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Application of thin-layer chromatographic data in quantitative structure–activity relationship assay of thiazole and benzothiazole derivatives with H_1-antihistamine activity. I

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Abstract

A quantitative structure–activity relationship analysis of H_1-antihistamine activity and chromatographic data of 2-[2-(phenylamino)thiazol-4-yl]ethanamine; 2-(2-benzyl-4-thiazolyl)ethanamine; 2-(2-benzhydrylthiazol-4-yl)ethylamine derivative; 2-(1-piperazinyl- and 2-(hexahydro-1*H*-1,4-diazepin-1-yl)benzothiazole derivatives was made. The RP2 thin-layer chromatography (TLC) plates (silica gel RP2 $60F_{254}$ silanised precoated), impregnated with solutions of selected amino acid mixtures (L-Asp, L-Asn, L-Thr and L-Lys), were used in two developing solvents as hH1R antagonistic interaction models. Using regression analysis, the relationships between chromatographic and biological activity data were found. The correlations obtained in regression analysis for the examined thiazole and benzothiazole derivatives with H_1-antihistamine activity [$pA_2(H_1)$] represent their interaction with all the proposed biochromatographic models (S1–S7). Some of the calculated equations can be applied to predict the pharmacological activity of new drug candidates. The best multivariate relationships useful in predicting the pharmacological activity of thiazole and benzothiazole derivatives were obtained under the condition of experiment with RP2 TLC plates using the developing solvent acetonitrile–methanol–buffer (40:40:20, v/v). The log *P* values of particular compounds are extremely important for this kind of activity.

Keywords: Structure–activity relationships; Regression analysis; Planar chromatography; Thiazoles; Benzothiazoles; Histamine; Organosulfur compounds

1. Introduction

Histamine exerts an immense diversity of physiological and pathophysiological effects and is known to mediate allergic and inflammatory responses through histamine H_1-receptors. It is released, e.g., from mast cells and basophiles by immunological and non-immunological mechanisms. By 1910 histamine had been found to have a profound contractile effect on smooth muscles [1]. Histamine plays an important role in atopic asthma [2], in the pathogenesis of coronary spasms [3–5], allergic rhinitis [6], urticaria [7], atopic dermatitis and eczema [8–10], in the control of sleep and waking state [11], or conjunctivitis [12]. Histamine seems to be an anticonvulsive inhibitory transmitter [13,14] and a

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central inhibitor of intestinal transit [15]. The histamine H_1-receptor plays an important role in allergic conditions and has been the therapeutic target for H_1-receptor antagonists [16]. With the recent cloning of the gene encoding the H_1-receptor [17–22] it became possible to study the interaction of a subtype of specific ligands with the receptor protein. The availability of the genetic information encoding histamine receptor proteins offers a great potential for detailed molecular investigations of ligand–receptor interaction [23]. From pharmacological studies important insights in the interaction of both selective agonists and antagonists were obtained [24]. Consequently, many histamine H_1-receptor antagonists have been developed. Unfortunately, for the H_1-receptor no detailed data on the three-dimensional structure of the receptor proteins is available.

The information necessary for developing new antihistamine H_1-receptor drugs is often obtained from the investigation of other potent agonists or antagonists using the structure–activity relationship, pharmacophoric models, selectivity, in vitro characterisation and their putative interaction with the helical transmembrane (TM) domains of the human histamine H_1-receptor (hH1R) [25–27].

Many processes of drug action and the processes that are basis of chromatographic separation have much in common. The process of receptor binding is dynamic in nature, as well as the solute's distribution processes in chromatography. The same intermolecular interactions determine the behaviour of chemical compounds in both biological and chromatographic environments, especially when the chromatographic systems contain bioactive chemical entities which are significant for biological interaction [28]. There are examples in the literature of biochromatographic data analyses, their implications for molecular pharmacology and application in predicting pharmacological activity of drugs [28–31]. It was demonstrated that systematic information extracted by chemometric analysis of behaviour of antihistamine drugs in diverse high-performance liquid chromatography (HPLC) systems is directly relevant to the pharmacological properties of the solutes [30]. Quantitative relationships between the structure of antihistamine drugs and their retention on an α_1-acid glycoprotein HPLC column were studied in order to identify characteristic structural features of the binding site for antihistamine drugs on α_1-acid glycoprotein [32]. The application of thin-layer chromatographic (TLC) data in quantitative structure–activity relationship (QSAR) assays was described in our previous papers [33,34] when the chromatographic models of β_2-adrenoreceptor interaction were proposed. We have applied the TLC plates, impregnated with solutions of amino acids important for β_2-binding of ligands as a β_2-agonistic and antagonistic interaction models.

Amino acid sequence alignments of the cloned histamine receptors with amino acid sequences of other aminergic receptors suggested that histamine binds to the third (TM3) and fifth (TM5) transmembrane domains of the receptor proteins [35,36]. The aspartic acid residue (Asp107), conserved within TM3 of other receptors [37–39] is one of the most crucial amino acids for the binding of histamine, other H_1 agonists, and H_1 antagonists to the H_1-receptor [25,40–42]. The negatively charged aspartate residue in TM3 is important for the binding of the agonists and antagonists by the ionic interaction with the protonated primary or tertiary amine function—N_α (e.g., ethylamine sidechain of histamine) of the H_1-receptor ligands [25,43,44].

Two residues within TM5 largely determine the specificity of aminergic receptors—the two Ser residues in the β_2-adrenergic receptor [45], and the Asp and Thr residues in the histamine H_2 receptor [39,45]. For the human H_1-receptor, the threonine (Thr194) and asparagine (Asn198) residues are present in the homologous positions [20]. It has been suggested that different histamine H_1-receptor agonists interact in different ways with the receptor protein [46]. The stereoselective binding of the two isomers might be caused by steric hindrance of the side chain of Thr194 [23,35,47]. In the MD simulation, histamine was able to form a stable hydrogen bond between the N_τ-H atom [43] of the imidazole ring and the carbonyl oxygen of the Asn198 side chain in TM5. Lys191 located in the fifth transmembrane domain of the human histamine H_1 receptor interacts with the histamine, but not with all H_1 agonists [23,46]. In the MD simulation, the N_π atom of the histamine imidazole ring interacts with Lys191 forming a hydrogen bond. This locates the N_π atom to the extracellular site of the receptor. This residue is important for the activation of the H_1-

receptor by histamine and the non-imidazole agonists [48,49].

The binding site of antagonists was defined as resulting from an orthogonal interaction between their aromatic ring with Phe199 of hH1R, and hydrophobic interaction between the other aromatic ring and the lipophilic amino acid of the upper part of TM4 and TM5 [50]. This is consistent with the structure–activity data of most described antagonists and can explain the important role of log *P* values of H_1-antagonists.

The proposed in the literature [20,23,25,35–50] binding site models of H_1-receptor for H_1-histaminergic and H_1-antihistamine drugs suggest that the key binding residues are amino acids: Asp107—within TM3 of the H_1-receptor, and Thr194, Asn198, Lys191—within TM5 of the H_1-receptor. In this study we investigated the role of the interaction between selected thiazole derivatives with L-Asp, L-Asn, L-Thr and L-Lys in a chromatographic environment.

The analysis of chromatographic data proposed in this paper is an attempt to find an analytical model of H_1-antihistamine activity of the thiazole and benzothiazole derivatives group. The approach should facilitate the pre-selection of drug candidates, at the same time reducing the costs and the use of laboratory animals.

2. Experimental

2.1. Examined compounds

The examined compounds (compounds **1**–**18**) were chosen from previously synthesised and pharmacologically evaluated thiazole derivatives. The synthesis method, analytical data and biological activity of the compounds: 2-[2-(phenylamino)-thiazol-4-yl]ethanamines (compounds **1** and **2**); 2-(2-benzyl-4-thiazolyl)ethanamines (compounds **3**–**6**); 2-(2-benzhydrylthiazol-4-yl)ethylamine derivatives (compounds **7**–**12**); 2-(1-piperazinyl- and 2-(hexahydro-1*H*-1,4-diazepin-1-yl)benzothiazole derivatives (compounds **13**–**18**) (see Fig. 1) were described in previous papers [51–53].

The thiazole and benzothiazole derivatives showed in pharmacological assays the antagonistic and partial agonistic activity on the H_1-receptor. Some of them showed the antagonistic activity both on the H_1- and H_3-receptors. All compounds were tested for H_1 agonistic or antagonistic activity, and H_3-antagonistic effect in vitro using standard methods [54–56] on isolated guinea pigs. Male guinea pigs weighing 300–400 g were sacrificed. The ileum was excised and placed in buffer (pH 7.4) at room temperature. The potency of an antagonist is expressed by its pA_2 value, calculated from the Schild regression analysis, with at least three concentrations used [51–53]. The pharmacology data of the examined compounds are collected in Table 1.

The other physicochemical parameters (as a log *P*) of the examined compounds were calculated by quantum chemical methods from their structures after geometry optimisation by the HyperChem 7.0 program.

2.2. Chromatography

In this study we investigated the role of the interaction between the examined thiazole derivatives and L-Asp, L-Asn, L-Thr and L-Lys (selected according to the literature [20,23,25,35–50] agonistic and antagonistic binding site models of hH1R) in a chromatographic environment.

For determination of the chromatographic data, TLC systems were used. For the chromatographic evaluation of compounds commercially available RP2 TLC silanised pre-coated plates (silica gel RP2 $60F_{254}$; Merck, Darmstadt, Germany) 20×20 cm were used. A 1-μl volume of methanol solutions (1 mg/ml) of the tested compounds was applied to the plate by the aid of micro-syringe (Hamilton GC). Starting points of the compounds were pointed 20 mm from the bottom edge of the plate, at least 25 mm from side of the plate with 5 mm mutual distance allowance.

Temporary investigations (data not shown) revealed that the best R_F values of compounds can be obtained under the following conditions: a silica gel RP2 $60F_{254}$ as a stationary phase and organic solvents in an 80:20 mixture with buffer (pH 7.4) as a developing solvent (DS). Under these conditions of chromatography the amino acids used for the impregnation of plates (L-Asp, L-Asn, L-Thr, L-Lys) are rather motionless (R_F solutes$>R_F$ amino acids).

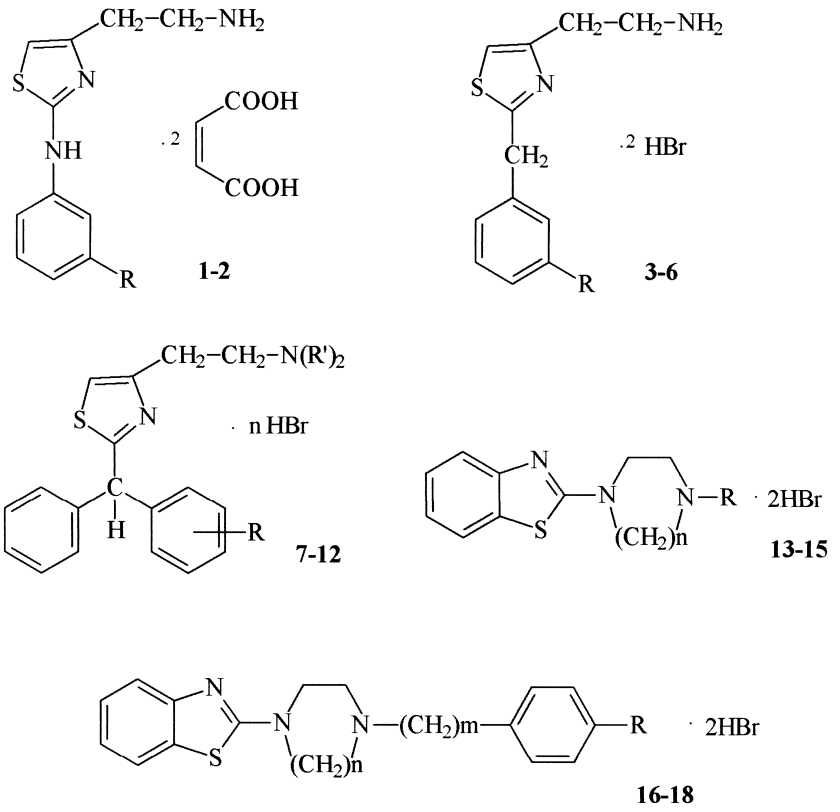

Fig. 1. Structures of the examined compounds **1**–**18**.

Acetonitrile, methanol and methylene chloride were used as a developing organic solvent and 0.02 mol/l ammonium acetate buffer (pH 7.4) as an inorganic solvent. Chromatography was carried out using two eluents: acetonitrile–methanol–buffer (40:40:20, v/v) (DS_A) and acetonitrile–methanol–methylene chloride–buffer (60:10:10:20, v/v) (DS_B).

As chromatographic models of H_1-interaction with solutes, impregnated RP2 TLC plates were used. The plates were impregnated with solutions of amino acids important for this interaction with ligands.

Seven solutions of L-amino acids in buffer were obtained for the preparation of the H_1-interaction models (S1–S7): (a) Asp (0.1 mol/l), S1, (b) Asn (0.1 mol/l), S2, (c) Thr (0.1 mol/l) S3, (d) Lys (0.1 mol/l) S4, (e) Asn (0.1 mol/l)+Thr (0.1 mol/l) S5, (f) Asn (0.1 mol/l)+Lys (0.1 mol/l), S6, (g) Asp (0.1 mol/l)+Asn (0.1 mol/l)+Thr (0.1 mol/l), S7.

The plates were impregnated with the solutions a–g, except the control plates, to give models S1–7. The control plates were prerun for 1 h with developing solvent in use and dried before application of test compounds. The other plates were first prerun for 1 h with the developing solvent and dried. Second, the plates were impregnated with solutions a–g by dip. The overabundance of solution was removed with blotting-paper and the plates were dried with air. Then the test compounds were applied. Running time amounted to 22±2 min (in experiments with eluent DS_B) and 30±2 min (in experiments with eluent DS_A) in which time the front reached 12 cm from the lower edge of the plate.

R_M values were calculated according to Bate-Smith and Westall [57]: $R_M = \log(1/R_F - 1)$.

$R_{M(S1-S7)}$ values represent the implication of interaction of used models S1–7 on test compounds, $R_{M(C)}$ values represent the behaviour of compounds

Table 1
The H_1- and H_3-antihistamine activity and lipophilicity data for the examined compounds **1**–**18**

No.[a]	R[b]	R′[c]	n[d]	m[e]	$pA_2(H_1)$	$pA_2(H_3)$	Log P
1	H	–	–	–	4.44	–	1.29
2	CH_3	–	–	–	4.0	–	1.76
3	F	–	–	–	4.53	–	1.15
4	Cl	–	–	–	4.82	–	1.53
5	Br	–	–	–	4.65	–	1.80
6	OCH_3	–	–	–	4.14	–	0.76
7	H	H	–	–	5.88	–	2.79
8	3-F	H	–	–	6.15	–	2.93
9	3-Cl	H	–	–	6.38	–	3.31
10	4-F	H	–	–	5.99	–	2.93
11	4-Br	H	–	–	5.87	–	3.58
12	H	CH_3	–	–	5.98	–	3.56
13	CH_3	–	3	–	5.70	5.96	2.47
14	H	–	2	–	5.82	5.43	2.05
15	CH_3	–	2	–	5.60	5.95	2.41
16	CH_3	–	3	1	5.99	5.45	4.71
17	CH_3	–	2	2	6.08	5.72	4.91
18	H	–	2	1	5.77	5.65	4.19

[a] Compounds numbered as in Fig. 1.
[b] Substituent of compounds **1**–**18**.
[c] Substituent of compounds **7**–**12**.
[d] Number of CH_2- groups in compounds **13**–**18**.
[e] Number of CH_2- groups in compounds **16**–**18**.

in the control chromatography environment (C). All chromatographic data are shown in Tables 2 and 3.

2.3. QSAR analysis

QSAR analysis of H_1-antihistamine activity and chromatographic data of **1**–**18** derivatives was made. A correlation between biological activity data (see Table 1) and behaviour of the examined compounds in chromatographic environment (S1–7) (see Tables 2 and 3) was investigated by linear and multivariate regression analysis method. The regression analysis was carried out using the STATISTICA 5.1 program. The use of more than one variable in a multivariate equation was justified by inter-correlation study. As data representing the interaction of test compounds with the environment of proposed models (the independent variables), the following descriptors derived from chromatographic data were used: $R_{M(S1-7)}$—as S1–7; $R_{M(C)} - R_{M(S1-7)}$—as C−S1–7; $R_{M(S1-7)}/R_{M(C)}$—as S1–7/C, and as data represent the H_1 and H_3 activity (dependent variable) we used pA_2 values. Additionally, the log P values (see Table 1) of solutes were applied as independent variables in the regression analysis.

3. Results and discussion

To answer the question whether there is any relationship between the behaviour of the examined thiazole and benzothiazole derivatives in chromatographic environments S1–7 (proposed as the analytical models of H_1-antihistamine activity) and their biological activity we used the linear and multivariate regression analysis method.

First, we analysed the relationship between the biological activity data and behaviour of the examined compounds in chromatographic environment of the control (C) (without amino acids). The analysis of the data from pharmacological and chromatographic studies led to the conclusion that there was no correlation between H_1-antihistamine activities of particular compounds **1**–**18** and their C-chromatographic data. The calculated correlation coefficients (R) in DS_A and DS_B were 0.22 and 0.35, respective-

Table 2
The R_M values for the experiment with RP2 TLC and DS_A developing solvent

No.[a]	RP2 TLC R_M (DS_A)							
	$R_{M(C)}$ (C)[b]	$R_{M(S1)}$ (S1)[c]	$R_{M(S2)}$ (S2)	$R_{M(S3)}$ (S3)	$R_{M(S4)}$ (S4)	$R_{M(S5)}$ (S5)	$R_{M(S6)}$ (S6)	$R_{M(S7)}$ (S7)
1	−0.310	−0.585	−0.613	−0.352	−0.278	−0.661	−0.501	−0.562
2	−0.254	−0.526	−0.630	−0.346	−0.240	−0.333	−0.489	−0.562
3	−0.346	−0.579	−0.346	−0.346	−0.269	−0.333	−0.489	−0.579
4	−0.293	−0.531	−0.334	−0.313	−0.236	−0.333	−0.454	−0.491
5	−0.285	−0.511	−0.293	−0.293	−0.222	−0.317	−0.432	−0.511
6	−0.372	−0.579	−0.522	−0.386	−0.298	−0.385	−0.525	−0.559
7	−0.253	−0.453	−0.261	−0.265	−0.208	−0.277	−0.389	−0.425
8	−0.704	−0.673	−0.740	−0.727	−0.865	−0.897	−0.886	−0.725
9	−0.214	−0.403	−0.199	−0.229	−0.167	−0.214	−0.327	−0.394
10	−0.269	−0.453	−0.280	−0.264	−0.222	−0.237	−0.399	−0.448
11	−0.206	−0.403	−0.207	−0.222	−0.167	−0.222	−0.317	−0.394
12	−0.187	−0.342	−0.165	−0.183	−0.043	−0.157	−0.259	−0.325
13	−0.161	−0.411	−0.162	−0.187	−0.105	−0.194	−0.298	−0.403
14	−0.206	−0.511	−0.222	−0.240	−0.231	−0.254	−0.399	−0.492
15	−0.180	−0.289	−0.180	−0.192	−0.213	−0.168	−0.288	−0.273
16	−0.157	−0.284	−0.153	−0.183	−0.122	−0.131	−0.222	−0.253
17	−0.143	−0.169	−0.138	−0.146	−0.140	−0.091	−0.176	−0.164
18	−0.180	−0.172	−0.169	−0.199	−0.203	−0.168	−0.231	−0.195

[a] Compounds numbered as in Fig. 1.
[b] $R_{M(C)}$ = Retention parameter of the compounds in the control environment of chromatography.
[c] $R_{M(S1–S7)}$ = Retention parameters of the compounds in the S1–S7 environment models of chromatography.

Table 3
The R_M values for the experiment with RP2 TLC and DS_B developing solvent

No.[a]	RP2 TLC R_M (DS_B)							
	$R_{M(C)}$ (C)[b]	$R_{M(S1)}$ (S1)[c]	$R_{M(S2)}$ (S2)	$R_{M(S3)}$ (S3)	$R_{M(S4)}$ (S4)	$R_{M(S5)}$ (S5)	$R_{M(S6)}$ (S6)	$R_{M(S7)}$ (S7)
1	−0.753	−0.792	−0.458	−0.417	−0.096	−0.408	−0.443	−0.548
2	−0.359	−0.797	−0.531	−0.434	−0.070	−0.408	−0.410	−0.559
3	−0.218	−1.046	−0.599	−0.542	−0.176	−0.439	−0.443	−0.767
4	−0.164	−0.861	−0.430	−0.477	−0.131	−0.404	−0.389	−0.566
5	−0.154	−0.769	−0.408	−0.393	−0.131	−0.394	−0.368	−0.508
6	−0.444	−1.165	−0.505	−0.541	−0.185	−0.507	−0.477	−0.687
7	−0.191	−0.765	−0.335	−0.365	−0.122	−0.325	−0.308	−0.491
8	−0.869	−1.243	−1.369	−1.151	−0.501	−0.760	−1.380	−1.113
9	−0.098	−0.663	−0.272	−0.336	−0.105	−0.249	−0.269	−0.431
10	−0.135	−0.712	−0.323	−0.373	−0.140	−0.288	−0.308	−0.468
11	−0.079	−0.666	−0.275	−0.320	−0.087	−0.294	−0.250	−0.446
12	0.004	−0.430	−0.110	−0.114	0.026	−0.051	−0.087	−0.235
13	0.007	−0.577	−0.211	−0.240	−0.017	−0.135	−0.176	−0.368
14	0.011	−0.637	−0.296	−0.325	−0.158	−0.183	−0.317	−0.484
15	−0.029	−0.446	−0.180	−0.189	−0.140	−0.116	−0.213	−0.239
16	−0.051	−0.416	−0.158	−0.187	−0.122	−0.131	−0.167	−0.246
17	−0.120	−0.340	−0.195	−0.248	−0.167	−0.154	−0.203	−0.223
18	−0.168	−0.331	−0.248	−0.284	−0.231	−0.195	−0.259	−0.240

[a] Compounds numbered as in Fig. 1.
[b] $R_{M(C)}$ = Retention parameter of the compounds in the control environment of chromatography.
[c] $R_{M(S1–S7)}$ = Retention parameters of the compounds in the S1–S7 environment models of chromatography.

ly. These results may indicate that the other significant relationships, written below (see Table 4), depend upon the specific biochromatographic environment.

A distinct relationship between $pA_2(H_1)$ values and interactions data of the examined compounds with the all models S1–7 can be observed.

Under the conditions of experiment with DS_A, good univariate relationships of the H_1-antihistamine effect involve interactions of solutes with environments of the models: S3, S2 (in the case of analysis for $n=18$) and additionally S1, S3 and S7 (in the case of analysis for $n=12$). The best univariate relationship, explaining the upper 70% of the total variance was obtained from model S3 (see Eqs. (1) and (6) in Table 4). The model **S3** describes this kind of interaction which is possible between the H_1-ligands and Thr194 in TM5 of hH1R. The other univariate relationships, explaining about 50% of the variance, were obtained from models S1, S2 and S7. The models describe the interaction of compounds with Asn198, Asp107 and Asp107, Asn198, Thr194 (in TM3 and TM5 of hH1R). In the case of analysis for the whole group of compounds significant multivariate relationships of the H_1-antihistamine effect involve log P values of solutes, explaining 84–88% of the variance, were obtained from all models S1–7 (some of them are shown in Table 4). Eqs. (2)–(5) may be useful in predicting the pharmacological activity of thiazole and benzothiazole drug candidates. The best multivariate relationship (Eq. (3), see Tables 4 and 5) explains 88% of variance and describes an interaction which is possible between the H_1-ligands and amino acids residues in TM5 of hH1R: Asn198 and Lys191:

$$pA_2 = -1.27(\pm 0.23)S2/C + 9.59(\pm 2.60)C - S6 + 0.63(\pm 0.09)\log P + 3.96(\pm 0.52) \quad (3)$$

Table 4
The relationships between $pA_2(H_1)$ values and descriptors obtained in the experiment with DS_A and DS_B developing solvents

Eq. No.	Independent variables in equation $pA_2 =$		R[a]	F[b]	S[c]	$P<$[d]	n[e]
Developing solvent DS_A							
1	$a + bS3/C$	(1)	0.84	38.497	0.42875	0.00001	18
2	$a + bS3/C - cS1 + d\log P$	(2)	0.93	29.012	0.31489	0.00000	18
3	$a - bS2/C + c(C - S6) + d\log P$		0.94	35.107	0.28976	0.00000	18
4	$a + bS3/C + c(C - S5) + d\log P$	(4)	0.92	25.641	0.33194	0.00001	18
5	$a + bS3/C - cS7 + d\log P$	(5)	0.92	24.943	0.33583	0.00001	18
6	$a + bS3/C$	(6)	0.83	22.918	0.50696	0.00074	12
7	$a - b(C - S1) + cS7$	(7)	0.91	22.195	0.39807	0.00033	12
8	$a - bS1/C + c\log P$	(8)	0.95	39.002	0.31183	0.00004	12
9	$a - bS2/C + c\log P$	(9)	0.95	43.117	0.29806	0.00002	12
10	$a + b(C - S4) - c(C - S6) + d\log P$	(10)	0.96	34.784	0.27441	0.00006	12
11	$a + b(C - S4) - c(C - S3) + d\log P$		0.97	43.826	0.24629	0.00003	12
12	$a - b(C - S3) + cS4/C + d\log P$		0.97	44.892	0.24351	0.00002	12
13	$a - bS6/C + c\log P$		0.96	48.848	0.28159	0.00001	12
14	$a - bS7/C + c\log P$		0.96	54.819	0.26704	0.00001	12
Developing solvent DS_B							
15	$a + bS6/C + c\log P$		0.92	24.447	0.38228	0.00023	12
16	$a - bS3 + c\log P$		0.93	28.497	0.35805	0.00013	12
17	$a - bS1 + c\log P$		0.93	30.788	0.34623	0.00009	12
18	$a - bS4 + c\log P$		0.94	33.694	0.33280	0.00007	12
19	$a - bS1 + c(C - S4) + d\log P$	(19)	0.94	19.981	0.35287	0.00045	12
20	$a - bS4 + c(C - S4) + d\log P$	(20)	0.94	21.881	0.33894	0.00033	12

[a] The correlation coefficient.
[b] The value of the F-test of significance.
[c] The standard error of estimate.
[d] The significance level of the equation.
[e] The number of compounds used to derive the regression equation.

Table 5
The obtained and predicted $pA_2(H_1)$ values of the examined compounds **1**–**18** (under the experimental conditions with RP2 TLC DS_A and DS_B)

Compound	pA_2 obtained	pA_2 predictions: Eq.					
		3	11	12	13	14	18
1	4.440	4.102	4.312	4.301	4.332	4.352	4.369
2	4.000	4.185	4.094	4.096	4.190	4.341	4.693
3	4.530	4.787	4.575	4.580	4.557	4.380	4.382
4	4.820	5.029	4.655	4.650	4.617	4.676	4.598
5	4.650	5.204	4.973	4.981	4.875	4.777	4.820
6	4.140	4.130	4.157	4.145	4.267	4.235	4.061
7	5.880	5.717	5.656	5.719	5.574	5.664	5.604
8	6.150	6.223	6.214	6.088	6.124	6.398	6.311
9	6.380	5.956	5.978	6.041	5.981	5.927	6.032
10	5.990	5.740	5.945	6.031	5.763	5.792	5.751
11	5.870	6.012	6.177	6.265	6.161	6.069	6.222
12	5.980	5.779	6.095	5.933	6.389	6.217	5.988
13	5.700	5.559	–	–	–	–	–
14	5.820	5.745	–	–	–	–	–
15	5.600	5.254	–	–	–	–	–
16	5.990	6.318	–	–	–	–	–
17	6.080	6.145	–	–	–	–	–
18	5.770	5.904	–	–	–	–	–

The correlation of calculated $pA_2(H_1)$ values of the tested compounds predicted by the use of Eq. (3) versus their $pA_2(H_1)$ obtained from the biological tests was significant ($R^2 = 0.88$).

After excluding compounds **13**–**18** from the calculations (under the conditions of experiment with DS_A), possessing both H_1- and H_3-antihistamine activity [53], we could conclude that the $pA_2(H_1)$ effect correlates better with the behaviour of the examined compounds in chromatographic environment of models S1–7. The significant bivariate and multivariate relationships (some of them are shown in Table 4) of the H_1-antihistamine effect involve interactions of solutes with environments of the models: S1 (Eqs. (7) and (8)); S2 (Eq. (9)); S3 (Eqs. (11) and (12)); S4 (Eqs. (10)–(12)); S6 (Eqs. (10) and (13)); S7 (Eqs. (7) and (14)) and explain 83–94% of variance.

The best multivariate relationships involve log *P* values of solutes (Eqs. (11)–(14)) and describe an interaction which is possible between the H_1-ligands and amino acids residues in TM3 and TM5 of hH1R: Asp107, Lys191, Thr194 and Asn198. These relationships can be expressed by the following equations:

$$pA_2(H_1) = 2.82(\pm 1.12)C - S4 - 11.67(\pm 3.15)C - S3 + 0.69(\pm 0.08)\log P + 4.00(\pm 0.60) \quad (11)$$

$$pA_2(H_1) = 0.95(\pm 0.37)S4/C - 11.94(\pm 3.14)C - S3 + 0.76(\pm 0.08)\log P + 2.97(\pm 0.36) \quad (12)$$

$$pA_2(H_1) = -1.60(\pm 3.15)S6/C + 0.74(\pm 0.09)\log P + 5.94(\pm 0.88) \quad (13)$$

$$pA_2(H_1) = -0.95(\pm 0.29)S7/C + 0.79(\pm 0.08)\log P + 5.06(\pm 0.54) \quad (14)$$

We can see clearly that the Eqs. (11)–(14) have the predictive value in new H_1-antihistamine drugs design (see Table 5 and Fig. 2). The correlations of calculated $pA_2(H_1)$ values of the tested compounds predicted by the use of Eqs. (11)–(14) versus their $pA_2(H_1)$ values obtained from the biological tests

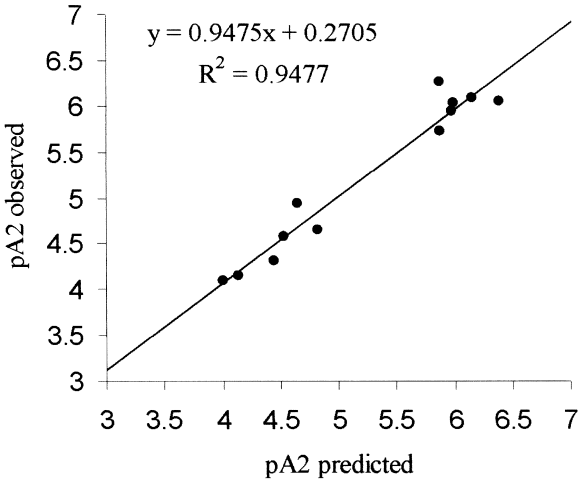

Fig. 2. Correlation of calculated $pA_2(H_1)$ values of the tested compounds predicted by the use of Eq. (12) versus their $pA_2(H_1)$ obtained from the biological tests.

were significant (R^2 0.92–0.95). However, the range of pA_2 data of the examined compounds obtained from the biological tests clustered around two sets (compounds **1**–**6** have pA_2 values between 4.00 and 4.82; compounds **7**–**18** have pA_2 values between 5.60 and 6.38). For the two-point data distribution the possibility of coincidence in the model presented in the figure cannot be eliminated.

Under the experimental conditions with DS_B, there were no good univariate relationships of the H_1-antihistamine effect. We could conclude that the DS_B developing solvent is not as good as the DS_A. The $pA_2(H_1)$ effect correlates less strongly with the behaviour of the examined compounds in chromatographic environment of models S1–7. The significant bivariate and multivariate relationships involve interactions of all the examined solutes (**1**–**18**) with environments of the all S1–7 models, but they explain only 74–77% of the variance (the equations not shown).

After excluding from the calculations compounds **13**–**18** (under the conditions of experiment with DS_B), with H_3-antihistamine activity good bivariate and multivariate relationships were found (see Eqs. (15)–(20) in Table 4). The significant bivariate and multivariate relationships describe interaction which is possible between the H_1-ligands and amino acids residues in TM3 and TM5 of hH1R: Asp107, Lys191, Thr194 and Asn198. These relationships can be expressed by following equations:

$$pA_2(H_1) = 0.02(\pm 0.01)S6/C + 0.83(\pm 0.12)\log P + 3.34(\pm 0.30) \quad (15)$$

$$pA_2(H_1) = -0.64(\pm 0.45)S3 + 0.82(\pm 0.11)\log P + 3.06(\pm 0.37) \quad (16)$$

$$pA_2(H_1) = -0.85(\pm 0.51)S1 + 0.92(\pm 0.13)\log P + 2.42(\pm 0.65) \quad (17)$$

$$pA_2(H_1) = -1.78(\pm 0.92)S4 + 0.82(\pm 0.10)\log P + 3.09(\pm 0.30) \quad (18)$$

The predictive role of the Eq. (18) is shown in Table 5. The correlation of calculated $pA_2(H_1)$ values of the tested compounds predicted by the use of Eq. (18) versus their $pA_2(H_1)$ obtained from the biological tests was significant ($R^2 = 0.88$).

There was no significant correlation between the chromatographic data and H_3-antihistamine activities of compounds **13**–**18**. Moderate H_3-antihistamine activity of derivatives **13**–**18** observed in the experiment with guinea pig ileum exhibited exclusive correlation within the interaction between these compounds and the model S3 (use of DS_A: $R = 0.50$; $n = 6$) and the model S4 (use of DS_B: $R = 0.50$; $n = 6$). These results indicate that the proposed S1–7 models cannot explain the intermolecular interaction between ligands and hH3R. Compounds **13**–**18**, with both H_1- and H_3-antihistamine activity [53] are not selective. Their H_1 affinities can be less strong than those of **1**–**12** [51,52]. This may explain the fact that correlation coefficients, which were found in this experiment for the group of compounds **1**–**12**, are better than these accounted for in the whole group.

On the basis of the described results, we can clearly see that the log *P* parameter is crucial for the H_1-antihistamine effect of the thiazole derivatives. An increase of the log *P* value favours higher biological activity of the tested compounds. A lot of significant multivariate relationships of the H_1-antihistamine effect involve log *P* values of solutes (Table 4). The calculated univariate relationships as the correlation coefficients between $pA_2(H_1)$ and log *P* values were: $R = 0.79$ for the whole group of

compounds ($n = 18$) and 0.91 for compounds **1**–**12** ($n = 12$).

4. Conclusion

It is evident in QSAR assay that all the best correlations obtained in regression analysis for thiazole and benzothiazole derivatives (**1**–**18**) with H_1-antihistamine activity [p$A_2(H_1)$] represent their interaction of them with the proposed biochromatographic models (S1–S7). The same intermolecular interactions can determine the behavior of the examined compounds in both biological and chromatographic environments, because the proposed chromatographic models contain bioactive chemical entities which are significant for biological interaction. It is possible that the Asn and Lys are able to form the hydrogen bonds with the N atoms of the solutes. The negatively charged aspartate residue may be important for the binding of the solutes by the ionic interaction with their protonated amine function. We can also see that the log *P* values of particular compounds are extremely important for this kind of activity.

Some of the calculated equations can be applied to predict the pharmacological activity of new drug candidates. It should facilitate their pre-selection, at the same time reducing the cost and the use of laboratory animals.

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