VILNIUS UNIVERSITY

Jurgis Sūdžius

SYNTHESIS AND PROPERTIES OF PYRIMIDINE DERIVATIVES – POTENT CARBONIC ANHYDRASE INHIBITORS

Summary of doctoral dissertation

Physical sciences, chemistry (03P)

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VILNIAUS UNIVERSITETAS

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PIRIMIDNO DARINIŲ – POTENCIALIŲ KARBOANHIDRAZIŲ SLOPIKLIŲ – SINTEZĖ IR SAVYBĖS

Daktaro disertacijos santrauka

Fiziniai mokslai, chemija (03P)

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Disertacija parengta 2006 – 2010 m. Vilniaus Univesitete, Chemijos fakultete

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Doc. dr. Algirdas Brukštus (Vilniaus Universitetas, fiziniai mokslai, chemija – 03P).

Disertacija bus ginama viešame chemijos mokslo krypties tarybos posėdyje 2011 m. gegužės 13 d. 13 val. Vilniaus Universiteto Chemijos fakulteto Neorganinės chemijos auditorijoje.

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Introduction

Reversible CO_2 hydration and conversion into bicarbonate and protons (eq. 1) is a simple but vital physiological reaction.

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \quad (1)$$

This reaction in human organism is essential in many physiological and pathological processes, such as respiration, CO_2 and bicarbonate distribution between metabolic tissues and lungs, pH and CO_2 homeostasis, exchange of electrolytes in various tissues and organs, biosynthetic reactions, resorbtion in bones, calcification, acidification of the extracellular environment around hypoxic tumor cells, etc. Reversible CO_2 hydration in organism occurs spontaneously yet at a low rate, thus, it is catalyzed by extremely efficient enzymes – carbonic anhydrases (CAs).

CAs also catalyze several other hydration and hydrolysis reactions (eq. 2 – 8), but it is still unclear if this catalysis plays important role in the organism [1]. On the other hand, the inhibition of CAs with certain compounds (thioxolone [2], cumarins and thiocumarins [3]) is based on esterase activity of CAs.

$$RCHO + H_2O \rightleftharpoons RCH(OH)_2 \quad (2)$$

$$RCO_2Ar + H_2O \rightleftharpoons RCO_2H + ArOH \quad (3)$$

$$RSO_3Ar + H_2O \rightleftharpoons RSO_3H + ArOH \quad (4)$$

$$ArOPO_3^{2-} + H_2O \rightleftharpoons HPO_4^{2-} + ArOH \quad (5)$$

$$ArF + H_2O \rightleftharpoons ArOH + HF \quad (6)$$

$$RSO_2Cl + H_2O \rightleftharpoons RSO_3H + HCl \quad (7)$$

$$NH_2CN + H_2O \rightleftharpoons CO(NH_2)_2 \quad (8)$$

Since CAs are ubiquitous enzymes, various malfunctions, especially hyperfunction cause various organism disorders and diseases [1, 4, 5]. The impairment of renal activity caused by cytosolic CAII and membrane bound or transmembrane CAIV, XII and XIV hyperfunction is treated by diuretics inhibiting these carbonic anhydrases. Glaucoma is usually treated by locally inhibiting CAII. Blood coagulation disorders are partially related to CAI, which is one of the many participants in this process. Recent research has demonstrated that inhibition of CAII and mitochondrial CAVA and CAVB during the lipogenesis, can be used for treatment of obesity. Inhibition of CAIX and CAXII, which play important role in tumour genesis (by acidifying the tissue medium), allows to fight oncogenic diseases. The hyperfunction of CAII, IV and XIV prevailing in the bone tissues causes increased transport of inorganic components via osteoclastic membrane to the exterior of the cell, thus causing the loss of calcium ions. Consequently, CA inhibitors can be used as preparations against osteoporosis.

There are approximately 30 clinically used drugs or agents in clinical development which show significant CA inhibitory activity [4]. High scientific relevance of CA inhibition can be illustrated by the fact that articles in journal Nature involving CA inhibition are published since the discovery of the first CA inhibitor in 1940 [6] until now [4].

Unfortunately, high level of CA homology and wide distribution of the CAs in organism are related to low selectivity of inhibitors. Consequently, most of inhibitors used in current medicinal practice exhibit various side effects, such as limb numbness and tingling, metallic taste, depression, fatigue, weight loss, decreased libido, renal stones, gastrointernal irritation, metabolic acidosis or temporal myopia. Taking into

account all these shortcomings, the greatest challenge in CA inhibitor synthesis is their selectivity to particular isoform.

Schematic structure of classical CA inhibitors is shown in Fig 1. Main components of CA inhibitors are the zinc binding headgroup, the ring and the tail fragment. The zinc binding group and ring fragment are mainly responsible for systematic inhibition of CAs and the tail fragment mainly affects selectivity to certain CA isoform.

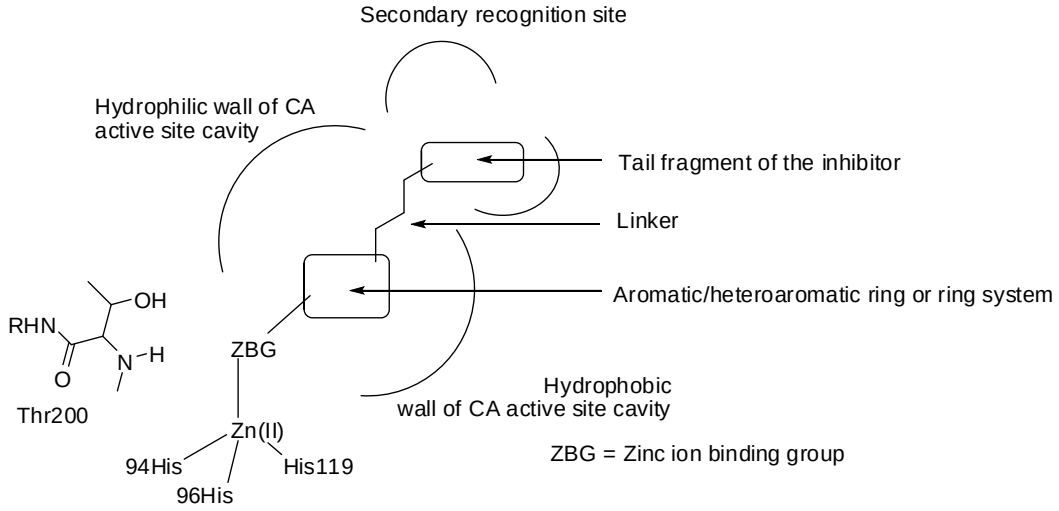


Fig. 1. Schematic structure of classical CA inhibitors

Aim of the work

Rational design, synthesis and study of hCA inhibitors based on pyrimidine heterocycles containing benzenesulfonamide moiety.

Tasks of the work

1) Theoretical investigation of interaction of 4-[*N*-(pyrimidin-4-yl)amino]-benzenesulfonamides with an active site of hCA isoforms. The main goals were to determine whether 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]benzenesulfonamides fit for the active site cavity of the enzyme and to approximately evaluatethe influence of various substituents at the pyrimidine ring to the binding capability of inhibitor to hCAs.

2) Synthesis of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]benzenesulfonamides and related heterocycles, developement of hCA inhibitors, simultaneously evaluating their binding properties to hCA isoforms. Modification of inhibitors by the variation of linker length between benezenesulfonamide and heterocyclic fragments and introduction of various substituents at the pyrimidine ring and constructing fused heterocyclic systems were also of interest.

Scientific novelty of the dissertation

Condensation of 2,4,6-substituted pyrimidine-5-carbaldehydes with indoline-2-thiones was investigated. It was shown that absence of active leaving groups at 4th and 6th position of 4,6-disubstituted pyrimidine-5-carbaldehyde results in a formation of *E*-[3-(5-pyrimidinylmethylene)indoline-2-thiones. Condensation of pyrimidine-5-carbaldehydes containing active leaving group, i.e. 4-chloropyrimidine-5-carbaldehydes with indoline-2-thiones results in a formation of novel heterocyclic systems – pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole and 5*H*-pyrimido[4′,5′:5,6][1,3]thiazino[3,2-*a*]indole. The reactivity of 4-chloro-2-methylthiopyrimido [4′,5′:5,6]thiopyrano[2,3-*b*]indole towards nucleophiles was also investigated.

Simple and effective procedures for synthesis of potencial hCA inhibitors – 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]benzenesulfonamides were developed.

Interaction of hCAI, II, VII and XIII with synthesized compounds was investigated. 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]benzenesulfonamides were found to be moderate – strong classical hCA inhibitors possessing increased affinity to hCAI, II or XIII.

Practical significance of the dissertation

4-[*N*-(pyrimidin-4-yl)amino]benzenesulfonamides represents a new class of CA inhibitors and may serve as a lead compounds for further development of selective hCAI, II or XIII inhibitors.

Main statements of the dissertation

- Reaction of indoline-2-thiones with pyrimidine-5-carbaldehydes that do not contain active leaving groups at positions 4 and 6 of the pyrimidine ring leads to the formation of (*E*)-3-(5-pyrimidinylmethylene)indoline-2-thiones. The latter can participate in Hetero-Diels-Alder reaction with 2-butyne-1,4-dicarboxylic acid dimethyl ester to form corresponding thiopyrano[2,3-*b*]indoles. Reaction between indoline-5-thiones and 4-chloropyrimidine-5-carbaldehydes yields pyrimido[4′,5′:5,6]-thiopyrano[2,3-*b*]indoles and 5*H*-pyrimido[4′,5′:5,6][1,3]thiazino[3,2-*a*]indoles.
- A convenient method for synthesis of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl) amino]benzenesulfonamides containing ciano, formyl and nitro groups at the position 5 of the pyrimidine ring is the substitution reaction of chloro group in the corresponding 4,6-dichloropyrimidines with 4-amino(alkyl)benzenesulfonamides. Further modification of the synthesized compounds can be performed by substitution of remaining chloro group at 6^{th} position of pyrimidine ring with various nucleophiles.
- Theoretical investigation of binding of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl) amino]benzenesulfonamides and 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-b]indol-4-yl)]amino}benzenesulfonamides to hCAs allows to evaluate interaction of these compounds with an active site of the enzymes.
- The synthesised 4-[*N*-(2,5,6-substituted pyrimidin-4-yl) amino]benzenesulfonamides bind to hCAs as classical CA inhibitors and their activity is determined by interaction of sulfonamide group with catalytic Zn ion and stacking of pyrimidine ring with phenyl group of Phe131.

Scientific approbation and publication of presented work

The results of this study have been published in 7 publications: 2 articles are published in journals included in the Thompson Reuters ISI database, 2 poster presentations at international conferences and 3 poster presentations at national conferences.

Contents of the dissertation

The dissertation is written in Lithuanian on 142 pages and includes 12 tables, 64 figures, graphs and schemes, 163 references and 1 appendix.

Results and Discussion

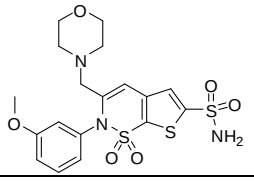
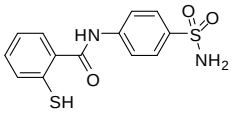
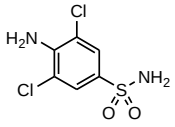
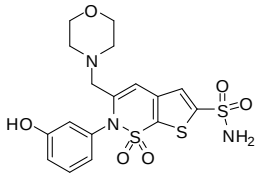
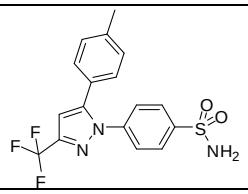
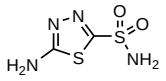
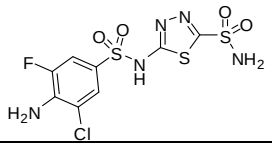
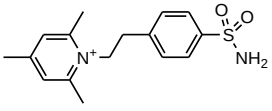
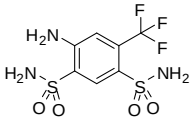
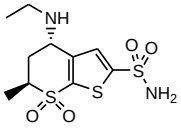
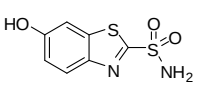
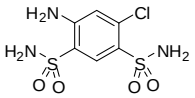
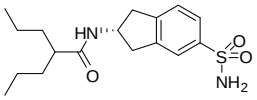
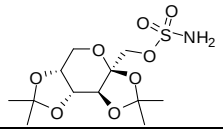
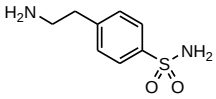
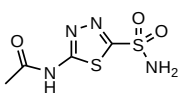
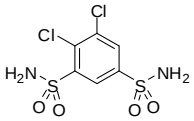
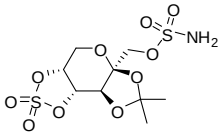
1. Theoretical investigation (docking) of binding of hCA inhibitors to the enzyme

Docking is a computational technique that samples conformations of small molecules in protein binding sites; scoring functions are used to assess which of these conformations best complements the protein binding site. Docking is often applied to screen large databases of potential drugs to identify compounds that are likely to bind target protein. There are many docking programs, both commercial and open source, that differ in docking algorithms and scoring functions, although none of them might be singled out as the best [7]. The selection of eHiTS [8, 9] program was caused by the free academic license and the capability to evaluate a protonation state of the ligand and the ease to handle it.

1.1. Docking of the protein – ligand complexes of known structure

In order to evaluate the capability of eHiTS to predict binding of ligands to carbonic anhydrases the following data was collected: experimental binding constants K_i or K_d, crystallographic data of ligand – protein complexes. There were 20 complexes (18 for hCAII, 1 for hCAI and 1 for hCAXII) for which both crystallographic and enzymologic data are available. The ligands were extracted from the complexes and the set 1 of compounds was built (table 1.1-1).

Table 1.1-1. Compound set 1.

No.	Compound	No.	Compound	No.	Compound
1		7		13	
2		8		14	
3		9		15	
4		10		16	
5		11		17	
6		12		18	

The selected compounds were docked using eHiTS v6.2 with default settings. An unoptimized protein and ligand structures extracted from the crystallographic data were used. The lowest energy pose of a ligand in an active site of protein does not always match the crystallographic data well. General orientation of the ligand is predicted correctly: the sulfonamide head of a ligand is directed towards the catalytic zinc ion of the carbonic anhydrase. Major inaccuracies were observed in the position of tail fragment of the inhibitor. Such inaccuracies are mainly caused by the lack of water molecules in the docked system. As an example might serve the hCAI – acetazolamide complex, where orientation of acetazolamide is determined by hydrogen bond network of the amide moiety, two water molecules and side chain of His200 (Fig 1.1-1). In this case whole acetazolamide – water system should be considered as a ligand. Unfortunately, it is impossible predict such interactions in advance.

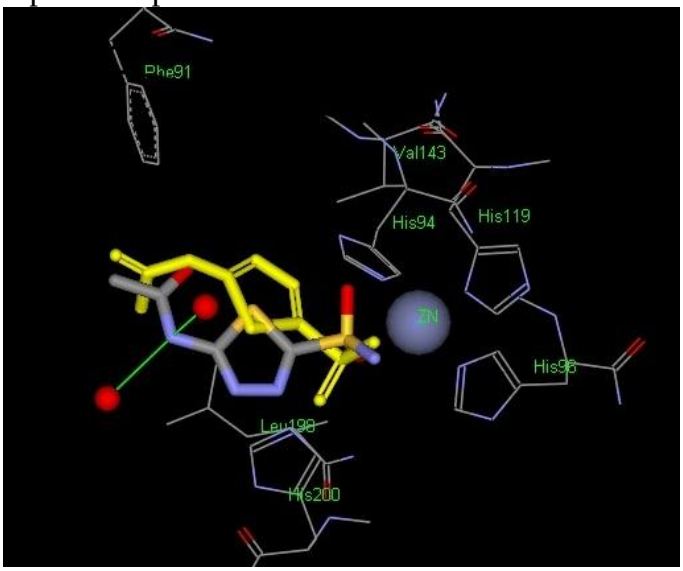


Fig. 1.1.-1. Pose of acetazolamide in hCAI active site. X-Ray determined (coloured, PDB entry 1azm) and produced by docking (yellow) lowest energy structure

If water does not play significant role in ligand binding to the active site of protein, program eHiTS evaluates pose of the ligand with acceptable level of similarity (Fig. 1.1.-2).

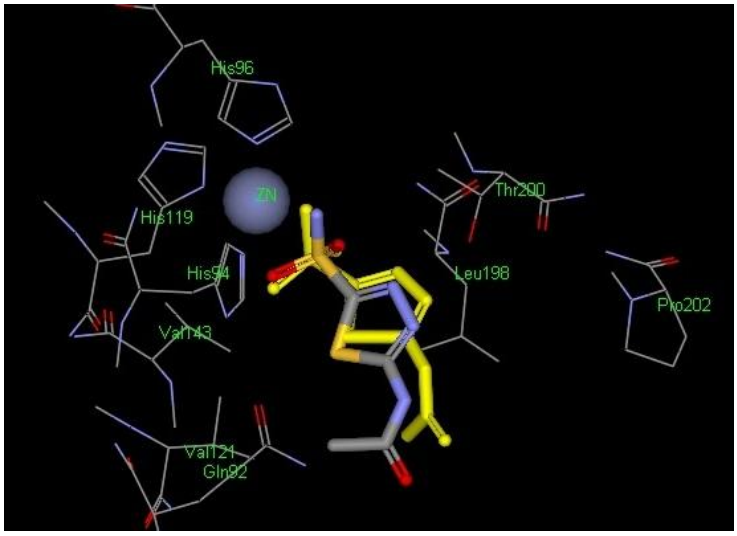


Fig.1.1.-2. Pose of acetazolamide in hCAXII active site. X-Ray determined (coloured, PDB entry 1jd0) and produced by docking (yellow) lowest energy structure

Since both systematic activity and selectivity of inhibitors are properties of interest, the ability of eHiTS to predict binding of compounds to active site of enzyme quantitatively was investigated. Thus the scores of three lowest energy water–free complexes produced by docking were compared to corresponding inhibition data (table 1.1-2).

Table 1.1-2. Experimentally determined inhibition constants (pK_{iE}) and produced by docking inhibition constants for the first (pK_{i1}), second (pK_{i2}) and third (pK_{i3}) lowest energy complexes for compound set 1.

Ligand	hCA	PDB entry	pK_{iE}	pK_{i1}	pK_{i2}	pK_{i3}
1	II	1i8z	-9.824	-9.582	-8.08	-7.944
2	II	1i91	-9.824	-10.225	-9.232	-8.119
3	II	2hoc	-9.523	-8.84	-8.814	-7.823
4	II	1cil	-8.046	-8.545	-8.47	-8.136
5	II	2qo8	-8.046	-7.62	-7.317	-6.842
6	II	1yda	-7.921	-8.009	-7.229	-7.194
7	II	2hd6	-7.796	-8.773	-7.844	-7.819
8	II	1oq5	-7.678	-9.717	-9.681	-9.602
9	II	1ze8	-7.678	-9.986	-9.652	-9.156
10	II	1zfg	-7.523	-9.199	-9.121	-8.664
11	II	1eou	-7.444	-6.498	-6.137	-5.855
12	II	2pou	-7.42	-10.612	-10.129	-10.071
13	II	1zge	-7.301	-7.815	-7.175	-7.127
14	II	2hnc	-7.222	-6.837	-6.075	-6.042
15	II	2pow	-7.201	-9.884	-9.758	-9.078
16	II	2pov	-7.125	-10.834	-10.336	-10.221
17	II	2nng	-6.796	-8.023	-7.918	-7.489
18	II	2h15	-5.671	-6.324	-6.28	-6.151
6	I	1azm	-6.602	-7.781	-7.382	-7.377
6	XII	1jd0	-8.244	-7.983	-7.708	-7.583

The correlation between experimental pK_i′s and pK_i′s produced by docking is low in all cases, although models of the lowest energy complexes represent experimental binding data the best $pK_{iE} = 0.5055 \times pK_{i1} - 4.7399$ ($R^2 = 0.1523$).

1.2. Crossdocking

Since program eHiTS treats protein as rigid structure some inaccuracies in docking may be caused by protein flexibility. Thus, it is important to determine available protein conformer which statistically gives the best correlation between experimental pK_i′s and pK_i′s produced by docking. The crossdocking experiment involves docking of the same compound set to different protein entries and allows select the most statistically suitable protein conformer. Crossdocking was applied to hCAII complexes which have no co-crystallized compounds other than water and ligand. This gave 6 protein entries. Additionally docking was performed into the complex whose structure was determined with the highest resolution (0.99 Å) and to the free enzyme. The set of ligands was the compound set 1. The docking data are given in Table 1.2.-1. Correlation between inhibition constants obtained experimentally and produced by docking was low or absent ($R^2 = 0.0036 – 0.2402$). Since protein from PDB entry 2fou gave the best correlation ($pK_{iE} = 0.5121 \times pK_{iD} - 4.7804$, $R^2 = 0.2402$), further docking to hCAII experiments were performed using this conformer.

Table 1.2.-1. Experimentally determined hCAII inhibition constants (pK_{iE}) and inhibition constants produced by docking compound set 1 to hCAII from various PDB entries (pK_{iD}).

No	pK_{iE}	pK_{iD} (1cil)*	pK_{iD} (1oq5)	pK_{iD} (1eou)	pK_{iD} (2pou)	pK_{iD} (2pov)	pK_{iD} (2pow)	pK_{iD} (2fou)	pK_{iD} (1ca2)
1	-9.824	-7.344	-8.939	-8.291	-8.276	-7.566	-8.07	-8.689	-8.882
2	-9.824	-10.265	-9.622	-5.864	-8.551	-10.016	-7.035	-10.062	-9.279
3	-9.523	-11.149	-9.035	-8.745	-8.812	-8.521	-8.408	-9.189	-8.545
4	-8.046	-8.545	-6.949	-6.604	-9.848	-8.284	-7.97	-8.895	-6.887
5	-8.046	-8.861	-6.884	-6.2	-8.229	-9.336	-9.611	-8.78	-8.752
6	-7.921	-7.104	-8.267	-7.155	-8.208	-8.71	-6.756	-8.577	-8.034
7	-7.796	-9.167	-9.215	-8.465	-9.105	-9.95	-9.602	-10.858	-9.55
8	-7.678	-11.256	-9.717	-8.292	-10.583	-9.766	-11.486	-9.209	-10.269
9	-7.678	-9.353	-8.555	-7.919	-9.77	-9.801	-9.131	-9.921	-9.286
10	-7.523	-7.738	-9.127	-7.845	-8.625	-8.176	-8.253	-8.689	-8.981
11	-7.444	-7.716	-7.715	-6.498	-6.869	-8.525	-8.229	-7.686	-6.481
12	-7.42	-8.052	-8.577	-7.62	-10.612	-7.131	-8.061	-8.852	-7.964
13	-7.301	-6.6	-6.796	-5.357	-7.903	-8.471	-6.406	-7.448	-6.766
14	-7.222	-7.255	-7.158	-6.172	-8.297	-8.565	-6.702	-7.563	-7.039
15	-7.201	-9.098	-10.023	-8.028	-9.138	-9.875	-9.884	-9.518	-8.668
16	-7.125	-7.991	-8.91	-8.279	-9.957	-10.834	-8.968	-9.237	-9.603
17	-6.796	-8.821	-8.193	-8.041	-9.393	-9.336	-9.03	-8.997	-8.191
18	-5.671	-7.214	-5.867	-6.205	-7.039	-8.481	-4.975	-6.02	-6.216

* PDB entry of the complex from which protein structure was extracted

The possible reasons of low correlation are: 1) errors of docking and 2) errors in enzymology and crystallography, i.e. errors in measurement of binding constants, variation of K_i measurement methods, errors in X-Ray analysis and the difference of structure of complex in solution and in a solid phase. Differences may also occur due to the absence of water in models of ligand – protein complexes.

In our case improvement of the docking results might be achieved by the refinement of protein – ligand models. Improving the docking algorithm *per se* is beyond the scope of this work. Modelling the ligands by several methods at different levels of theory (molecular mechanics, semi-empirical, *ab initio*) might eliminate the errors caused by distortions of ligand geometry. The variation of docking parameters and applying filters, which would eliminate the ligands containing residues/fragments the algorithm is incapable to deal with, might increase the docking accuracy.

1.3. Preparation of hCA tertiary structure models

In order to perform docking experiments to all hCAs, models of remaining CA isoforms were built. At the moment of experiment crystal structures of hCAI, hCAII, hCAIII, hCAIV and hCAXII, and the primary structures of the hCAVA, VB, VI, VII, IX, XIII, XIV were available. The models of hCAI, hCAII, hCAIII, hCAIV and hCAXII were built by removing all cocrystallized compounds from crystallographically determined structures of free protein or protein – ligand complexes (hCAI PDB ID 1azm, hCAIII PDB ID 2hfw, hCAIV PDB ID 1znc and hCAXII PDB ID 1jd0). In order to perform docking to other isoforms of hCA, their models had to be prepared at first. Models were built using program DeepView/Swiss-PdbViewer v.3.7 and final tertiary structure built in An Automated Comparative Protein Modelling Server. hCAVB, hCAVI, hCAVII, hCAIX, hCAXIII structures were built using hCAII (PDB ID 2fou) as a template, hCAVA – using mCAVA (PDB ID 1dmy) as a template, and hCAXIV –

using mCAXIV (PDB ID 1rj6) as a template. Zinc ion was added after tertiary protein structure modelling.

1.4. Ligand Modelling

Geometry of ligands from the compound set 1 was optimised using three methods: MM+ (molecular mechanics) [10], PM3 (semi-empirical) [11] and AM1 (semi-empirical) [12]. Application of *ab initio*/DFT methods for ligand modelling was rejected because of enormous consumption of CPU time. The obtained ligand models were docked to hCAII model. While docking using models produced by semi-empirical methods gave random binding energies, docking using models produced by molecular mechanics showed better correlation with experimental binding data ($pK_{iE} = 1.1540 \times pK_{iD} + 0.8357$ $R^2 = 0.6734$). Experimental and modelled binding constants are given in Table 1.4.-1.

Table 1.4.-1. Experimentally determined inhibition constants and inhibition constants produced by docking compound set 1 of ligands modelled using MM+, PM3 and AM1 methods to hCAII.

Compound	Experimentally determined pK_i	pK_i prduced by docking ligands modelled using methods		
		MM+	PM3	AM1
1	-9.824	-10.259	-7.127	-6.131
2	-9.824	-10.165	-7.818	-7.174
3	-9.523	-10.827	-9.507	-9.645
4	-8.046	-8.5	-7.27	-6.972
5	-8.046	-8.623	-9.065	-7.945
6	-7.921	-6.902	-7.474	-7.008
7	-7.796	-8.415	-7.18	-9.541
8	-7.678	-7.913	-7.672	-9.964
9	-7.678	-9.003	-10.331	-8.538
10	-7.523	-8.504	-7.618	-7.926
11	-7.444	-6.881	-5.318	-5.377
12	-7.42	-7.534	-7.675	-8.928
13	-7.301	-6.447	-6.52	-6.991
14	-7.222	-6.531	-7.703	-7.191
15	-7.201	-7.702	-10.782	-8.769
16	-7.125	-9.151	-8.629	-9.229
17	-6.796	-8.148	-7.226	-8.512
18	-5.671	-5.063	-5.428	-5.52

On the basis of these results, the ligands with known K_i were modelled using MM+ method (geometry optimization was performed using conjugated gradient method until convergence of 0.05 kcal/Å was reached). The compound set 2, containing models of 328 compounds with known experimental binding to hCA constants was built and was docked to all hCA isoforms. Analysis was performed only on the data which has experimental coverage. There are enough experimental binding data for evaluation of binding prediction only for isozymes hCAI, hCAII, hCAVA, hCAVB and hCAIX. The rest of isozymes were investigated insufficiently thus the analysis was not performed. Scores produced by docking of the compound set 2 to the isoforms hCAI, hCAII, hCAVA, hCAVB and hCAIX did not correlate with experimental binding data. Several attempts to improve those results were made.

1.5. Variation of docking parameters

Accuracy test was performed using both compound sets 1 and 2. In both cases increase of accuracy (from 6720 solutions out of rigid dock and 300 solutions out of pose match (accuracy level 3) to 22080 solutions out of rigid dock and 1050 solutions out of pose match (accuracy level 6)) did not affect correlation significantly.

1.6. Application of filters

There might be two main reasons for diminished correlation between experimental pK_i′s and pK_i′s produced by docking when the compound set is increased. One reason might be false positive correlation of pK_i′s produced by docking of compound set 1. The other reason might be the incapability of eHiTS to deal with compounds containing certain fragments. In order to eliminate such a possibility some compound filters were applied.

Since eHiTS considers non-aromatic rings as rigid structures and docking algorithm cannot change their conformation, the selection of conformer is ambiguous. Thus, Filter 1 was applied to remove compounds with larger than 3-membered non-aromatic rings from the compound set and the compound set 2F1 (243 compounds) was built.

Filters 2, 3, 4 and 5 additionally removed compounds with more than 10, 8, 6 and 5 rotatable bonds, respectively. These filters might improve results because eHiTS does only restricted conformational search (depends on the accuracy level) and eliminating compounds with large conformational freedom should help avoid errors related to conformational search. In this way compound sets 2F2 (215 compounds), 2F3 (176 compounds), 2F4 (134 compounds) and 2F5 (110 compounds) were built.

Nevertheless, even filtered data did not correlate in acceptable level – the correlation coefficient for compound set 2F4 (hCAII) is $R^2 = 0.1531$ and for compound set 2F5 (hCAII) was $R^2 = 0.1799$.

1.7. Correction of binding constants

The size and other properties of ligand molecules may cause the error of calculated binding constant in several ways: the conformational freedom and parameterization related problems. Large molecules may have more fragments which can interact with side chains of amino acids and, depending on the parameterization, the scoring function may underestimate or overestimate their influence to total binding energy.

In order to evaluate parameterization of eHiTS for hCAs, the dependence between error of binding constants and the size (represented by molecular weight) of ligand molecules was examined. Indeed, the error of binding constants depends on the molecular size ($R^2 = 0.4702$ (hCAI), $R^2 = 0.2756$ (hCAII), $R^2 = 0.1847$ (hCAVA), $R^2 = 0.3727$ (hCAVB)). Slightly better correlation between binding constants pK'_{iD} produced by docking and experimental binding data was achieved (compound set 2F4 $R^2 = 0.4544$ (hCAI), $R^2 = 0.3255$ (hCAII), $R^2 = 0.2232$ (hCAVA), $R^2 = 0.2214$ (hCAVB)), when correction of binding constants produced by docking was applied ($pK'_{iD} = pK_{iD} + y$, where $y = -0.0117 \times M(lig) + 4.3378$ (hCAI), $y = 0.007 \times M(lig) - 0.9441$ (hCAII), $y = 0.0081 \times M(lig) + 1.0298$ (hCAVA), $y = -0.0111 \times M(lig) - 1.4239$ (hCAVB). Still, correlation in case of hCAIX was not observed.

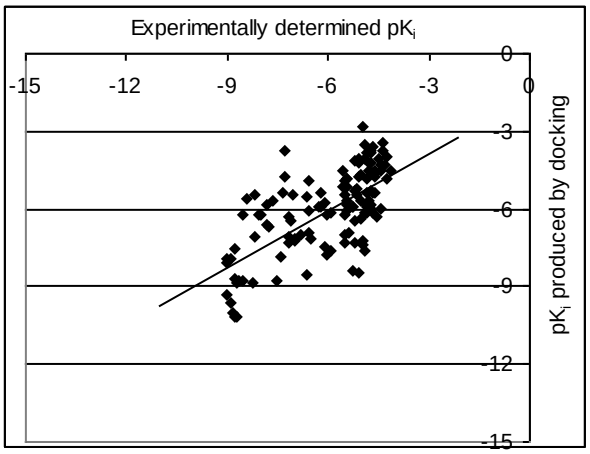
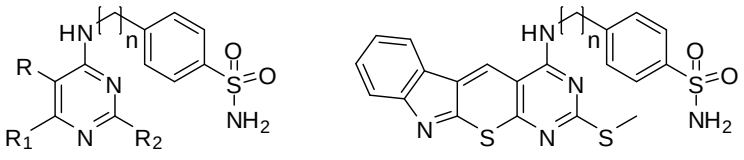


Fig. 1.7-1. Correlation between experimentally determined inhibition constants and inhibition constants produced by docking compound set 2F4 of ligands to hCAI after correction by mass was applied. $pK_{iE} = 0.7354 \times pK'_{iD} - 1.6673$ ($R^2 = 0.4544$)

The poor correlation between experimental and calculated binding constants for hCAVA, hCAVB and hCAIX isn′t surprising, because structures of these proteins were not determined experimentally but were modelled using other hCAs as a template. Despite our efforts to prepare method for prediction of hCA inhibition constants, eHiTS is incapable to predict it quantitatively. On the other hand, the position of ligand in active site of protein, the interaction of sulfonamide headgroup with zinc ion and interactions of tail fragment of inhibitor with polar and non-polar side chains of amino acids can be predicted.

*1.8. Docking of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]- and 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indol-4-yl)amino]}benzenesulfonamides*

Despite the eHiTS lack of ability to predict interaction of inhibitors with hCAs quantitatively, the theoretical studies of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]benzenesulfonamides (**A**) and 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indol-4-yl)amino]}benzenesulfonamides (**B**) (compound set 3, 56 compounds) interaction with hCAI, II, VA, VB and IX were performed in order to evaluate their binding qualitatively.



R = NO_2, CHO, CN; R_1 = Cl, OCH_3, $NHCH_2Ph$; R_2 = H, SCH_3
n = 0, 1, 2

Fig. 1.8.-1. Structure of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]benzenesulfonamides (**A**) and 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indol-4-yl)amino]}benzenesulfonamides (**B**)

Investigation of pyrimidine derivatives - 4-[*N*-(2,5,6-substituted pyrimidin-4-yl)amino]benzenesulfonamides (**A**) and 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indol-4-yl)amino]}benzenesulfonamides (**B**) (fig 1.8-1) as hCA inhibitors was encouraged by reported succesful inhibition of hCA with 4-[*N*-(4,6-substituted 1,3,5-tiazino-2-yl)amino]benzenesulfonamides [13, 14]. The pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole fragment is especially interesting due to its structure properties.

First, it can be considered as 4,5-disubstituted pyrimidine. Second, rigid relatively nonpolar four ring system may interact with side chains of amino acids present in nonpolar wall of hCA active site cavity. The size of heterocyclic system might increase selectivity of binding to certain hCA isozyme.

Analysis of resulting complex models shows that all tested compounds should occupy a typical for classical CA inhibitors position in active center of hCAs, pointing the sulfonamide headgroup to catalytic zinc ion. Pyrimidine ring and substituents attached to it can participate in additional interactions with side chains of aminoacids. Figure 1.8.-2 exhibits an example of superimposed structures of docked and X-Ray determined (see Sec. 2.5) ligand – hCAII complexes. The pose of benzenesulfonamide moiety of docked 4-[*N*-(pyrimidin-4-yl)-amino]benzenesulfonamides is similar to the experimentally determined one. The pose of pyrimidine moiety is determined by its edge to face stacking with the Phe131 benzene ring. However, the underestimated interaction of either pyrimidine ring by itself or substituents attached to it with Gln92 side chain amide residue results stacking with the opposite side of Phe 131 benzene ring in the models.

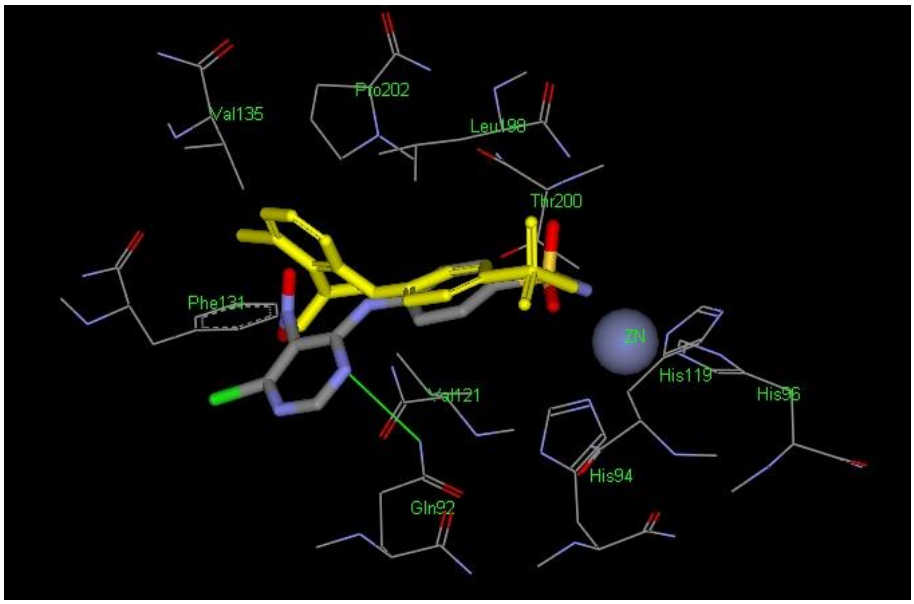


Fig. 1.8-2. Pose of 4-[*N*-(6-chloro-5-nitropyrimidin-4-yl)amino]benzenesulfonamide in active site of hCAII. X-Ray determined and (coloured, PDB entry 3m40) and produced by docking (yellow) structure.

Analysis of 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indol-4-yl)amino]}benzene-sulfonamides (**B**) bound to hCA models confirms that compounds of structure **B** also should fit into active site of hCAs, their sulfonamide headgroups should participate in interaction with catalytic zinc ion and surrounding side chains of amino acids (Fig 1.8-3). Also the interaction of pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole fragment with non-polar side chains of aminoacids (in case of hCAII Val121, Phe131, Val135, Leu198, Pro202, Leu204) is predicted. Additional hydrogen bond of 2-methylthio group with side chain of Gln92 should increase the stability of such complex.

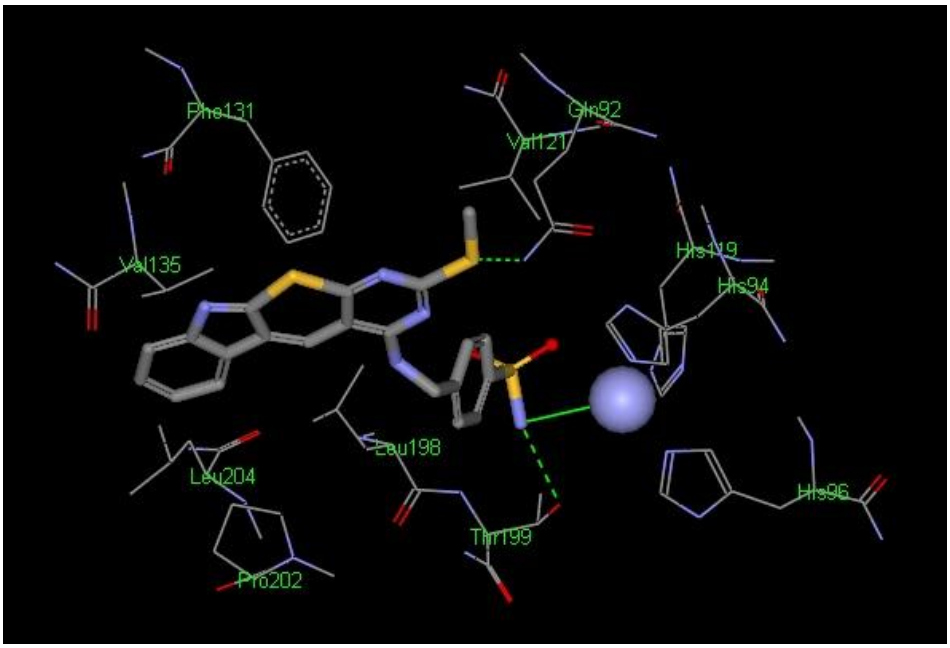


Fig. 1.8-3. Pose of 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indol-4-yl)amino]methyl}benzenesulfonamide in active site of hCAII produced by docking.

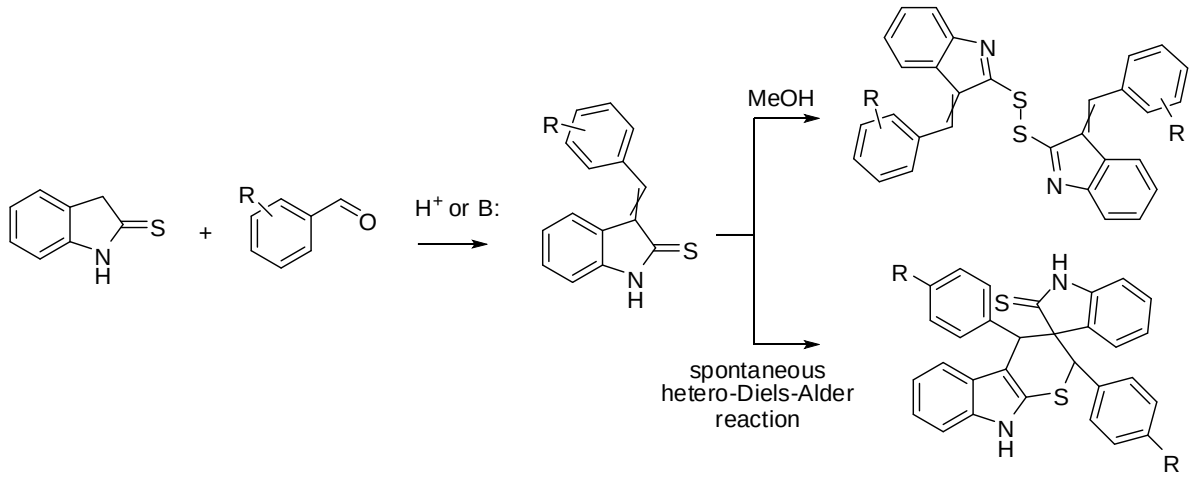
The docking results confirm that the compounds of structure **A** and **B** can be potential hCA inhibitors. Thus the synthesis compunds **A** and **B**, their hCA inhibition activity and interaction with active site of hCAII were further investigated.

2. Synthesis and properties of carbonic anhydrase inhibitors based on pyrimidine heterocycles

2.1. Condensation of indoline-2-thiones with pyrimidine-5-carbaldehydes.

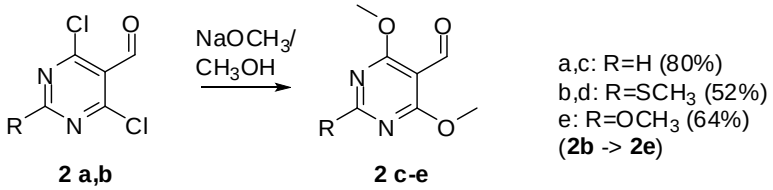
In contrast to oxindole, condensation of indoline-2-thione with aromatic/heteroaromatic aldehydes can give various products. For example, condensation of indoline-2-thione with benzaldehydes under acidic or basic conditions yields 3-benzylidenindoline-2-thiones, which participate in an intermolecular [4+2] cycloaddition reaction yielding spiro(indolo)thiopyrano[2,3-*b*]indole derivatives even at room temperature [15]. Other authors reported the formation of 3-arylideneindoline-2-thiones when reaction was carried out in ethanol at 90 °C using piperidine as a base [16, 17]. 3-Arylideneindoline-2-thiones in methanol solution undergo spontaneous oxidation at room temperature yielding corresponding bis(3-aryliden-1,3-dihydroindol-2-yl)disulfides [16] (Scheme 2.1-1).

Our investigation of the reaction of indoline-2-thione (**1a**) with 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde (**2b**) in various alcohols (methanol, ethanol or 2-propanol) using piperidine, triethylamine, or 2-propoxide in 2-propanol as a base showed that the reaction proceeds ambiguously and inseparable mixture of products was formed. A variety of side reactions is probably caused by active chloro groups at 4th and 6th positions of the pyrimidine ring. Reactions of O-alkylation of solvent molecules or S-alkylation of indoline-2-thione may occur. The Cannizzaro reaction and Hetero- Diels – Alder reaction cannot be excluded, too.



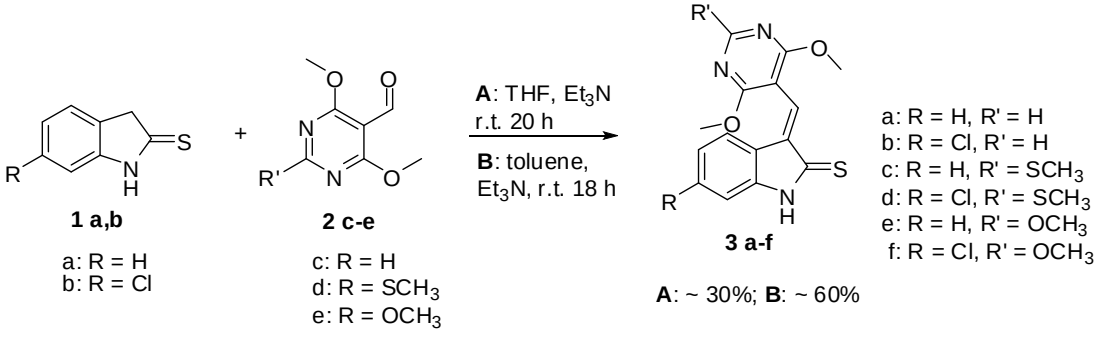
Scheme 2.1-1.

In order to reduce the probability of side reactions and evaluate whether dimerization plays important role in the formation of side products, we decided to use pyrimidine-5-carbaldehydes without active groups at 4th and 6th positions of the pyrimidine ring in the reaction with indoline-2-thione. Thus, a series of 4,6-dimethoxypyrimidine-5-carbaldehydes (**2c-e**) were synthesised by substitution of chloro groups with methoxy groups in 4,6-dichloropyrimidine-5-carbaldehydes **2a,b**. Reaction was performed in methanol at room temperature with 2 equivalents of sodium methoxide (Scheme 2.1-2). In the case of synthesis of dimethoxypyrimidine **2d** partial substitution of methylthio group was observed. Full conversion of **2b** to trimethoxypyrimidine **2e** was achieved by prolonged treatment of dichloropyrimidine **2b** with 4 equivalents of sodium methoxide.



Scheme 2.1.-2.

Taking into account that 2-methyltio group in 4,6-dimethoxy-2-methylthiopyrimidine-5-carbaldehyde in the presence of base in alcohol solutions can be substituted by alkoxy group, condensation with indolin-2-thiones (**1a,b**) was carried out in tetrahydrofuran solutions at room temperature using triethylamine as a base. Under these conditions 3-[(4,6-dimethoxypyrimidin-5-yl)methylene]indoline-2-thiones (**3a-f**) were obtained in approx. 30% yields (Scheme 2.1-3). The change of solvent from tetrahydrofuran to toluene increased yields of **3a-f** up to 60 %.



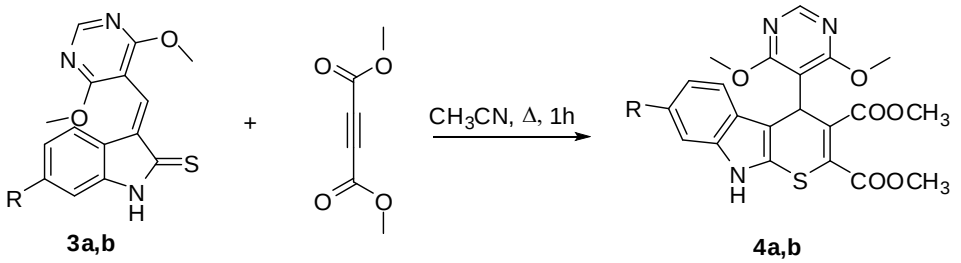
Scheme 2.1-3.

In the NMR spectra of compounds **3a-f** only one set of signals is observed, confirming that only one isomer was formed. Since it is rather difficult to determine the configuration of resulting 3-[(4,6-dimethoxypyrimidin-5-yl)methylen]indoline-2-thiones (**3a-f**) only from the NMR data, molecular modelling experiments were performed. The geometry of both *Z*- and *E*-isomers was optimized using B3LYP/6G31(d,p) method [18, 19]. The energy of *E*-isomers favours over the energy of *Z*-isomers more than 3 kcal/mol. So, *E*-configuration was assigned to the synthesised compounds **3a-f** (the data is given in the Table 2.1-1).

Table 2.1.-1. Difference in the formation energy between *Z*- and *E*- isomers of 3-[(4,6-dimethoxypyrimidin-5-yl)methylen]indolin-2-thiones (**3 a-f**).

Compound	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
ΔE (E_E-E_Z), kcal/mol	-3.086	-3.128	-3.137	-3.224	-3.136	-3.268

Formation of dimers of *E*-3-[(4,6-dimethoxypyrimidin-5-yl)methyliden]indoline-2-thiones during the condensation reaction was not observed, as well. Moreover, *E*-3-[(4,6-dimethoxypyrimidin-5-yl)methylene]indoline-2-thiones (**3a-f**) were found to be stable in refluxing toluene. Nevertheless, the ability of *E*-3-[(4,6-dimethoxypyrimidin-5-yl)methyliden]indoline-2-thiones **3a,b** to participate in hetero- Diels – Alder reaction was demostrated. Reaction of **3a,b** with butyne-1,4-dicarboxylic acid dimethyl ester in acetonitrile under reflux for 1 hour yielded the corresponding dimethyl-4,9-dihydrothiopyrano[2,3-*b*]indole-2,3-dicarboxylates (**4a,b**) (Scheme 2.1-4).



3a,b
a: R=H; b: R=Cl

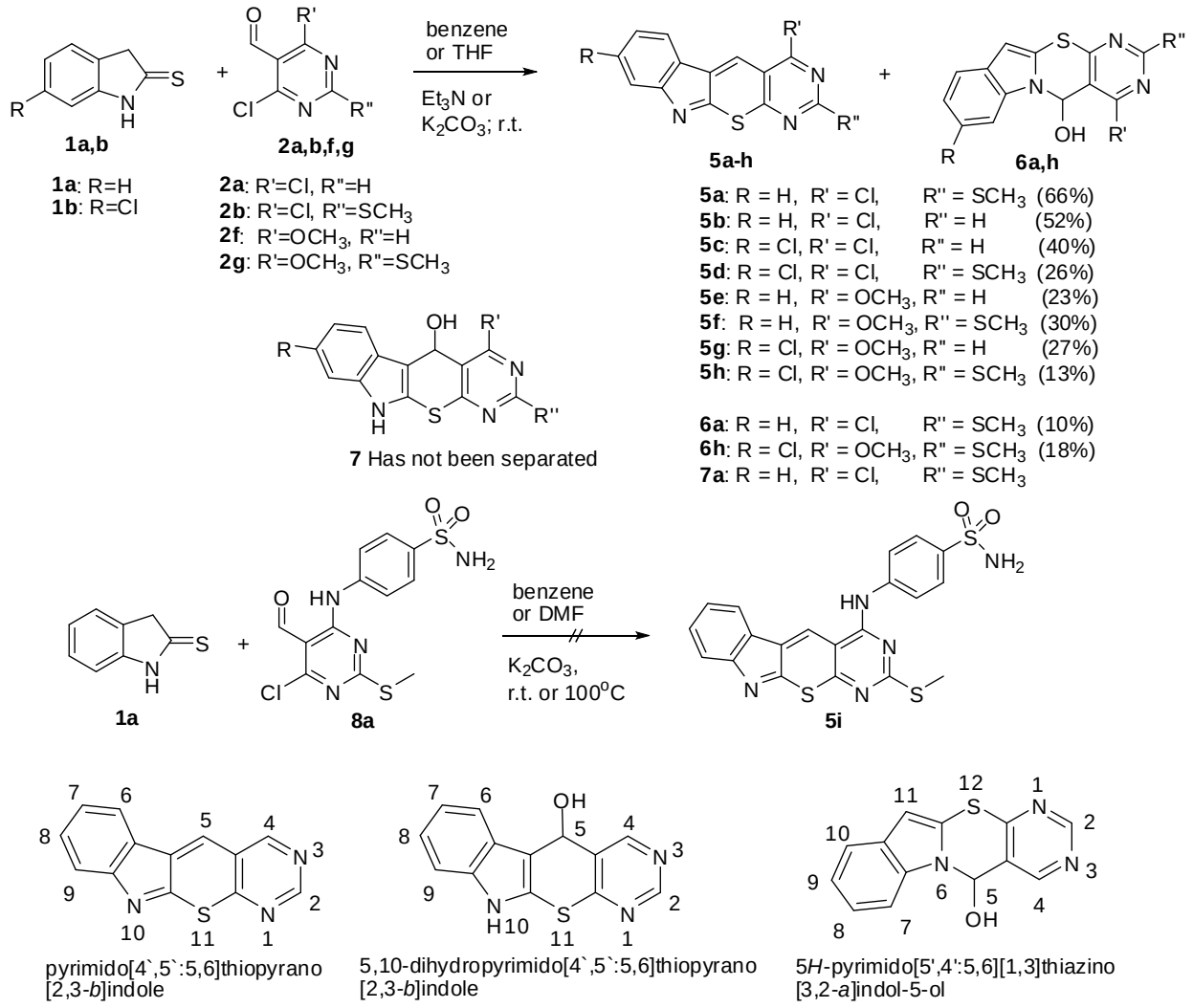
4a,b
a: R=H (60%); b: R=Cl (56%)

Scheme 2.1-4.

In summary, condensation of indoline-2-thiones with pyrimidine-5-carbaldehydes occurs in mild conditions yielding *E*-3-(pyrimidin-5-yl-methylen)indoline-2-thiones, capable to react as heterodienes in Diels – Alder reaction.

*2.2. Synthesis of pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indoles*

Presence of active chloro group in 4th and / or 6th position of pyrimidine-5-carbaldehyde may turn condensation with indoline-2-thiones in other direction, i.e. S-alkylation of thione can take place. Condensation of indoline-2-thione (**1a**) with 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde (**2b**) performed in benzene in the presence of potassium carbonate or triethylamine as a base yielded 4-chloro-2-methylthiopyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole (**5a**) (Scheme 2.2-1), that is both reactions - S-alkylation of indoline-2-thione and condensation with aldehyde group occured. The structure of compound **5a** was confirmed by spectroscopic data. The ^{1}H- and ^{13}C-NMR spectra were consistent with the structure **5a**. There are no absorption bands typical for NH or OH vibrations in the IR spectrum.

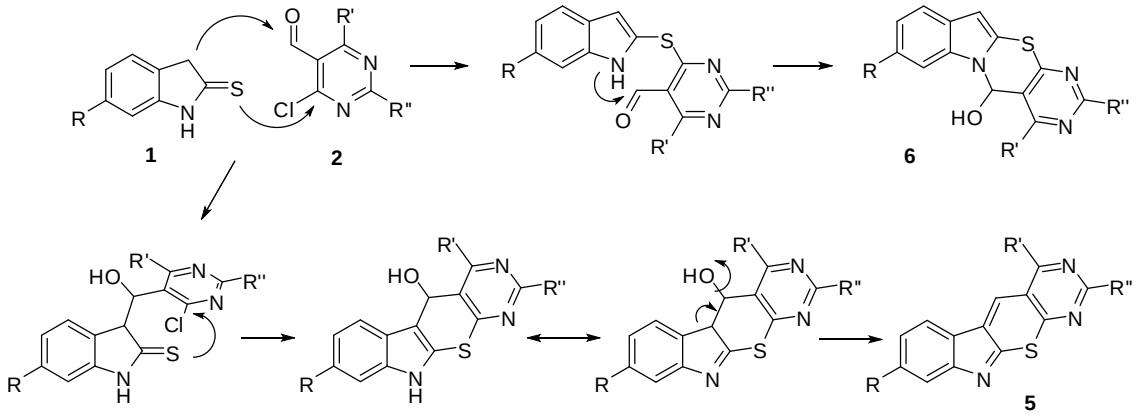


Scheme 2.2-1.

When the reaction of indoline-2-thione (**1a**) with aldehyde **2b** was performed in tetrahydrofuran solution using triethylamine as a base, an additional product was formed. Formation of **6a** or **7a** could be envisaged, however the structure could not be elucidated from ^{1}H-NMR spectrum alone. The obtained ^{1}H-NMR spectrum of product may represent both **6a** and **7a** structures. A broad singlet at 6.75 ppm in the ^{1}H-NMR spectrum can be assigned to NH group of compound **7a** or C(11)H of **6a**. CH-OH fragment of both compounds should give the similar set of signals. The DEPT experiment reveals seven carbons bonded with hydrogen, resonance signals of which are observed at 14.6, 72.2, 102.0, 111.1, 120.1, 121.9 and 122.3 ppm. The signal at 14.6 ppm corresponds to methylthio group, the resonance signal at 72.2 ppm represents CH-OH fragment, whereas resonances in 111.1 – 122.3 ppm area can be assigned to C(7), C(8), C(9) and C(10) carbon atoms of the indole fragment, respectively. The resonance signal at 102.0 ppm corresponds to C(11) of indole moiety and this proves that the compound obtained has a structure **6a** - 4-chloro-2-methylthio-5*H*-pyrimido[5′,4′:5,6][1,3]tiazino[3,2-*a*]indole-5-ol. All ^{1}H-NMR peaks of compound **6a** were unequivocally assigned based on the correlation in HETCOR spectrum and coupling constants *J* in the ^{1}H-NMR spectrum.

The formation of 4-chloro-2-methylthio-5*H*-pyrimido[5′,4′:5,6][1,3-*a*]indol-5-ol (**6a**) can be explained by increased rate of thione alkylation in more polar solvents. If thione

alkylation occurs first, the indoline ring becomes aromatic indole ring. The presence of a base in the reaction mixture causes deprotonation of indole NH moiety and the so formed N- nucleophile further reacts with aldehyde to yield compound **6a**. The Scheme 2.2-1 illustrates the proposed reaction mechanism for the formation of both 5*H*-pyrimido[5′,4′:5,6][1,3-*a*]indole-5-ols **6** and pyrimido[5′,4′:5,6]thiopyrano[2,3-*b*]indoles **5**.



Scheme 2.2-2.

Attempts to synthesize other 4-chloropyrimidin[4′,5′:5,6]thiopyrano[2,3-*b*]indoles from starting compounds **1a,b** and **2a,b** using the same procedure as for compound **5a** were made. Complex mixtures of products have formed and 4-chloropyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole (**5b**) and 4,8-dichloropyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole (**5c**) were isolated in less than 20% yields. The formation of 4,8-dichloro-2-methylthiopyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole (**5d**) was not observed. An alternative procedure, involving dropwise addition of aldehyde solution to initially prepared indoline-2-thione and base solution was elaborated. The essence of such a procedure is to keep an excess of indoline-2-thione anion in the solution and thus avoiding premature alkylation of thione. By using this methodology the yields of compounds **5b-d** were increased up to 26 – 50%. Since 6-chloroindoline-2-thione is less soluble in nonpolar solvents, the cyclization was carried out in tetrahydrofuran.

The possibility to synthesize pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indoles containing other substituents at 4th position was also investigated. For this purpose 6-methoxypyrimidine-5-carbaldehydes **2f,g** and 4-[*N*-(6-chloro-5-formyl-2-methylthiopyrimidin-4-yl)]aminobenzenesulfonamide (**8a**) were synthesised. Aldehydes **2f,g** were obtained by reflux 4,6-dichloropyrimidines **2a,b** in methanol in the presence of one equivalent of triethylamine for 3 hours. Synthesis of compound **8a** is described in Section 2.4.

Several procedures of cyclocondensation were applied and 4-methoxypyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indoles (**5e-h**) were synthesized (Scheme 2.2-1). However, cyclocondensation of indoline-2-thione (**1a**) with 4-[*N*-(6-chloro-5-formyl-2-methylthiopyrimidin-4-yl)]aminobenzenesulfonamide (**8a**) to give compound **5i** was unsuccessful. On the other hand 4-substituted 2-methylthiopyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indoles can be synthesized in good yields by substitution of chloro group with various N-, O-, S- nucleophiles in compound **5a** (see Sec. 2.3).

For additional structure confirmation of the synthesized pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indoles **5a-h** the shielding constants of nuclei for each compound were calculated using B3LYP/6G31(d,p)//B3LYP/6G31(d,p) method. TMS was used as the reference compound for conversion of schielding constants to chemical shifts. Experimental and calculated ^{1}H and ^{13}C chemical shifts and errors are shown in the Table 2.2-3. Experimental and calculated chemical shifts correlate well. Largest deviations in ^{1}H-NMR shifts occur when experimental spectrum is recorded in DMSO solution. Such results are not unexpected, because the molecular geometry was optimized in vacuum and interaction with solvent molecules was not modelled. Due to interaction with solvent molecules resonance signals of some protons are shifted upfield. For example, for compound **5h** in DMSO-D_6, the resonance signals ofC(5)-**H** and C(6)-**H** are shifted ca. 0.5 ppm upfield in comparison with the corresponding signals in the NMR spectrum recorded in $CDCl_3$. Also, typical error of c.a. 0.3 ppm for methylthio group chemical shift seems to be systematic.

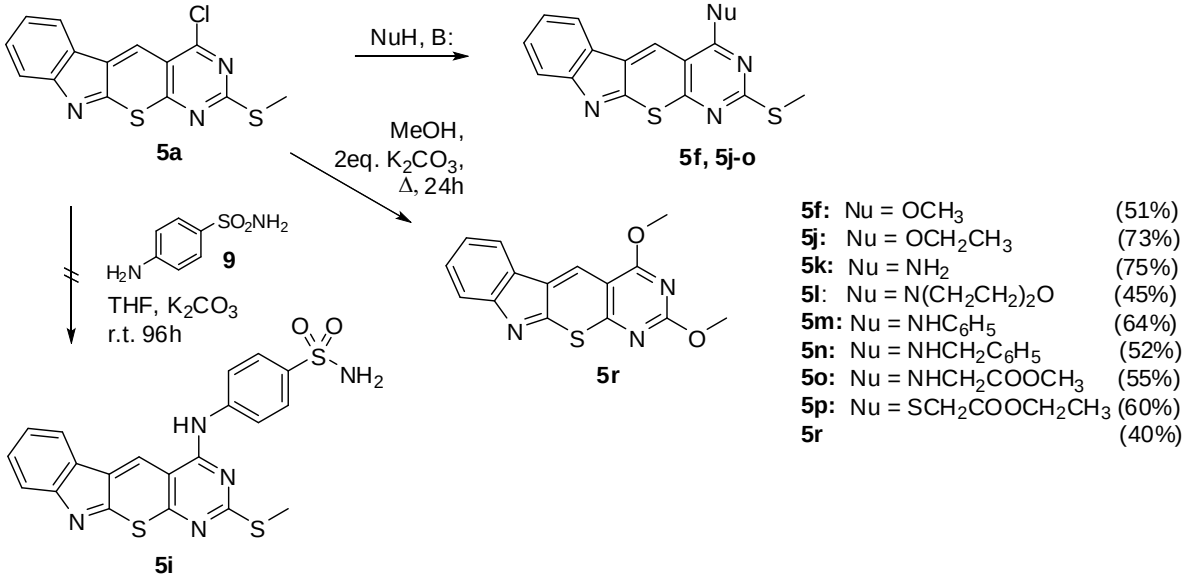
Table 2.2-3. . Experimentally observed and calculated ^{1}H and ^{13}C-NMR chemical shifts and errors of pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indoles **5a-h**.

	5a			**5b**			**5c**			**5d**			**5e**			**5f**			**5g**			**5h**			**5h**		
	δ(E), ppm†	δ(M), ppm	Δ(δ), ppm	δ(E), ppm†	δ(M), ppm	Δ(δ), ppm	δ(E), ppm††	δ(M), ppm	Δ(δ), ppm	δ(E), ppm††	δ(M), ppm	Δ(δ), ppm	δ(E), ppm†	δ(M), ppm	Δ(δ), ppm	δ(E), ppm†	δ(M), ppm	Δ(δ), ppm	δ(E), ppm†	δ(M), ppm	Δ(δ), ppm	δ(E), ppm††	δ(M), ppm	Δ(δ), ppm	δ(E), ppm†	δ(M), ppm	Δ(δ), ppm
C5-H	8.62	8.25	0.37	8.66	8.42	0.24	8.55	8.39	0.16	8.79	8.22	0.57	8.61	8.48	0.13	8.55	8.32	0.23	8.58	8.46	0.12	9.01	8.30	0.71	8.54	8.30	0.24
C6-H	8.09	7.98	0.11	8.13	8.08	0.05	8.40	7.94	0.46	8.20	7.84	0.36	8.05	8.00	0.05	8.01	7.92	0.09	7.91	7.86	0.05	8.41	7.78	0.63	7.90	7.78	0.12
C7-H	7.43	7.36	0.07	7.43	7.40	0.03	7.38	7.26	0.12	7.26	7.21	0.05	7.38	7.33	0.05	7.36	7.30	0.06	7.32	7.19	0.13	7.42	7.15	0.27	7.33	7.15	0.18
C8-H	7.63	7.62	0.01	7.66	7.69	0.03	-	-	-	-	-	-	7.60	7.62	0.02	7.56	7.56	0.00	-	-	-	-	-	-	-	-	-
C9-H	7.79	7.71	0.08	7.79	7.76	0.03	7.71	7.63	0.08	7.57	7.58	0.01	7.78	7.74	0.04	7.76	7.67	0.09	7.71	7.61	0.10	7.74	7.56	0.18	7.72	7.56	0.16
C2-H	-	-	-	8.98	8.93	0.05	9.21	8.95	0.26	-	-	-	8.76	8.84	0.08	-	-	-	8.79	8.85	0.06	-	-	-	-	-	-
SMe	2.72	2.36	0.36	-	-	-	-	-	-	2.60	2.36	0.24	-	-	-	2.67	2.37	0.30	-	-	-	2.67	2.37	0.30	2.69	2.37	0.32
OMe	-	-	-	-	-	-	-	-	-	-	-	-	4.27	4.12	0.15	4.23	4.04	0.19	4.29	4.12	0.17	4.21	4.04	0.17	4.25	4.04	0.21
C2	171.9	171.3	0.6	155.6	148.5	7.1	151.3	148.7	2.6	164.4	171.8	7.4	155.4	150.0	5.4	172.3	172.2	0.1	156.2	150.3	5.9	-	-	-	172.7	172.7	0
C4	160.7	163.0	2.3	161.7	164.8	3.1	160.8	165.0	4.2	161.2	163.2	2.0	163.5	160.0	3.5	161.1	158.3	2.8	163.1	160.2	2.9	-	-	-	162.7	158.3	4.4
C4a	115.3	113.5	1.8	120.2	118.3	1.9	124.3	118.2	6.1	112.4	113.4	1.0	110.2	108.6	1.6	106.4	104.9	1.5	110.2	108.5	1.7	-	-	-	106.4	104.9	1.5
C5	123.9	117.5	6.4	123.1	116.8	6.3	123.8	117.4	6.4	123.5	118.1	5.4	122.2	116.0	6.2	123.4	116.5	6.9	123.0	116.7	6.3	-	-	-	123.5	117.3	6.2
C5a	132.2	129.8	2.4	134.9	131.3	3.6	133.0	130.2	2.8	129.4	127.8	1.6	132.4	128.6	3.8	130.0	126.6	3.4	131.4	127.5	3.9	-	-	-	135.4	125.5	9.9
C5b	125.5	121.1	4.4	125.4	120.7	4.7	127.4	119.0	8.4	126.7	119.3	7.4	125.8	121.0	4.8	125.9	121.2	4.7	124.1	119.3	4.8	-	-	-	124.5	119.6	4.9
C6	121.6	115.9	5.7	122.1	116.6	5.5	117.3	116.6	0.7	123.2	115.9	7.3	121.4	115.8	5.6	121.0	115.2	5.8	122.0	115.8	6.2	-	-	-	119.5	115.3	4.2
C7	124.1	117.8	6.3	124.4	117.9	6.5	124.1	118.6	5.5	124.5	118.4	6.1	123.6	117.0	6.6	123.5	116.9	6.6	123.7	117.7	6.0	-	-	-	124.1	117.5	6.6
C8	130.6	124.9	5.7	131.7	125.8	5.9	134.6	142.2	7.6	133.8	141.2	7.4	130.5	124.5	6.0	129.8	123.7	6.1	136.2	140.7	4.5	-	-	-	138.1	139.7	1.6
C9	119.2	115.5	3.7	119.8	115.7	4.1	117.2	116.5	0.7	118.2	116.2	2.0	119.4	115.3	4.1	119.1	115.1	4.0	119.6	116.1	3.5	-	-	-	121.6	115.8	5.8
C9a	154.9	150.7	4.2	155.5	151.0	4.5	151.0	151.6	0.6	154.2	151.3	2.9	156.5	150.7	5.8	155.1	150.4	4.7	156.8	151.3	5.5	-	-	-	155.9	151.0	4.9
C10a	160.4	158.6	1.8	161.1	159.5	1.6	159.3	161.2	1.9	159.5	160.2	0.7	161.6	159.8	1.8	164.0	158.9	5.1	163.4	161.5	1.9	-	-	-	164.1	160.4	3.7
C11a	165.0	166.4	1.4	165.0	166.7	1.7	163.9	166.4	2.5	162.9	166.2	3.3	167.0	165.8	1.2	165.7	165.3	0.4	167.1	165.7	1.4	-	-	-	165.8	165.2	0.6
SMe	14.7	19.2	4.5	-	-	-	-	-	-	14.0	19.2	5.2	-	-	-	14.7	19.0	4.3	-	-	-	-	-	-	14.8	19.0	4.2
OMe	-	-	-	-	-	-	-	-	-	-	-	-	55.4	52.8	2.6	55.4	52.9	2.5	55.5	53.0	2.5	-	-	-	55.5	53.0	2.5

† Experimental spectra recorded in $CDCl_3$ solutions.
†† Experimental spectra recorded in DMSO-D_6 solutions.

*2.3. Reaction of 4-chloro-2-methylthiopyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole with N-, O- and S- nucleophiles*

Since pyrimido[5′,4′:5,6]thiopyrano[2,3-*b*]indole is a new heterocyclic system, it was important to investigate the possibility to functionalize 4-chloropyrimido[5′,4′:5,6]thiopyrano[2,3-*b*]indoles via substitution of chloro group with various nucleophiles and apply this route for synthesis of 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indol-4-yl)amino]}benzenesulfonamides. In order to evaluate these properties, the reactions of several N-, O- and S- nucleophiles with 4-chloro-2-methylthiopyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole were investigated (Scheme 2.3-1). Synthesis of compounds **5l-p** was carried out at room temperature in THF solutions using potassium carbonate as a base. In case of synthesis of compound **5k,** excess of ammonia was used. In case of synthesis of compounds **5e** and **5j**, reaction was carried out in the corresponding alcohol and potassium carbonate was used as a base.



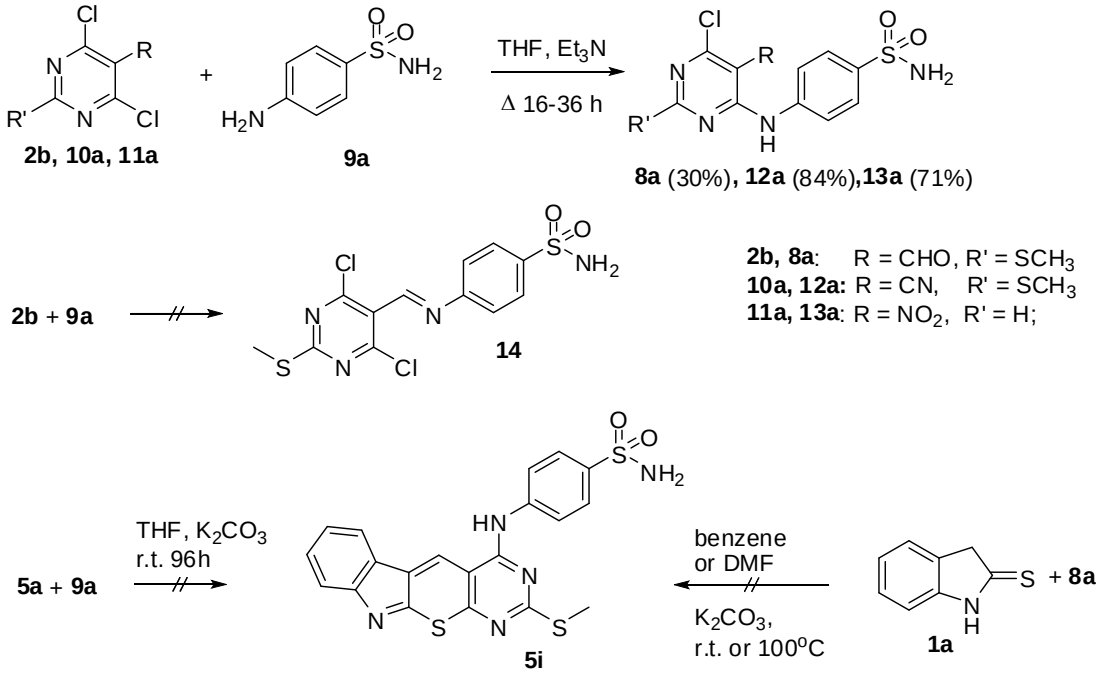
Scheme 2.3-1.

Partial substitution of methylthio group was observed only during the reaction with methanol. Full conversion of 4-chloro-2-methylthiopyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole (**5a**) to 2,4-dimethoxypyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole (**5r**) could be achieved by prolonged treatment of compound **5a** with methanol in the presence of excess of base. Substitution of chloro group with 4-aminobenzenesulfonamide resulted in mixture of products, which could not be separated into individual compounds due to their low solubility in most solvents.

2.4. Synthesis of hCA inhibitors

Docking results and known high affinity of benzenesulfonamides containing heterocyclic fragment suggest that benzenesulfonamides containing pyrimidine and pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole fragments can be good hCA inhibitors. Introduction of various substituents in positions 2, 4 and 5 of pyrimidine ring can increase affinity to certain hCA isoform. Synthesis of the first compounds **8a**, **12a** and **13a** for hCA inhibition involved substitution of chloro group of pyrimidines **2b**, **10a** and

11a by 4-aminobenzenesulfonamide **9a** and was carried out by refluxing the reaction mixture for 16-36 hours in tetrahydrofuran in the presence of triethylamine as a base.



Scheme 2.4-1.

Formation of substitution products was confirmed by spectral data. In the ^{1}H-NMR spectra of compounds **2b**, **10a** and **11a,** the signal corresponding to the sulfonamide group appears as a singlet at 7.36 – 7.38 ppm, the signals at 7.36 – 7.92 ppm are assigned to four benzene ring protons of benzenesulfonamide and a singlet at 10.25 – 10.44 ppm is attributed to NH group. In case of compound **8a** this signal proves formation of the substitution product instead of imine **14**. Monosubstitution is confirmed by ratio of protons of the benzenesulfonamide moiety with protons of substituents at the pyrimidine ring. The ^{13}C-NMR spectra also confirm non-equivalence of carbons at 4- and 6-position of the pyrimidine ring. There are absorption bands at 3240 – 3340 cm^{-1} typical for stretching of NH group in the IR spectra of compounds **8a**, **12a** and **13a**. Additionally there are absorption bands typical for aldehyde CO stretching in pyrimidine-5-carbaldehydes at 1638 cm^{-1} and CN stretching at 2220 cm^{-1} in the IR specta of compounds **8a** and **12a,** correspondingly.

Binding of compounds **8a**, **12a** and **13a** to hCAI, hCAII, hCAVII and hCAXIII was measured using thermal shift assay (TSA) and isothermal titration callorimetry (ITC) techniques at the Institute of Biotechnology. Binding data of the synthesized compounds as well as standart reference compound azetazolamide (**AZM**) and very active but unstable compound trifluoromethanesulfonamide (**TFM**) [20] (Fig. 2.4-1) are shown in Table 2.4-1.

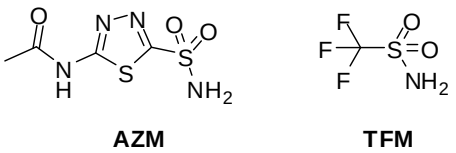


Fig. 2.4-1. Formula of acetazolamide (**AZM**) and trifluoromethanesulfonamide (**TFM**)

The activity of compounds **8a**, **12a** and **13a** was high – binding constants were micro- and submicromolar order and were similar to those of **AZM**. Especially

interesting compound is 4-[*N*-(6-chloro-5-nitropyrimidin-4-yl)amino]benzenesulfonamide **13a**, which selectively inhibited hCAXIII in nanomolar concentration.

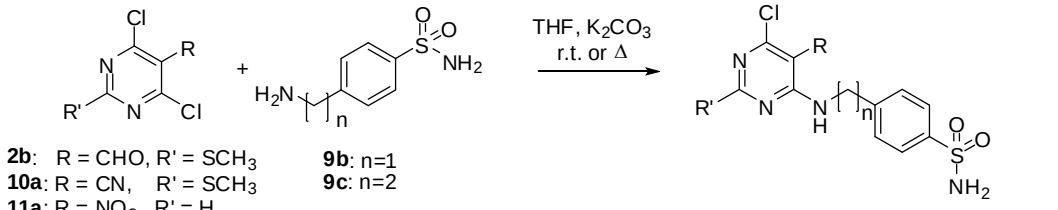
Table 2.4.-1. Binding to hCAI, II, VII and XIII constants of compounds **8a**, **12a** and **13a** determined by TSA and ITC, in μM at 37°C.

No.	Compound	hCA I		hCA II		hCA VII		hCA XIII	
		TSA	ITC	TSA	ITC	TSA	ITC	TSA	ITC
1	**8a**	1.0	2.8	0.17	0.32	4.0	ND	ND	ND
2	**12a**	1.4	20	0.071	0.22	0.83	1.7	0.091	0.12
3	**13a**	0.13	0.26	0.091	0.17	0.13	0.77	0.002	0.014
	AZM	1.4	0.78	0.017	0.018	ND	ND	0.050	0.065
	TFM	0.05	ND	0.13	0.091	0.036	0.029	0.020	0.027

ND – data nor available mostly due to insufficient solubility of compounds.
Average standard deviations for both methods are ±25%.

Further modification of inhibitors was performed by increasing length of a linker between heterocyclic and benzenesulfonamide fragments. This can affect binding of inhibitors to hCA in two ways. Firstly, the distance between benzenesulfonamide and heterocyclic fragment is changed. Thus, heterocyclic fragment can interact with side chains of other amino acids. Secondly, increased conformational freedom makes inhibitor more flexibe and it can take better position for interaction with side chains of amino acids.

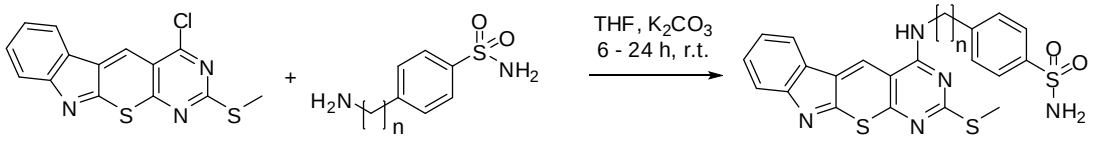
Compounds **8b,c**, **12b,c**, **13b,c** and **5s,t**, the homologues of pyrimidines **8a**, **12a**, **13a**, and pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole **5i** were synthesized in 38 – 73 % yield by substitution of chloro group in pyrimidines **2b**, **10a** and **11a** or pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole **5a** with 4-(aminomethyl)benzenesulfonamide (**9b**) or 4-(2-aminoethyl)benzenesulfonamide (**9c**). Substitution of both chloro groups in pyrimidines **2b**, **10a** and **11a** was not observed. In contrast to the synthesis of 4-[*N*-(pyrimido[5′,4′:5,6]thiopyrano[2,3-*b*]indol-4-il)]aminobenzenesulfonamide (**5i**), its homologues - pyrimido[5′,4′:5,6]thiopyrano[2,3-*b*]indoles **5s** and **5t** formed unambiguously and purification of products was much easier.



2b: R = CHO, R' = SCH_3
10a: R = CN, R' = SCH_3
11a: R = NO_2, R' = H

9b: n=1
9c: n=2

8b: R=CHO, R'=SCH_3, n = 1 (70%)
8c: R=CHO, R'=SCH_3, n = 2 (61%)
12b: R=CN, R'=SCH_3, n = 1 (38%)
12c: R=CN, R'=SCH_3, n = 2 (73%)
13b: R=NO_2, R'=H, n = 1 (76%)
13c: R=NO_2, R'=H, n = 2 (64%)



5a

9b: n=1
9c: n=2

5s n = 1 (75%)
5t n = 2 (42%)

Scheme 2.4-2.

The solubility of pyrimido[5′,4′:5,6]thiopyrano[2,3-*b*]indoles **5s** and **5t** in buffer solution was lower than 100 μM and was too low for measuring binding to hCA. Thus benzenesulfonamides with pyrimido[5′,4′:5,6]thiopyrano[2,3-*b*]indole substituents were eliminated from further hCA inhibition study.

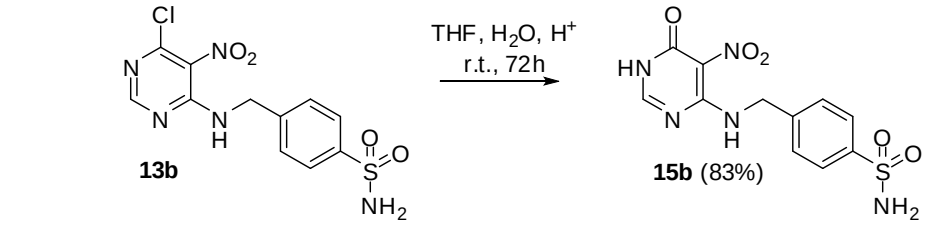
Variation of linker length between benzenesulfonamide and pyrimidine fragments did not influence significantly hCA inhibition with 5-cianopyrimidines **11a-c**. On the contrary, 4-(5-formylpyrimidin-4-yl)benzenesulfonamide **8b** with methyleneamino linker possessed more than tenfold increased affinity to all tested hCAs in comparison with 4-(5-formylpyrimidin-4-yl)benzenesulfonamides **8a** and **8c**, bearing amino or ethyleneamino linkers.

Table 2.4-2. Binding to hCAI, II, VII and XIII constants of compounds **8a-c**, **12a-c**, **13a-c** and **15b** determined by TSA and ITC, in μM at 37 °C.

No.	Compound	hCA I		hCA II		hCA VII		hCA XIII	
		TSA	ITC	TSA	ITC	TSA	ITC	TSA	ITC
1	**8a**	1.0	2.8	0.17	0.32	4.0	ND	ND	ND
2	**12a**	1.4	20	0.071	0.22	0.83	1.7	0.095	0.12
3	**13a**	0.13	0.26	0.091	0.17	0.13	0.78	0.002	0.014
4	**8b**	0.0071	0.083	0.024	0.043	0.10	0.10	0.028	0.13
5	**8c**	0.11	0.48	0.11	0.35	1.0	0.77	0.033	0.19
6	**12b**	0.10	ND	0.17	ND	0.10	ND	0.14	ND
7	**12c**	0.33	ND	0.42	ND	0.10	ND	0.10	ND
8	**13b**†	0.013	0.099	0.0002	0.043	0.005	0.40	0.0001	0.01
9	**13c**†	0.063	0.39	0.17	0.22	0.0017	0.41	0.0002	0.39
10	**15b**	0.17	ND	0.20	ND	0.25	ND	0.020	ND
	AZM	1.4	0.78	0.017	0.018	ND	ND	0.050	0.065
	TFM	0.05	ND	0.13	0.091	0.036	0.029	0.020	0.027

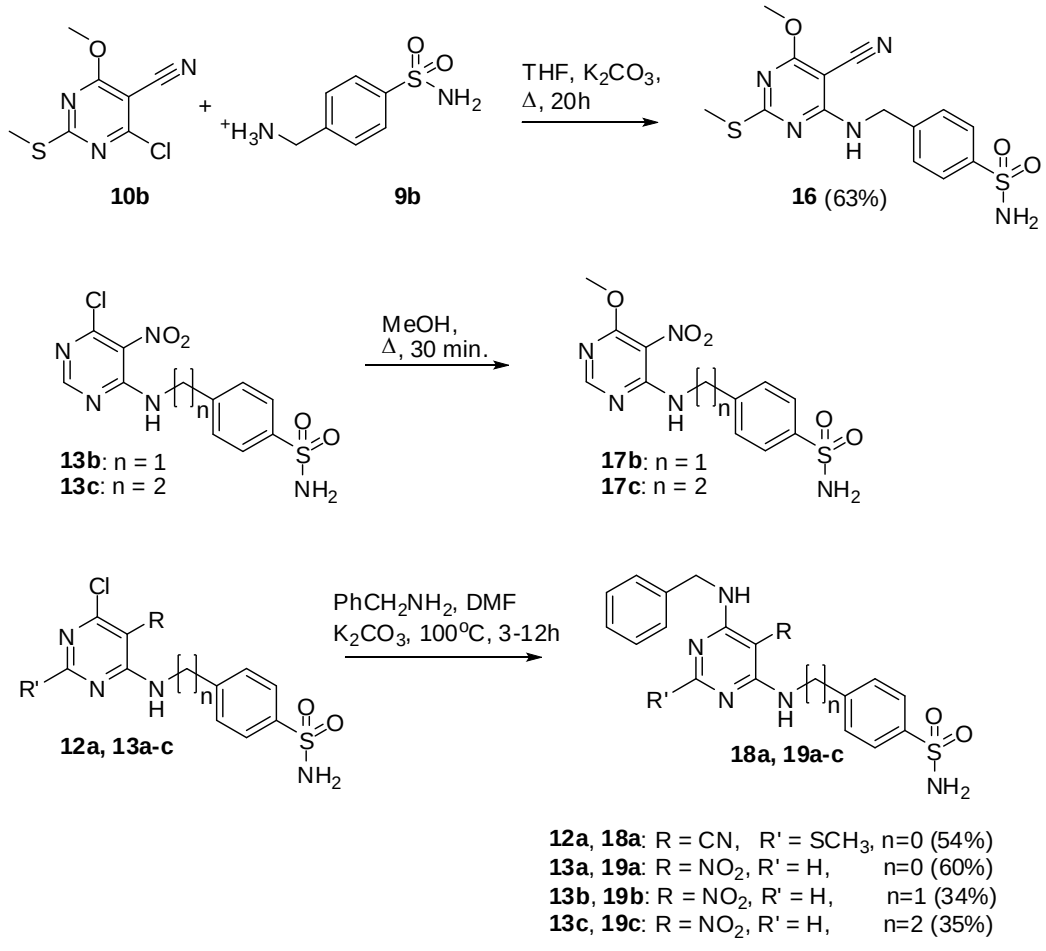
† Compounds are unstable in the solution under storage conditions and binding constants vary depending on solution storage period.
ND – data nor available mostly due to insufficient solubility of compounds.
Average standard deviations for both methods are ±25%.

The affinity of 4-(6-chloro-5-nitropyrimidin-4-yl)benzenesulfonamides **13b** and **13c** to hCA droped after several days of preparation of the sample solution. This could be caused by hydrolysis of chloro group to form oxopyrimidines **15**. In order to prove this presumption the 4-{[(5-nitro-6-oxo-1,6-dihydropyrimidin-4-yl)amino]methyl}benzenesulfonamide **15b** was synthesized (Scheme 2.4-3) as model compound and binding data to hCAs were collected. Affinity of compound **13b** was 10 - 10^3 fold higher than affinity of compound **15b**. It is still unclear if it is due to the leap of affinity by itself or due to possible covalent binding of inhibitor to side chains of amino acids. 4-{[(5-nitro-6-oxo-1,6-dihydropyrimidin-4-yl)amino]methyl}benzenesulfonamide (**18a**) similarly to 4-[*N*-(6-chloro-5-nitropyrimidin-4-yl)-4-amino]benzenesulfonamide (**13a**) exhibited specific binding to hCAXIII.



Scheme 2.4-3.

Substitution of chloro group in 6-position of the pyrimidine ring may also occur in other tested compounds in lower rates. Thus, further modification of inhibitors involved introduction of less reactive substituents in this position. Methoxy group and benzylamino group were selected for this purpose. Methoxy group is relatively small substituent, thus, modified inhibitor should occupy the same position in active site of the enzyme as the inhibitor with chloro group. Benzylamino group is large and more flexible substituent which may participate in an additional interaction with nonpolar side chains of amino acids. Since formyl group might also participate in covalent binding with enzyme and, thus, inhibition data might be ambiguous, compounds possessing this group were considered unsuitable as hCA inhibitors.



Scheme 2.4-4.

4-{[*N*-(5-ciano-6-methoxy-2-methylthiopyrimidin-4-yl)amino]methyl}benzene-sulfonamide (**16**) was synthesized by substitution of chloro group in 4-chloro-6-methoxy-2-methylthiopyrimidin-5-carbonitrile (**10b**) with 4-(aminomethyl)benzene-sulfonamide (**9b**) using the same procedure as for the synthesis of compound **12a**. Compounds **17** – **19** were synthesized by modifying previously synthesized 5-cyano- or

5-nitropyrimidines **12** or **13**. Introduction of methoxy group in nitropyrimidines was carried out by refluxing compounds **13** in methanol for 30 min. Substitution of chloro group with benzylamine in compounds **12a** and **13a-c** required more vigorous conditions. Compounds **18a**, **19a-c** were synthesised by heating compounds **12a**, **13a-c** with benzylamine in DMF at 100 °C for several hours in the presence of potassium carbonate.

Introduction of benzylamino substituents into pyrimidines **12a** and **13a-c** or introduction of methoxy substituent into pyrimidine **12b** highly reduced their solubility in water. The solubility of compounds **18a**, **19a-c** was too low for study of their hCA inhibition using ITC method. Due to low solubility inhibition of hCA with compound **16** could not be measured either by ITC or by TSA method.

Substitution of chloro group by benzylamino group in 5-cianopyrimidine **12a** reduced its binding to hCAs more than 100 fold. Activity of 4-[N-(6-methoxy- or 6-benzylamino-5-nitropyrimidin-4-yl)]aminobenzenesulfonamides **17b,c** and **19a-c** was found to be comparable with that of compound **13a**, although compounds **19a-c** had increased affinity for other hCAs (**19a,b** – hCAI and **19c** - hCAII).

Table 2.4-3. Binding to hCAI, II, VII and XIII constants of compounds synthesized for hCA inhibition determined by TSA and ITC, in μM at 37°C.

No.	Compound	hCA I		hCA II		hCA VII		hCA XIII	
		TSA	ITC	TSA	ITC	TSA	ITC	TSA	ITC
1	**8a**	1.0	2.8	0.17	0.32	4.0	ND	ND	ND
2	**12a**	1.4	20	0.071	0.22	0.83	1.7	0.095	0.12
3	**13a**	0.13	0.26	0.091	0.17	0.13	0.78	0.002	0.014
4	**8b**	0.0071	0.083	0.024	0.043	0.10	0.10	0.028	0.13
5	**8c**	0.11	0.48	0.11	0.35	1.0	0.77	0.033	0.19
6	**12b**	0.10	ND	0.17	ND	0.10	ND	0.14	ND
7	**12c**	0.33	ND	0.42	ND	0.10	ND	0.10	ND
8	**13b**†	0.013	0.099	0.0002	0.043	0.005	0.40	0.0001	0.01
9	**13c**†	0.063	0.39	0.17	0.22	0.0017	0.41	0.0002	0.39
10	**15b**	0.17	ND	0.20	ND	0.25	ND	0.020	ND
11	**17b**	0.014	0.11	0.050	0.056	0.83	0.83	0.067	0.24
12	**17c**	0.067	0.28	0.071	0.15	0.13	0.44	0.13	0.23
13	**18a**	100	ND	100	ND	3300	ND	100	ND
14	**19a**	0.071	ND	0.17	ND	10	ND	0.50	ND
15	**19b**	0.025	ND	0.10	ND	4.2	ND	0.33	ND
16	**19c**	0.63	ND	0.016	ND	1.4	ND	0.50	ND
	AZM	1.4	0.78	0.017	0.018	ND	ND	0.050	0.065
	TFM	0.05	ND	0.13	0.091	0.036	0.029	0.020	0.027

† Compounds are unstable in the solution under storage conditions and binding constants vary depending on solution storage period.
ND – data nor available mostly due to insufficient solubility of compounds.
Average standard deviations for both methods are ±25%.

2.5. X-Ray analysis of hCAII-inhibitor complexes

Crystal structures of compounds **8a,b**, **13a**, **15b**, **17b,c** and **19b** bound to hCA II were determined by X-ray crystallography at the Institute of Biotechnology (PDB entries 3mho, 3m5e, 3m40, 3mhi, 3mhl, 3m3x, 3mhm).

The benzenesulfonamide moiety of all inhibitors in all crystal structures is found in the same orientation. The ring is fixed by sulfonamide bound to catalytic Zn ion. Rotations of the ring are restricted by van der Waals contacts with amino acids of active site cavity. Side chains of Val121 and Thr200 restrict the mobility from the sides, whereas Leu198 supports the ring plane.

Compounds **8a,b**, **15b**, **17b,c** and **19b** make an intramolecular hydrogen bond between the nitro group at the C(5) atom of the pyrimidine ring and the amino group of the linker.

The pyrimidine ring is observed in three different orientations in the crystal structures. The pyrimidine ring of compound **8a** is fixed by hydrophobic interaction with Pro202, Val135, Trp5, His64, and Phe131. Similarly to compound **8a**, the pyrimidine ring of **15b** appears to be trapped in this orientation mostly by the van der Waals interactions with Pro202, Phe131, and Val135 (Fig. 2.5-1). In both crystal structures, the DMSO molecule, which originates from the stock solution of the inhibitor, is found between Phe131 and Gln92.

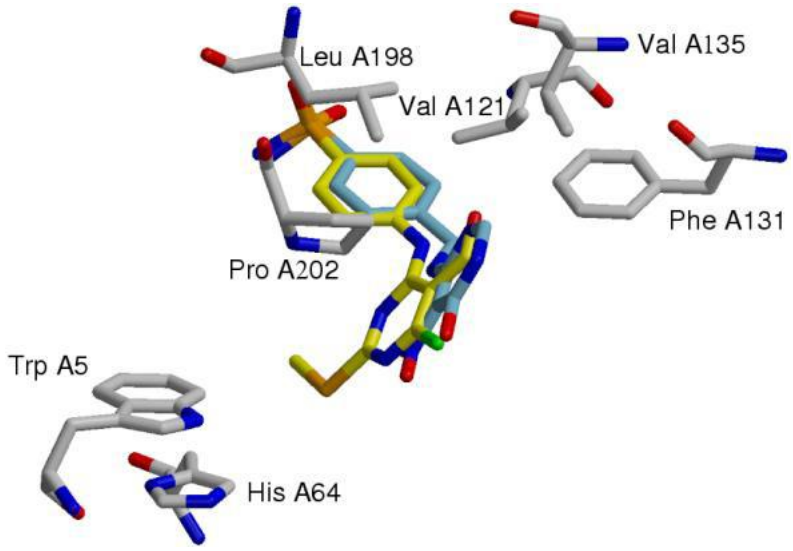


Fig. 2.5-1. View of 4-[*N*-(6-chloro-5-formyl-2-methylthiopyrimidin-4-yl)amino]benzenesulfonamide **8a** (yellow) and 4-{[(5-nitro-6-okso-1,6-dihydropirimidin-4-yl)amino]methyl}benzenesulfonamide **15b** (blue)) bound in active center of hCAII. Protein residues are shown in gray.

Figure 2.5-2 shows the position of several superimposed ligands. The pyrimidine rings of compounds **8b**, **13a**, **17b** and **19b** form a plane that is nearly orthogonal to that formed by **8a** and **15b**. This conformation is fixed in the protein active center by a stacking with Phe131 and by a hydrogen bond between the amino group of Gln92 and a nitro or formyl group at C(5) of the pyrimidine ring (or with the N(3) atom of the pyrimidine ring in compound **13a**). Phe131 restrains the rotation of ligands by forming edge to face stacking with pyrimidine ring. An inferred intramolecular hydrogen bond between the nitro group and the amino group of the linker can render molecules more rigid because it can prevent rotation of the pyrimidine ring.

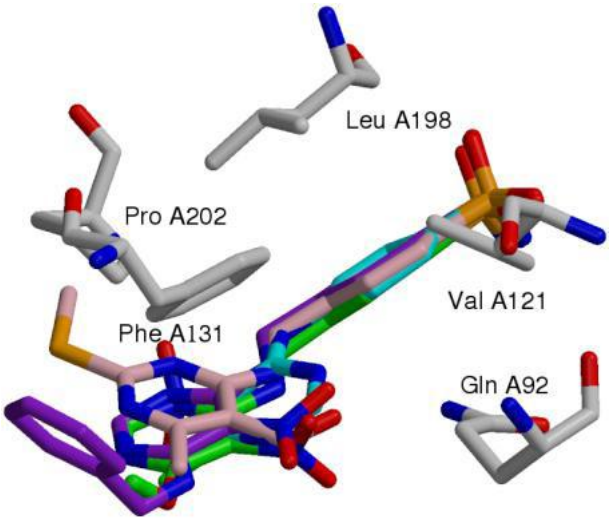


Fig. 2.5-2. View of 4-[*N*-(6-chloro-5-nitropyrimidin-4-yl)amino]benzenesulfonamide **13a** (cyan), 4-{[*N*-(6-chloro-5-formyl-2-methylthiopyrimidin-4-yl)amino]methyl}benzenesulfonamide **8b** (pink), 4-{[N-(6-methoxy-5-nitropyrimidin-4-yl)amino]methyl}benzenesulfonamide **17b** (green) and 4-{[*N*-(6-benzylamino-5-nitropyrimidin-4-yl)amino]methyl}benzenesulfonamide **19b** (purple) bound in active center of hCAII. Protein residues are shown in gray.

Compound **17c** has the longest linker of ethylamino group, and therefore higher intrinsic flexibility could be expected. Nevertheless, it is resolved very well in the crystal structure of the corresponding complex with hCA II. It appears that in the binding of this ligand, hydrophobic interactions play the most important role. Therefore, the nitro group is exposed to bulk solvent without contacts with the protein. The linker and the pyrimidine ring adopt a conformation that places them equidistantly between hydrophobic side chains (Pro202, Val135, and Phe131). Orientation of the pyrimidine ring of **17c** differs from that of other inhibitors.

The benzylamino moiety at 6-position of pyrimidine ring (compound **19b,** Fig. 2.5-2) goes out from the cavity of the active hCAII site thus does not affect binding of inhibitors to the enzyme.

Conclusions

1. Reaction of indoline-2-thiones with pyrimidine-5-carbaldehydes without active nucleofuges at positions 4 and 6 of the pyrimidine ring proceeds under mild conditions to yield (*E*)-3-(5-pyrimidinylmethylene)indoline-2-thiones. The obtained products were shown to participate in Hetero- Diels-Alder reaction with butyne-1,4-dicarboxylic acid dimethyl ester to form the corresponding thiopyrano[2,3-*b*]indoles.
2. Reaction between indoline-5-thiones and 4,6-dichloropyrimidine-5-carbaldehydes leads to the formation of tetracyclic novel heteocycles – pyrimido[4′,5′-5,6]thiopyrano[2,3-*b*]indole and 5*H*-pyrimido[4′,5′:5,6][1,3]thiazino[3,2-*a*]indole. Chloro group of 4-chloro-2-methylthiopyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indole was demonstrated to undergo substitution reactions with various N-, O- or S-nucleophiles to give the corresponding 4-substituted pyrimido[4′,5′:5,6]thiopyrano[2,3-*b*]indoles.
3. Simple and efficient method for synthesis of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl) amino]benzenesulfonamides containing cyano, formyl and nitro groups at position 5 of the pyrimidine ring was prepared. The method involves selective monosubstitution of chloro group in 5-cyano-, 5-formyl- and 5-nitro-4,6-dichloropyrimidines with 4-amino(alkyl)benzene sulfonamides and further modification of 4-[*N*-(2,5-substituted 6-chloropyrimidin-4-yl) amino]benzenesulfonamides at 6^{th} position of pyrimidine ring by substitution of chloro group with nucleophiles.
4. Possibilities of application of quantitative prediction of inhibitory activity constants of inhibitors towards human carbonic anhydrases and prediction of an interaction of inhibitors with protein active site using eHiTS programme has been studied. It was determined that quantitative prediction of binding of inhibitors to hCAs cannot be achieved in satisfactory accuracy by eHiTS programme. However, structures of inhibitor – hCA complexes can be predicted well enough and this method can be used for a design of hCA inhibitors.
5. Docking of 4-[*N*-(2,5,6-substituted pyrimidin-4-yl) amino]benzenesulfonamides and 4-{*N*-[(pyrimido[4′,5′:5,6]thiopyrano[2,3-b]indol-4-yl)]amino}benzenesulfonamides to hCAI, II, VA, VB and IX showed that the compounds should interact with CAs as a classical inhibitors.
6. The synthesised compounds were subjected to the studies of hCAI, II, VII and XIII inhibition and binding to the enzyme active centers. 4-[*N*-(2,5,6-substituted pyrimidin-4-yl) amino]benzenesulfonamides containing ciano, formyl or nitro groups at position 5 and chloro, oxo, methoxy or benzylamino groups at position 6 of the pyrimidine ring were found to inhibit hCAs in nano – micromolar concentration. Some of the synthesised compounds exhibited selectivity towards hCAI, hCAII or hCAXIII enzymes.
7. X-Ray analysis of some of the compounds bound to hCA II showed that the synthesised compounds bind to hCAs as classical CA inhibitors. Their inhibitory activity is determined by interaction of sulfonamide group with a catalytic Zn ion and stacking of the pyrimidine ring with Phe131 phenyl group.

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Reziume

Grįžtamasis CO_2 hidratavimas ir virsmas į hidrokarbonato jonus ir protonus yra paprasta, tačiau gyvybiškai svarbi fiziologinė reakcija, kurią organizme katalizuoja ypatingai efektyvūs metalofermentai karboanhidrazės (CA arba EC 4.2.1.1). CA pasiskirstymas organizme – organuose ir audiniuose bei ląstelės viduje – yra labai įvairus, tad įvairūs CA veiklos nukrypimai, ypač hiperfunkcija, lemia rimtus sutrikimus. Sutrikusi citozolinės CAII ir membraninių CAIV, XII ir XIV veikla inkstuose gydoma diuretikais, slopinančiais šias karboanhidrazes. Glaukoma gydoma lokaliai slopinant CAII. Kraujo krešėjimo sutrikimai iš dalies yra susiję su CAI, kuri yra vienas iš daugelio šio proceso dalyvių. Pastaruoju metu atlikti tyrimai rodo, kad slopinant CAII bei mitochondrines CAVA ir CAVB, dalyvaujančias lipogenezėje, galima kovoti su nutukimu. Slopinant CAIX ir CAXII, kurios yra svarbios auglių vystymuisi (rūgština terpę), galima kovoti su vėžiniais susirgimais. Kaulų audiniuose gausiai esančių CAII, IV ir XIV hiperfunkcija lemia neorganinių komponentų transportą per osteoklastų membraną į išorę, taip prarandant kalcio jonus. Tad šių fermentų slopikliai ateityje gali būti naudojami kaip preparatai nuo osteoporozės.

Šiuo metu apie 30 karboanhidrazių slopiklių yra naudojami kaip klinikiniai preparatai arba yra klinikinių tyrimų stadijoje. Deja, su dideliu α-CA homologiškumu ir plačiu pasiskirstymu organizme susijęs mažas slopiklių atrankumas. Todėl daugelis šiuo metu medicininėje praktikoje naudojamų CA slopiklių turi įvairių šalutinių poveikių: galimas galūnių tirpimas ir dilgčiojimas, metalinis prieskonis, depresija, nuovargis, svorio kritimas, sumažėjęs libido, inkstų akmenligė, virškinamojo trakto sudirgimas, metabolinė acidozė ar trumpalaikė trumparegystė. Tad šiuo metu didžiausias CA slopiklių sintezės iššūkis yra jų atrankumas konkrečiai izoformai.

Šio darbo tikslas – potencialių žmogaus karboanhidrazių slopiklių – 4-[*N*-(pirimidin-4-il)]aminobenzensulfonamidų – kūrimas. Tikslo buvo siekiama dviem etapais.

Pirmasis etapas yra teorinis šių junginių sąveikos su aktyviuoju įvairių hCA izoformų centru tyrimas, skirtas įvertinti, ar 4-[*N*-(pirimidin-4-il)]aminobenzensulfonamidai, turintys pakaitus 2-, 5- ir 6-oje pirimidino žiedo padėtyje, gali įsiterpti į aktyvųjį baltymo centrą, ir apytiksliai įvertinti įvairių funkcinių grupių įtaką jungimuisi su juo. Atlikti dokinimo eksperimentai parodė, kad galima kokybiškai įvertinti šių junginių sąveiką su hCA, o tiriamieji junginiai su hCA turėtų sąveikauti kaip tipiški klasikiniai hCA slopikliai.

Antrojo etapo metu buvo sintetinami potencialūs hCA slopikliai - 4-[*N*-(2,5,6-pakeisti pirimidin-4-il)]aminobenzensulfonamidai. Tiksliniai junginiai sintetinti 4,6-dichlorpirimidinuose, 5-oje padėtyje turinčiuose cian-, formil- arba nitrogrupes chloro atomą keičiant 4-aminobenzensulfonamidu. Bendradarbiaujant su Biotechnologijos instituto mokslininkais, kurie atliko hCA slopinimo susintetintais junginiais tyrimus, tobulintos šių junginių hCA slopinimo savybės. Slopiklių struktūros modifikuotos keičiant jungtuko tarp benzensulfonamido ir heterociklinio fragmento ilgį ir įvedant naujus pakaitus pirimidino žiede, kai kuriais atvejais taip sudarant naujas heterociklines sistemas. Šiuo tikslu ištyrinėta pirimidin-5-karbaldehidų kondensacija su indolin-2-tionais, kurios metu, priklausomai nuo pakaitų pirimidino 4- ir 6- padėtyse, susidaro (*E*)-3-(5-pirimidinilmetilen)indolin-2-tionai arba pirimido[4′,5′:5,6]tiopirano[2,3-*b*]indolai ir 5*H*-pirimido[4′,5′:5,6][1,3]tiazino[3,2-*a*]indolai. 4-chlor-2-metiltiopirimido[4′,5′:5,6]-

tiopirano[2,3-*b*]indolo pavyzdžiu išnagrinėtas šios heterociklinės sistemos 4-os padėties chloro atomo aktyvumas N-, O-, ir S- nukleofilų atžvilgiu.

Ištyrus 4-[*N*-(2,5,6-pakeistų pirimidin-4-il)amino](metil-,etil-)benzensulfonamidų, 5-oje pirimidino žiedo padėtyje turinčių cian-, formil- arba nitrogrupes, o 6-oje pirimidino žiedo padėtyje turinčių benzilamino-, chlor-, metoksi- arba oksogrupes, parodyta, kad jie yra nano- ir mikromolinės eilės slopikliai, galintys atrankiai slopinti hCAI, hCAII arba hCAXIII. Atlikti kai kurių iš šių junginių kompleksų su hCAII rentgenostruktūriniai tyrimai patvirtino, kad 4-[*N*-(2,5,6-pakeisti pirimidin-4-il)amino](metil-,etil-)benzensulfonamidai su hCA jungiasi kaip tipiški klasikiniai hCA slopikliai, o jų hCA slopinimo aktyvumą lemia sulfonamido grupės sąveika su katalitiniu cinko jonu ir pirimidino fragmento sanglauda su aminorūgšties Phe131 šoninės grandinės benzeno žiedu.

Curriculum Vitae

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