments. **S. M. D.**: editing. **M. V. I.**: NMR correlation experiments. **V. A. C.** conceptualization, supervision, writing - review & editing.

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Переключаємі за рахунок умов багатокомпонентні реакції 5-аміно-3-(метилтіо)-1,2,4-триазолу, ароматичних альдегідів та піровиноградної кислоти

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Резюме: Вивчено багатокомпонентні реакції 5-аміно-3-метилтіо-1,2,4-триазолу з ароматичними альдегідами та піровиноградною кислотою із застосуванням звичайного термічного нагріву та при використанні ультразвукової активації при кімнатній температурі у тому ж самому розчиннику. При звичайному нагріванні в оцтовій кислоті утворювались 2-(метилтіо)-7-арил-4,7-дигідро-[1,2,4]триазоло-[1,5-*a*]піримідин-5-карбонові кислоти, як при трикомпонентній реакції, так і при послідовній двохкомпонентній реакції, шляхом попереднього синтезу ариліденпіровиноградних кислот. У випадку ультразвукової активації багатокомпонентна реакція в оцтовій кислоті за кімнатної температури приводила до утворення 7-гідрокси-2-(метилтіо)-5-арил-4,5,6,7-тетрагідро-[1,2,4]триазоло-[1,5-*a*]піримідин-7-карбонових кислот. Подальше вивчення особливостей утворення даних речовин має суттєве фундаментальне значення для детального розуміння механізмів формування азолоазинових систем, що є важливим для цілеспрямованого синтезу гетероциклічних систем, які мають заздалегідь задані властивості. Показано перспективність синтезу нових гетероциклічних сполук цього класу, які прогнозовано мають широкий спектр біологічної активності.

Ключові слова: 5-аміно-3-(метилтіо)-1,2,4-триазол, багатокомпонентна реакція, ультразвук, піровиноградна кислота, гетероциклізація.

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RESEARCH ARTICLE

Amino acid sulfonamides based on 4-(1-oxo-1*H*-isochromen-3-yl)-benzenesulfonyl chloride

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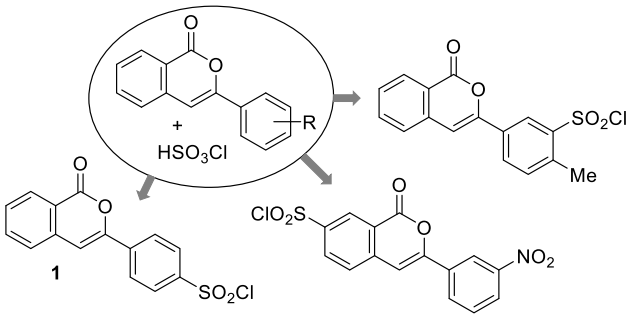
Abstract: The creation of new amino acid derivatives of 4-(1-oxo-1*H*-isochromen-3-yl)benzenesulfonyl chloride **1** was investigated. The interaction of the sulfonyl chloride **1** with amino acid methyl esters (hydrochlorides) in 1,4-dioxane in the presence of triethylamine led to the corresponding amino acid sulfonamide derivatives of isocoumarin. The reaction of the sulfonyl chloride **1** with phenylalanine in the basic aqueous solution was complicated by the lactone system disclosure and led to 2'-carboxydeoxybenzoin ultimately (namely, 2-(2-(4-(*N*-(1-carboxy-2-phenylethyl)sulfamoyl)phenyl)-2-oxoethyl)benzoic acid). Similar product was obtained by the alkali hydrolysis of methyl ((4-(1-oxo-1*H*-isochromen-3-yl)phenyl)sulfonyl)leucinate.

Keywords: sulfonamides, amino acids, 3-phenylisocoumarin, sulfochlorination.

Introduction

Synthetic [1-2] and natural [2-3] compounds that contain the isocoumarin (1*H*-isochromen-1-one) fragment are well known group of oxygen containing heterocycle. These substances have a tremendous potential as starting materials in synthetic organic chemistry (synthesis of isoquinolines [4]) or as bioactive compounds [5-6]. However, isocoumarins can be found in the scientific literature not as often as isomeric chromones and coumarins. It can be explained by the fact that the existing methods for synthesis of isocoumarins and their derivatives [1, 7] do not allow obtaining structures with certain functional groups. Moreover, different polyfunctionalized chromones and coumarins can be easily constructed using phenols as a starting material [8, 9] whereas the synthesis of isocoumarins requires prefunctionalized and not readily available compounds. The electrophilic substitution reactions for isocoumarin compounds have not been widely explored as a possible route for their chemical modification. It can be viewed as an opportunity to tackle the problem. [10-12].

One of the promising electrophilic substitution reactions for the isocoumarin system is a chlorosulfonation. The reaction medium for the chlorosulfonation combines the high activity of the electrophilic particle and weak oxidizing properties, that is especially important for the electron deficient and disposed to oxidative degradation of isocoumarin system. The possibility of preparative



Scheme 1. The basic directions of chlorosulfonation in the 3-aryl-isocoumarins [14].

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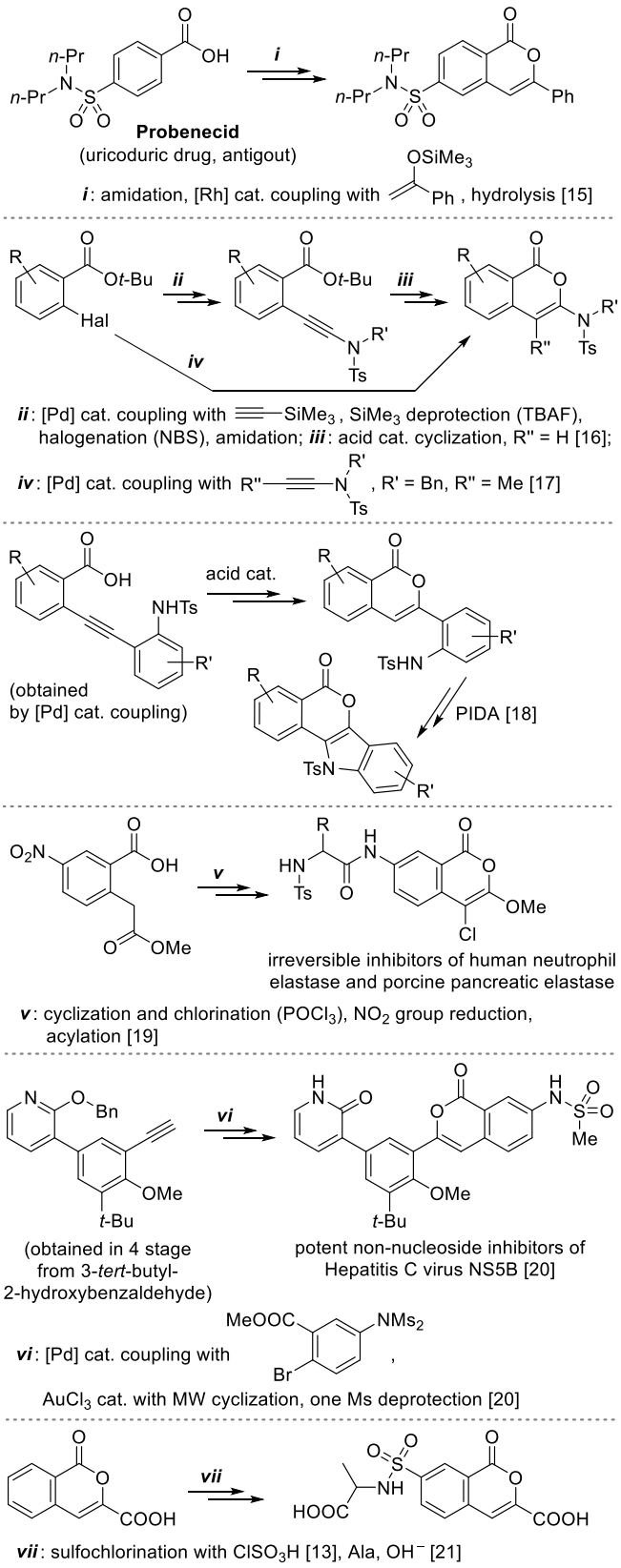
chlorosulfonation of isocoumarin derivatives was confirmed by the preparation of 7-chlorosulfonylisocoumarin-3-carboxylic acid in this way [13].



Scheme 2. Previously reported methods for the synthesis of sulfonamide-containing isocoumarins [15-21].

Earlier, we investigated the basic directions of chlorosulfonation in the 3-arylisocoumarins [14]. For unsubstituted 3-phenylisocoumarin, this reaction proceeds on the 4-position of the phenyl substituent and leads to the sulfonyl chloride **1** (Scheme 1).

The formation of sulfonamides in the reaction of sulfonyl chlorides with selected aromatic and aliphatic amines was also reported [14]. Only several examples of isocoumarins with a sulfonamide group are known to date. The synthetic methods that was developed for obtaining such compounds often required specific substrates, numerous steps, expensive reagents and catalysts (Scheme 2) [15-20]. Moreover, the sulfonamide fragment is attached to isocoumarin mainly by the nitrogen atom and just a few cases of reaction of chlorosulfonyl isocoumarins with amines have been described [21].

The isocoumarins with fragments of amino acid have been attracting significant attention due to their high biological activity [22]. The most known representatives of this class of compounds are ochratoxins [5] and amicoumacins [6] (Figure 1). Therefore, we chose amino acids as an amino component to continue developing synthetic methods and to understand the reactivity of isocoumarins in the following row: isocoumarin – chlorosulfonyl isocoumarin – sulfonamide isocoumarin.

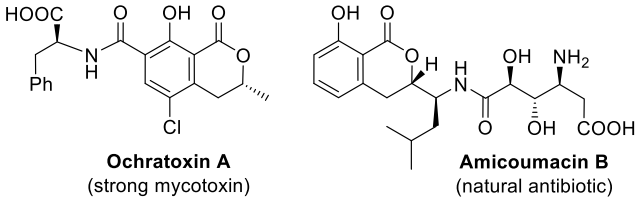


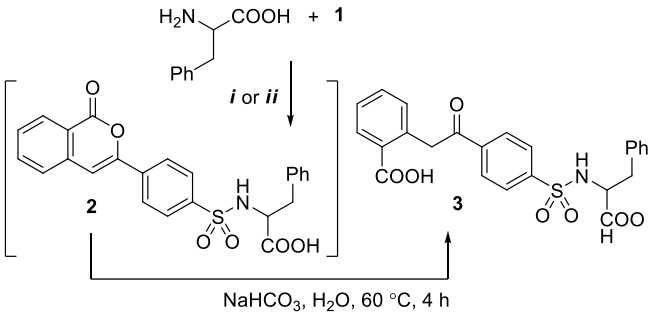
Figure 1. Natural amino-acids derivatives of 3,4-dihydroisocoumarins.

Results and Discussion

The simple 3-phenylisocoumarin (Scheme 1, R = H) as a convenient and available substrate for its transformation into sulfonamide derivative was selected. The chlorosulfonation of this substance occurs easily over a wide temperature range (from room temperature up to 60 °C) [14], and the sulfonyl chloride **1** was obtained without any by-products (Scheme 1). Only the mechanical losses during the isolation process can decrease the reaction yield at this stage.

However, the formation of sulfonamides by the reaction of sulfochloride **1** with phenylalanine in a slightly alkaline solution at room temperature is accompanied by a partial opening of the lactone ring. To increase solubility of amino acids during reaction water has to be used as a solvent. At first, we carried out this reaction in an aqueous solution using sodium bicarbonate as a base, so the observed hydrolysis of the lactone was not surprising. However, when a 1,4-dioxane-water solution (5 : 1, respectively) in the presence of triethylamine was used the mixture of

isocoumarin **2** and deoxybenzoin **3** (Scheme 3) was obtained.

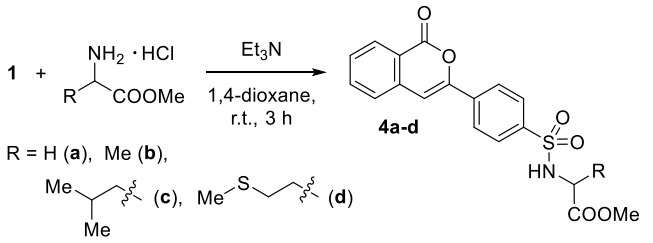


Scheme 3. The interaction of 4-(1-oxo-1*H*-isochromen-3-yl)-benzenesulfonyl chloride (**1**) with glycine.

The fast hydrolysis of **2** is atypical for these lactones. It can be explained by both the electron-withdrawing effect of sulfonyl group and the higher solubility of **2** due to the amino acid fragment. Interestingly, the addition of racemic alanine to 7-chlorosulfonylisocoumarin-3-carboxylic acid under mild basic conditions (K_2CO_3) in the water-acetone mixture occured without opening the lactone cycle (Scheme 2) [21].

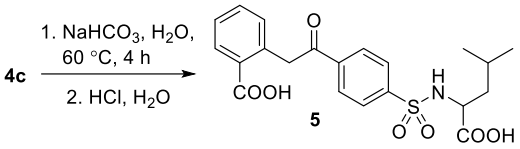
According to LCMS analysis, substances **2** and **3** are formed in approximately equal proportions. The two are very difficult to separate because they have similar physicochemical properties. The additional heating of this mixture in an aqueous solution with the presence of sodium bicarbonate yields the pure deoxybenzoin **3** (Scheme 3). It is worth noting that the isocoumarin cycle of the sulfonamide derivative was opened under stronger conditions (heating with KOH in a water-alcohol solution) while saving of the sulfonamide fragment [15].

Therefore, making the reaction of sulfonyl chloride **1** with methyl esters of amino acids in an anhydrous 1,4-dioxane in the presence of Et_3N solved this problem and gave sulfonamides **4a-d** easily and without undesirable by-products (Scheme 4). The yields of compounds **4** did not depend on the nature of substituent in the amino acid fragment.



Scheme 4. Reaction of 4-(1-oxo-1*H*-isochromen-3-yl)-benzenesulfonyl chloride (**1**) with amino acid esters.

To confirm the higher hydrolytic stability of sulfonamide group compare to ester or lactone fragments, the leucine methyl ester derivative **4c** was heated in an aqueous solution with a large excess of $NaHCO_3$ for 4 h. After acidification the sulfonamide **5** with two carboxylic groups was isolated with high yield (Scheme 5).



Scheme 5. Hydrolysis reaction of isochromone cycle of the compound **4c**.

The sulfonamides **4a** as well as deoxybenzoins **3** and **5** are the colorless crystalline solids. The structures of compounds **4a-d** were confirmed ^1H, ^{13}C and IR spectroscopic data that correlated with a previously known data for isochromone derivatives. The ^1H NMR resonance signal for the sulfonamide proton appeared as a broad dublet or broad singlet in a weak field at ~ 8.4 ppm and the methyl ester group appeared as a singlet at 3.4-3.5 ppm. Characteristically, the ^1H NMR spectra of deoxybenzoins **3** and **5** showed a resonance signal for the CH_2 group of $CH_2C{=}O$ fragment at ~ 4.7 ppm.

The characteristic absorption bands associated with N–H, C=O, and S=O bonds of compounds **3-4** can be easily identified in the IR spectra; the absorption bands associated with two (compounds **4**) or three (acids **3**, **5**) C=O groups can appears as two separated bands or appears as one very strong broad band at 1693 (compound **3**) or 1721 (compounds **4b,d**) cm^{-1}.

Conclusions

The reaction of 4-(1-oxo-1*H*-isochromen-3-yl)benzenesulfonyl chloride with amino acids in the alkaline water-containing solution occurred with the opening of the lactone ring and can be recommended for synthesis of 2'-carboxydeoxybenzoins that contains the amino acid sulfamide group. At the same time, the reaction of this sulfochloride with amino acid esters in 1,4-dioxane in the presence of triethylamine yielded the corresponding isocoumarin sulfonamide derivatives.

Experimental section

All solvents were purified according to the standard procedures [23]. All materials were purchased from commercial sources and used without further purification. ^1H and ^{13}C NMR spectra were recorded on a Mercury-400 spectrometer (400 and 100 MHz respectively) in DMSO-d_6 solutions. Chemical shifts are reported in ppm downfield from TMS as internal standards. FT-IR (KBr pellet) spectra were performed on a Bruker VERTEX 70 spectrometer. Melting points were determined by using a Kofler-type Leica Galen III micro hot stage microscope and are uncorrected. LC-MS/MS analyses were performed using an Agilent 1200 LCMSD SL system equipped with DAD/ELSD/LCMS-6120 diode matrix and mass-selective detector (chemical ionization (APCI), electrospray

ionization (ESI)). Elemental analyses for C, H, and N were determined using Perkin-Elmer CHN Analyzer. All the experiment values are in a good agreement (±0.4%) with the calculation results.

*Synthesis of the mixture of isocoumarin **2** and deoxybenzoin **3**.*

Method 1. To a solution of 330 mg (2 mmol) of phenylalanine and 504 mg (6 mmol) of $NaHCO_3$ in 10 mL of water 321 mg (1 mmol) of sulfonyl chloride **1** was added by three portion with a vigorous stirring at room temperature. The reaction mixture was stirred at room temperature for 3 h to a clear solution formation; the medium should remain alkaline all the time. The solution was acidified by 1N HCl to pH 4-5 and leaved for a night. The precipitate was filtered, washed with water, and dried.

Method 2. To a solution of 321 mg (1 mmol) of sulfonyl chloride **1** in 7.5 mL of 1,4-dioxane 330 mg (2 mmol) of phenylalanine, 1.5 mL of water, and 0.55 mL (4 mmol) of Et_3N were added. The reaction mixture was heated slightly (up to 40 °C) with stirring to a homogeneous condition, and was stirred at room temperature for 30 min. Then the mixture was poured into ice and acidified by 1N HCl to pH 4-5. The precipitate was filtered, washed with water, and dried.

*2-(2-(4-(N-(1-carboxy-2-phenylethyl)sulfamoyl)phenyl)-2-oxoethyl)benzoic acid (**3**).*

The obtained mixture of isocoumarin **2** and deoxybenzoin **3** was suspended in 20 mL water with 336 mg (4 mmol) of $NaHCO_3$, and was stirred at 60 °C to a clear solution formation. The solution was cooled to room temperature and acidified by 1N HCl to pH 4-5. The precipitate was filtered and washed with water obtaining pure deoxybenzoin **3**. Yield: 257 mg, 55% (after two stage through *Method 1*) or 313 mg, 68% (after two stage through *Method 2*), mp 114-115 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (br d, *J* 8.2 Hz, 1H, N*H*), 8.01 (d, *J* 8.0 Hz, 2H, *H*-3',5'), 7.97 (d, *J* 7.8 Hz, 1H, *H*-6), 7.66 (d, *J* 8.0 Hz, 2H, *H*-2',6'), 7.55 (t, *J* 7.4 Hz, 1H, *H*-4), 7.42 (t, *J* 7.4 Hz, 1H, *H*-5), 7.37 (d, *J* 7.4 Hz, 1H, *H*-3), 7.20-7.14 (m, 3H, C_6H_5), 7.14-7.08 (m, 2H, C_6H_5), 4.75 (s, 2H, CH_2CO), 3.99-3.89 (m, 1H, CH_2CH), 3.01-2.92 (m, 1H, CH_2CH), 2.78-2.65 (m, 1H, CH_2CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.2, 172.7, 168.6, 145.1, 139.8, 137.1, 133.3, 132.5, 131.0, 129.6, 128.8, 128.6, 127.6, 127.0, 126.9, 58.0, 45.3, 38.2. IR (KBr) *v* 3539 (br), 3304 (br, NH), 3069, 3034, 2963, 2928, 2911, 1693 (vs, br, C=O), 1602, 1577, 1496, 1454, 1423, 1400, 1349 (S=O), 1294, 1235, 1211, 1162 (S=O), 1094, 1079, 999, 952, 832, 749, 727, 702, 651, 621, 584, 549. LC/MS (CI) m/z $(M+H)^+$ 468.

*General procedure for preparation of sulfonamides **4a-d**.*

To a solution of 321 mg (1 mmol) of sulfonyl chloride **1** in 5 mL of 1,4-dioxane 2 mmol of hydrochloride of amino acid methyl ester, and 0.55 mL (4 mmol) of Et_3N were added. The reaction mixture was stirred vigorously at room temperature for 3 h; then 30 mL of water was added. The forming precipitate was filtered, washed with water, and recrystallized from isopropyl alcohol (5-10 mL).

*Methyl ((4-(1-oxo-1H-isochromen-3-yl)phenyl)sulfonyl)-glycinate (**4a**).*

Yield: 209 mg, 56 %, mp 196-197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (br s, 1H, N*H*), 8.19 (d, *J* 7.5 Hz, 1H, *H*-8), 8.09 (d, *J* 8.5 Hz, 2H, *H*-3',5'), 7.95-7.85 (m, 3H, *H*-6,2',6'), 7.74 (d, *J* 7.6 Hz, 1H, *H*-5), 7.70-7.60 (m, 2H, *H*-4,7), 3.76 (s, 2H, CH_2), 3.51 (s, 3H, COO*Me*). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 160.8, 151.3, 142.1, 137.3, 136.0, 135.7, 129.8, 129.5, 127.7, 127.5, 126.0, 120.7, 104.7, 52.3, 44.2. IR (KBr) *v* 3335 (br, NH), 3100, 2987, 2955, 1751 (s, C=O), 1720 (vs, C=O), 1637, 1603, 1485, 1455, 1407, 1328 (S=O), 1239, 1207, 1162 (S=O), 1112, 1066, 977, 830, 756, 727, 686, 630, 612, 540. LC/MS (CI) m/z $(M+H)^+$ 374.

*Methyl ((4-(1-oxo-1H-isochromen-3-yl)phenyl)sulfonyl)-alaninate (**4b**).*

Yield: 195 mg, 50 %, mp 177-178 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (br d, *J* 5.6 Hz, 1H, N*H*), 8.19 (d, *J* 8.1 Hz, 1H, *H*-8), 8.09 (d, *J* 7.6 Hz, 2H, *H*-3',5'), 7.93-7.84 (m, 3H, *H*-6,2',6'), 7.74 (d, *J* 7.6 Hz, 1H, *H*-5), 7.70-7.61 (m, 2H, *H*-4,7), 3.99-3.89 (m, 1H, $CHCH_3$), 3.39 (s, 3H, COO*Me*), 1.18 (d, *J* 6.4 Hz, 3H, $CHCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 161.3, 151.4, 142.1, 137.1, 136.1, 135.5, 129.8, 129.5, 127.7, 127.5, 125.7, 120.6, 104.8, 54.5, 52.4, 17.1. IR (KBr) *v* 3286 (br, NH), 3097, 2957, 1721 (vs, C=O), 1638, 1604, 1485, 1454, 1434, 1408, 1339 (S=O), 1237, 1212, 1168 (S=O), 1131, 1093, 1065, 1014, 972, 834, 756, 727, 686, 631, 614, 560. LC/MS (CI) m/z $(M+H)^+$ 388.

*Methyl ((4-(1-oxo-1H-isochromen-3-yl)phenyl)sulfonyl)-leucinate (**4c**).*

Yield: 338 mg, 79 %, mp 194-195 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (br d, *J* 8.2 Hz, 1H, N*H*), 8.20 (d, *J* 7.9 Hz, 1H, *H*-8), 8.10 (d, *J* 8.4 Hz, 2H, *H*-3',5'), 7.94-7.84 (m, 3H, *H*-6,2',6'), 7.75 (d, *J* 7.8 Hz, 1H, *H*-5), 7.69 (s, 1H, *H*-4), 7.65 (t, *J* 8.1 Hz, 1H, *H*-7), 3.87-3.73 (m, 1H, NHC*H*), 3.37 (s, 3H, COO*Me*), 1.62-1.50 (m, 1H, $CH(CH_3)_2$), 1.50-1.33 (m, 2H, CH_2), 0.82 (d, *J* 6.5 Hz, 3H, $CHCH_3$), 0.72 (d, *J* 6.4 Hz, 3H, $CHCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 161.5, 151.2, 142.0, 137.2, 136.0, 135.5, 129.7, 129.4, 127.6, 127.4, 125.8, 120.7, 104.7, 54.4, 52.3, 41.0, 24.3, 22.9, 21.4. IR (KBr) *v* 3314 (br, NH), 3281 (br, NH), 3097, 2956, 2935, 2870, 1739 (s, C=O), 1720 (vs, C=O), 1617, 1586, 1512, 1469, 1414, 1340 (S=O), 1311, 1297, 1237, 1169 (S=O), 1141, 1091, 1065, 1012, 966, 887, 833, 756, 685, 634, 569, 537. LC/MS (CI) m/z $(M+H)^+$ 430.

*Methyl ((4-(1-oxo-1H-isochromen-3-yl)phenyl)sulfonyl) methioninate (**4d**).*

Yield: 288 mg, 64 %, mp 182-183 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (br. d, *J* 8.8 Hz, 1H, N*H*), 8.20 (d, *J* 7.6 Hz, 1H, *H*-8), 8.11 (d, *J* 8.2 Hz, 2H, *H*-3',5'), 7.92-7.84 (m, 3H, *H*-6,2',6'), 7.75 (d, *J* 7.6 Hz, 1H, *H*-5), 7.70-7.61 (m, 2H, *H*-4,7), 4.04-3.98 (m, 1H, NHC*H*), 3.38

(s, 3H, COO*Me*), 2.44-2.26 (2H, m, CH_2CH$_2$SCH$_3$), 1.93 (s, 3H, SCH_3), 1.88-1.72 (2H, m, CH$_2$CH_2SCH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 161.6, 151.1, 141.8, 137.1, 135.8, 135.6, 129.6, 129.5, 127.6, 127.2, 126.0, 120.6, 104.8, 54.2, 52.0, 24.4, 22.8, 21.3. IR (KBr) ν 3272 (br, NH), 3094, 2956, 2917, 1721 (vs, C=O), 1638, 1604, 1485, 1451, 1430, 1408, 1341 (S=O), 1282, 1233, 1209, 1162 (S=O), 1092, 1066, 974, 864, 834, 755, 728, 685, 633, 615, 564. LC/MS (CI) m/z (M+H)$^+$ 448.

*2-(2-(4-(N-(1-carboxy-3-methylbutyl)sulfamoyl)phenyl)-2-oxoethyl)benzoic acid (**5**).*

The sulfonamide **4c** (200 mg, 0.47 mmol) was suspended in 20 mL water with 2 mL of ethanol and 672 mg (8 mmol) of NaHCO$_3$, and was stirred at 60 °C near 4 h to a clear solution formation. The solution was cooled down to room temperature and acidified by 1N HCl to pH 4-5. The precipitate was filtered, washed with water, dried to give deoxybenzoin **5**. Yield: 192 mg, 95 %, mp 188-189 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20-8.10 (m, 3H, *H*-3',5', N*H*), 8.01 (d, *J* 7.5 Hz, 1H, *H*-6), 7.90 (d, *J* 8.2 Hz, 2H, *H*-2',6'), 7.51 (t, *J* 7.0 Hz, 1H, *H*-4), 7.38 (t, *J* 7.0 Hz, 1H, *H*-5), 7.30 (d, *J* 7.2 Hz, 1H, *H*-3), 4.73 (s, 2H, CH_2CO), 3.80-3.70 (m, 1H, NHC*H*), 1.79-1.66 (m, 1H, C*H*(CH$_3$)$_2$), 1.55-1.35 (m, 2H, CH_2), 0.91 (d, *J* 6.5 Hz, 3H, CHCH_3), 0.85 (d, *J* 6.4 Hz, 3H, CHCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.6, 173.3, 168.4, 145.4, 140.1, 137.2, 133.0, 132.0, 131.1, 130.6, 128.5, 127.2, 127.1, 54.5, 45.2, 41.5, 24.4, 23.1, 21.4. IR (KBr) ν 3604, 3525, 3395 (br), 3240 (br), 2963. 2874, 2629, 1711 (s, C=O), 1691 (vs, C=O), 1400, 1330 (S=O), 1294, 1251, 1214, 1168 (S=O), 1146, 1081, 1000, 934, 842, 811, 752, 729, 715, 624, 582. LC/MS (CI) m/z (M+H)$^+$ 434.

Notes

The authors declare no conflict of interest.

Author contributions. **A. A. R.**: synthesis of compounds, investigation, formal analysis, writing experimental section. **S. V. S.**: synthesis of compounds, investigation, editing. **O. V. S.**: formal analysis, writing of the most part of the manuscript, editing. **S. A. C.**: synthesis of compounds, investigation, spectral analysis. **V. P. K.**: conceptualization, supervision, writing review & editing**.**

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Одержання амінокислотних сульфонамідів на основі 4-(1-оксо-1*H*-ізохромен-3-іл)бензенсульфонілхлориду

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Резюме: Представлено дослідження особливостей взаємодії 4-(1-оксо-1*H*-ізохромен-3-іл)бензенсульфонілхлориду (**1**) з похідними амінокислот, що веде до утворення відповідних сульфонамінів. На початку статті подано короткий огляд способів синтезу рідкісних на сьогодні 1*H*-ізохромонів (ізокумаринів) із сульфонамідними фрагментами. Ізокумарини та споріднені до них молекули, як синтетичного, так і природного походження – давно знана група оксигеновмісних гетероциклів. Ці речовини цікаві з точки зору їх біоактивності, а також мають дуже великий потенціал використання синтетичній органічній хімії (наприклад, для отримання ізохінолінів). На жаль, існуючі методи синтезу ізокумаринів обмежують можливості побудови молекул з певними функціональними групами. Зокрема, відомо дуже небагато прикладів одержання ізокумаринів із сульфамідними групами; переважно методи синтезу таких похідних базуються на паладій-каталізованих сполученнях із використанням досить вартісних вихідних речовин, а пряма послідовність сульфохлорування ізокумарину – амідування використовувалась лише кілька разів. Раніше нами було показано, що сульфохлорування із наступним утворенням сульфамідів може бути з препаративними виходами проведено в ряду 3-арилізокумаринів. В даній роботі сульфонілхлорид **1** було залучено в реакцію з амінокислотними похідними. Внаслідок взаємодії сульфонілхлориду **1** із фенілаланіном в присутності основи та при наявності навіть невеликих кількостей води в реакційному середовищі утворення сульфонамідного фрагменту ускладнюється частковим розкриттям хромонового циклу. Додаткова обробка такої суміші слабколужним водним розчином завершує розкриття циклу та приводить до утворення похідної дезоксибензоїну **3** – 2-(2-(4-(*N*-(1-карбокси-2-фенілетил)сульфамоїл)феніл)-2-оксоетил)бензойної кислоти. Натомість в результаті реакції сульфонілхлориду **1** з метиловими естерами амінокислот (у формі гідрохлоридів), що була проведена у безводному 1,4-діоксані та з додаванням триетиламіну у якості основи, легко та без побічних продуктів утворюються відповідні сульфонаміди; внаслідок чого було синтезовано ізокумаринові сульфонаміди із фрагментами гліцину, аланіну, лейцину та метіоніну. Щоб продемонструвати більшу, ніж у естеру або лактону, гідролітичну стабільність сульфонамідної групи у синтезованих сполуках, один із естерів **4** (похідна лейцину **4c**) був оброблений водним розчином $NaHCO_3$ при нагріванні. Після підкислення було виділено з майже кількісним виходом сульфонамід **5** з двома карбоксильними групами, спорідненим сполуці **3**.

Ключові слова: сульфонаміди; амінокислоти; 3-фенілізокумарин; сульфохлорування.

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RESEARCH ARTICLE

Synthesis and evaluation of new thiazole-containing rhodanine-3-alkanoic acids as inhibitors of protein tyrosine phosphatases and glutathione *S*-transferases

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Abstract: Thiazole-containing derivatives of rhodanine-3-alkanoic acids with propanoic or undecanoic acid groups were synthesized and evaluated as inhibitors of some protein tyrosine phosphatases and glutathione *S*-transferases. The rhodanines bearing longer carboxylated *N*-alkyl chain were found to inhibit PTP1B, MEG1, MEG2, and VE-PTP as well as GST from equine liver and GSTA1-1 with IC_{50} values in the low micromolar range. The inhibitory effect on protein tyrosine phosphatase activity depends on substituent at position 2 of the thiazole ring. The best compound showed a competitive type of VE-PTP inhibition. In case of GST from equine liver, the inhibition was of mixed or non-competitive type with respect to glutathione or CDNB substrate, respectively. Possible binding modes of the inhibitors were discussed based on molecular docking calculations.

Keywords: rhodanine; thiazole; protein tyrosine phosphatase, glutathione *S*-transferase; enzyme inhibition; molecular docking.

Introduction

Thiazole derivatives represent a number of natural and synthetic biologically active compounds with anticancer [1], antibacterial [2], and antiviral activities [3]. Many of rhodanine-based compounds turned out to have low toxicity, exhibit antidiabetic, anti-inflammatory, anti-Alzheimer's, anticancer, antibacterial, antifungal, and antiviral activities [4]. The best known anti-hyperglycemic, hypoglycemic and hypolipidemic effects of rhodanine derivatives are based on their agonist activity against PPARs and FFAR1 receptors, as well as inhibition of ALR2, PTP1B, and α-glucosidase [5]. Anticancer activity of the compounds can be attributed to the inhibition capacity against pan-PIM kinases [6-7], protooncogene transcription factor *c*-Myc [8-9], protein disulfide isomerase (PDI) [10], histone acetyltransferases [11], topoisomerase II [11], as well as Bcl-XL and Mcl-1 families of the proteins [13]. Antibacterial effects of rhodanine derivatives can be realized *via* inhibition of bacterial penicillin-binding proteins [14] and β-lactamases [15-16].

The protein tyrosine dephosphorylation is a fundamental regulatory mechanism of many signal transduction pathways in processes of growth, proliferation, differentiation, or survival of eukaryotic cells. It was shown that elevated activity of classical non-receptor and receptor-like protein tyrosine phosphatases (PTPs) coincides with a number of pathologies [17-18]. PTP1B, being a negative regulator of insulin and leptin signaling is considered as a promising therapeutic target for treatment of type 2 diabetes and obesity [19]. Overexpression of this phosphatase was also found to contribute to tumorigenesis of cells [20-21]. Megakaryocyte protein tyrosine phosphatase MEG-2 which involved in regulating of hematopoietic signaling and blood glucose homeostasis is of interest as a therapeutic target for treatment of type 2 diabetes and myeloproliferative disorders [23-25]. PTPβ, also called as vascular endothelial-protein tyrosine phosphatase (VE-PTP), downregulates Tie2 signaling and often associated with development of variety endothelial dysfunctions. In this connection, a number of chemical compounds were developed as inhibitors of the protein tyrosine phosphatases.

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Among them, aryl and hetaryl compounds [26], including derivatives of rhodanine [27-29] and its closely related analog, thiazolidinedione [30-31], were studied as PTPs inhibitors. Compounds bearing carboxylic [32], phosphonic [33-34], sulfonic groups [35] were designed as pTyr mimetics for inhibition of PTP1B. As a drug for treatment of diabetic macular edema, diabetic retinopathy, and ocular hypertension, the inhibitor of VE-PTP AKB-9778/Razuprotafib was developed [36-38].

It was reported previously that thiazolyl-2,4-thiazolidinedione/rhodanine compounds possess anticancer activities against hepatocellular carcinoma cell lines having resistance to chemotherapeutic agents [39]. The drug resistance caused by drug-metabolizing enzymes is considered a serious problem in treatment of cancer diseases [40]. Glutathione *S*-transferases (GSTs) comprise a superfamily of multifunctional phase II detoxification enzymes catalyzing the conjugation of glutathione (GSH) with a variety of exogenous and endogenous xenobiotics. The glutathione conjugates having less cytotoxicity and greater hydrophilicity excreted from the cells *via* the mercapturic acid pathway [41]. Overexpression of GSTs was noticed in a number of tumor cases [42-44]. Chemotherapeutic alkylating agents such as busulfan, melphalan, chlorambucil, brostallicin, and immunosuppressant azathioprine described as the substrates of these enzymes. Therefore, many compounds including analogs of glutathione and its conjugates as well as derivatives of benzoquinone, benzophenone, nitrobenzoxadiazole, and curcumin were designed as inhibitors of GSTs [41-44].

Previously, a series of rhodanine-3-alkanoic acid derivatives were described as potential inhibitors of protein tyrosine phosphatase [45]. In the current study, we report synthesis of thiazole-containing rhodanine-3-alkanoic acids and their *in vitro* evaluation as inhibitors of PTPs and GSTs.

Results and discussion

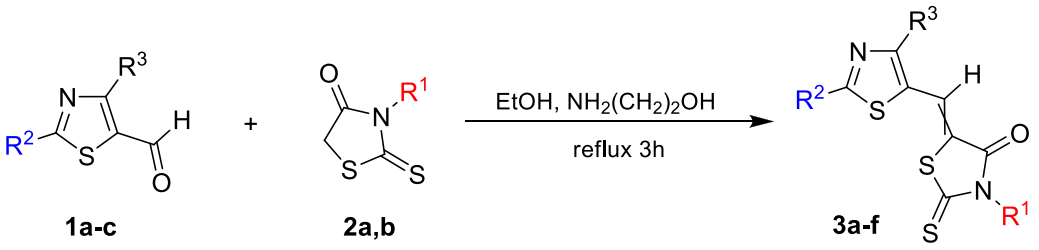
Synthesis of 5-(thiazol-5-ylmethylene)-2-thioxothiazolidin-4-one derivative

The synthesis of thiazole-containing rhodanine-3-carboxyalkyl acids **3a-f** is outlined in Scheme 1. The compounds were obtained by Knoevenagel condensation of corresponding rhodanines with aldehydes. Ethanol solution containing a proper 1,3-thiazolecarbaldehyde (compounds **1a-c**), *N*-alkyl carboxylated rhodanine (compounds **2a**, **b**), and 2-aminoethanol was heated for 3h. The obtained precipitate was filtered off and recrystallized from ethanol [46]. The compounds were obtained in moderate to good yield and were characterized by ^{1}H NMR, ^{13}C NMR and mass spectra. The data of NMR spectra showed that the newly synthesized compounds are represented by one of two Z/E isomeric forms. It should be noted that similar thiazole-contained rhodanine derivatives were described as *Z*-isomers [39].

Biological evaluation of thiazole-containing rhodanine-3-alkanoic acids as inhibitors of protein tyrosine phosphatases and glutathione S-transferases

Human recombinant protein tyrosine phosphatases PTP1B, VE-PTP, MEG1, and MEG2 were used for *in vitro* assays of the synthesized thiazole-containing rhodanine-3-carboxyalkyl acids **3a-f**. Values of the half maximal inhibitory concentrations (IC_{50}) for the compounds are presented in Table 1.

As can be seen from Table 1, rhodanine derivatives **3a-c** containing shorter alkyl carboxylated group were less effective inhibitors of PTPs than derivatives **3d-f** bearing longer *N*-alkyl chain. Compound **3a**, which possesses butyric acid fragment in *N*-3 position of rhodanine scaffold and phenyl substituent at *C*-2 position of 1,3-thiazol-5-ylmethylene moiety, was found to be a weak inhibitor of



	R^1	R^2	R^3
3a	$(CH_2)_3$-COOH	Ph	H
3b	$(CH_2)_3$-COOH	C_6H_4-p-Cl	H
3c	$(CH_2)_3$-COOH	$N(CH_3)_2$	Ph
3d	$(CH_2)_{10}$-COOH	Ph	H
3e	$(CH_2)_{10}$-COOH	C_6H_4-p-Cl	H
3f	$(CH_2)_{10}$-COOH	$N(CH_3)_2$	Ph

Scheme 1. Synthesis of thiazole-containing rhodanine-3-alkanoic acids.

Table 1. IC_{50} values of thiazole-containing rhodanine derivatives **3a-f** as inhibitors of protein tyrosine phosphatases*

Compd	IC_{50}, μM			
	PTP1B	MEG1	MEG2	VE-PTP
3a	> 25	> 25	> 25	> 25
3b	17.0 ± 4.4	>25	18.7±3.2	> 25
3c	> 25	> 25	> 25	> 25
3d	4.1 ± 1.2	23.1 ± 3.9	2.4 ± 0.4	2.4 ± 0.7
3e	0.82 ± 0.17	12.4 ± 3.5	0.54 ± 0.12	0.43 ± 0.07
3f	2.5 ± 0.6	11.0 ± 1.4	2.4 ± 0.5	3.1 ± 0.6

*IC_{50} values represent the mean of 2–3 assays ± standard deviation.

PTPs. The introduction of a chlorine substituent into the *para*-position of phenyl group of this rhodanine derivative slightly increases inhibitory effects of compound **3b** against PTP1B and MEG2. However, the replacement of 4-chlorophenyl residue by dimethylamino one, as well as introduction of phenyl group at *C*-4 position of 1,3-thiazol-5-ylmethylene moiety (compound **3c**) caused a decrease in inhibitory effects. The rhodanine derivative **3d** bearing undecanoic acid fragment at position *N*-3 showed significant increase in inhibition of PTP1B, VE-PTP, and MEG2 as compared to structure of compound **3a** with butyric acid fragment. Similarly to the effects obtained for rhodanine derivative **3b**, the introduction of chlorine atom in the *para*-position of phenyl group of 1,3-thiazol-5-ylmethylene moiety of compound **3d** led to increased inhibitory potential of rhodanine derivative **3e**. IC_{50} values of this compound were 0.82 μM, 0.54, and 0.43 μM μM for PTP1B, MEG2, and VE-PTP, respectively.

Kinetic studies were carried out to elucidate the possible mechanism of protein tyrosine phosphatases inhibition by the thiazole-containing rhodanine derivatives. According to Lineweaver-Burk plots (Figure 1), compound **3e** is a competitive-type inhibitor of protein tyrosine phosphatase VE-PTP with the calculated value of inhibition constant K_i of 0.20 μM.

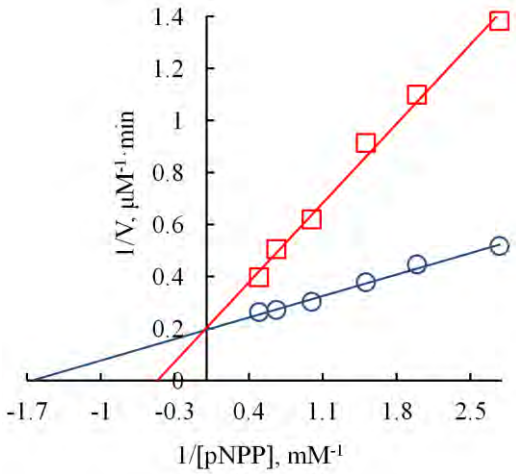


Figure 1. Lineweaver-Burk plots for inhibition of VE-PTP by compound **3e**. The inhibitor concentrations were 0 (○) and 0.5 μM (□).

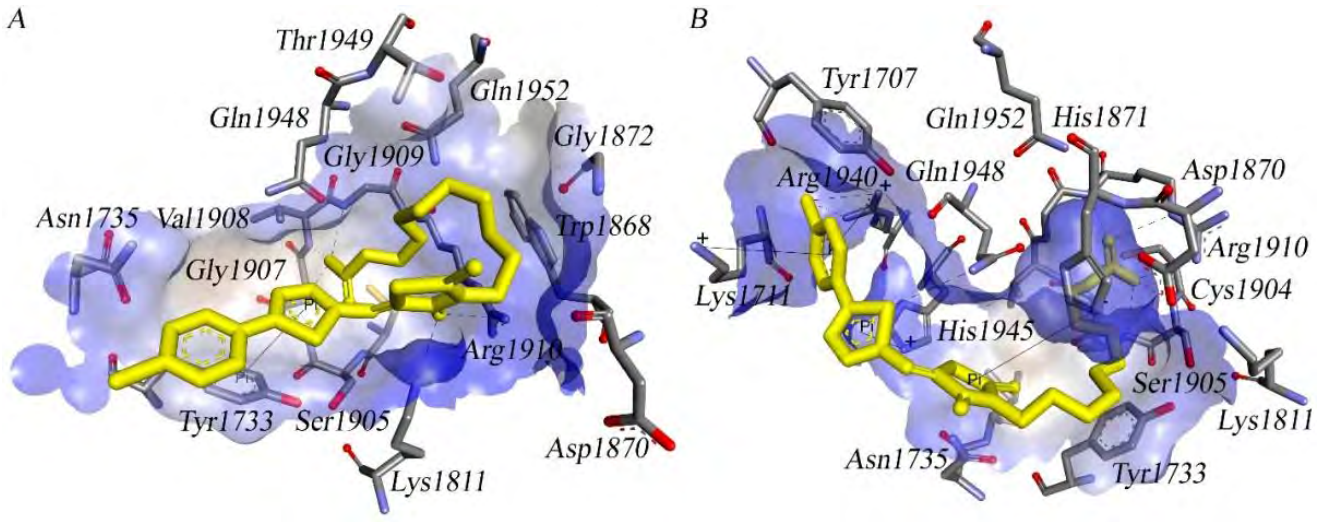


Figure 2. Possible binding modes of rhodanine derivative **3e** to human recombinant VE-PTP with open (A) and closed (B) conformations.

Molecular docking was performed to predict possible binding mode of carboxylated rhodanine derivative with PTPs. Synthetic studies described 5-ene-rhodanines mainly as *Z*-isomeres [4, 39]. In this connection, *Z*-isomer of compound **3e** was chosen to be docked into the active site region of the open and closed conformations of human VE-PTP. The estimated affinity of carboxylated rhodanine derivative **3e** to the active site of VE-PTP in open conformation was found to be -5.9 kcal/mol. The rhodanine scaffold of the inhibitor formed hydrogen bonds with amino acids residues of Lys1811 and Arg1910. The carboxylic group of the alkyl chain occupies active site near catalytic Cys1904 forming hydrogen bonds with amino acid residues Ala1906, Gly1906, Val1908, and Gly1909, while thiazole ring provided π-stacking interaction with Tyr1733 (Figure 2A).

In case of closed enzyme conformation, the estimated binding energy was -6.4 kcal/mol. The obtained model indicates that compound may be oriented into the active site of VE-PTP by carboxylic group of alkyl chain. This fragment form hydrogen bonds with amino acid residues Ser1905, Ala1906, and Arg1910. The thiazole ring of compound **3e** is involved in hydrogen bond formation with Gln1948 and π-cation interaction with His1945. The 2-chlorophenyl group forms π-cation interactions with Lys1711 and Arg1940 as well as halogen bond of chlorine atom with Arg1940 (Figure 2B).

The thiazole-containing rhodanine-3-carboxyalkyl acids were studied as inhibitors of GST from equine liver and human recombinant GSTA1-1. The obtained results (Table 2) demonstrated that compounds **3d-f** were more potent inhibitors in comparison with derivatives **3a-c** bearing butyric acid fragment at *N*-3 position of rhodanine scaffold. Better inhibition effects against GST from equine liver were observed in the case of compounds **3d-f** modified at *N*-3 position of rhodanine ring with undecanoic acid group. Further studies showed that these compounds can also inhibit the recombinant form of human GSTA1-1 with IC_{50} values in the low micromolar range.

Table 2. Inhibition activity of 5-(thiazol-5-ylmethylene)-2-thioxothiazolidin-4-one derivatives **3a-f** against GST from equine liver and human recombinant GSTA1-1*.

Compd	IC_{50}, μM	
	GST from equine liver	GSTA1-1
3a	> 25	-
3b	24.1 ± 2.7	-
3c	> 25	-
3d	5.2 ± 1.4	1.1 ± 0.2
3e	4.5 ± 0.5	0.83 ± 0.22
3f	6.2 ± 0.9	2.7 ± 0.7

*IC_{50} values are the means of 2-3 assays ± standard deviation.

Lineweaver-Burk plots (Figure 3) showed mixed-type or non-competitive inhibition of GST from equine liver by compound **3d** toward glutathione or CDNB substrate. According to the mixed-type inhibition, the calculated values of inhibition constants K_i and K_i' were 12.9±3.7 μM and 20.2±5.8 μM, respectively, while the non-competitive inhibition constant K_i was 6.8±1.8 μM.

The results of computer modeling suggest that *Z*-isomer of rhodanine derivative **3d** may occupy interdomain cavity near active site of human GSTA1-1 (Figure 4A) with the calculated docking energy of -9.3 kcal/mol. Deregulation of the interdomain contacts in structure of GSTA1-1 was shown to lead to disruption of the enzyme catalytic functions [47] which may explain the inhibition of the enzyme. The compound position (Figure 4B) is characterized by interaction of the inhibitor with hydrophobic amino acid residues Thr68, Leu72, Ile96, Ile99, Ala100, Ile106, Leu107, and Leu163. Thiazole ring at *C*-5 position of rhodanine scaffold formed π-stacking interaction with Tyr166, while phenyl fragment at *C*-2 position of the thiazole ring provided π-cation interaction with Arg13. Hydrogen bond was observed between oxygen atom of rhodanine scaffold and Ser18. Carboxylate group of the inhibitor is adjacent to the active G-site and provides

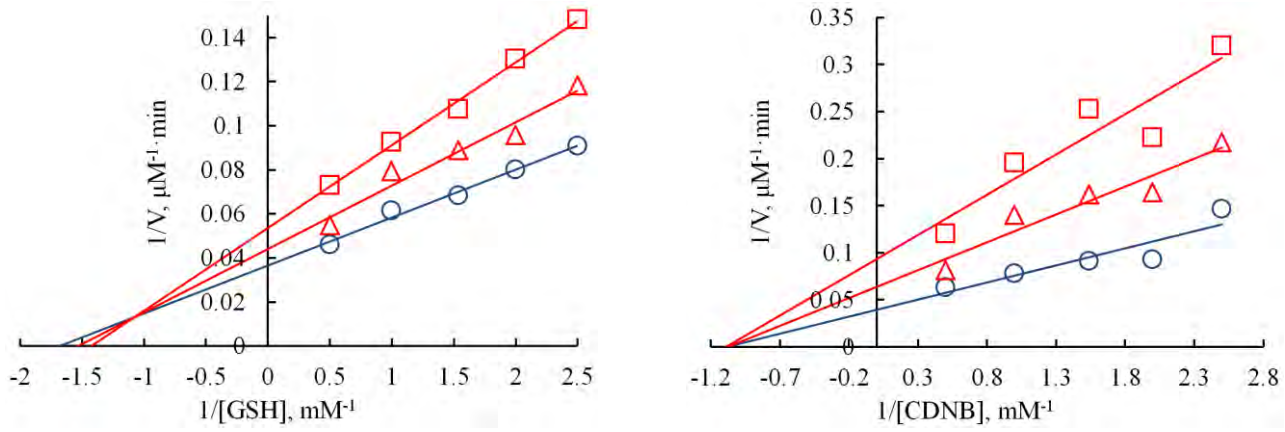


Figure 3. Lineweaver-Burk plots for inhibition of GST from equine liver by compound **3d**. The inhibitor concentrations were 0 (○), 5 μM (Δ) and 7.5 μM (□).

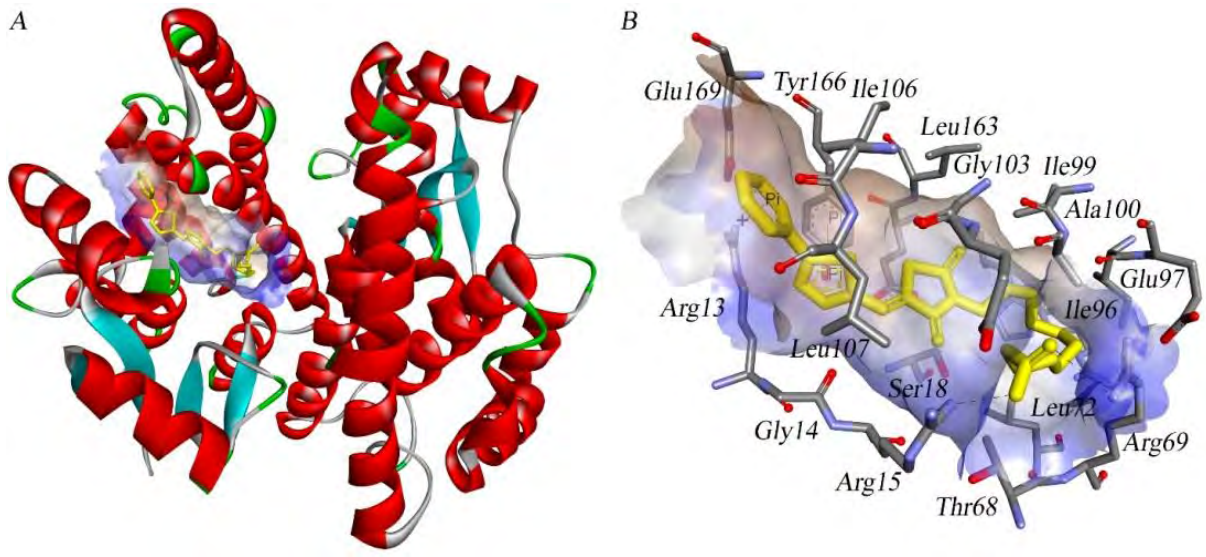


Figure 4. Possible binding mode of rhodanine derivative **3d** to homodimer structure of human GSTA1-1.

hydrogen bonds with amino acid residues Arg69 and Arg15 which is involved in interaction with GSH.

Conclusions

The study reported synthesis of new rhodanine-3-alkanoic acids with propanoic or undecanoic acid groups. The data obtained showed that compounds **3d-f** bearing undecanoic acid group at *N*-3 position of rhodanine scaffold possess good inhibitory effects against PTP1B, MEG1, MEG2, and VE-PTP as well as GSTA1-1. According to kinetic data, thiazole-containing rhodanines can be competitive-type inhibitors of VE-PTP. In case of GST from equine liver, the compounds can be considered as mixed-type inhibitors toward GSH and non-competitive toward CDNB substrate. Molecular docking results indicate that the inhibitors may occupy VE-PTP active site, while inhibition of GSTA1-1 might be explained by the location of rhodanine derivative between *C*- and *N*-terminal subunits of the enzyme.

Experimental section

Chemistry

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Bruker Avance DRX 500 spectrometer in DMSO-d_6 solution. IR spectra were recorded on a Vertex 70 spectrometer from KBr pellets. Melting points were measured with a Büchi melting point apparatus and are uncorrected. LC-MS spectra were obtained using HPLC apparatus, Agilent 1100 Series, equipped with diode-matrix and mass-selective detector Agilent LC/MSD SL.

*General procedure for synthesis of compounds **3a-f***

A solution of 0.002 mol of rhodanine derivative **2a** or **2b** in 5 mL of ethanol and 0.02 mL of 2-aminoethanol were added to a solution of 0.002 mol of corresponding aldehyde (**1a-c**) in 5 mL of ethanol. The mixture was refluxed for 3 h and cooled. The precipitate was filtered off and recrystallized from EtOH.

*4-{4-Oxo-5-[(2-phenyl-1,3-thiazol-5-yl)methylidene]-2-sulfanylidene-1,3-thiazolidin-3-yl}butanoic acid (**3a**).*

Yield: 0.531 g (68%); yelow crystals; mp 190-191 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (br s, 1H, COO*H*), 8.48 (s, 1H, C*H*), 8.12 (s, 1H, C^4-$H_{thiazol}$), 8.03 (d, *J* 7.7 Hz, 2H, Ph), 7.49-7.59 (m, 3H, Ph), 4.06 (t, *J* 6.8 Hz, 2H, CH_2), 2.30 (t, *J* 7.2 Hz, 2H, CH_2), 1.84-1.95 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 192.5, 174.1, 172.8, 166.8, 151.2, 133.7, 132.6, 132.0, 129.9, 127.1, 123.7, 123.2, 44.4, 31.4, 22.4. LC/MS (CI) m/z 391 (M)$^+$. Anal. Calcd. for $C_{17}H_{14}N_2O_3S_3$: C, 52.29; H, 3.61; N, 7.17; S, 24.63. Found: C, 52.35; H, 3.60; N, 7.10; S, 24.64.

*4-(5-{[2-(4-Chlorophenyl)-1,3-thiazol-5-yl]methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)butanoic acid (**3b**).*

Yield: 0.484 g (57%); yelow crystals; mp 201-202 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.12 (br s, 1H, COO*H*), 8.47 (s, 1H, C*H*), 8.10 (s, 1H, C^4-$H_{thiazol}$), 8.02 (d, *J* 8.5 Hz, 2H, C$_6H_4$-p-Cl), 7.56 (d, *J* 8.5 Hz, 2H, C$_6H_4$-p-Cl), 4.05 (t, *J* 6.8 Hz, 2H, CH_2), 2.31 (t, *J* 7.1 Hz, 2H, CH_2), 1.83-1.93 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 190.2, 171.9, 169.0, 164.6, 148.9, 134.4, 131.8, 129.2, 127.7, 126.5, 121.7, 120.8, 42.2, 29.2, 20.1. LC/MS (CI) m/z 426 (M)$^+$. Anal. Calcd. for $C_{17}H_{13}ClN_2O_3S_3$: C, 48.05; H, 3.08; N, 6.59; S, 22.64. Found: C, 48.14; H, 3.04; N, 6.53; S, 22.54.

*4-(5-{[2-(Dimethylamino)-4-phenyl-1,3-thiazol-5-yl]methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)butanoic acid (**3c**).*

Yield: 0.702 g (81%); yelow crystals; mp 179-180 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.63 (s, 1H, C*H*), 7.50-7.62 (m, 5H, Ph), 3.98 (t, *J* 6.5 Hz, 2H, CH_2), 3.20 (s, 6H, N(CH_3)$_2$), 2.16 (t, *J* 7.2 Hz, 2H, CH_2), 1.74-1.87 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 191.2, 174.2,

172.2, 166.4, 161.8, 133.6, 129.7, 129.4, 128.7, 125.4, 116.9, 113.8, 44.0, 41.7, 32.0, 22.5. LC/MS (CI) m/z 434 $(M)^+$. Anal. Calcd. for $C_{19}H_{19}N_3O_3S_3$: C, 52.64; H, 4.42; N, 9.69; S, 22.19. Found: C, 52.68; H, 4.39; N, 9.68; S, 22.12.

*11-{4-Oxo-5-[(2-phenyl-1,3-thiazol-5-yl)methylidene]-2-sulfanylidene-1,3-thiazolidin-3-yl}undecanoic acid (**3d**).*

Yield: 0.733 g (75%); yelow crystals; mp 141-142 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43 (s, 1H, C*H*), 8.07 (s, 1H, C^4-$H_{thiazol}$), 7.99 (d, *J* 8.1 Hz, 2H, Ph), 7.46-7.56 (m, 3H, Ph), 3.94 (t, *J* 7.3 Hz, 2H, CH_2), 2.16 (t, *J* 7.4 Hz, 2H, CH_2), 1.54-1.64 (m, 2H, CH_2), 1.41-1.51 (m, 2H, CH_2), 1.16-1.29 (m, 12H, (CH_2)$_6$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 192.1, 174.9, 172.8, 166.6, 151.3, 133.6, 132.6, 132.0, 129.8, 127.1, 123.5, 123.4, 44.9, 34.1, 29.3, 29.3, 29.2, 29.0, 29.0, 26.7, 26.6, 25.0. LC/MS (CI) m/z 489 $(M)^+$. Anal. Calcd. for $C_{24}H_{28}N_2O_3S_3$: C, 58.99; H, 5.78; N, 5.73; S, 19.68. Found: C, 59.10; H, 5.73; N, 5.70; S, 19.69.

*11-(5-{[2-(4-Chlorophenyl)-1, 3-thiazol-5-yl]methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)undecanoic acid (**3e**).*

Yield: 0.764 g (73%); yelow crystals; mp 173-174 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (br s, 1H, COO*H*), 8.41 (s, 1H, C*H*), 8.06 (s, 1H, C^4-$H_{thiazol}$), 7.99 (d, *J* 7.7 Hz, 2H, C$_6H_4$-p-Cl), 7.54 (d, *J* 7.7 Hz, 2H, C$_6H_4$-p-Cl), 3.94-4.00 (m, 2H, CH_2), 2.16 (t, *J* 7.4 Hz, 2H, CH_2), 1.58-1.65 (m, 2H, CH_2), 1.44-1.51 (m, 2H, CH_2), 1.19-1.30 (m, 12H, (CH_2)$_6$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.8, 172.4, 169.1, 164.4, 148.9, 134.4, 131.8, 129.3, 127.7, 126.5, 121.7, 120.9, 42.7, 31.9, 27.0, 26.9, 26.9, 26.7, 26.7, 24.4, 24.3, 22.7. LC/MS (CI) m/z 524 $(M)^+$. Anal. Calcd. for $C_{24}H_{27}ClN_2O_3S_3$: C, 55.10; H, 5.20; N, 5.35; S, 18.39. Found: C, 55.14; H, 5.15; N, 5.32; S, 18.37.

*11-(5-{[2-(Dimethylamino)-4-phenyl-1,3-thiazol-5-yl]-methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)undecanoic acid (**3f**).*

Yield: 0.744 g (70%); yelow crystals; mp 114-115 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.67 (s, 1H, C*H*), 7.47-7.63 (m, 5H, Ph), 3.89-3.99 (m, 2H, CH_2), 3.21 (s, 6H, N(CH_3)$_2$), 2.15 (t, *J* 6.6 Hz, 2H, CH_2), 1.54-1.62 (m, 2H, CH_2), 1.42-1.50 (m, 2H, CH_2), 1.16-1.29 (m, 12H, (CH_2)$_6$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.3, 172.5, 170.5, 164.5, 160.0, 131.9, 127.6, 126.9, 123.7, 123.6, 115.1, 112.0, 42.5, 32.0, 26.9, 26.9, 26.8, 26.8, 26.7, 26.6, 24.5, 24.3, 22.8. LC/MS (CI) m/z 532 $(M)^+$. Anal. Calcd. for $C_{26}H_{33}N_3O_3S_3$: C, 58.73; H, 6.26; N, 7.90; S, 18.09. Found: C, 58.79; H, 6.26; N, 7.84; S, 18.03.

Biological tests

In vitro study of thiazole-containing rhodanine-3-alkanoic acid derivatives as inhibitors of protein tyrosine phosphatases and glutathione S-transferases

Protein tyrosine phosphatases were purchased in Sigma-Aldrich. Prior to experiments, the defined volume of PTP1B, VE-PTP, MEG1 and MEG2 were diluted in a solution of 50 mM Bis-Tris buffer (pH 7.2) containing 30% glycerol, 3 mM EDTA, 2 mM DTT, 75 mM NaCl, and 0.05% Tween-20. The system for inhibition study consisted of 50 mM Bis-Tris buffer (pH 7.2), 100 mM NaCl, 3 mM EDTA, 1 mM DTT, 1 vol. % DMSO, inhibitor and enzyme. The mixture was thermostated at 30 ºC during 5 min and reaction was started by adding the substrate (pNPP) at concentration near K_m value for each of the enzymes. The activity of enzymes was measured spectrophotometrically at 410 nm. The molar extinction coefficient of 18300 $M^{-1}cm^{-1}$ was used for calculation of *p*-nitrophenol concentration.

GST from equine liver and human recombinant GSTA1-1 was purchased from Sigma-Aldrich. Before use in the experiments, 0.25 mg of GST from equine liver was diluted in 1 ml of distillated water, and 25 µL of GSTA1-1 was diluted in 1 ml of solution consisted of 50 mM Tris-HCl buffer (ph 7.5), 50 mM NaCl, 1 mM DTT, 5 mM EDTA and 50 vol. % glycerol. The rhodanine derivatives were dissolved in DMSO. *In vitro* studies were carried out in system consisting of 0.1 M sodium-phosphate buffer (pH 6.5), 0.1 mM EDTA, 2.5 vol. % DMSO, water, 20 µl of enzyme solution and inhibitor. After incubation of this mixture at 25 ºC during 5 min, the reaction was started by addition 200 µL of 10 mM reduced L-glutathione (GSH) and 20 µL of 100 mM 1-chloro-2,4-dinitrobenzene (CDNB). The enzyme activity was monitored spectrophotometrically at 340 nm. The molar extinction coefficient of 9600 $M^{-1}cm^{-1}$ was used for calculation of dinitrophenyl-*S*-glutathione concentration [48].

Molecular docking calculation

Crystal structures of open and closed conformation of VE-PTP (PDB code 2AHS and 2H02, respectively) and GSTA1-1 (PDB code 6ATO) were downloaded from PDB server (https://www.rcsb.org) [49]. Before docking calculation, the ligands, water molecules and amino acids conformers were removed from obtained PDB files. The structure of thiazole-containing rhodanine derivatives were drawn using MarvinSketch [50] and optimized with MMFF94s force field in Avogadro software [51]. Docking files were prepared using AutoDockTools (version 1.5.6) [52]. The docking calculations were carried out by Autodock Vina software [53]. The models visualizations and analysis was performed using Discovery Studio 3.5 Visualizer (Accelrys Inc., San Diego, CA, USA).

Notes

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The authors declare no conflict of interest.

Author contributions. **O. L. K.**: conceptualization, supervision, molecular docking simulation, writing-original draft. **V. O. S.**: synthesis of compounds, investigation, formal analysis. **Y. V. S.**: investigation of bioactivity. **V. M. B.**: investigation of bioactivity. **D. M. H.**:

investigation. **S. G. P.**: synthesis of compounds, analysis. **V. S. B.**: supervision, writing-review and editing. **A. I. V.** conceptualization, supervision, writing-review and editing.

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Синтез та оцінка нових тіазоловмісних роданін-3-алканових кислот як інгібіторів протеїнтирозинфосфатаз та глутатіон-*S*-трансфераз

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Резюме: Тіазоловмісні похідні роданін-3-алканової кислоти, модифіковані залишками пропанової чи ундеканової кислот, синтезовано та оцінено як інгібітори деяких протеїнтирозинфосфатаз та глутатіон-*S*-трансфераз. Сполуки отримано за реакцією Кновевенагеля взаємодією відповідних роданінів з альдегідами. Встановлено, що роданіни з довшим карбоксильованим *N*-алкільним ланцюгом інгібують активність PTP1B, MEG1, MEG2 та VE-PTP, а також GST з печінки коня та людську рекомбінантну GSTA1-1 зі значеннями IC_{50} у низькому мікромолярному діапазоні. Інгібувальний ефект на активність протеїнтирозинфосфатаз залежав від замісника в 2 положенні тіазолового кільця, тоді як природа замісників у положенні 2 та 4 мала незначний вплив на інгібувальну активність сполук щодо глутатіон-*S*-трансфераз. Найкраща сполука, 11-(5-{[2-(4-хлорфеніл)-1,3-тіазол-5-іл]метиліден}}-4-оксо-2-сульфаніліден-1,3-тіазолідин-3-іл)ундеканова кислота, продемонструвала конкурентний тип інгібування VE-PTP. У випадку GST з печінки коня сполука виявилась змішаним інгібітором при використанні GSH як субстрату та неконкурентним інгібітором у разі CDNB. Результати молекулярного докінгу вказують на те, що інгібітор може займати активний центр протеїнтирозинфосфатази VE-PTP, тоді як інгібування людської рекомбінантної GSTA1-1 може бути пояснено розташуванням похідної роданіну між *C*- та *N*-кінцевими доменами однієї з субодиниць ферменту.

Ключові слова: роданін; тіазол; протеїнтирозинфосфатаза; глутатіон-*S*-трансфераза; інгібування ферменту; молекулярний докінг.

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RESEARCH ARTICLE

Some pharmacological properties of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one

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Abstract: A series of 3,5-diaryl pyrazolyl thiazolinones were designed and synthesized as potential biologically active compounds. The study of anticancer activity of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) revealed its high antiproliferative activity against a panel of cancer cells with the lowest growth inhibition concentration (GI_{50}) towards leukemic cell line SR (0.0351 μM) and ovarian cancer cell line OVCAR-3 (0.248 μM). It was also found that pyrazolyl thiazolinone **1** inhibited growth of *Trypanosoma brucei brucei* by 98,8% at a concentration of 10 μg/mL. The in-depth cytotoxicity study of compound **1** on human hepatocellular carcinoma HepG2 cells and non-tumorigenic murine fibroblast Balb/c 3T3 in MTT, NRU, TPC and LDH assays showed that normal cells were less sensitive to compound **1** than the cancer cells; its action had led to a disintegration of the cell membrane, inhibition of mitochondrial and lysosomal activity, and proliferation of cancer cells. The highest selectivity were detected in the LDH assay.

Keywords: pyrazolyl thiazolinone hybrids, antitumor activity, antitrypanosomal activity, cytotoxicity.

Introduction

The hybrid pharmacophore approach has been used to develop a drug-like molecules with anticancer properties [1]. Pyrazole and thiazole/thiazolidinone cycles are considered as favored scaffolds and have been used to design novel drug-like molecules possessing antiproliferative activity [2-3]. A combination of the above heterocycles in one molecule is likely to provide more efficient pyrazolyl-thiazole/thiazolidone conjugates via synergistic anticancer effects. For example, a 2-amino-iminothiazolidinone derivative with a pyrazole moiety at position 5 inhibited necroptosis [4], pyrazolyl-4-thiazolidinones exhibited dose-dependent cytotoxic effect in human breast cancer cells (MCF-7 line) [5], a series of 2-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)ones inhibited growth of leukemia cell lines and non-small cell lung cancer cell lines [6]. Cytotoxicity of pyrazole derivatives includes inhibition of the topoisomerase I and II activity for pyrazoloacridine [7] or inhibition of the Janus-activated kinase (JAK1/2) for the Ruxolitinib [8]. The pyrazolyl thiazol/thiazolidinone-based hybrids as biologically active compounds have been studied for anti-parasitic activity [9-10]. For example, some of the 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones showed IC_{50} level of activity within 0.6-0.7 μM *in vitro* assay towards *Trypanosoma brucei gambiense* [11]; 5-[5-aryl-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-thiazolidine-2,4-dione and 2-{5-[5-aryl-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2,4-dioxothiazolidin-3-yl}-N-arylacetamides inhibited growth of the *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* at micromolar concentrations [12]. Thiazole/thiazolidinone phenylindole/imidazothiadiazoles comprise another highly active class of antitrypanosomals [13]. The combination of different pharmacophores in one molecule may lead to novel drug-like molecules that will exhibit biological activity. Thus, the above mentioned polypharmacological [14] and hybrid-pharmacophore approaches [1] inspire the design and synthesis of pyrazolyl-thiazolinones as potential anticancer and antiparasitic agents.

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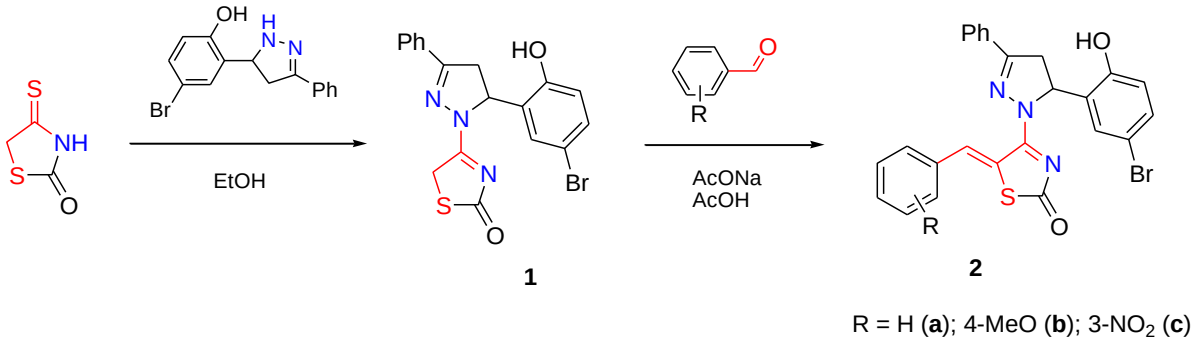

Scheme 1. Synthesis of a pyrazolyl-thiazolinone **1** and its 5-arylidene derivatives **2a-c**.

Results and Discussion

A 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) was synthesized in the reaction of 3,5-diarylpyrazoline with 4-thioxo-2-thiazolidinone (isorhodanine) in the ethanol by known approach [6]. 5-arylidene derivatives **2a-c** were synthesized in high yields by the Knoevenagel reaction with corresponding aldehydes (Scheme 1). 4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydro-pyrazol-2-yl]-5*H*-thiazol-2-one (**1**) was selected by National Cancer Institute (NCI, Bethesda, USA) Developmental Therapeutic Program (DTP) for anticancer screening at one dose assay (10^{-5} M) using a panel of 59 cancer cell lines that represented different types of cancer (leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers). The anticancer activity of compound **1** showed the mean growth percent for the whole cancer cell panel and it was about 25% [15-17]. Moreover, the tested pyrazolyl thiazolinone **1** demonstrated the cytotoxic effects to five leukemic cell lines (CCRF-CEM, HL-60(TB), MOLT-4, RPMI-8226, SR), a non-small cell lung cancer cell line (HOP-92), a CNS cancer cell line (SF-295), and an ovarian cancer cell line (IGROV1). The high growth inhibition rates of compound **1** justified the in-depth screening at a range of concentrations using 55 cell lines. The percentage of growth was evaluated spectrophotometrically versus control that were not treated with tested compound after 48 h of exposure. The SRB protein assay was used to estimate cells' viability or growth. Three dose-response parameters for antitumor activity were found for each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI – molar concentration of the compound leading to the total inhibition; and LC_{50} – molar concentration of the compound leading to 50% net cell death. The high antiproliferative activity of compound **1** against the majority of cell is reported in Table 1. The lowest inhibitory concentrations for compound **1** were observed against the leukemia panel. The most sensitive leukemic cell line was the SR line (GI_{50} = 0.0351 μM). It inhibited growth of CCRF-CEM, HL-60, MOLT-4 and SR leukemia lines at submicromolar concentrations (TGI). The high cytotoxic activity of **1** was found against non-small cell lung cancer line NCI-H322M and EKVX as well as colon cancer line HCT-116, ovarian cancer OVCAR-3, and breast cancer MCF7 lines.

The antitrypanosomal activity of pyrazolyl-thiazolinone **1** on against *T. brucei brucei* was investigated *in vivo* by the polypharmacological approach. A combination of pyrazoline and thiazolinone cycles in one molecule did not only contribute to the anticancer properties, but also increased the antiparasitic activity. It was found that 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihyd-ropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) inhibited growth of *T. brucei brucei* by 98,8% at a concentration of 10 μg/mL. Lowering concentration to 1 μg/mL led to sharp decrease in the inhibition activity (11,87 % of inhibition).

The biological assays demonstrated high antitumor and antitrypanosomal activities of pyrazolyl thiazolinone **1**. High activities inspired further in-depth studies of **1** against human hepatocellular carcinoma HepG2 cells and non-tumorigenic murine fibroblast Balb/c 3T3. Four different biochemical endpoints were measured: mitochondrial activity, lysosomal activity, total protein content, and cellular membrane integrity in MTT assay, NRU assay, TPC assay and LDH assay respectively (after 24, 48 and 72 h exposition).

The cells viability after the exposure to tested compound was found to be a time-, a concentration- and a cell line-dependent (Figure 1). Human hepatoma HepG2 cells were more sensitive to the compound **1** compared to normal murine Balb/c 3T3 fibroblast cells. The first sign of anticancer activity of compound **1** was seen at the lowest concentrations after 24 h of exposure (LDH assay). Moreover, the toxic effect for normal fibroblasts cell line in the same assay was observed at a concentration of 0.9 μM (CC_{20}) after 48 h of exposure (Figure 1). The calculated cytotoxic concentrations values (CC_{20}, CC_{50}, and CC_{80}) for the cancer HepG2 cell line and for the non-tumorigenic immortalized Balb/c 3T3 cell line are shown in Table 2. It is worth to note that the lowest cytotoxic concentrations (CC_{20}) that inhibits 20% of cell growth were calculated for all tests regardless of the exposure time. CC_{50} values indicated that the first step of anticancer action of compound **1** was the first disintegration of cellular membranes leading to the inhibition of mitochondrial activity with further inhibition of proliferation and lysosomal activity. It is noteworthy that compound **1** exhibits low toxicity against normal murine embryonic fibroblast cell line (Balb/c 3T3). Its toxic action led to dis-

Table 1. Influence of the 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) on the growth of the most sensitive tumor cell lines in *in vitro* test (10^{-4}-10^{-8}M).

Cancer cell lines		GI_{50}, μM	TGI, μM	LC_{50}, μM
Leukemia	*CCRF-CEM*	0.339	2.70	>100.0
	HL-60	0.306	0.923	>100.0
	K-562	0.959	>100.0	>100.0
	MOLT-4	0.397	17.7	>100.0
	SR	0.0351	0.282	>100.0
Non-small cell lung cancer	*A549/ATCC*	6.55	>100.0	>100.0
	EKVX	0.382	>100.0	>100.0
	NCI-H23	0.819	>100.0	>100.0
	NCI-H322M	0.306	15.3	>100.0
	NCI-H460	0.619	>100.0	>100.0
Colon cancer	*HCC-2998*	1.94	8.58	>100.0
	HCT-116	0.302	>100.0	>100.0
	HCT-15	0.767	>100.0	>100.0
	SW-620	0.694	>100.0	>100.0
CNS cancer	*SF-268*	0.805	>100.0	>100.0
	SF-295	0.636	>100.0	>100.0
Melanoma	*MALME-3M*	0.843	22.3	>100.0
	M14	0.907	28.4	>100.0
	MDA-MB-435	1.92	>100.0	>100.0
	SK-MEL-5	1.18	20.8	>100.0
Ovarian cancer	*OVCAR-3*	0.248	0.682	>100.0
	OVCAR-4	0.464	>100.0	>100.0
	NCI/ADR-RES	0.830	20.7	>100.0
	SK-OV-3	1.84	36.0	>100.0
Renal cancer	*ACHN*	1.44	>100.0	>100.0
	CAKI-1	0.723	4.06	>100.0
	TK-10	1.62	>100.0	>100.0
Prostate cancer	*DU-145*	2.24	>100.0	>100.0
Breast cancer	*MCF7*	0.301	-	>100.0
	T-47D	0.377	>100.0	>100.0
	MDA-MB-468	0.631	67.6	>100.0

integration of the cell membrane, inhibition of mitochondrial and lysosomal activity of cancer cells.

The selectivity indexes (SI), which indicates the cytotoxic selectivity (i.e. drug safety) were calculated for HepG2 cell line against corresponding normal cell line (Figure 2). Higher values of SI indicate higher anticancer specificity. Compounds with SI above 3.0 are considered highly selective [18]. The highest values of SI were detected in the LDH assay for compound **1**. The SI increased from 167 to 4975 with increase of the exposure time from 24 h to 72 h whereas SI values for the cisplatin increased only from 13 to 1429 (Figure 2).

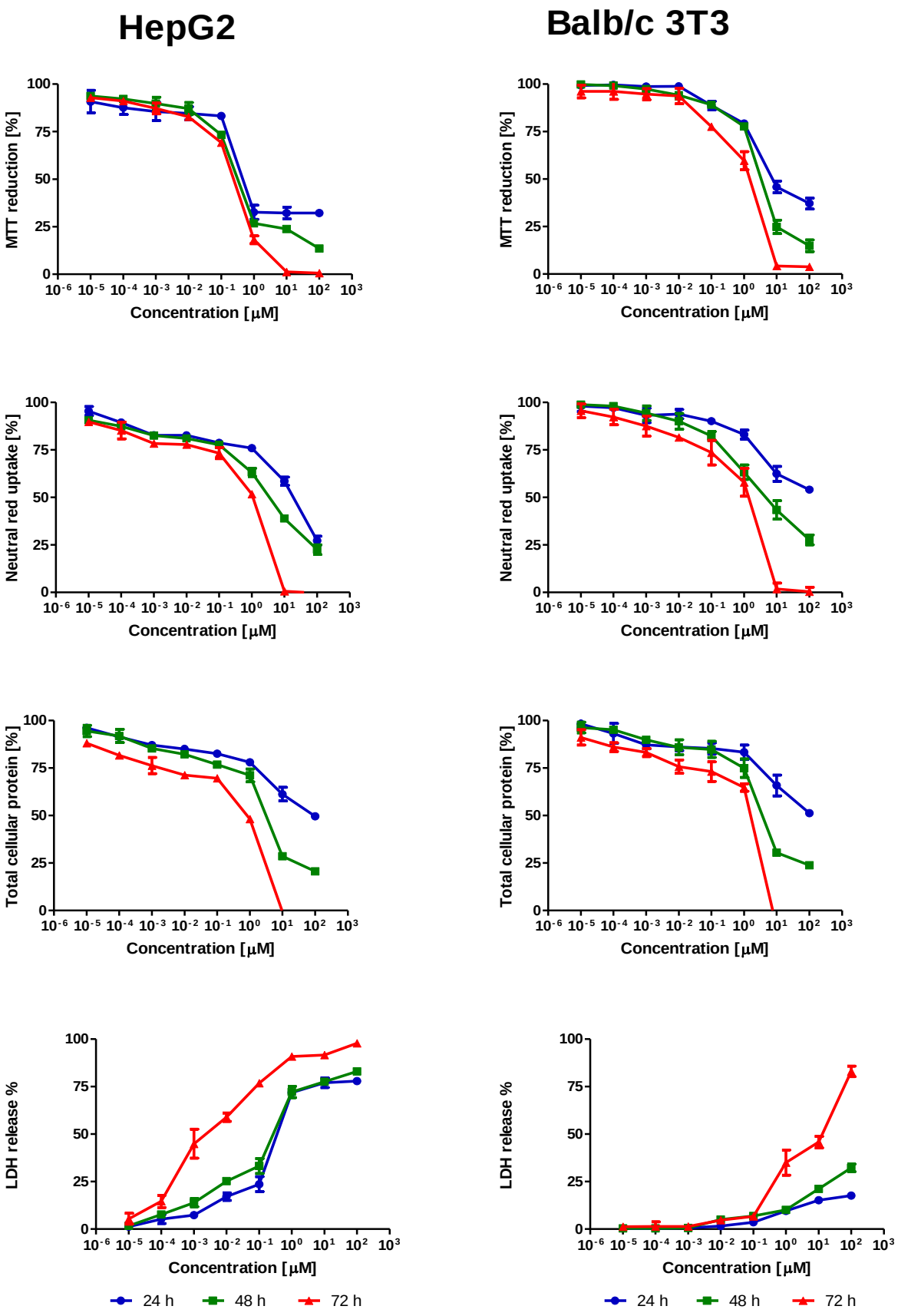

Figure 1. Cytotoxic concentrations (CC_{20}; CC_{50}; CC_{80}) (μM) calculated for compound **1** in normal and cancer cells.

Conclusions

High anticancer activity of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) against a series of cancer cell lines with increased selectivity against leukemic cell lines had been demonstrated. (3,4-Dihydropyrazol-2-yl)-5*H*-thiazol-2-one (**1**) showed a good antitrypanosomal activity in the *in vitro* experiment against *T. brucei brucei*. The dual anticancer and antitrypanosomal activity along with high selectivity indices as well as low cytotoxicity would justify further in-depth studies of compound **1** as a perpective drug candidate.

Table 2. The values of cytotoxic concentrations (CC_{20}; CC_{50}; CC_{80}) (μM) calculated for compound **1** in MTT, NRU, TPC and LDH assays.

Cell line	Method	Time (h)	CC_{20}	CC_{50}	CC_{80}*
Balb/c 3T3	MTT	24	0.9±0.3	8.9±0.9	
		48	0.8±0.2	5.8±0.6	52.1±5.2
		72	0.1±0.1	2.5±0.8	7.4±0.5
	NRU	24	2.4±1.1	-	-
		48	0.2±0.1	7.1±1.5	
		72	0.05±0.002	2.1±1.0	7.1±0.3
	TPC	24	2.3±1.0	-	-
		48	0.6±0.3	6.0±0.5	
		72	0.006±0.002	2.8±0.7	6.5±0.9
	LDH	24	-	-	-
		48	9.2±0.6	-	-
		72	0.5±0.1	19.9±2.6	92.9±2.5
HepG2	MTT	24	0.9±0.09	6.9±0.5	-
		48	0.1±0.04	5.5±0.2	42.1±2.5
		72	0.03±0.01	0.44±0.02	0.97±0.04
	NRU	24	0.07±0.02	34.2±1.7	-
		48	0.04±0.01	5.9±0.7	-
		72	0.001±0.0003	1.3±0.1	6.6±0.1
	TPC	24	0.7±0.2	95.5±0.5	-
		48	0.05±0.02	5.4±0.4	-
		72	0.001±0.0002	0.9±0.04	6.2±0.1
	LDH	24	0.06±0.01	0.6±0.02	-
		48	0.006±0.001	0.5±0.07	47.9±4.9
		72	0.0003±0.0001	0.004±0.001	0.3±0.07

* CC_{20}, CC_{50} and CC_{80} (μM) represents the concentrations of compound **1** that is required for 20%, 50% and 80 % inhibition using the MTT, NRU, TPC, and LDH assays. Data are expressed as the mean ± SEM and were calculated from the dose response curves of at least three independent experiments.

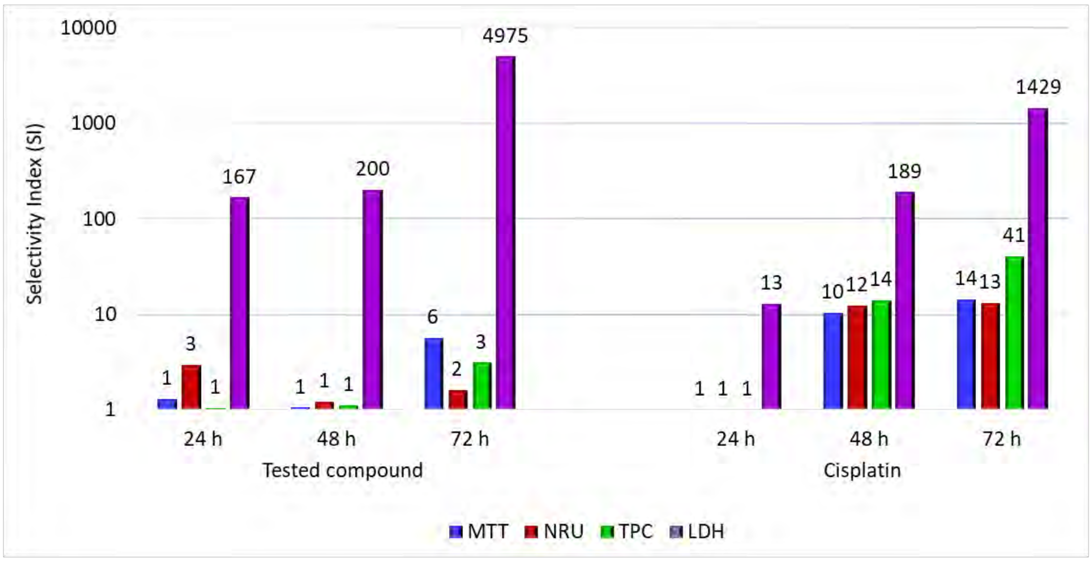

Figure 2. Selectivity indices (SI) calculated for compound **1** and cisplatin against HepG2 cells.

Experimental section

Chemistry

Commercial reagents were purchased from Merck and used without purification. Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses were performed using the Perkin-Elmer 2400 CHN analyzer. The ^{1}H NMR spectra were recorded on Varian Gemini (^{1}H at 400 and ^{13}C at 100 MHz) instrument in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard.

The starting 3,5-diaryl-4,5-dihydropyrazole was synthesized according to known method from chalcone [19].

*4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5H-thiazol-2-one (**1**).*

A mixture of 4-thioxo-2-thiazolidinone (0.01 mol) and 4-bromo-2-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol (0.01 mol) was refluxed in 100 ml of ethanol for 1.5 h. After cooling the reaction mixture to room temperature, formed precipitate was filtered off, washed with methanol and recrystallized. Yield: 65%; pale yellow solid; mp 232-234 °C, (DMF-EtOH 1:2). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.86 (d, *J* 8.2 Hz, 2H, Ar), 7.47-7.56 (m, 3H, Ar), 7.24 (dd, *J* 2.7, 8.2 Hz, 1H, Ar), 7.10 (d, *J* 2.7 Hz, 1H, Ar), 6.83 (d, *J* 8.2 Hz, 1H, Ar), 5.75 (dd, *J* 3.3, 20.0 Hz, 1H, C$_5$*H*-pyraz.), 4.87 (d, *J* 16.8 Hz, 1H, C$_4$*H*-thiaz.), 4.65 (d, *J* 16.8 Hz, 1H, C$_4$*H*-thiaz.), 4.00 (dd, *J* 11.1, 20.0 Hz, 1H, C$_4$*H*-pyraz.), 3.25 (dd, *J* 3.3, 20.0 Hz, 1H, C$_4$*H*-pyraz.). Anal. Calcd. for $C_{18}H_{14}BrN_3O_2S$: C, 51.93; H, 3.39; N, 10.09. Found: C, 52.05; H, 3.45; N, 9.93.

*General procedure for synthesis of compounds **2a-c***

The equimolar amounts of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) (0.01 mol) with the appropriate aromatic aldehyde (0.01 mol) and sodium acetate (0.01 mol) in the acetic acid medium were refluxed for 3-4 hours. After cooling the reaction mixture formed precipitate was filtered off and recrystallized.

*5-Benzylidene-4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]thiazol-2-one (**2a**).*

Yield: 72%; yellow solid; mp 242-244 °C, (DMF-EtOH 1:3). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.86 (d, 2H, *J* 8.2 Hz, Ar), 7.66 (m, 4H, Ar), 7.40-7.55 (m, 5H, Ar, C*H*=), 7.19 (d, 1H, Ar), 7.04 (d, *J* 7.3 Hz, 1H, Ar), 6.84 (d, *J* 8.0 Hz, 1H, Ar), 6.04 (dd, *J* 3.3, 20.0 Hz, 1H, C$_5$*H*-pyraz.), 3.98 (dd, *J* 11.1, 20.0 Hz, 1H, C$_4$*H*-pyraz.), 3.26 (dd, *J* 3.3, 20.0 Hz, 1H, C$_4$*H*-pyraz.). Anal. Calcd. for $C_{25}H_{18}BrN_3O_2S$: C, 59.53; H, 3.60; N, 8.33. Found: C, 59.62; H, 3.68; N, 8.29.

*4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5-[(4-methoxyphenyl)methylene]thiazol-2-one (**2b**).*

Yield: 78%; yellow-orange solid; mp > 250 °C, (DMF-EtOH 1:3). ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.89 (s, 1H, O*H*), 7.88-7.93 (m, 2H, Ar), 7.64 (d, *J* 8.0 Hz, 2H, Ar), 7.50-7.54 (m, 3H, Ar, C*H*=), 7.19 (d, 1H, Ar), 7.06 (d, *J* 7.3 Hz, 3H, Ar), 6.83 (d, *J* 8.0 Hz, 1H, Ar), 6.03 (dd, *J* 3.3, 20.0 Hz, 1H, C$_5$*H*-pyraz.), 3.98 (dd, *J* 11.1, 20.0 Hz, 1H, C$_4$*H*-pyraz.), 3.29 (dd, *J* 5.0, 10.0 Hz, 1H, C$_4$*H*-pyraz.), 1.91 (s, 3H, C*H*$_3$). Anal. Calcd. for $C_{28}H_{20}BrN_3O_3S$: C, 58.43; H, 3.77; N, 7.86. Found: C, 58.50; H, 3.85; N, 7.80.

*4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5-[(3-nitrophenyl)methylene]thiazol-2-one (**2c**).*

Yield: 69%; yellow-orange solid; mp > 250 °C, (DMF-EtOH 1:3). ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.26 (s, 1H, O*H*), 9.16 (s, 1H, Ar), 8.36 (d, 2H, *J* 8.0 Hz, Ar), 7.98-8.10 (m, 4H, Ar), 7.60 (m, 3H, Ar, C*H*=), 7.31 (d, 1H, Ar), 7.20 (s, 1H, Ar), 6.86 (d, *J* 8.0 Hz, 1H, Ar), 6.02 (dd, *J* 3.3, 20.0 Hz, 1H, C$_5$*H*-pyraz.), 4.09 (dd, *J* 11.1, 20.0 Hz, 1H, C$_4$*H*-pyraz.), 3.39 (dd, *J* 5.0, 10.0 Hz, 1H, C$_4$*H*-pyraz.). Anal.

Calcd. for $C_{25}H_{19}BrN_4O_4S$: C, 54.45; H, 3.47; N, 10.16. Found: C, 54.60; H, 3.40; N, 10.03.

Biological tests

Anticancer in vitro screening methodology as well as data interpretation are described in details at the NCI Development Therapeutics Program site [20].

Cytotoxicity assays

The human hepatoma cell line (HepG2) was purchased from the American Type Culture Collection (ATCC HB-8065). The cells were cultured in Minimum Essential Medium Eagle (MEME) (ATCC, USA). The murine fibroblasts cell line (Balb/c 3T3 clone A31) (gift from Department of Swine Diseases of the National Veterinary Research Institute in Pulawy, Poland) was cultured in Dulbecco`s Modified Eagle`s Medium (DMEM) (ATCC, USA). The media were supplemented with 10% BCS (Balb/c 3T3), 10% FBS (HepG2), 1% L-glutamine, 1% antibiotic solution. The cells were maintained in 75 cm^2 cell culture flasks (NUNC) in a humidified incubator at 37 °C, in an atmosphere of 5% CO_2. The medium was refreshed every two or three days and the cells were trypsinized by 0.25% trypsin-0.02% EDTA after reaching 70-80% confluence. Single cell suspensions were prepared and adjusted to a density of $2x10^5$ cell/mL (HepG2) and $1x10^5$ cell/mL for 24h, 48h exposition or $5x10^4$ cell/mL for 72 h exposition (Balb/c 3T3). The cell suspension was transferred to 96-well plates (100 µl/well) and incubated for 24 h before the exposure to the studied compound. Stock solution of the studied compound was prepared in DMSO and diluted with culture medium to obtain a concentration range from 10^{-5}-10^2 µM. The cells were also exposed to the reference drug – cisplatin used as internal laboratory control. The final concentration of DMSO was 0.1% in the medium and had no influence on cell growth. The medium used for test solutions and in control preparation did not contain serum and antibiotics. As negative control, cultured cells were grown in the absence of study compound. Each concentration was tested in six replicates with three independent experiments. Cytotoxicity was assessed after 24, 48 and 72 h of exposure the cells to tested compound. The medium was not changed during the incubation time.

MTT assay. The metabolic activity of living cells was assessed by the measurement of the activity of dehydrogenases [21].

NRU assay. The method is based on staining living cells with neutral red which readily diffuses through the plasma membrane and accumulates in lysosomes [22].

TCP assay. The assay was based upon staining total cellular protein (proliferation) [23].

LDH leakage assay. The integrity of the plasma membrane was assessed through the test of lactate dehydrogenase (LDH) release [24], which was monitored using the commercially available Cytotoxicity Detection Kit (LDH) (Roche Diagnostics, Poland). The absorbance was measured at microplate reader (Synergy HTXmulti-mode reader (BioTek® Instruments Inc., USA)) at 570 nm, using blank as a references. Cytotoxicity was expressed as a percentage of the negative control (0.1% DMSO) [25].

Selectivity index.

To determine the cytotoxic selectivity of the tested compound, the selectivity index (SI) was calculated according to the following equation:

$$SI = CC_{50}^{\text{no cancer cells}}/CC_{50}^{\text{cancer cells}}$$

If a SI was found to be ≥ 3 the compound can be considered to be selective [18].

A beneficial SI > 3.0 indicates a drug with efficacy against tumor cells greater than toxicity against normal cells. The results of the cytotoxicity assessment were expressed as mean arithmetic values from three independent experiments. The percentage of viability inhibition was calculated in comparison with the untreated controls. The CC_{20}, CC_{50}, CC_{80} values (cytotoxicity concentrations) are the compound's concentrations that inhibit the cell viability by 20%, 50% and 80%were calculated by GraphPad Prism 5 software (San Diego, CA, USA) using nonlinear regression.

Notes

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Деякі фармакологічні властивості 4-[3-(5-бромо-2-гідроксифеніл)-5-феніл-3,4-дигідропіразол-2-іл]-5H-тіазол-2-ону

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Резюме: Розроблено та синтезовано ряд піразоліл-тіазолідинонів як потенційних біологічно активних сполук. Дослідження протипухлинної активності 4-[3-(5-бром-2-гідроксифеніл)-5-феніл-3,4-дигідропіразол-2-іл]-5*H*-тіазол-2-ону (**1**) виявило його високі антипроліферативні властивості щодо панелі більш, ніж 50-ти ракових клітинних ліній з найнижчою концентрацією пригнічення росту (GI_{50}) обчисленою для лінії лейкемії SR (0,0351 мкМ) та лінії раку яйників OVCAR-3 (0,248 мкМ). Крім того, піразоліл-тіазолідинон **1** повністю пригнічував ріст (TGI) ліній лейкемії CCRF-CEM, HL-60, MOLT-4 та SR при субмікромолярних значеннях концентрації. Крім ліній лейкемії, чутливими до дії сполуки **1** були лінії недрібноклітинного раку легень *NCI-H322M* та *EKVX*, лінія раку товстого кишківника *HCT-116*, раку яйників *OVCAR-3* та раку молочної залози MCF7. Також було встановлено, що піразоліл-тіазолідинон **1** інгібує ріст *Trypanosoma brucei brucei* на 98,8% у концентрації 10 мкг/мл, що свідчить про перспективність розробки даного агенту в рамках концепції «поліфармакологічних лікарських засобів». Поглиблене дослідження цитотоксичності сполуки **1** на клітинах гепатоцелюлярної карциноми людини HepG2 та нормальних мишачих фібробластах Balb/c3T3 у MTT, NRU, TPC та LDH тестах показало, що нормальні клітини менш чутливі до дії піразоліл-тіазолідинону **1**, ніж ракові. Відповідно до значень CC_{50}, першим етапом протипухлинної дії сполуки **1** було пошкодження клітинних мембран, що призводить до інгібування мітохондріальної активності, з послідовним інгібуванням проліферації та лізосомальної активності ракових клітин. Для 4-[3-(5-бром-2-гідроксифеніл)-5-феніл-3,4-дигідропіразол-2-іл]-5*H*-тіазол-2-ону (**1**) обчислено індекси селективності, що були вищими у тесті LDH, ніж такі для препарату порівняння цисплатину.

Ключові слова: піразолін-тіазолідинонові гібриди, протипухлинна активність, антитрипаносомна активність, цитотоксичність.

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RESEARCH ARTICLE

In silico study of binding affinity of nitrogenous bicyclic heterocycles: fragment-to-fragment approach

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Abstract: The binding affinity of model aromatic amino acids and heterocycles and their derivatives condensed with pyridine were carried out *in silico* and are presented in the framework of fragment-to-fragment approach. The presented model describes interaction between pharmacophores and biomolecules. Scrupulous data analysis shows that expansion of the π-electron system by heterocycles annelation causes the shifting up of high energy levels, while the appearance of new the dicoordinated nitrogen atom is accompanied by decreasing of the donor-acceptor properties. Density Functional Theory (DFT) wB97XD/6-31(d,p)/calculations of possible π-complexes of the heterocycles **1-3** with model fragments of aromatic amino acids, which were formed by π-stack interaction, show an increase in the stabilization energy of π-complexes during the moving from phenylalanine to tryptophan. DFT calculation of pharmacophore complexes with model proton-donor amino acid by the hydrogen bonding mechanism (H-B complex) shows that stabilization energy (ΔE) increases from monoheterocycles to their condensed derivatives. The expansion of the π-electron system of compounds **1a-c** by the pyridine cycle reduced the stabilization energy of π-complexes and H-B complexes in comparison with the expansion of the π-electronic system by introducing phenyl radicals at positions 2 and 5 of the oxazole ring [18].

Keywords: fragment-to-fragment approach, binding affinity, [*Pharm-BioM*] complex, π-stacking interaction, hydrogen bonds.

Introduction

Pharmacologically active compounds, which are based on the nitrogenous conjugated bicyclic compounds, are well-known by their vital role in the metabolism of all living cells [1-3]. These compounds are suitable to design new perspective molecules using so-called Fragment-Based Drug Discovery (FBDD) method [4-5]. The simplest nitrogenous heterocycles (oxazole, pyrazole and their heterosubsituted and annelated derivatives) were found to be convenient synthetic intermediates and were often used as perspective scaffolds in combinatorial medical chemistry [6-7]. Recently, series of new nitrogenous conjugated bicyclic molecules were synthesized and were evaluated *in vitro* for anti-cancer and other biological activities [8-9].

The preliminary search of new perspective pharmacophores requires information about the chemical composition, spatial and electron structure, and other properties, including the affinity to the biomolecules. There is a growing interest in the *in silico* studies in search of novel biologically active molecules. Particularly, development in the field of quantitative structure-reactivity relationships (QSAR) [10-12] and molecular docking [7, 10, 13] are gaining traction. As the next step in the development of the *in silico* approach, the approximation based non-empirical quantum-chemical calculation using the fragment-to-fragment approach. This technique is used as opposed to the traditional methods and is able to evaluate the biological activity by the chemical structures of the pharmacophore molecules and the involved fragments of biomolecules [14]. Specifically, the proposed method allows to quantitatively evaluate a donor/acceptor property of conjugated molecules. It takes into consideration the relative position of the frontier molecular levels and non-

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bonding *n*-levels. All required parameters can be experimentally measured by photo-electron and absorption spectroscopies [15-17]. Similar *in silico* approaches discover ways for better analysis of the binding affinities of the bioactive molecules.

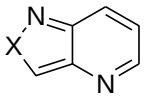
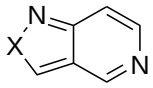
A biological activity can unambiguously depend on the chemical structures of both a pharmacophore and a target molecules that includes their 3D geometry and electron structure. Recently, we reported [18] the influence of the various conjugated groups (donors, acceptors as well as ambivalent phenyls) in a mono-cyclic oxazole platform on the electron densities of substituted derivatives and on their biological activity using the fragment-to-fragment approach. It was found that the expansion of the π-system by introducing the conjugated substituents to oxazole platform influenced their biological activity. Moreover, it was demonstrated that the molecules that contain acceptor conjugated substituent (-SO_2R) increase the biological activity while donor substituents (-NR_2 or -SR) decrease their activity [8,14].

In the present work we use *in silico* approach to study conjugated system that was formed by condensation of an oxazole cycle (or its heteroanalogues) with pyridine cycle. Similar substitution was shown to be effective to increase anticancer efficiency and other biological activity [9, 19].

Materials and calculation method

Many 1,2-oxazoles have been reported to have a variety of interesting and significant biological activities [20-22]. Oxazoles conjugated with pyridine at 2-position have been synthesized and shown antibacterial activities [23]. The antibacterial and antifungal activities of oxazoles condensed with a benzene ring was also studied [21, 24]. Here, we would like to report *in silico* study of oxazole and its heteroanalogues **1a-c** as well as their annelated derivatives with nitrogen heterocycles **2** and **3** (Table 1).

Table 1. Structure of compounds studied **1-3(a-c)**.

Compd			
	1	**2**	**3**
X=NH	a	a	a
X=O	b	b	b
X=S	c	c	c

The influence of a heteroatom X on the electronic structure of the conjugated system and the formation of hydrogen bonds by dicoordinated nitrogen atom (including the energies and shape π- and *n*-molecular orbitals (MOs), donor/acceptor property) will be discussed.

The biological affinity of the potential pharmacophore (*Pharm*) should be connected to its ability to form a stable complex with biomolecule [*Pharm-BioM*], where *BioM* is the fragment of the biomolecule (polypeptide chain). The complex stability depends on the interaction energy between both components in [*Pharm-BioM*] complex. In our study, the following interaction types of interactions will be considered: (1) the π-stack interaction between the conjugated systems of the components and (2) the formation of hydrogen bonds with the corresponding functional groups of the amino acids.

The characteristics of the electron structure (optimized molecular geometry, charge distribution, energies and molecular orbital shapes) as well as the energy of their interactions with peptide fragments were calculated using DFT method with wB97XD function and 6-31 (d.p.) basis set (package GAUSSIAN 03 [25]).

Results and Discussion

Intermolecular characteristics of the pharmacophore molecules

All studied molecules **1-3** are conjugated system. Therefore, it was no surprise that DFT optimization of molecular geometry give a planar geometry of the above-mentioned compounds. The thickness of π-electron shell is ≈ 3.4 Å. The dimensions of the molecules **1-3** do not exceed the dimensions of protein fragments. The main regions of amino acid (-CO-NH-) forms hydrogen bonds in the polypeptide chain of the protein helix and, therefore, are inaccessible for the formation of a complex with the molecules of pharmacophores. Therefore, molecules of pharmacophores **1-3** should interact with protein fragments that contain flat "aromatic" amino acid groups by the π-stacking mechanism.

In addition, due to the presence of dicoordinated nitrogen atoms (*trtrtr*π^2 configuration) the *n*-MO occurs among the highest occupied π-orbitals in the electron shell. The detailed description of these MOs will be communicated later during hydrogen bonds discussion.

The oxazole ligand can form complexes with peptide fragments thought protein-ligand complex [*Pharm-BioM*] interaction [12]. Moreover, it was shown [12, 14] that biological activity is connected to the frontier orbitals (the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). The DFT calculations showed that the HOMO and LUMO are the delocalized π-orbitals: one orbital *n*-MO in the monocycle compounds **1**, and two – orbitals *n*-MO in compounds **2**, **3** (Figure 1) are among highest MOs and they correspond to the lone electron pair (LEP) of these molecules.

As shown in Figure 1a, the changing the nitrogen atom (compound **1a**) with oxygen (**1b**) or sulfur (**1c**) leads that second σ-orbital appears much higher: HOMO-5 ⇒ HOMO-3, which directly effects to the ability of generating hydrogen bonds with the corresponding amino acid residues in protein molecules by substances **1a**, **1b**, and **1c**. Annelation with a pyridine ring in heterocycles **2a-2c** (Figure 1b) leads to a redistribution of electron density in the σ-orbitals in such a way that the nitrogen atom

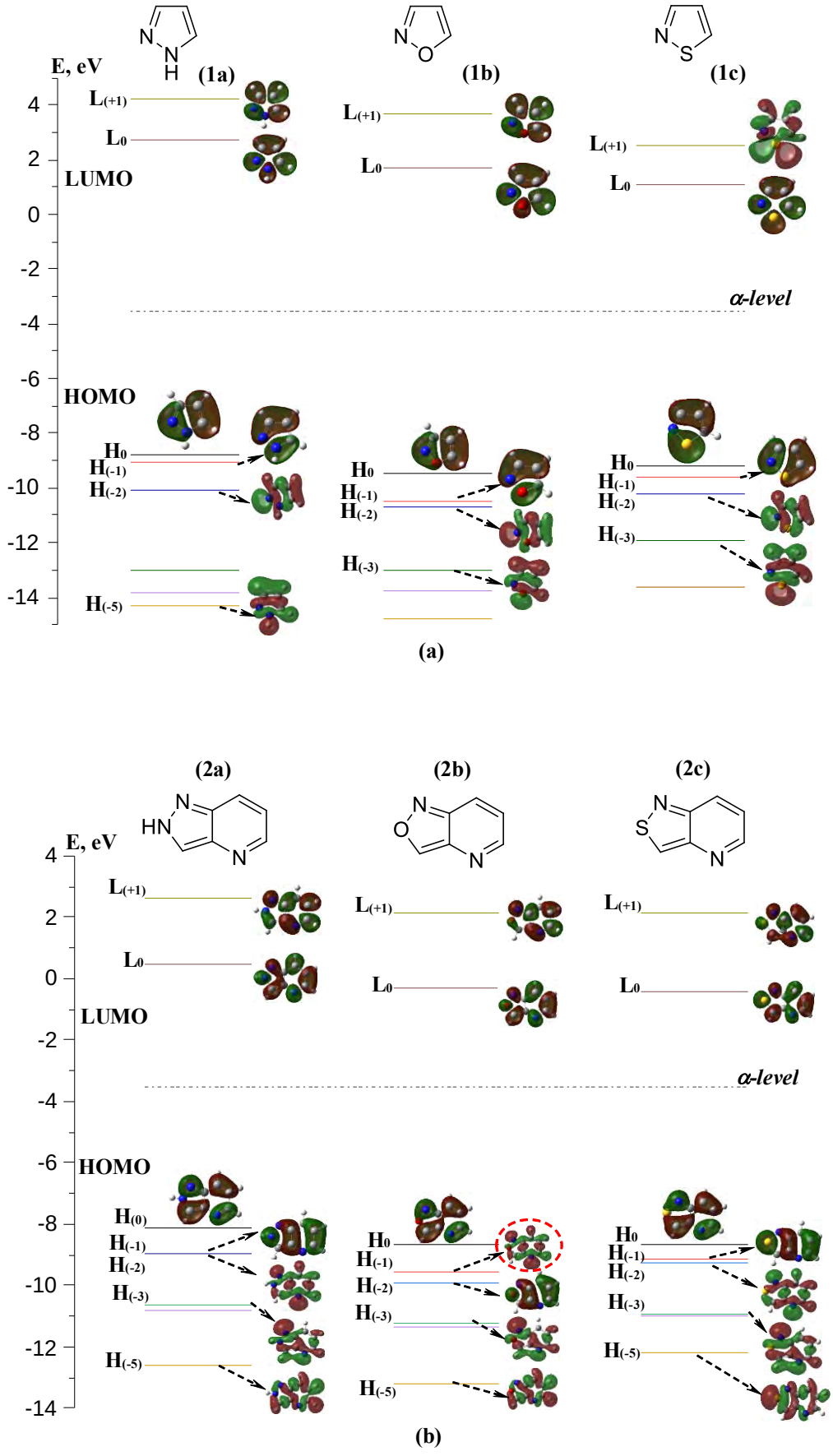


Figure 1. Shape of frontier and nearest MO in compounds **1a-1c** (a) and compounds **2a-2c** (b).

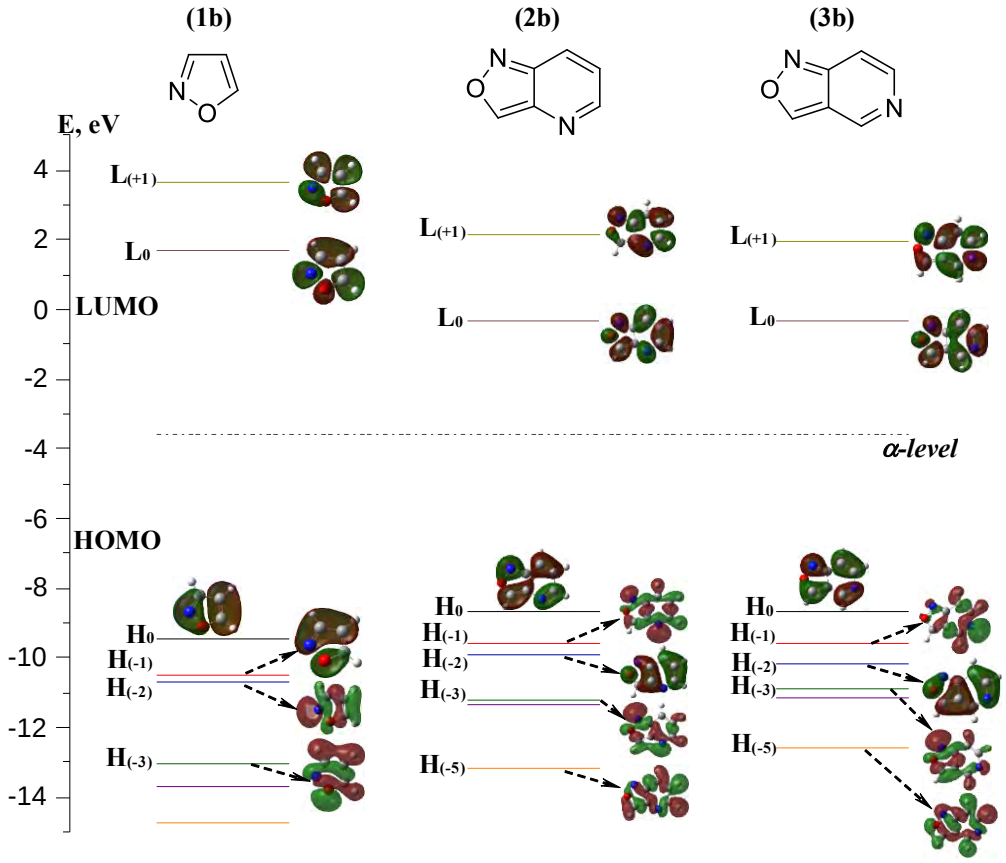


Figure 2. Shape of frontier and nearest MO in compounds **1b**, **2b**, **3b**.

(HOMO-2) of the pyridine ring is more sensitive to the formation of a hydrogen bond compared to the nitrogen atom of the five-membered ring (HOMO-3) of substances **2a-2c**. The shift of the first orbital is observed in oxazolo pyridine **2b**, in comparison with compounds **2a** and **2c**: HOMO-2 ⇒ HOMO-1. This suggests that the probability of hydrogen bonding by oxazolopyridine **2b** is higher compared to compounds **2a** and **2c**.

When the oxazole ring is annelated with various pyridine isomers (compounds **2b** and **3b**), insufficient of HOMO-2 in isomer **3b** is observed. It should also be noted that in isomer **3b**, the electron density of the σ-orbital (HOMO-1) is more concentrated on the nitrogen atom of the pyridine ring than in substance **2b**. It can be assumed that the stabilization energy of the H-B complexes is higher for isomer **3b**.

Also, Figures 1 and 2 shows that the LUMO is totally delocalized π-orbital. The energy of both frontier MOs of the studied molecules are collected in Table 2.

It is seen from Table 2, that change of the heteroatom X (in series X = NH, O, S) causes the regular shifting up of the highest occupied level, but this effect decreases upon expansion of the conjugated system (**1** ⇒ **2**, **3**). Similarly, the lowest vacant level shifts down in both initial molecules **1** and in annelated derivatives **2**,**3**. Also, the energy gap (distance between highest occupied level and lowest vacant level, Δ) becoming essentially lesser. Besides, the annelation effects differ for the both isomers **2**, **3**.

Table 2. Electron characteristics of compounds **1-3**.

Compd	X	ε[a], eV		Δ[b]	φ_0[c]
		HOMO	LUMO		
1a	NH	-8.70	2.51	11.21	0.541
1b	O	-9.43	1.54	10.97	0.465
1c	S	-9.15	1.15	10.30	0.458
2a	NH	-8.10	0.36	8.46	0.463
2b	O	-8.67	-0.43	8.24	0.380
2c	S	-8.66	-0.37	8.29	0.384
3a	NH	-8.01	0.41	8.42	0.472
3b	O	-8.59	-0.44	8.15	0.383
3c	S	-8.56	-0.42	8.14	0.386
	Polyene-15[15]	-6.21	-0.91	5.30	0.500

[a]ε is energy of orbital;
[b]Δ = ε(LUMO) - ε(HOMO);
[c]φ_0 = [ε(LUMO) - α]/Δ [15]; α= -3.56137 eV [17].

This could indicate the essential influence on the stacking interaction between the π-systems of both complex components [*Pharm-BioM*]. We could suppose that

biological activity should be connected firstly with the donor-acceptor properties of the interacted components. Naturally, the frontier molecular orbitals contribute to the determinative effects. Besides, the position of the frontier levels affects directly on the donor and acceptor ability of the conjugated molecules.

Also, a variation of the chemical constitution causes the relative position of the energy gap. Numerically, the energy gap can be characterized by its position in respect to the non-bonding level (Fermi level of π-electron) [17]; such a method will correlate with donor-acceptor property. Previously, basing on the frontier MO energies, it was proposed to call it as a donor-acceptor parameter (DAP); this parameter (signed as an index φ_0) can be estimated quantitatively [15].

By definition, a value α of neutral conjugated molecules (for example, for the long unsubstituted polyenes or acene series), corresponds to such dispositions of the frontier levels when the donor and acceptor properties are mutually balanced and hence: $\varphi_0 = 0.5$, i.e., the energy gap is situated symmetrically in respect to the imaginary level α [17]. If the energy gap is shifted up, then the parameter increases and $\varphi_0 > 0.5$; these relative positions of the frontier levels indicate on the predominately donor properties of the conjugated molecules. Inversely, if the parameter $\varphi_0 < 0.5$ and the energy gap is shifted down, then the molecule is predominately acceptor [14-17]. The calculated values φ_0 for the compounds **1-3** are collected in Table 2.

Firstly, analysis shows that only compound **1a**, X = NH is the true donor system: the energy gap middle is shifted up relatively the non-bonding level α and hence $\varphi_0 > 0.5$. In the heteroanalogues **1b** (X = O) and **1c** (X = S) energy gap is shifted down so that they are weak acceptor ($\varphi_0 \approx 0.46$). Table 2 shows that the nitrogenous cycle leads to the energy gap shift down so that all derivatives **2**,**3** become acceptor molecules. The difference between both isomers **2** and **3** is seen from Table 2 to be negligible: comparing of the parameter φ_0 for both corresponding molecules with the same heteroatom X shows that their acceptor capacity should be not considerably distinguished one each other.

As far as the dicoordinated nitrogen atoms with their LEPs can be involved in formation of the hydrogen bonds, let us their characteristics. Their atomic charges are presented in Table 3. Firstly, the atomic charge nitrogen atom depends considerably on the heteroatom X caused redistribution of the electron densities at the atoms of the compounds **1-3**: the calculations give the minimal negative charge at the dicoordinated nitrogen atom in the oxazole (X = O), while the charge maximum is obtained in the thiazole (X = S). Going to the corresponding annelated derivatives **2,3** is accompanied by a considerable increase of the atomic charges at the dicoordinated nitrogen of the five-membered cycle: the difference between both isomers is negligible. Also, the performed calculations give that that charges at the nitrogen atom in the six-membered cycle differ from the charges in the five-membered cycle and are weakly sensitive to the nature of the heteroatom X. Besides, these charges are practically no sensitive to isomerization.

Table 3. Charges at nitrogen atoms and energies of *n*-MOs in compounds **1-3**.

Compd	N(5)[a]			N(6)[b]		
	z[c], e.u.	n-MO	ε[d], eV	z[c], e.u.	n-MO	ε[d], eV
1a	-0.29	HOMO-2	-10.05	-	-	-
1b	-0.16	HOMO-2	-10.69	-	-	-
1c	-0.49	HOMO-2	-10.23	-	-	-
2a	-0.39	HOMO-3	-10.63	-0.50	HOMO-2	-8.99
2b	-0.26	HOMO-3	-11.22	-0.49	HOMO-1	-9.56
2c	-0.58	HOMO-3	-10.92	-0.48	HOMO-2	-9.28
3a	-0.39	HOMO-3	-10.41	-0.46	HOMO-1	-9.06
3b	-0.26	HOMO-3	-10.91	-0.45	HOMO-1	-9.60
3c	-0.57	HOMO-3	-10.49	-0.45	HOMO-1	-9.41

[a]N(5) is Nitrogen atom in five-membered cycle compounds 1-3;
[b]N(6) is Nitrogen atom in six-membered cycle compounds 2-3;
[c]z is charge at atoms N(5) and N(6) respectively;
[d]ε_n is energy of corresponding n-MO
e.u. is electron units.

Also, the position of the level corresponding to n-MO (LEP) was calculated. One can see from Figure 2 and Figure 3 that this orbital is directed perpendicularly to π-MOs and hence can interact with the σ-orbitals. When two *n*-MOs appear in bicyclic molecules **2**, **3**, they interact between them (and with σ-MOs); then *n*-MOs are mixed and are localized at both dicoordinated nitrogen atoms. The positions and energies of corresponding *n*-levels are collected in Table 3. It shows that the *n*-level is the HOMO-2 in the monocyclic molecules **1**; the expansion of the π-system upon going to the bicyclic molecules **2**, **3** (and hence the appearance of the new π-levels near the energy gap) positions and energies of the *n*-levels can differ.

In the monocyclic molecules **1**, the energy of the *n*-MO decreases in the series X = NH, O, S. In the bicyclic systems **2**, **3** with two LEPs, the level splitting causes an appearance of two splitted levels; their energies are weakly sensitive to isomerization, especially second splitted *n*-level. The first splitting levels in the isomers **2** are somewhat shifted lower than in the corresponding isomers **3**. Thus, the sensitivity of the electronic properties of the heterocycles **1-3** to their chemical constitution should be manifested in the interaction with the biomolecules, i.e. in the biological affinity of the heterocycles **1-3**.

Intermolecular binding affinity: interaction between pharmacophore and biomolecule fragments

In this paper, the biological affinity of any potential pharmacophore will be understood as its capacity to effectively interact with certain fragments of a biological molecule, so that the pharmacophore *(Pharm)* and biomolecule *(BioM)* could generate the stable complex: [*Pharm*] + [*BioM*] $\leftrightarrow$ [*Pharm-BioM*]; then the pharmacological effect can occur. The effectiveness of such an effect should depend on the [*Pharm-BioM*] complex stability, which in turn depends on the geometric complementarity of

both complex components. It is well known [26] that proteins usually form branched polypeptide chains connected by numerical hydrogen bonds (mutual interactions of fragments -CO-NH-). Therefore, the oxygen and nitrogen atoms of these groups cannot interact with foreign molecules (pharmacophores). But there are residues of proteinogenic amino acids available for intermolecular interactions with the pharmacophore.

The main feature of the studied heterocycles **1-3** is their branched system of π-electrons. Then, the effective electron interaction with the non-conjugated amino acid residues is evidently no effective; we could assume that only the water environment enforces all organic molecules to approach together. In contrast, the interactions with conjugated molecular fragments can give the additional contribution (by stacking interaction) in the total stabilization of [*Pharm-BioM*] complex. There are only four aromatic amino acids: Phe, Tyr, Trp and His. These peptide fragments are spatially commensurable with the conjugated molecules **1-3**, and hence just their interactions can additionally stabilized the [*Pharm-BioM*] complex, i.e., increases the binding affinity.

Also, one can see from the chemical formulae 1-3 that the studied molecules contain one (compounds 1) or two (compounds 2,3) dicoordinated nitrogen atoms with LEPs; these electron pairs (situated perpendicularly to the conjugated system) can effectively generate the hydrogen bonds with that amino-acid rests containing the OH or $-NH_2$ or -SH groups and hence can produce the specific complex – H-B complex (a complex formed with the help of hydrogen bonds). Thereafter, a capacity to form the π-complex will be treated as a *π-electron affinity* component; similarly, a capacity to form the complex by the hydrogen bonds can be named as a *H-B affinity* component.

These properties above can be estimated by direct quantum-chemical modeling. Thus, the many events of the [*Pharm-BioM*] interactions can be modeled by elementary interactions between the pharmacophore and some fragments of the biological molecules, taking into consideration the complementarity of the *Pharm* components. Similarly, to the well-known FBDD approach [4] taking into consideration the namely molecular fragments of the pharmacophore, we will call our method a fragment-to-fragments approach, so as not only the chemical constitution of the pharmacophore fragments is considered in detail, but also the chemical constitution of the biomolecule fragments takes similarly into account. Then, just approach is used in this paper.

π-π-Interaction in stacking [Pharm-BioM] complex

Here, we will examine the intermolecular interaction in the π-electron complex' generated by oxazole and its heteroanalogues **1** as well as by both isomers **2** and **3**; their chemical constitution should influence evidently on the stability of such complex. Generally, the interaction of two π-electron systems is estimated by the relative positions of the molecular levels of both molecules as well as the overlapping of their π-systems; in MO approximation, the interaction energy ΔE is quantitatively calculated in the approximation of the interaction of MO [18].

Here, we will restrict only two amino acid residues: phenyl-alanine (Phe) and tryptophan (Trp) acids. Then, in our study of the π-π-density interaction, we simulate the outside radicals by the model molecules: Het-CH_3, where Het is the conjugated heterocycle of the corresponding amino acids. So, the phenyl-alanine is modeled by the toluene (Phe-CH_3); its π-affinity parameter φ_0 = 0.52 (φ_0 = 0.53 for the phenyl alanine acid). The tryptophan is modeled by 3-methyl benzoindolenine (BIn-CH_3); its π-affinity parameter φ_0 = 0.60 (φ_0 = 0.62 for the corresponding tryptophan acid).

In the studied [*Pharm-BioM*] complex**,** the distance between the components (i.e., between planes of the molecules **1-3** and plane of the aromatic residues is initially 3.4 Å) as in DNA helix [27] or in polymethine dye aggregates [28]. The initial mutual arrangements in the π-complex of the molecule **1** (X = O) and model fragments are pictured in Figure 4 (in two planes):

To simplify, the binding energy ($E_{binding}$) in the complex is estimated as the difference of the total energy of the generated [*Pharm-BioM*] complex and energies of both its components, i.e., in the stable complex, the binding energy is additional stabilization energy [29-30]. The calculated binding energies for the optimized complex are collected and presented in Table 4; so as far the areas of the bicycle molecules **1,2** and monocycle **3** are incommensurable, then comparing of their stabilizations did not carry out.

The performed calculations show that all complexes should be stable: formation of the complexes with pharmacophore and model biofragments leads to decreasing of total energy of the generated complex in compare with energies of the initial components. Perhaps, absolute values of the are stabilization energies, ΔE, could be somewhat overestimated, nevertheless, they are sensitive to chemical constitution. Thus, the proposed approach enables to study the dependence of the biological affinity on features of molecular topology, and hence, to establish the general regularities between chemical structure of pharmacophores and different components of their biological activity. So, can conclude that the presence of the branched conjugated system increases the stability of the possible π-complexes with the appropriate fragments of the biomolecules.

As regard to the chemical constitution of the molecules studied here, the data in Table 4 show that the influence of replacing of the heteroatom X on the calculated stabilization energy in the [oxazole:Phe-CH_3] complex is negligible, while the similar [oxazole:BIn-CH_3] complex with model are more sensitive to nature of heteroatom X. Perhaps, it connects with the more overlapping of the conjugated system of both complex components in [oxazole: BIn-CH_3]. Besides, one should take into consideration, that

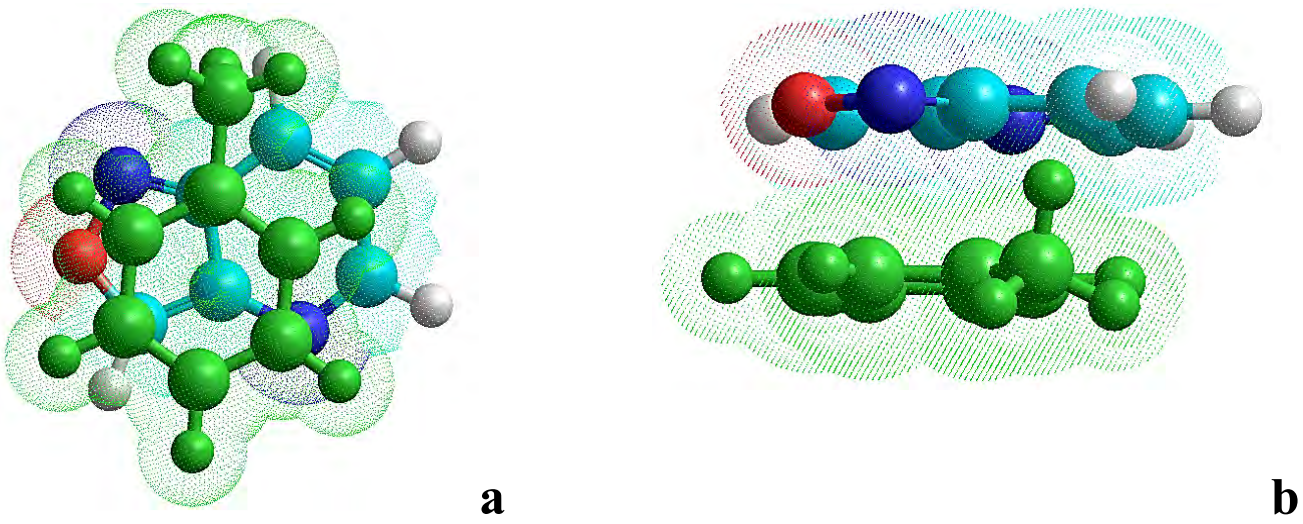


Figure 4. Mutual arrangement of both components in π-π-complex [*Pharm-BioM*]: a) compound **2** with phenylalanine residue in X-Y plane; b) compound **2** with a phenylalanine residue in X-Z plane.

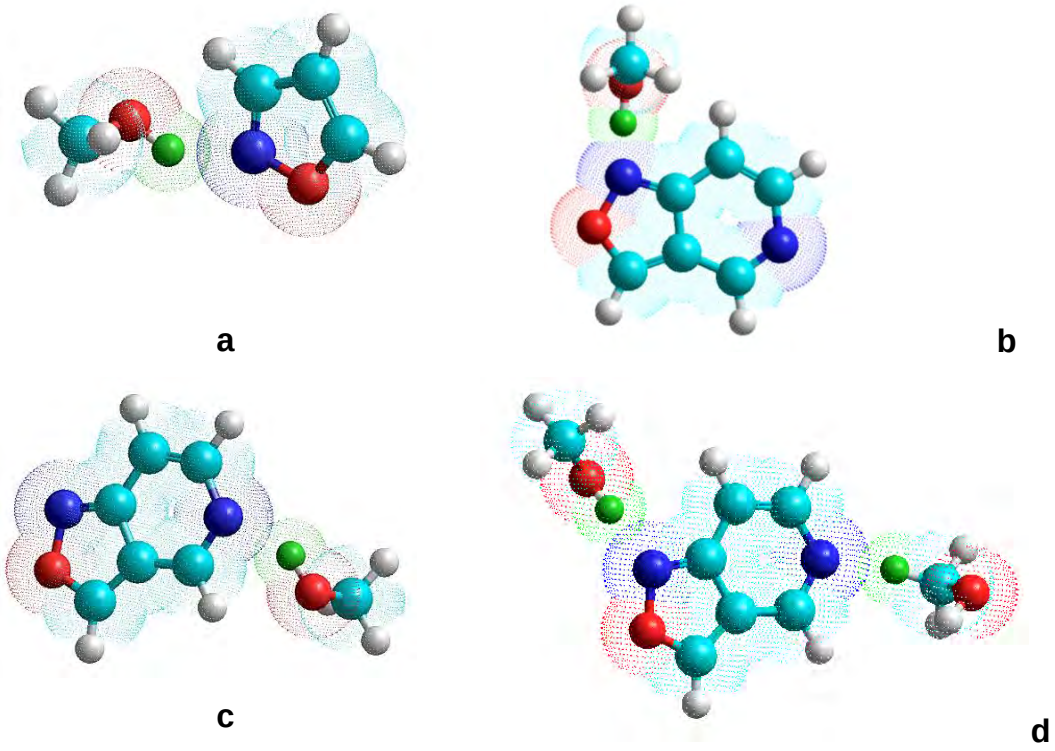


Figure 5. Possible types of H-B complex with CH_3OH with oxazoles – [oxazole:H-X].

Table 4. Stability of π-complexes compounds **2**, **3** with model biofragments.

Compd	X	E_{mol}[a], a.u.	[Oxazole:Phe-CH_3]		[Oxazole: BIn-CH_3]	
			E_{compl}[b], a.u.	ΔE[c], kcal/mol	E_{compl}, a.u.	ΔE, kcal/mol
2a	NH	-395.7	-667.2	-7.15	-798.8	-9.55
2b	O	-415.6	-687.0	-7.93	-818.6	-10.80
2c	S	-738.6	-1010.1	-7.65	-1141.6	-8.82
3a	NH	-395.7	-667.2	-8.89	-798.8	-12.88
3b	O	-415.6	-687.0	-9.21	-818.6	-9.36
3c	S	-738.6	-1010.1	-9.41	-1141.6	-12.49
Phe-CH_3		-271.5				
BIn-CH_3		-403.0				

[a]E_{compd} is total energy of compounds;
[b]E_{compl} is total energy of [*Pharm-BioM*] complex;
[c]ΔE is binding energy increases only the stability of the formed complex.

tryptophan shows more donor properties than phenylalanine, then the generated [oxazole:BIn-CH_3] complex with the acceptor molecules **2**, **3** should be more stable than the [oxazole:Phe-CH_3] complex with the same pharmacophores. Indeed, the performed calculations confirm this assumption: going from the complex with model phenylalanine to the complex with model tryptophan increases every time the stabilization energy both complexes. Isomers **3** form more stable π-complexes with model phenylalanine than isomers **2**. In contrast to model tryptophan, where the stabilization energy of the π-complex is sensitive to the heteroatom rather than to the isomer.

Hydrogen bonding

There are some amino acids which contain their residues with groups X-H can potentially form the hydrogen bonds, for example, lysine, arginine, histidine, or other groups containing active hydrogen (-NH, -OH and -SH) can provide the required proton. On the other hand, the oxazolo-pyridines **2-3** contain the dicoordinated nitrogen atoms with LEP which can promote such non-covalent bonds as an acceptor. For sake a comparison, the hydrogen bonds of the oxazole **1** (and its hetaryl-containing analogues) were also calculated. The residues of the potential donor components were modeled by the methyl groups, proposing optimistically that the influence of the non-conjugated part the amino acids is negligible, i.e., the biocomponent in H-B complexes is modeled by the simpler molecule: H_3C-Y where Y = OH, NH, SH.

For annelated molecules **2**, **3** two possible ways of generation of the model H-B complex fixed by the hydrogen bonds. The possible complexes of the model molecule H_3C-O-H with oxazoles **1b**, **2b**, **3b** as well as with its annelated derivatives are pictured in Figure 5.

At the beginning, let us consider the H-B complex with the simpler molecules **1**; for them one hydrogen bond can be generated with the dicoordinated nitrogen atom. The calculated stabilization energies of the possible H-B complex was collected in Table 4.

The length of hydrogen bond in the generated [oxazole **1**:H-X] complexes depends weakly on nature of the compounds **1a**, **1b**, **1c** and/or H-X (l = ≈ 2 ÷ 2.3 Å); although the negative charge at the dicoordinated nitrogen atom changes depends considerably on nature of the heteroatom X in the oxazole hetaryl-containing analogues (see Table 2).

Analysis of the calculated energies of the hydrogen bonds in the [oxazole **1**:H-X] complexes shows the oxazole **1b** gives the maximum value for the complex with the model molecule CH_3OH. Going to pyrazole **1a** increases stabilization of the similar complex, while going to thiazole **1c** decreases the stability of the generated complex on ≈ 0.7 kcal/mol.

In the same time, replacing of the proton-donor molecule CH_3OH by the amino-analog (CH_3NH_2) or on the thiol analog (CH_3SH) is seen from Table 4 to be accompanied by appreciable decreasing of the bonding energy in [oxazole **1**:H-X] complex.

The chemical modification of the oxazoles **2,3** by the condensed acceptor pyridine cycle influences essentially on the affinity of the pharmacophore; the calculated binding energies of possible H-B complexes with model donor component H_3C-OH are collected in Table 5.

First of all, let us compare the calculated values ΔE for the [oxazole **2**:H-O-CH_3] complex and [oxazole **3**: H-O-CH_3] complex with the hydrogen bonding involved the di-coordinated nitrogen atom in five-membered oxazole cycle presented in Table 5 with the corresponding complex [oxazole **1**:H-O-CH_3] complex in Table 4. One can see that such annelation of the pyrazole **1a** giving the isomer **2** does practically not change binding energy for the H-B complex, while the similar H-B complex which the corresponding isomer **3** is appreciable destabilized on ≈2.5 kcal/mol.

Table 5. Hydrogen bond energy of complex [oxazole **1**:H-X] with model methanol, methylamine, and methanethiol.

Complex	H-X	l^a, Å	$E_{compd}{}^b$, a.u.	$E_{compl}{}^c$, a.u.	ΔE^d, kcal/mol
[oxazole **1b**:H-X]	H-O-CH_3	2.067	-245.9	-361.6	**-8.09**
[oxazole **1b**:H-X]	H_2N-CH_3	2.295	-245.9	-341.8	**-4.16**
[oxazole **1b**:H-X]	H-S-CH_3	2.268	-245.9	-684.6	**-3.72**
[oxazole **1a**:H-X]	H-O-CH_3	2.062	-226.1	-341.8	**-12.06**
[oxazole **1c**:H-X]	H-O-CH_3	1.988	-569.0	-684.7	**-7.30**
	H_2N-CH_3		-95.8	-	-
	H-O-CH_3		-115.7	-	-
	H-S-CH_3		-438.7	-	-

[a]*l* is the length of the hydrogen bond;
[b]E_{compd} is the energy of the compounds;
[c]E_{compl} is the energy of the [oxazole:H-X] complex;
ΔE is the stabilization energy.

Table 6. Stability of hydrogen bond energy [oxazole:H-O-CH_3] complex with compounds **2**, **3**.

Complex	[Oxazole:H-O-CH_3] by N(5)[a]			[Oxazole:H-O-CH_3] by N(6)[b]		
	l, Å	E_{compl}, a.u.	ΔE, kcal/mol	*l*, Å	E_{compl}, a.u.	ΔE, kcal/mol
[oxazole **2a**:H-O-CH_3]	2.062	-511.4	-12.31	1.955	-511.4	-12.22
[oxazole **2b**:H-O-CH_3]	2.040	-531.3	-10.11	1.992	-531.3	-8.91
[oxazole **2c**:H-O-CH_3]	2.003	-854.3	-10.43	1.973	-854.3	-11.65
[oxazole **3a**:H-O-CH_3]	1.983	-511.4	-9.93	1.937	-511.4	-9.96
[oxazole **3b**:H-O-CH_3]	2.047	-531.3	-9.58	1.966	-531.3	-9.65
[oxazole **3c**:H-O-CH_3]	2.012	-854.3	-9.98	1.954	-854.3	-9.82

[a][oxazole:H-O-CH_3] by N(5) involves dicoordinated nitrogen in five-membered cycle of compounds **2**,**3**
[b][oxazole:H-O-CH_3] by N(6) involves dicoordinated nitrogen in six-membered cycle of compounds **2**,**3**.

The annelation of the azoles **1** is sensitive to the expansion of the conjugation system of the pharmacophore; so, going from molecule **1b** (X = O) to corresponding **2b** or its isomer **3b** is accompanied by increasing of generated H-B complex with same place of the formation of the hydrogen bonding approximately on ≈ 2 kcal/mol. At the same time, for the thiol-containing heteroanalogues, annelation by the pyridine cycle leads to the essential increasing of the stabilization of the corresponding H-B complex practically on the 3.0 kcal/mol.

In compare with the five-membered nitrogenous circle, the compounds **2**, **3** contain the additional LEP at the di-coordinated nitrogen atom in the pyridine cycle. Comparing of two possible ways of generated H-B complex by N(5) and N(6), presented in Table 6, one can see that the stabilities of the H-B complex for both isomers **2** and **3** are close each other both in N(5) and according to N(6).

Analyzing the energy for the [oxazole **2**:H-O-CH_3] complexes and the [oxazole **3**:H-O-CH_3] complexes by N(5) and N(6), one can see that complexes **2** are more stable than complexes **3**, except for H-B complex by N(6) oxazole derivative: isomer **3b** is more stable (≈ 1 kcal/mol).

It should be noted that the annelation and the heterosubstitution have little effect on the length of the formed hydrogen bond, especially in isomers **2** and **3**. Thus, it can be assumed that annelation of heterocycles **1a-1c** with a pyridine ring leads to a greater stabilization of the complex, and, consequently, to an increase in on the binding affinity.

Conclusions

Scrupulous *in silico* study of the conjugated pharmacophores based on heteroazoles and their pyridocondenced derivatives, in the framework of fragment-to-fragment approach, shows that expansion of the π-electron system by annelation causes the shifting up of the high levels, while the appearance of new the di-coordinated nitrogen atom is accompanied by decreasing of the donor-acceptor property overall system. The numeral calculations of the possible complexes of the studied

heterocycles **1-3** with model fragments of the biomolecules give that stabilization energy [*Pharm-BioM*] π-complex is increases upon going from the phenyl alanine to the high donor triptophan. Annelation of compounds **1** with the pyridine ring reduced the stabilization energy of the π-complexes in comparison with the expansion of the π-electron system by introducing phenyl radicals into positions 2 and 5 of the oxazole ring.

Calculations of the stabilization energies of *H-B complexes* with model proton-donor biomolecules show that the ΔE increases upon annelation of oxazole (and its heteroanalogues) with pyridine rings. It should also be noted that the expansion of the π-electron system by introducing phenyl radicals into positions 2 and 5 of the oxazole ring leads to a decrease in the stabilization energy of the complexes in comparison with the annelation of the heterocycles by the pyridine ring [18].

Notes

The authors declare no conflict of interest.

Author contributions. **Ye. S. V.**: provision of study materials, computing resources, or other analysis tools. **N. V. O.**: formulation or evolution of overarching research goals and aims, application of statistical, mathematical, computational, or other formal techniques to analyze study data. **S. G. P.**: development and design of methodology; creation of models, provision of study materials, computing resources, or other analysis tools. **M. V. K.**: preparation, creation and presentation of the published work, specifically visualization. **O. D. K.**: ideas; formulation or evolution of overarching research goals and aims, development or design of methodology; creation of models. **V. S. B.** ideas; formulation or development of common goals and objectives of the research, verification of results, responsibility for managing and coordinating the planning and implementation of research activities.

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In silico дослідження афінності зв'язування азотистих біциклічних гетероциклів: пофрагментний підхід

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Резюме: В рамках підходу «фрагмент до фрагменту» представлені *in silico* результати біологічної спорідненості гетероциклів та їх похідних, конденсованих з піридином. Така модель використовується для дослідження взаємодії між фармакофорами та біомолекулами. Детальний аналіз показує, що розширення π-електронної системи шляхом конденсації гетероциклічних систем молекулою піридину викликає зміщення вищих зайнятих молекулярних рівнів, тоді як поява додаткового двох-координованого атома азоту супроводжується зменшенням їх донорно-акцепторних властивостей. Розрахунки можливих π-комплексів досліджуваних гетероциклів **1-3** із модельними фрагментами ароматичних амінокислот, утворених за механізмом π-стекової взаємодії, показують збільшення енергії стабілізації π-комплексів при переході від фенілаланіну до триптофану. Розрахунок енергій стабілізації комплексів фармакофорів з модельними протон-донорними залишками амінокислот за механізмом водневого зв'язку (H-B комплекс) показує, що ΔE збільшується при переході від моногетероциклів до їх конденсованих похідних. Розширення π-електронної системи сполук **1** піридиновим циклом зменшило енергію стабілізації π-комплексів та H-B комплексів у порівнянні з розширенням π-електронної системи шляхом введення фенільних радикалів у положення 2 та 5 оксазольного кільця [18].

Ключові слова: підхід «фрагмент до фрагмента», афінність зв'язування, комплекс [*Фармакофор-Біомолекула*], π-стекінгова взаємодія, водневі зв'язки.