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Normal and reversed phase thin layer chromatography data in quantitative structure–activity relationship study of compounds with affinity for serotonin (5-HT) receptors

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ABSTRACT

Quantitative structure–activity relationship (QSAR) analysis of 20 drugs with affinity for serotonin (5-HT) receptors was carried out. A set of physicochemical parameters calculated by HyperChem 7.0 and ACDLabs 8.0 programs and chromatographic data were applied in the analysis. Thin layer chromatography was performed on silica gel NP $60F_{254}$ and silica gel RP2 $60F_{254}$ (silanized) plates impregnated with solutions of aspartic acid, serine, phenylalanine, tryptophan, tyrosine, asparagine, threonine and their mixtures (denoted as S1–S11 models), with two mobile phases – the systems were chosen as models of drug-5-HT-receptor interaction. Relationships between chromatographic data and molecular descriptors and biological activity data were found by means of regression analysis. The correlations obtained for the compounds with serotoninergic activity represent their interaction with the proposed biochromatographic models (S1–S11). The presented regression models based on biochromatographic studies can be an efficient tool in the QSAR analysis for initial prediction of compounds activity direction within 5-HT receptors.

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1. Introduction

Determination of biological activity of a specific chemical substance is particularly important in the research aimed at the development of new drugs. Experimental determination of such activity is a time-consuming and costly process, based on the knowledge and experience of highly qualified experts (biologists, pharmacologists). Computer-aided methods, allowing to predict in parallel even over ten types of biological activity of novel, potentially therapeutic compounds, are used in this field in addition to standard biological tests [1–4]. Such methods are based mainly on analysis of the structure–activity correlations and comparisons with the database, consisting of substances with known biological activity. The discovery, dating back to the 19th century, that the biological activity of compounds is determined by their structure, a dependence currently referred to as QSAR (Quantitative Structure Activity Relationship), allows to identify specific characteristics of the analyzed compound molecule affecting its biological activity. The classic QSAR analysis utilizes regression techniques enabling to develop predictive models, which can be used for prediction of biological activity of potential new drugs with similar structure and mechanism of action. Such models make use both of calculated parameters, resulting from the chemical structure of the compounds, and determined ones, obtained by their interaction with the experimental medium. Chromatographic systems containing the chemical elements of biological environment, which simulate the conditions of interaction of the studied compounds with a living organism are used here [5–11]. The knowledge of structure and function of a specific biological target (e.g. receptor, enzyme) can provide the basis for construction of an analytical model for indirect observation of the activity of chemical compounds in biological environment. So-called biochromatographic medium, devised on the basis of data concerning the structure of the biological target and serving as a laboratory imitation of the natural environment in which the potential drug will act, can be used for this purpose.

The available information concerning the ligand-binding sites within serotonin (5-HT) receptors allow to equip the biochromatographic model with the chemical components of biological environment directly responsible for the formation of a drug–receptor complex [12–22]. Serotonin receptors are present both in the central and in the peripheral nervous system, thus play an important role in regulation of many physiological processes. They have a common origin and similar mechanisms of action. So far, seven families of such receptors (5-HT_1–5-HT_7) were distinguished and several subtypes within each family. They are all classified as metabotropic receptors, except for the 5-HT_3 family, which belongs to the ionotropic receptor class [23]. As established on the basis of literature data, the following amino

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acids play an essential role in formation of drug-serotonin (5-HT) receptor complex: aspartic acid (Asp155), serine (Ser159), phenylalanine (Phe340), asparagine (Asn333), tryptophan (Trp200, 236, 367), tyrosine (Tyr370) and threonine (Thr196) [12–22]. A detailed description of the model of binding sites was presented in an earlier study [24]. This study is a continuation of research aiming to check the possibility of application of the data obtained from thin layer chromatography and computer-aided calculations of physicochemical parameters in correlation equations allowing to predict the receptor binding affinity (pK_i), as well as agonistic (pD_2) or antagonistic (pA_2) activity of chemical compounds interacting with serotonin receptors [24].

2. Experimental

2.1. Examined compounds

The compounds studied in this work (comp. **1**–**20**) were purchased at a pharmacy, in the form of a pharmaceutical preparation, or acquired in the pharmaceutical and chemical company as a standard substance. All compounds have the biological activity directed at the serotonin receptors: tiapride (**1**) (*Tiapridal*; Synthelabo Groupe Quetigny, Quetigny, France), clopenthixol (**2**) (*Clopixol Depot*; H. Lundbeck A/S, Copenhagen, Denmark), flupentixol hydrochloride (**3**) (*Fluanxol*; H. Lundbeck A/S, Copenhagen, Denmark), trifluoperazine (**4**) (*Apo-Trifluoperazine*; Apotex Inc., Weston Ontario, Canada), clozapine (**5**) (*Leponex*; Novartis Pharma AG, Basle, Switzerland), risperidone (**6**) (*Rispolept*; Janssen Pharmaceutica N. V., Beerse, Belgium), olanzapine (**7**) (*Zolafren*; Adamed, Czosnów, Poland), tropisetron (**8**) (*Novoban*; Novartis Pharma AG, Basle, Switzerland), cyproheptadine hydrochloride (**9**) (*Peritol*; Egis Pharmaceuticals LTD, Budapest, Hungary), trazodone hydrochloride (**10**) (*Trittico CR*; Aziente Chimiche Riunite Angelini Francesco ACRAF S.p.A, Viale Amelia, Italy), mianserin hydrochloride (**11**) (*Lerivon*; N.V. Organon, Oss, Netherlands), pizotifene (**12**) (*Polomigran*; Polon, Łódź, Poland), mirtazapine (**13**) (*Remeron*; N.V. Organon, Oss, Netherlands), buspirone hydrochloride (**14**) (Sigma–Aldrich, St. Louis, USA), sumatriptan succinas (**15**) (*Sumamigren*; Polpharma, Starogard Gdański, Poland), rizatriptan benzoate (**16**) (Merck Research Laboratories, Rahway, USA), zolmitriptan (**17**) (*Zomig*; Astra Zeneca UK, Macclesfield, Great Britain), cisapride (**18**) (*Gasprid*; Polfa, Kutno, Poland), serotonin hydrochloride (**19**) (Sigma–Aldrich, USA), propranolol hydrochloride (**20**) (*Propranolol*; Warszawskie Zakłady Farmaceutyczne Polfa, Warszawa, Poland). The active substances were isolated from pharmaceutical products with methods described according to specific monographs presented in Polish Pharmacopoeia and information available in The Merck Index Twelfth Edition, 1996. The data concerning pharmacological properties and activity profiles of the particular compounds are presented in Table 1.

Table 1
Biological activity for compounds **1**–**20**.

Comp.	pK_i [a]	pD_2 [b]	pA_2 [c]	Ref.
1	–	7.97	5.10	[25]
2	7.60	7.99	7.30	[25]
3	7.00	7.88	6.90	[25]
4	7.90	8.04	5.70	[22,25]
5	6.00	–	7.50	[26,27]
6	9.90	8.16	6.69	[25]
7	8.10	8.10	7.20	[25]
8	8.40	–	6.60	[26,28]
9	8.22	–	8.73	[26,29]
10	7.40	–	8.79	[30,31]
11	9.70	8.09	7.50	[25]
12	7.40	–	9.20	[30,32]
13	8.40	6.88	7.99	[25]
14	7.70	7.70	6.35	[25]
15	6.60	5.80	–	[30,33]
16	6.40	6.30	–	[30,34]
17	6.60	6.20	–	[30,34]
18	7.40	7.60	7.30	[25,30]
19	6.40	–	–	[26]
20	7.50	–	6.08	[35,36]

[a] pK_i: 5-HT receptor binding affinity.
[b] pD_2: agonistic activity.
[c] pA_2: antagonistic activity.

2.2. Chromatography

The compounds **1**–**20** were subjected to chromatographic analysis under reproducible conditions. Acetonitrile, methanol and methylene chloride were used as a developing organic solvents and 0.02 mol/L ammonium acetate buffer of pH 7.4 as an inorganic solvent. The analysis was carried out in normal (NP TLC) and reversed (RP2 TLC) phase system, for two variants of the mobile phase (denoted as DS_A and DS_B): acetonitrile–methanol–buffer (40:40:20, v/v/v; DS_A) and acetonitrile–methanol–methylene chloride–buffer (60:10:10:20, v/v/v/v; DS_B). Aluminium TLC silica gel 60 F_{254} sheets (20 cm × 20 cm, Merck, Darmstadt, Germany) and glass TLC silica gel 60 RP2 F_{254} plates (silanized; 20 cm × 20 cm, Merck, Darmstadt, Germany) were used as the stationary phase. Each plate was submitted to initial passaging (in the presence of an appropriate mobile phase – DS_A or DS_B) under chromatographic conditions for 1.5 h, then the plates were air-dried. The stationary phase was modified by impregnation with 0.03 mol/L binding L-amino acids solutions to obtain the designed biochromatographic models that were denoted as: (a) Asp – S1, (b) Ser – S2, (c) Phe – S3, (d) Trp – S4, (e) Tyr – S5, (f) Asn – S6, (g) Thr – S7, (h) Asp + Ser (1:1; v/v) – S8, (i) Asp + Ser + Phe (1:1:1; v/v/v) – S9, (j) Trp + Tyr (1:1; v/v) – S10, (k) Asn + Thr (1:1; v/v) – S11.

The plates were impregnated with the solutions (a)–(k) by spraying (GS1apparatus, Desaga, Germany), then air-dried. Additional plates (two for each type of mobile and stationary phase) were left clean for control analysis (C – without amino acids solutions). The compounds **1**–**20** were weighed on analytical laboratory scales with 0.1 mg accuracy, and then dissolved in methanol to obtain 1.0 mg/mL concentrations. The compounds in 1.0 μL quantities were applied onto the previously prepared plates by means of a Nanomat 4 applicator (Camag, Switzerland), at 0.8 cm intervals. The distance from the lateral edges was 2 cm. The start line was set at the level of 2 cm from the lower edge of the plate. The chromatograms were developed in a horizontal chromatographic chamber with an eluent dispenser, DS-II-20x20 (CHROMDES, Lublin, Poland) to the height of 12 cm above the lower edge of the plate. The duration of chromatogram development was 45 ± 2 min and 38 ± 2 min (NP TLC system, for eluents DS_A and DS_B, respectively), and 35 ± 2 min and 28 ± 2 min (RP2 TLC system, for eluents DS_A and DS_B, respectively). The plates were scanned densitometrically at 280 nm by means of a Desaga CD 60 densitometer with Windows-compatible ProQuant software (Desaga, Germany). The R_f values for the particular compounds were read, and then the R_M values were calculated according to Bate-Smith and Westall [37]: $R_M = \log(1/R_f - 1)$.

The R_M values used for analysis constituted a mean from two reproducible experiments. $R_{M(S1)} - R_{M(S11)}$ and $R_{M(C)}$ values for the analytes were presented in the course of the described quantitative analysis as S1–S11 and C, respectively, whereas the derivatives of these results were denoted with symbols: C-S (1–11) and S (1–11)/C.

C-S (1–11) parameters describe the retention difference obtained for the compounds between the control group and the examination conducted in individual models environments. S (1–11)/C parameters describe the relation between particular R_M

Table 2
The R_M values for the experiment with NP TLC system.

NP TLC R_M (DS_A and DS_B)												
Comp.	$R_{M(C)}$ (C)[a]	$R_{M(S1)}$ (S1)[b]	$R_{M(S2)}$ (S2)	$R_{M(S3)}$ (S3)	$R_{M(S4)}$ (S4)	$R_{M(S5)}$ (S5)	$R_{M(S6)}$ (S6)	$R_{M(S7)}$ (S7)	$R_{M(S8)}$ (S8)	$R_{M(S9)}$ (S9)	$R_{M(S10)}$ (S10)	$R_{M(S11)}$ (S11)
Developing solvent DS_A												
1	0.720	0.421	0.644	0.689	0.689	0.659	0.589	0.644	0.537	0.575	0.704	0.644
2	0.096	−0.026	0.131	0.185	−0.026	−0.061	−0.114	0.114	−0.052	−0.026	0.078	0.087
3	0.061	−0.087	0.052	0.131	−0.122	−0.176	−0.317	−0.213	−0.122	−0.140	0.035	0.017
4	0.410	0.176	0.378	0.432	0.389	0.327	0.288	0.337	0.203	0.231	0.368	0.347
5	0.114	0.052	0.140	0.185	0.043	0.017	0.087	0.105	0.185	0.017	0.105	0.122
6	0.259	0.240	0.176	0.185	0.185	0.149	0.122	0.131	0.176	0.122	0.176	0.131
7	0.432	0.477	0.537	0.537	0.489	0.501	0.443	0.454	0.410	0.443	0.443	0.477
8	0.673	0.231	0.575	0.659	0.589	0.513	0.454	0.513	0.358	0.399	0.630	0.537
9	0.358	−0.035	0.288	0.337	0.009	−0.035	−0.288	0.222	−0.009	−0.087	0.269	0.185
10	−0.537	−0.399	−0.443	−0.443	−0.421	−0.489	−0.562	−0.501	−0.477	−0.525	−0.489	−0.513
11	−0.043	−0.087	−0.009	0.017	−0.149	−0.176	−0.317	−0.250	−0.122	−0.213	−0.096	−0.096
12	0.368	−0.035	0.000	0.358	0.061	0.000	−0.231	−0.240	0.061	0.017	0.308	0.185
13	0.140	0.149	0.213	0.213	0.140	0.131	0.122	0.140	0.131	0.061	0.149	0.167
14	−0.358	−0.288	−0.185	−0.298	−0.298	−0.337	−0.443	−0.389	−0.327	−0.454	−0.575	−0.389
15	0.562	0.176	0.489	0.537	0.501	0.389	0.368	0.432	0.222	0.213	0.513	0.432
16	0.865	0.616	0.807	0.865	0.788	0.771	0.689	0.737	0.659	0.513	0.845	0.753
17	0.644	0.222	0.562	0.616	0.562	0.489	0.443	0.501	0.337	0.231	0.602	0.525
18	−0.501	−0.432	−0.203	−0.432	−0.358	−0.537	−0.753	−0.616	−0.550	−0.845	−0.575	−0.644
19	0.432	−0.043	0.358	0.443	0.525	0.158	−0.347	0.278	−0.061	−0.250	0.213	0.250
20	0.327	−0.347	0.096	0.140	0.035	−0.562	−0.704	−0.443	−0.421	−0.704	−0.704	−0.616
Developing solvent DS_B												
1	0.550	0.443	0.550	0.616	0.537	0.489	0.399	0.421	0.443	0.432	0.525	0.550
2	−0.194	−0.105	−0.087	0.009	−0.087	−0.149	−0.317	−0.347	−0.203	−0.140	−0.278	−0.250
3	−0.278	−0.167	−0.176	−0.061	−0.140	−0.240	−0.410	−0.432	−0.259	−0.203	0.070	−0.347
4	−0.078	−0.078	−0.035	0.070	0.035	−0.078	−0.259	−0.317	−0.222	−0.131	−0.017	−0.231
5	−0.140	−0.061	−0.078	−0.009	−0.009	−0.105	−0.231	−0.259	−0.149	−0.096	−0.213	−0.203
6	0.368	0.240	0.250	0.298	0.327	0.259	0.149	0.131	0.203	0.203	0.213	0.176
7	0.308	0.337	0.347	0.389	0.347	0.288	0.203	0.176	0.222	0.259	0.185	0.213
8	0.222	0.167	0.278	0.399	0.288	0.222	0.087	0.052	0.122	0.140	0.149	0.122
9	−0.259	−0.158	−0.140	−0.035	−0.096	−0.231	−0.443	−0.443	−0.288	−0.231	−0.358	−0.358
10	−0.616	−0.410	−0.537	−0.466	−0.399	−0.575	−0.659	−0.704	−0.443	−0.466	−0.489	−0.550
11	−0.389	−0.250	−0.308	−0.278	−0.317	−0.389	−0.525	−0.550	−0.368	−0.347	−0.410	−0.432
12	−0.194	−0.327	−0.096	−0.753	−0.078	−0.240	−0.399	−0.454	−0.288	−0.176	−0.358	−0.454
13	0.026	0.078	0.070	0.105	0.122	0.026	−0.052	−0.087	−0.009	−0.009	−0.070	−0.026
14	−0.337	−0.213	−0.278	−0.278	−0.176	−0.337	−0.432	−0.489	−0.298	−0.337	−0.389	−0.368
15	0.410	0.250	0.432	0.466	0.347	0.269	0.167	0.131	0.259	0.269	0.278	0.231
16	0.720	0.589	0.788	0.807	0.826	0.753	0.659	0.644	0.630	0.443	0.753	0.720
17	0.477	0.327	0.537	0.550	0.432	0.347	0.250	0.240	0.347	0.222	0.421	0.327
18	−0.562	−0.432	−0.513	−0.477	−0.399	−0.644	−0.753	−0.771	−0.489	−0.644	−0.537	−0.575
19	0.368	0.250	0.410	0.501	0.317	0.278	0.140	0.114	0.250	0.105	0.213	0.203
20	−0.259	−0.140	−0.122	−0.070	−0.513	−0.259	−0.432	−0.477	−0.259	−0.317	−0.337	−0.317

[a] $R_{M(C)}$: retention parameter of the compounds in control environment of chromatography.
[b] $R_{M(S1–S11)}$: retention parameters of the compounds in S1–S11 models environment of chromatography.

values. These parameters better reflect the influence caused by appropriate modifying factors (amino acids) presence then simple $R_{M(S1–S11)}$ measurements.

The results of chromatographic analysis are presented in Tables 2 and 3.

2.3. Calculation of the molecular descriptors

The molecular descriptors were calculated with HyperChem 7.0 [38] and ACD/Labs 8.0 [39] programs and they are collected in Table 4. HyperChem 7.0 software, utilizing semi-empirical AM1 method with Polak-Ribiere'a algorithm, was used for calculation of the following molecular descriptors: the total energy (E_T, kcal mol^{-1}), the binding energy (E_b, kcal mol^{-1}), the heat of formation (ΔH_F, kcal mol^{-1}), the total dipole moment (μ, D), the energy of the highest occupied molecular orbital (ε_{HOMO}, eV), the energy of the lowest unoccupied molecular orbital (ε_{LUMO}, eV), the grid surface area (A_S, Å^2), the molar volume (V_m, Å^3), the hydration energy (E_H, kcal mol^{-1}), the logarithm of the octanol/water partition coefficient ($\log P$), the molar refractivity (R_m, Å^3), polarizability (α, Å^3), the molecular weight (M_W, g mol^{-1}) and the net atomic charge on the nitrogen atom (Q_N). The distribution coefficient ($\log D$), the polar surface area (*PSA*, Å^2), the dissociation constant (pK_a), the number of H-bond donors (HD) and the number of H-bond acceptors (HA) were calculated using ACD/Labs 8.0.

2.4. Statistical analysis

The chromatographic data together with physicochemical parameters were submitted to chemometric analysis. The relationships between the behavior of compounds **1**–**20** in chromatographic environments (proposed as analytical models of serotoninergic activity), their physicochemical properties and their biological activity (pK_i, pD_2, pA_2) were tested using stepwise multiple linear regression (MLR) analysis and correlation analysis. Those analyses were carried out using *STATISTICA 8.0* program [40]. Values of biological activity (pK_i, pD_2 and pA_2) of the analyzed compounds were used as dependent variables (regressand), as independent variables (regressors) were applied the chromatographic data and the calculated physicochemical descriptors.

The statistical quality of the obtained mathematical models was estimated with the help of the following statistical indicators: the correlation coefficient (R), the squared correlation coefficient

Table 3
The R_M values for the experiment with RP2 TLC system.

RP2 TLC R_M (DS_A and DS_B)												
Comp.	$R_{M(C)}$ (C)	$R_{M(S1)}$ (S1)	$R_{M(S2)}$ (S2)	$R_{M(S3)}$ (S3)	$R_{M(S4)}$ (S4)	$R_{M(S5)}$ (S5)	$R_{M(S6)}$ (S6)	$R_{M(S7)}$ (S7)	$R_{M(S8)}$ (S8)	$R_{M(S9)}$ (S9)	$R_{M(S10)}$ (S10)	$R_{M(S11)}$ (S11)
Developing solvent DS_A												
1	−0.185	−1.235	−0.489	0.105	0.140	−0.194	−0.213	−0.278	−0.673	−0.432	−0.213	−0.231
2	0.035	−0.052	0.087	0.087	0.122	0.122	0.043	0.043	0.026	0.043	0.043	0.043
3	0.070	−0.035	0.096	0.087	0.131	0.114	0.052	0.061	0.043	0.043	0.052	0.035
4	0.337	0.231	0.327	0.308	0.389	0.368	0.317	0.327	0.259	0.298	0.317	0.288
5	0.009	−0.105	0.009	0.009	0.052	0.052	−0.009	0.000	−0.043	0.000	−0.009	−0.017
6	−0.194	−0.908	−0.269	−0.167	−0.149	−0.269	−0.389	−0.327	−0.389	−0.399	−0.288	−0.337
7	0.114	0.078	0.122	0.140	0.122	0.140	0.052	0.078	0.070	0.087	0.078	0.070
8	0.052	−0.689	−0.009	0.105	0.070	0.078	0.009	0.052	−0.213	−0.078	0.052	0.035
9	0.269	0.052	0.203	0.259	0.288	0.250	0.231	0.250	0.105	0.185	0.231	0.222
10	−0.378	−0.489	−0.389	−0.432	−0.378	−0.432	−0.378	−0.410	−0.399	−0.432	−0.399	−0.410
11	0.026	−0.131	−0.026	0.000	0.035	−0.017	0.009	0.000	−0.043	−0.035	−0.035	−0.017
12	0.308	0.052	0.259	0.087	0.278	0.259	0.269	0.278	0.096	0.167	0.222	0.231
13	−0.078	−0.194	−0.087	−0.096	−0.061	−0.105	−0.078	−0.105	−0.149	−0.140	−0.122	−0.122
14	−0.288	−0.466	−0.269	−0.317	−0.250	−0.298	−0.288	−0.308	−0.378	−0.378	−0.308	−0.317
15	−0.149	−1.279	−0.288	−0.185	−0.131	−0.194	−0.269	−0.194	−0.240	−0.602	−0.259	−0.185
16	0.194	−1.032	0.061	0.410	0.259	0.327	0.000	0.131	−0.096	0.096	0.269	0.140
17	−0.122	−1.195	−0.250	−0.087	−0.087	−0.167	−0.176	−0.096	−0.087	−0.477	−0.203	−0.149
18	−0.176	−0.466	−0.203	−0.203	−0.140	−0.167	−0.203	−0.203	−0.308	−0.269	−0.203	−0.213
19	0.009	−1.380	−0.035	0.140	0.061	0.105	−0.035	0.114	−0.288	−0.259	0.070	0.105
20	0.070	−0.477	0.017	0.096	0.061	0.078	0.017	0.035	−0.203	−0.017	0.043	0.035
Developing solvent DS_B												
1	0.035	−0.298	0.043	0.035	0.026	0.078	0.035	0.017	−0.078	−0.078	−0.035	−0.017
2	0.213	0.035	0.298	0.259	0.278	0.317	0.240	0.240	0.122	0.158	0.194	0.203
3	0.231	0.009	0.317	0.259	0.288	0.308	0.185	0.231	0.114	0.140	0.185	0.213
4	0.477	0.203	0.513	0.525	0.550	0.213	0.454	0.501	0.399	0.466	0.432	0.454
5	0.140	−0.017	0.185	0.149	0.222	0.194	0.131	0.149	0.087	0.070	0.114	0.149
6	0.096	−0.158	0.026	0.078	0.096	0.087	−0.052	−0.052	−0.087	−0.070	−0.043	−0.070
7	0.288	0.158	0.378	0.317	0.337	0.337	0.240	0.317	0.240	0.250	0.259	0.269
8	0.122	−0.158	0.131	0.078	0.158	0.140	0.078	0.096	0.026	0.009	0.052	0.043
9	0.389	0.122	0.399	0.378	0.443	0.399	0.317	0.358	0.240	0.269	0.337	0.337
10	−0.288	−0.259	−0.250	−0.298	−0.250	−0.278	−0.308	−0.327	−0.213	−0.368	−0.337	−0.308
11	0.176	0.070	0.203	0.194	0.222	0.231	0.140	0.194	0.167	0.114	0.122	0.149
12	0.432	0.158	0.421	0.432	0.410	0.432	0.288	0.298	0.278	0.288	0.337	0.317
13	0.114	0.009	0.131	0.114	0.096	0.131	0.087	0.096	0.105	0.035	0.070	0.087
14	−0.070	−0.140	−0.009	−0.061	−0.026	−0.043	−0.078	−0.043	−0.078	−0.158	−0.149	−0.140
15	−0.026	−0.327	−0.052	−0.043	−0.026	−0.017	−0.078	−0.026	−0.078	−0.176	−0.087	−0.061
16	0.240	0.017	0.259	0.250	0.240	0.278	0.203	0.278	0.122	0.149	0.203	0.213
17	0.009	−0.317	0.000	−0.017	0.000	0.061	−0.035	0.009	−0.096	−0.167	−0.061	−0.035
18	0.087	−0.078	0.131	0.070	0.114	0.149	0.052	0.096	0.009	−0.035	0.035	0.061
19	0.131	−0.213	0.158	0.105	0.096	0.213	0.213	0.194	0.026	−0.009	0.122	0.167
20	0.194	−0.114	0.185	0.203	0.158	0.222	0.131	0.176	0.009	−0.017	0.114	0.114

Table 4
The calculated molecular descriptors for compounds **1**–**20**.

Comp.	E_T	E_b	ΔH_F	μ	ε_{HOMO}	ε_{LUMO}	A_S	V_m	E_H	log *P*	R_m	α	M_W	Q_N	log *D*	pK_a	PSA	HD	HA
1	−95,473.516	(4458.85	(114.41802	5.419	−9.223006	−0.8138675	363.122	299.487	−5.377	−1.561	91.230	31.512	328.426	−0.283	−1.480	9.660	84.090	1	6
2	−102,871.32	−5376.37	66.706707	1.296	−7.679547	−0.8795735	390.649	362.081	−7.202	−0.058	125.836	44.971	400.966	−0.264	5.060	3.400	52.010	1	3
3	−130,832.64	−5768.48	−126.83314	3.341	−8.185675	−0.6107714	428.401	375.931	−7.158	0.734	126.334	44.605	434.519	−0.260	4.420	3.400	52.010	1	3
4	−121,985.47	−5378.24	−77.028567	3.373	−7.748451	−0.5433432	397.66	353.227	−1.068	−0.189	119.657	41.841	407.497	−0.243	4.210	8.210	35.020	0	3
5	−86,841.847	−4443.93	103.02242	4.266	−8.120341	−0.3728039	332.898	297.108	−2.846	−0.730	103.527	36.471	326.829	−0.257	3.280	7.140	30.870	1	4
6	−121,479.92	−5930.86	−3.6329411	2.832	−8.884932	−0.6652046	417.973	374.259	−3.954	0.631	118.480	43.543	410.491	−0.251	2.270	7.890	61.940	0	6
7	−80,076.438	−4363.58	101.99217	2.471	−8.203828	−0.4581621	534.025	908.509	−2.750	−0.268	100.059	35.900	312.433	−0.258	2.680	6.080	59.110	1	4
8	−80,841.486	−4306.45	−14.166268	5.200	−8.832393	−0.1467351	306.24	268.608	−4.731	−0.379	85.350	31.530	284.358	−0.219	1.070	10.000	45.330	1	4
9	−73,412.657	−4717.17	78.665389	0.930	−8.51944	−0.2251996	320.589	290.432	−1.137	1.772	102.757	36.028	287.404	−0.243	4.860	8.950	3.240	0	1
10	−102,872.02	−4942.24	104.46058	3.651	−8.490873	−0.5326552	386.683	336.505	−3.394	0.398	109.910	39.935	371.869	−0.253	1.580	6.730	42.390	0	6
11	−69,008.062	−4266.06	78.003214	1.693	−8.560602	0.4595214	294.118	264.061	−0.671	0.940	91.207	32.258	264.370	−0.254	2.760	8.260	6.480	0	2
12	−72,005.618	−4460.87	59.580266	0.518	−8.663204	0.05507502	315.584	285.550	−0.785	0.498	99.089	35.742	295.442	−0.259	4.490	9.040	31.480	0	1
13	−70,511.878	−4151.08	82.984902	1.400	−8.712882	0.1338292	289.582	259.656	−1.557	1.159	87.078	31.549	265.358	−0.247	1.970	8.100	19.370	0	3
14	−110,495.96	−5922.35	−34.379971	4.108	−8.767308	0.1302918	427.837	371.810	−1.853	1.179	109.352	42.114	385.509	−0.261	3.350	6.730	69.640	0	7
15	−81,555.823	−4027.14	−16.01921	2.301	−8.354092	−0.5024017	327.288	270.642	−7.570	−1.533	85.919	29.339	295.400	−0.268	−1.380	9.490	73.580	2	5
16	−74,102.912	−3978.93	139.35663	5.473	−8.535871	0.05110605	312.82	264.074	−6.377	−1.307	87.268	31.130	269.349	−0.266	−1.100	9.490	44.810	1	5
17	−82,988.146	−4308.72	−22.21843	6.508	−8.489959	−0.00465511	320.462	275.043	−6.126	−1.013	86.026	31.820	287.362	−0.280	−0.440	9.520	57.360	2	5
18	−140,797.97	−6228.62	−162.07634	3.887	−8.756364	−0.1129853	480.4	412.469	−8.507	2.246	122.436	47.392	465.952	−0.267	2.600	7.770	86.050	3	7
19	−50,258.522	−2618.46	1.2205941	3.411	−8.307039	0.13555862	204.407	168.406	−15.763	−2.208	56.325	20.147	176.218	−0.350	−2.200	10.310	62.040	4	3
20	−73,460.184	−4116.22	−55.71855	2.205	−8.774205	−0.5060177	311.485	260.891	−7.250	0.680	83.380	30.251	259.348	−0.302	1.370	9.140	41.490	2	3

(determination coefficient, R^2), the variance ratio *F* and the standard error of estimate (*s*). The statistical significance (*p*-level) of the results was determined as $p \leq 0.05$. The results are presented in Table 5.

The correlation matrix was used to correlate the biological activities with the various variables. If two descriptors showed a correlation coefficient greater than 0.5, one of them was removed. A quantitative overview of the collinearities existing between the parameters occurring in the established regression models is shown in Table 6, where the respective intercorrelation coefficients are given.

Evaluation of the best correlation models was carried out by validation of each model using general internal cross-validation procedures such as the 'leave-one-out' (LOO) and 'leave-*N*-out' (LNO). These kinds of internal validation are recommended, if the number of compounds is small [41,42]. In the 'leave-one-out' approach, the whole set of data was divided many times into two subsets, with one subset used in model construction consisting of $n-1$ elements and the element not involved in model construction used for its verification. The cross-validated squared correlation coefficient (Q^2), predicted residual sum of squares (PRESS), standard deviation based on PRESS (S_{PRESS}) and standard deviation of error of prediction (SDEP) were used to evaluate the predictive power the developed models. Finally, 'leave-*N*-out' cross-validation, known also as 'leave-many-out' (LMO), was applied on the final equations by deleting 20% of the compounds in 5 cycles and predicting the biological activity of the deleted compounds in each cycle from the corresponding equation derived from the reduced data set. The best model is obtained when $R^2 \geq Q^2_{LO(N)O}$ and the cross-validated squared correlation coefficients are similar $Q^2_{LOO} \approx Q^2_{LNO}$. For a reliable model, the validated squared correlation coefficient $Q^2 > 0.5$ and $R^2 > 0.6$ [43–47].

3. Results and discussion

In the literature there are examples of biochromatographic data analyses, their implications for molecular pharmacology and application in predicting pharmacological activity of drugs [5,48–55]. On the basis of this information and our previous studies [56–61] this research explored the possibility to use chromatographic methods and physicochemical data for determination of receptor-binding potential (pK_i), agonistic (pD_2) and antagonistic (pA_2) activity of 20 compounds with proven affinity to serotonin receptors. The study took advantage of the data concerning the structure and function of this receptor [12–22]. As indicated by these data, amino acids such as: aspartic acid (Asp155), serine (Ser159), phenylalanine (Phe340), asparagine (Asn333), tyrosine (Tyr370), threonine (Thr196), and tryptophan (Trp200, 236, 367) located within 5-HT receptors play the most important role in ligands binding. This information made it possible to devise a hypothetical model of drug-serotonin receptor interaction, in which amino acids were introduced into the stationary phase of chromatographic environment.

To answer the question whether there is any relationship between the behaviour of the compounds **1**–**20** in chromatographic environments (proposed as the analytical models serotoninergic activity) and their biological activity we used the stepwise multiple linear regression analysis.

First, we analyzed 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 serotoninergic activities of particular compounds **1–20** and their C-chromatographic data.

Table 5
Regression models for the correlation between values of biological activity (pK_i, pD_2, and pA_2) and chromatographic data and molecular descriptors.

Eq. no.		R[e]	R^2[f]	F[g]	s[h]	p[i]	n[j]
(1)[a]	$pK_i = a + b$S9/C + cC-S4 − dC-S1 + eC-S11	0.64	0.42	2.4968	0.90477	0.09031	19
(2)[b]	$pK_i = a + b$C-S11 + cS3/C − dC-S1 − eS2/C	0.83	0.68	7.5142	0.66761	0.00189	19
(3)[c]	$pK_i = a + b$C-S2 − cS10 + dS4/C + eS3/C	0.70	0.49	3.3506	0.84651	0.04019	19
(4)[d]	$pK_i = a - b$S7/C − cC-S9	0.67	0.45	6.6748	0.81795	0.00780	19
(5)	$pK_i = a + bQ_N - c\mu - d\log D$	0.83	0.69	11.047	0.63866	0.00044	19
(6)[a]	$pK_i = a + bQ_N - c\mu + d$S4/C + eC-S3	0.87	0.76	11.007	0.58171	0.00030	19
(7)[b]	$pK_i = a + bQ_N - c\mu - d$S1 − eS2/C	0.88	0.77	11.510	0.57189	0.00024	19
(8)[c]	$pK_i = a + bQ_N - c\mu + d$C-S4 + eC-S2	0.89	0.79	13.528	0.53925	0.00010	19
(9)[d]	$pK_i = a - b$S7/C − $c\mu + dQ_N - e\alpha$	0.87	0.76	11.101	0.57984	0.00029	19
(10)[a]	$pD_2 = a - b$C-S9 + cC-S6	0.83	0.69	10.983	0.51405	0.00300	13
(11)[b]	$pD_2 = a - b$C-S1 + cC-S8	0.83	0.70	11.457	0.50660	0.00259	13
(12)[c]	$pD_2 = a - b$C-S9 + cC-S2	0.77	0.59	7.1137	0.59047	0.01198	13
(13)[d]	$pD_2 = a - b$C-S4 − cS7/C	0.82	0.67	10.174	0.52757	0.00380	13
(14)	$pD_2 = a + b\log D - c\varepsilon_{LUMO}$	0.77	0.57	6.7003	0.60081	0.01425	13
(15)[a]	$pD_2 = a - b$C-S9 − cE_T	0.86	0.74	14.265	0.46823	0.00118	13
(16)[b]	$pD_2 = a + b\log D + c$C-S8	0.89	0.79	18.403	0.42482	0.00044	13
(17)[c]	$pD_2 = a + b\log D + c$C-S2	0.77	0.59	7.1868	0.58870	0.01163	13
(18)[d]	$pD_2 = a + b\log D + c$S3/C	0.79	0.62	8.0787	0.56827	0.00817	13
(19)[a]	$pA_2 = a - b$S4 − cC-S10 + dC-S2	0.77	0.59	5.6766	0.81628	0.01175	16
(20)[b]	$pA_2 = a + b$C-S3	0.70	0.49	13.687	0.83584	0.00238	16
(21)[c]	$pA_2 = a - b$S3 − cS9/C − dC-S9	0.82	0.67	8.1660	0.72799	0.00319	16
(22)[d]	$pA_2 = a - b$S3/C + cC-S7 − dC-S5	0.79	0.62	6.5726	0.78093	0.00707	16
(23)	$pA_2 = a + b\Delta H_F - c\mu + dR_m$	0.83	0.69	8.8893	0.70727	0.00224	16
(24)[a]	$pA_2 = a + b\Delta H_F - c\mu - d$C-S10	0.86	0.75	11.761	0.63960	0.00069	16
(25)[b]	$pA_2 = a + b$C-S3 + $c\Delta H_F - dE_H$	0.86	0.73	10.682	0.66268	0.00105	16
(26)[c]	$pA_2 = a + b\Delta H_F - c$S3 − dS9/C	0.90	0.81	17.279	0.55046	0.00012	16
(27)[d]	$pA_2 = a + b\Delta H_F - c\mu + d$C-S3	0.83	0.69	9.0717	0.70232	0.00207	16

[a] Chromatographic parameters from the experiment in NP TLC DS_A system.
[b] Chromatographic parameters from the experiment in RP2 TLC DS_A system.
[c] Chromatographic parameters from the experiment in NP TLC DS_B system.
[d] Chromatographic parameters from the experiment in RP2 TLC DS_B system.
[e] The correlation coefficient.
[f] The determination coefficient.
[g] The variance ratio F.
[h] The standard error of estimate.
[i] The significance level of the equation.
[j] The number of compounds used to derive the regression equation.

The calculated correlation coefficient values (R) were (the regression equations are not presented in the text):

(i) for NP TLC system: 0.17 and 0.19 (pK_i, n = 19, for eluents DS_A and DS_B, respectively), 0.44 and 0.49 (pD_2, n = 13, for eluents DS_A and DS_B, respectively), 0.35 and 0.48 (pA_2, n = 16, for eluents DS_A and DS_B, respectively);
(ii) for RP2 TLC system: 0.03 and 0.12 (pK_i, n = 19, for eluents DS_A and DS_B, respectively), 0.12 and 0.39 (pD_2, n = 13, for eluents DS_A and DS_B, respectively), 0.13 and 0.04 (pA_2, n = 16, for eluents DS_A and DS_B, respectively).

These results may indicate that the other significant relationships, written below (see Table 5), depend upon the specific biochromatographic environment. A distinct relationships between values of biological activity and interactions data of the compounds **1**–**20** with the all models (S1–S11) can be observed (see Table 5).

Table 6
Correlation matrix of the biological activity (pA_2 and pK_i) and molecular descriptors used in (A) Eqs. (8) and (26), and (B) Eq. (16).

	S3	C-S2	C-S4	S9/C	ΔH_F	μ	Q_N	pA_2	pK_i
(A)									
S3	1.00								
C-S2	0.40	1.00							
C-S4	0.35	0.08	1.00						
S9/C	−0.25	−0.23	0.10	1.00					
ΔH_F	0.06	−0.03	−0.14	−0.45	1.00				
μ	0.35	0.13	−0.20	0.11	−0.16	1.00			
Q_N	0.36	0.48	−0.41	−0.11	0.15	0.41	1.00		
pA_2	−0.51	−0.25	−0.47	−0.39	0.61	−0.52	−0.08	1.00	
pK_i	0.32	0.53	0.27	−0.18	0.12	−0.29	0.51	−0.11	1.00

	C-S8	$\log D$	pD_2
(B)			
C-S8	1.00		
$\log D$	−0.49	1.00	
pD_2	0.08	0.69	1.00

Table 7
Observed and predicted values of activity for Eqs. (8), (16) and (26).

Comp.	pK_i (Eq. (8))			pD_2 (Eq. (16))			pA_2 (Eq. (26))		
	Obser.	Pred.	Residual	Obser.	Pred.	Residual	Obser.	Pred.	Residual
1	–	–	–	7.97	7.60	0.37	5.10	5.21	−0.11
2	7.60	7.78	−0.18	7.99	8.22	−0.23	7.30	7.28	0.02
3	7.00	6.89	0.11	7.88	8.04	−0.16	6.90	6.50	0.40
4	7.90	8.04	−0.14	8.04	8.17	−0.13	5.70	5.87	−0.17
5	6.00	6.85	−0.85	–	–	–	7.50	7.83	−0.33
6	9.90	9.92	−0.02	8.16	7.88	0.28	6.69	6.68	0.01
7	8.10	8.26	−0.16	8.10	7.43	0.67	7.20	6.95	0.25
8	8.40	8.29	0.11	–	–	–	6.60	6.33	0.27
9	8.22	7.88	0.34	–	–	–	8.73	7.61	1.12
10	7.40	7.54	−0.14	–	–	–	8.79	8.71	0.08
11	9.70	8.42	1.28	8.09	7.57	0.52	7.50	8.04	−0.54
12	7.40	8.00	−0.60	–	–	–	9.20	8.88	0.32
13	8.40	8.30	0.10	6.88	7.27	−0.39	7.99	8.29	−0.30
14	7.70	7.16	0.54	7.70	7.88	−0.18	6.35	7.31	−0.96
15	6.60	6.64	−0.04	5.80	6.04	−0.24	–	–	–
16	6.40	6.30	0.10	6.30	6.95	−0.65	–	–	–
17	6.60	6.96	−0.36	6.20	5.90	0.30	–	–	–
18	7.40	7.88	−0.48	7.60	7.76	−0.16	7.30	6.84	0.46
19	6.40	5.79	0.61	–	–	–	–	–	–
20	7.50	7.75	−0.25	–	–	–	6.08	6.60	−0.52

Under the conditions of experiment with DS_A, a distinct relationships were found for the compounds with acknowledged binding affinity (pK_i) to the receptor ($R = 0.83$ – RP2 TLC system; Eq. (2)) and determined agonistic activity pD_2 ($R = 0.83$ – NP and RP2 TLC system; Eqs. (10) and (11)). For that mobile phase, the correlations concerning binding affinity (pK_i) and chromatographic parameters with NP TLC system (Eq. (1)) were no statistically significant, however for antagonistic activity pA_2 and chromatographic parameters with NP and RP2 TLC systems (Eqs. (19) and (20)), the determination coefficient R^2 explained only 49–59% of the overall variance. The binding affinity (pK_i) of the compounds to 5-HT receptor (RP2 TLC system) was described on the basis of models S1, S2, S3 and S11. The correlation explains 68% of the variance and simultaneously describes the potential interactions between the ligands and amino acid residues: Asp155, Ser159, Phe340, Asn333 and Thr196. In the case of agonistic activity pD_2, chromatographic models S6 and S9 (NP TLC system; Eq. (10)) as well as S1and S8 (RP2 TLC system; Eq. (11)) demonstrate a significant effect on correlation. The correlations explain ca. 70% of the variance and simultaneously describe the potential interactions between the ligands and amino acid residues: Asp, Ser, Phe and Asn. All types of interactions between the structural elements of the receptor hydrophobic pocket and the chemical substance in the drug–receptor complex are represented here – ionic and hydrogen bonds, as well as stabilization of aromatic ligands rings by hydrophobic forces.

The analysis of correlation between the data characterizing biological activity of the investigated compounds towards serotonin receptor and chromatographic parameters for the DS_B phase yielded satisfactory results for compounds with determined antagonistic activity (pA_2, $R = 0.82$ – NP TLC system and 0.79 – RP2 TLC system; Eqs. (21) and (22)). In the case of binding affinity (pK_i) to the receptor, both for NP and RP2 TLC system, the relationships demonstrated values of the determination coefficient below 49% (Eqs. (3) and (4)). For compounds with agonistic activity pD_2, the correlations explain 59–67% of the total variance (Eqs. (12) and (13)). For compounds with determined antagonistic activity pA_2, the relationships (Eqs. (21) and (22)) explain over 62% of the total variance and describe interactions of the ligands with amino acids: phenylalanine, tyrosine, threonine, aspartic acid and serine (S3, S5, S7 and S9 models).

At the next stage of the study, the molecular descriptors (see Table 4) were included in the multiple regression analysis as regressors, affecting the affinity to the receptors of the 5-HT group. The obtained equations revealed clearly the influence of molecular (Q_N, μ, ε_{LUMO}, ΔH_F) and thermodynamic (R_m, $\log D$) properties of examined compounds. The final mathematical models for biological activity (pK_i and pA_2) explain 69% of the total variance (Eqs. (5) and (23)) and model for pD_2 explains only 57% of total variance (Eq. (14)).

Considering the role of molecular descriptors in prediction of biological activity, these parameters were included in the regression analysis together with chromatographic data. The contribution of molecular descriptors can supplement the observation of cases with factors not revealed by chromatographic analysis (energetic parameters, stability, distribution of charges, steric parameters). As a result, more effective tools for designing new drugs may become available.

First, we analyzed the contribution of physicochemical parameters in the analysis of chromatographic models under the conditions of experiment with DS_A. The results of this analysis, for the subsequent regressands, were presented as Eqs. (6), (7), (15), (16), (24) and (25). Statistically significant correlations were obtained for all types of biological activity, and the mathematical models explain over 70% of the total variance.

Testing correlation for the development phase DS_B yielded good results of regression analysis for biological activity pK_i and pA_2 (Eqs. (8) and (9), (26) and (27)). For compounds with agonistic activity pD_2, the correlations explain only 59–62% of the total variance (Eqs. (17) and (18)).

It can be seen that combining chromatographic data with physicochemical parameters has improved the results of QSAR analysis. Eqs. (5)–(9) indicate that the net atomic charge on the nitrogen atom and the total dipole moment are the main independent variables determining the biological activity pK_i. The distribution coefficient and the heat of formation are the main independent variables determining the agonistic and antagonistic activity, respectively (Eqs. (14), (16)–(18) and (23)–(27)). As can be seen, Q_N, $\log D$ and ΔH_F contributes positively and μ contributes negatively to pK_i and pA_2 activity. Moreover, in all equations, notes the influence of biochromatographic environments as the proposed models of drug–receptor interaction.

On the basis of such analyses, mathematical equations describing all the types of ligands interactions with 5-HT receptors (affinity, stimulation and inhibition) can be proposed – Eqs. (8), (16) and (26). The models, together with the statistical and validation parameters, are given by:

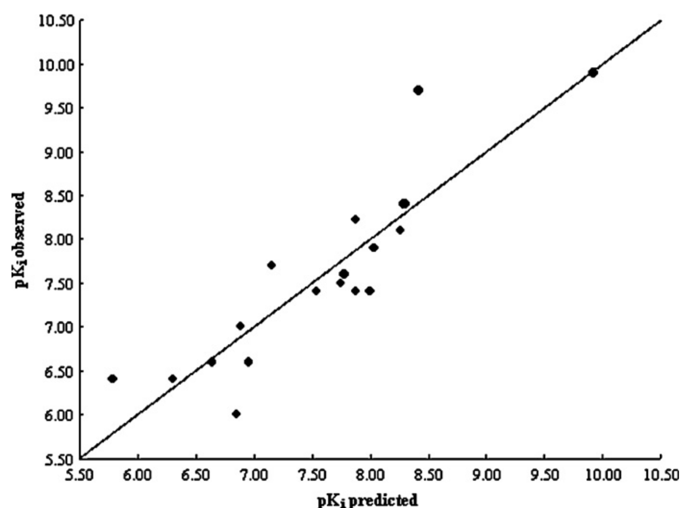

Fig. 1. Observed versus predicted values of serotoninergic binding affinity pK_i according to Eq. (8).

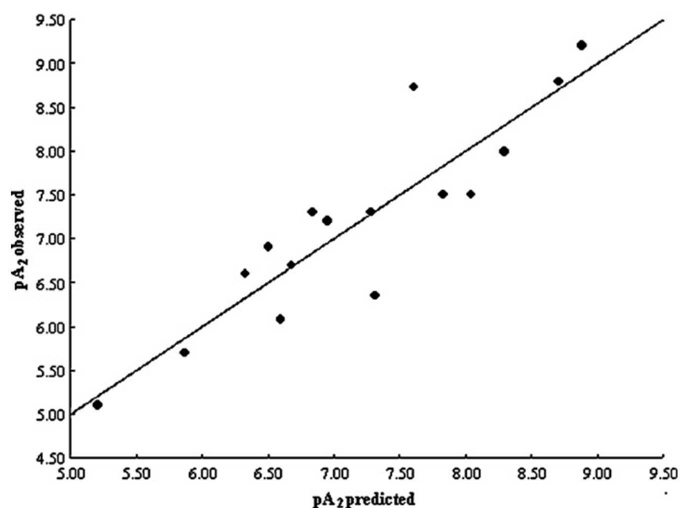

Fig. 3. Observed versus predicted values of antagonistic activity pA_2 according to Eq. (26).

$pK_i = 14.944(\pm1.354) + 22.281(\pm5.160)Q_N - 0.370(\pm0.075)\mu + 3.150(\pm1.347)$C-S4 + $5.713(\pm2.605)$C-S2 (Eq. 8)

$n = 19$, $R = 0.89$, $R^2 = 0.79$, $R^2_{adj} = 0.74$, $F = 13.528$, $s = 0.53925$, $p < 0.00010$, $Q_{LOO}{}^2 = 0.60$, SDEP = 0.68310, PRESS = 9.919286, $S_{PRESS} = 0.72254$, $Q_{LNO}{}^2 = 0.66$

$pD_2 = 6.212(\pm0.250) + 0.390(\pm0.069)\log D + 4.020(\pm1.064)$C-S8 (Eq. 16)

$n = 13$, $R = 0.89$, $R^2 = 0.79$, $R^2_{adj} = 0.74$, $F = 18.403$, $s = 0.42482$, $p < 0.00044$, $Q_{LOO}{}^2 = 0.57$, SDEP = 0.57155, PRESS = 3.940419, $S_{PRESS} = 0.55055$, $Q_{LNO}{}^2 = 0.56$

$pA_2 = 7.723(\pm0.350) + 0.006(\pm0.002)\Delta H_F - 1.968(\pm0.415)$S3 $- 0.743(\pm0.398)$S9/C (Eq. *26*)

$n = 16$, $R = 0.90$, $R^2 = 0.81$, $R^2_{adj} = 0.77$, $F = 17.2790$, $s = 0.55046$, $p < 0.00012$, $Q_{LOO}{}^2 = 0.70$, SDEP = 0.63910, PRESS = 5.875394, $S_{PRESS} = 0.60598$, $Q_{LNO}{}^2 = 0.74$

where n is the number of compounds included in the analysis, R the correlation coefficient, R^2 the squared correlation coefficient, R^2_{adj} the adjusted squared correlation coefficient, s the standard error of estimation, F the variance ratio, p the significance of the variables in the model, Q^2_{LOO} an Q^2_{LNO} the squared correlation coefficients of the LOO and LNO validation procedures, respectively, SDEP the standard deviation of error of prediction, PRESS the predicted residual sum of squares and S_{PRESS} standard deviation based on PRESS.

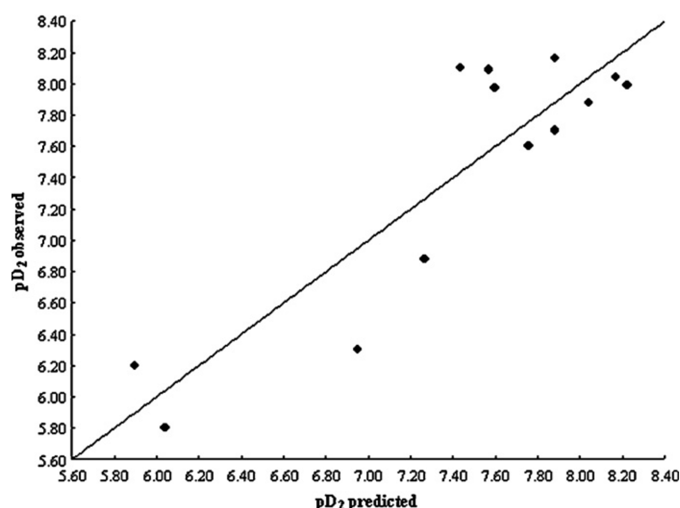

Fig. 2. Observed versus predicted values of agonistic activity pD_2 according to Eq. (16).

Table 6 presents the correlation matrix, where is shown that the selected descriptors from the above equations are not highly correlated. Table 7 and Figs. 1–3 report the comparison of observed and predicted values of biological activity for Eqs. (8), (16) and (26).

The relation $R^2_{adj} < R^2$ confirms that models are not overparameterized. According to Wold [46], Kiralj and Ferreira [47] and group of Tropsha [43–45], terms for a reliable model: $Q^2 > 0.5$ and $R^2 > 0.6$, $Q^2_{LOO} \le R^2 \ge Q^2_{LNO}$ and $Q^2_{LOO} \approx Q^2_{LNO}$ are fulfilled in the above equations.

These equations can be proposed as the tools for prediction of 5-HT activity of novel compounds characterized by various structures with 79–86% probability of obtaining a reliable result.

4. Conclusions

Statistical analysis was carried out to obtain multiple regression models describing the variations of 5-HT affinity in a group of compounds characterized by various structures (comp. **1–20**). Such models can be construed on the basis of interactions of the compounds with the chromatographic environment containing chemical elements of drug-binding structures, characteristic of serotonin receptors. Thus, the independent variables used in this analysis described the activity of the compounds in the biochromatographic environment containing the elementary chemical structures responsible for drug-5-HT receptor interaction (L-amino acids: Asp, Ser, Phe, Asn, Thr, Trp and Tyr). Additionally, independent variables in the form of physicochemical parameter values, characterizing hydrophobic, electron and steric properties of the analytes were included in the analysis. In all the types of chromatographic systems described above, regression models based on interaction of the examined compounds with substances modifying the stationary phase composition were found. Thus, it was established that the proposed biochromatographic systems can describe an interaction which is possible between the ligands and the appropriate amino acids. On the other hand, no correlation was found between the activity of the compounds and their behavior in the control chromatographic environment (without amino acids), which confirmed the important role of the presence of compounds modifying the stationary phase of chromatographic systems in construction of analytical drug-receptor interaction models. The presented regression models were selected on the basis of most favorable values of R^2 discrimination coefficient and statistical tests

(F, p), as representatives of each group of chromatographic experiments and type of the investigated activity limited to only one equation. They constitute a proposal for application of specific methods to predict the activity of 5-HT receptor ligands.

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