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Original article

Evaluation of electronic, lipophilic and membrane affinity effects on antiproliferative activity of 5-substituted-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles against various human cancer cells

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Abstract

The QSAR studies of 5-substituted-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles set of antiproliferative activity against human cancer cell lines have been performed. Electronic properties of compounds were estimated by the Hartree–Fock method at 6-31G** level. Lipophilicity and membrane affinity parameters were determined by the chromatographic methods RP-8 OPLC and IAM HPLC, respectively. Mono- and multivariable regression analyses were performed. The principle factor for determination of activity of compounds is partial charge of nitrogen (q_{N3}, q_{N4}) and carbon (q_{C5}) atoms of the 1,3,4-thiadiazole ring. Biological effect is also connected with molar refractivity (CMR) and lipophilicity of derivatives obtained by RP-8 chromatography. The analysis of the QSAR equations for individual cell lines indicates both similarities and differences of electron, steric factors and hydrophobic–hydrophilic character of the analogues of the tested set affecting the antiproliferative activity.

Keywords: 1,3,4-Thiadiazoles; QSAR; Electronic descriptors; Lipophilicity; Membrane affinity; Mono- and multivariable regression analyses

1. Introduction

One of the rational approaches to design novel antitumour and antiproliferative strategies is based on the QSAR studies [1–3]. The analysis of many QSAR equations indicates that hydrophobicity, experimentally determined or calculated partition coefficient in the octanol/water system, log P, is the most essential factor which is most frequently used in studies. The dependences between antitumour activity and log P values both linear and parabolic with both positive and negative contributions of this parameter, which is related to the potential mechanism of action [4,5], are obtained. The atypical reversed parabola system where at first activity decreases as the values of these parameters increase, but at a specific point turns around, is also observed. This may be due to a change in the receptor structure termed allosteric reaction. These kinds of effects, among others, for bisbenzimidazoles against the LOX IMVI melanoma cell line, isoquinoline-4,6-dione analogues in relation to A549 non-small-cell drug resistant lung carcinoma cells were found [6–8].

Another factor of essential effect on antitumour activity is a steric parameter. As a rule, it is expressed as molar refractivity estimated from the Lorentz–Lorentz equation, for individual substituents or the whole molecule taking both volume and polarizability into account. The Verloop's sterimol parameters: B5, estimated the maximum width of the substituent, B1, being a measure of the width of the first atom and L, substituent length are also commonly used [9–13]. For many groups of compounds simple, mono-parameter, linear relationships with a high correlation coefficient were obtained, among others, against various melanoma cells [9].

Other descriptors also appear in the QSAR equations of anticancer activity possessing electronic character. They are

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usually the Hammet parameters σ and σ^- that apply to substituent effects on aromatic systems. However, their application in the model equations describing the dependences between structure and antitumour activity is definitely smaller compared with lipophilicity [9,10]. Recently, the new parameter, NVE including a number of valence electrons has been introduced by Hansch and co-workers and applied with success in the QSAR studies, including inhibition of various human cancer cells [14,15].

The aim of the paper is the analysis of the factor affecting antiproliferative activity of compounds from the 5-substituted-2,4-dihydroxyphenylo-1,3,4-thiadizoles set against four human cancer cell lines. As phase affinity descriptors, retention parameters from the chromatographic system using both octyl (RP-8) and immobilized artificial membrane (IAM) stationary phases were applied. The frontier orbital energies and the atom partial charges of the thiadiazole ring were considered as an electronic effect and the molar refractivity as a steric parameter.

2. Results

The structures of 5-substituted-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles used in the studies are presented in Table 1. Activity of derivatives against four human cell lines was expressed as the logarithmically converted $1/ID_{50}$ values and was used for subsequent analyses as the response variable (Tables 1 and 2) [16].

Phase affinity of compounds was determined from two different chromatographic approaches using Reversed-Phase Overpressured Layer Chromatography (RP-8 OPLC) and Immobilized Artificial Membrane Chromatography (IAM HPLC). The methods based on the linear relationship between the retention parameters ($\log k$ or R_M) and the concentration (φ) of organic modifier (acetonitrile or methanol) in the aqueous mobile phase are described by the Soczewiński–Wachtmeister equation [17,18]:

$$\log k = \log k_w + S\varphi \quad (1)$$

Log k_w (R_{Mw}) represents the retention factor of a solute with pure water as the mobile phase (intercept) and S is the slope of the regression curve. The obtained parameters for two chromatographic systems are presented in Table 1. Log k_w (R_{Mw}) values obtained from a suitable chromatographic system are commonly applied descriptors for phase affinity determination of biologically active compounds including the QSAR studies [19–22].

Log $k_{w(IAM)}$ values are determined on chromatographic surfaces prepared by covalently immobilized cell membrane phospholipids to solid surfaces at monolayer densities. They express the membrane affinity of compounds and reflect not only the effect of hydrophobic interactions between the solute and the solid phase but also other interactions with membrane like hydrogen bond formation, electrostatic and so on [23–28]. Therefore log $k_{w(IAM)}$ can be considered as the other parameter than lipophilicity obtained from the RP-8 chromatographic system, especially for compounds with a hydrophilic groups. For comparison log P values calculated according to the Ghose approach were used (Table 1).

Electronic properties of compounds were expressed as partial charges of atoms of thiadiazole ring and as the energies of frontier orbitals, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), which direct the nucleophilic and electrophilic reactivity of a molecule (Table 3). Hardness (η), including both parameters, obtained from the equation $\eta = (E_{LUMO} - E_{HOMO})/2$ was additionally calculated. Molar refractivity (CMR) was used as the steric parameter (Table 3).

Using mono- and multivariable analyses we try to find the best models for prediction of antiproliferative activity of compounds. Taking into account diverse influence of the descriptors on the activity against various cells, analyses were performed for individual cell lines independently.

To investigate the equivalency between the obtained MLR equations, the correlation coefficient matrix was calculated for the descriptors used in the equations. The results are presented in Table 4. Relatively higher correlation coefficients were found between partial charges of nitrogen (q_{N3}, q_{N4}) and carbon (q_{C2}) atoms of the thiadiazole ring, and between log $k_{w(IAM)}$, molar refractivity (CMR) and log P values.

2.1. HCV29T cell line

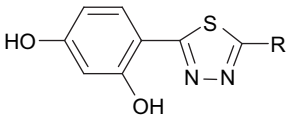
No close one-parameter dependence between the activity of compounds against HCV29T cells and any parameter describing hydrophilic–hydrophobic character, electron or steric properties was found. Multi-parameter analysis provides the best results applying molar refractivity, CMR, and partial charge of nitrogen atom (q_{N4}) as independent variables described by the equation:

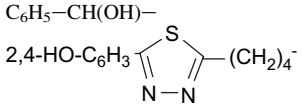
$$\begin{aligned}\log 1/ID_{50} = {} & 1.011(\pm 0.402)q_{N4} + 0.013(\pm 0.001)\mathrm{CMR} \\ & - 2.821(\pm 0.162) \\ & n = 25,\ r = 0.901,\ r^2 = 0.811, \\ & s = 0.097,\ F = 74.49\ (\mathrm{df}2, 22)\end{aligned} \quad (2)$$

The equation does not include compounds of the greatest activity **7** and **26** and additionally derivatives **24** and **25**. The effect of phase affinity estimated by both IAM HPLC, RP OPLC techniques and by application of computational methods (log P) on activity of the presented series of analogues against the HCV29T cells is insignificant.

The predicted values of compounds' activity based on Eq. (2) are given in Table 1. Misfit of compound **2** to the proposed QSAR equation (Eq. (2)), which was not taken into consideration in model formation due to the activity above the applied concentrations, should be associated with its relatively high lipophilicity. Despite insignificant effect of lipophilicity on activity (it was neglected in the general model) with such long hydrophobic chain, the effect of transport and distribution

Table 1
Structure of 5-substituted-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles, antiproliferative effect against the HCV29T cells (log $1/ID_{50}$) observed and calculated, respectively, the phase affinity parameters: log $k_{w(IAM)}$, R_{Mw}, S and φ_0 obtained from linear equation (1) for IAM HPLC and RP-8 OPLC and calculated log P values obtained from the Ghose approach



No.	Substituent	HCV29T, log $1/ID_{50}$		R_{Mw}	$S_{(RP-8)}$	$\varphi_{0(RP-8)}$	log $k_{w(IAM)}$	S_{IAM}	$\varphi_{0(IAM)}$	log P
		Observed	Calculated Eq. (2)							
1	H–	−2.250	−2.341	3.089	−4.572	0.676	0.960	−3.757	0.256	2.71
2	$CH_3(CH_2)_{14}$–	<−2.393[a]	−1.526	7.038	−8.178	0.861	4.086	−6.071	0.673	9.46
3	CH_3O–	−2.343	−2.359	3.107	−4.659	0.667	0.948	−7.170	0.132	3.09
4	C_2H_5O–	−2.305	−2.301	3.479	−5.776	0.602	1.180	−7.215	0.163	3.16
5	$HO(CH_2)_3$–	−2.232	−2.187	3.300	−5.062	0.652	3.266	−4.430	0.737	3.25
6	C_6H_5–	−2.276	−2.261	3.517	−4.663	0.754	2.426	−4.406	0.551	5.28
7	4-$(CH_3)_3CC_6H_4$–	−1.057[a]	−1.902	5.348	−6.633	0.806	3.274	−5.166	0.634	6.98
8	2-BrC_6H_4–	−1.919	–[b]	3.618	−4.673	0.774	2.527	−4.191	0.603	–[b]
9	3,5-di-CH_3O-4-$HO-C_6H_2$–	−2.027	−1.927	3.211	−4.709	0.682	2.407	−3.510	0.686	4.63
10	3-C_2H_5-OC_6H_4–	−2.040	−1.955	4.246	−5.435	0.781	2.685	−4.618	0.582	5.49
11	4-C_2H_5-OC_6H_4–	−2.016	−1.970	4.295	−5.539	0.775	2.748	−4.765	0.577	5.49
12	3-CH_3–2-$HO-C_6H_3$–	−1.745	−1.978	4.134	−5.116	0.808	2.861	−4.074	0.702	5.37
13	2-$HO-C_6H_4$–	−2.083	−2.204	3.550	−4.773	0.744	2.552	−4.073	0.626	4.89
14	2,4-di-$HO-C_6H_3$–	−2.045	−2.026	3.222	−4.727	0.682	2.369	−3.401	0.697	4.50
15	3,5-di-$HO-C_6H_3$–	−2.182	−2.053	2.969	−4.787	0.620	2.061	−3.608	0.571	4.50
16	4-$C_6H_5-C_6H_4$–	−1.693	−1.774	6.662	−8.541	0.780	3.505	−5.316	0.659	6.95
17	1-Naphthyl–	−1.871	−1.852	5.082	−6.598	0.770	2.825	−4.466	0.633	6.27
18	$C_6H_5-CH_2$–	−2.090	−2.013	3.537	−4.935	0.717	2.403	−5.308	0.453	5.22
19	1-Naphthylmethyl–	−1.923	−1.810	4.081	−5.223	0.781	2.772	−5.294	0.523	6.22
20	3-$F-C_6H_4-OCH_2$–	−2.093	−1.989	3.940	−5.328	0.739	2.452	−5.199	0.472	5.02
21	4-$F-C_6H_4-OCH_2$–	−1.999	−2.006	3.734	−5.071	0.736	2.459	−5.211	0.472	5.02
22	4-Cl–2-$CH_3-C_6H_3-OCH_2$–	−1.816	−1.859	4.284	−5.304	0.808	3.062	−5.424	0.565	5.90
23	2-Cl–$C_6H_4-OCH_2$–	−2.061	−1.930	3.981	−5.165	0.771	2.780	−5.402	0.515	5.42
24	2,4-di-Cl–$C_6H_3-OCH_2$–	−2.410[a]	−1.895	4.310	−5.342	0.807	3.054	−5.360	0.570	5.97
25	4-$NO_2-C_6H_4-OCH_2$–	−2.429[a]	−1.921	3.075	−4.189	0.734	2.393	−5.161	0.464	4.89
26	3-$CH_3O-C_6H_4-CH_2O$–	−0.522[a]	−2.021	3.684	−4.672	0.789	2.319	−5.626	0.412	4.70
27	$C_6H_5-CH(OH)$–	−1.857	−2.000	2.987	−4.575	0.653	3.070	−4.715	0.651	4.56
28		−1.421	−1.534	1.789	−2.518	0.710	2.611	−4.938	0.529	7.56
29	2-Furyl–	−2.178	−2.193	3.007	−4.016	0.749	1.938	−4.104	0.473	–[b]
30	2-Pyridyl–	−2.152	−2.131	3.111	−3.861	0.806	1.933	−3.952	0.490	4.36
31	4-Pyridyl–	<−2.567	−2.146	3.082	−3.828	0.805	1.632	−3.961	0.412	3.94
32	Benzo(*b*)thiophen-2-yl–	−1.848	−1.893	3.830	−4.685	0.818	2.767	−3.933	0.703	6.26

[a] Compounds were not included in the analysis.
[b] Descriptors were not obtained at 6-31G** level of calculations.

will most probably constitute a factor inhibiting activity of this kind of connection.

Relatively high activity of compound **7** can be related to the presence of the *tert*-butyl substituent in the *para*-position characterized by capability of interactions with a lipophilic pocket of the active site probably. Discrepancies with the model of compound **25** can result from a quite different distribution of frontier orbitals for this derivative. Fig. 1 presents the frontier orbitals' distribution for compound **26** which is characteristic of the whole series of analogues and derivative **25**. The differences include mainly localization of the orbital LUMO. As a rule it is localized on 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole moiety, but in compound **25** on 4-nitrophenyle moiety (Fig. 1).

High activity of compound **26** is connected with quite different distribution of charge in the thiadiazole ring compared to other analogues, particularly evident in the case of charge carbon atom q_{C5} (Table 3). The analogy with *N*-substituted-2-amino-1,3,4-thiadiazoles, of relatively high antiproliferative effect, where C-5 ring of thiadiazole is characterized by a large electron gap, can be found. Therefore, considering the activity mechanism the compound can be similar to aminothiadiazole derivatives where the additional factor affecting activity is large negative charge accumulated on amine nitrogen atom which in the case of derivative **26** can be replaced by the isoster –O– combined directly with the heterocyclic ring.

Table 2
Antiproliferative effect against T47D, SW707 and A549 cells (log 1/ID_{50}) observed and calculated, respectively, for some 5-substituted-2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles

No.	T47D, log 1/ID_{50}		SW707, log 1/ID_{50}		A549, log 1/ID_{50}	
	Observed	Calculated Eq. (4)	Observed	Calculated Eq. (6)	Observed	Calculated Eq. (8)
7	−1.088	−1.092	−1.139	−1.198	−1.593	−1.453
8	−1.444	–[b]	−1.890	–[b]	−1.912	–[b]
12	−1.273	−1.463	−1.746	−1.711	−1.292[a]	−1.697
16	−1.407	−1.403	−1.161	−1.021	−1.280	−1.328
17	−1.180	−1.194	−1.766	−1.556	−1.828	−1.700
19	−1.629	−1.388	−1.926	−1.968	−1.865	−1.982
22	−1.402	−1.378	−2.117	−1.936	−1.964	−1.962
23	−1.494	−1.523	−2.037	−2.018	−2.108	−2.021
26	−0.958	−0.991	−1.180	−1.388	−1.379	−1.586
27	−2.148	−2.169	−2.027	−2.182	−2.139	−2.136
28	<−2.354[a]	−3.458	−1.025	−1.148	−1.420	−1.408
29	−2.196	−2.174	<−2.585[a]	−1.952	<−2.585[a]	−1.862

[a] Compounds were not included in the analysis.
[b] Descriptors were not obtained at 6-31G** level of calculations.

Table 3
Electronic properties of compounds: the atomic partial charges from a Mulliken population analysis of 1,3,4-thiadiazole ring atoms and first carbon atom of resorcinol moiety (q_{C1}), energies of frontier orbitals as well as hardness (η) and molar refractivity (CMR)

No.	q_S	q_{C2}	q_{N3}	q_{N4}	q_{C5}	q_{C1}	E_{HOMO} (eV)	E_{LUMO} (eV)	η (eV)	CMR
1	0.286	0.163	−0.472	−0.205	−0.102	−0.126	−8.330	2.189	5.259	51.43
2	0.282	0.159	−0.458	−0.269	0.048	−0.128	−8.176	2.393	5.284	120.31
3	0.258	0.148	−0.451	−0.301	0.457	−0.119	−8.139	2.509	5.324	57.37
4	0.257	0.146	−0.451	−0.306	0.464	−0.119	−8.108	2.547	5.327	62.11
5	0.284	0.165	−0.465	−0.258	0.053	−0.130	−8.237	2.304	5.270	67.03
6	0.308	0.068	−0.299	−0.450	0.153	−0.128	−8.033	1.814	4.923	76.04
7	0.306	0.153	−0.450	−0.302	0.072	−0.129	−7.932	1.896	4.914	94.17
8	–[a]	–[a]	–[a]	–[a]	–[a]	–[a]	–[a]	–[a]	–[a]	83.66
9	0.317	0.151	−0.448	−0.313	0.074	−0.128	−7.786	1.948	4.867	90.66
10	0.314	0.153	−0.447	−0.296	0.067	−0.131	−7.998	1.841	4.919	87.25
11	0.302	0.151	−0.450	−0.311	0.075	−0.126	−7.816	2.002	4.909	87.25
12	0.304	0.159	−0.455	−0.260	0.006	−0.131	−8.131	1.933	5.032	82.78
13	0.325	0.145	−0.427	−0.411	0.134	−0.128	−8.088	1.700	4.894	77.34
14	0.289	0.161	−0.453	−0.263	0.009	−0.131	−8.055	2.003	5.029	79.43
15	0.320	0.152	−0.449	−0.290	0.059	−0.130	−8.057	1.775	4.916	79.43
16	0.308	0.153	−0.450	−0.301	0.070	−0.129	−7.916	1.737	4.826	101.18
17	0.304	0.162	−0.457	−0.263	0.014	−0.132	−7.872	1.866	4.869	92.49
18	0.294	0.164	−0.466	−0.265	0.066	−0.130	−8.186	2.342	5.264	80.59
19	0.300	0.159	−0.468	−0.265	0.071	−0.128	−7.998	2.317	5.157	95.77
20	0.327	0.161	−0.465	−0.261	0.019	−0.132	−8.289	2.211	5.250	82.05
21	0.324	0.161	−0.465	−0.261	0.021	−0.132	−8.289	2.213	5.251	80.78
22	0.323	0.161	−0.465	−0.259	0.021	−0.133	−8.330	2.162	5.246	91.68
23	0.329	0.156	−0.464	−0.263	0.014	−0.131	−8.198	2.333	5.265	86.64
24	0.340	0.160	−0.465	−0.260	0.015	−0.133	−8.310	2.170	5.240	91.44
25	0.325	0.164	−0.464	−0.258	0.021	−0.133	−8.446	1.701	5.073	89.16
26	0.293	0.150	−0.451	−0.346	0.472	−0.121	−8.108	2.585	5.346	88.44
27	0.318	0.161	−0.465	−0.270	0.031	−0.132	−8.250	2.246	5.248	81.93
28	0.278	0.162	−0.461	−0.257	0.086	−0.113	−8.284	2.246	5.265	115.80
29	0.335	0.156	−0.452	−0.283	0.017	−0.113	−7.935	1.877	4.906	68.43
30	0.350	0.150	−0.456	−0.288	0.046	−0.132	−8.059	1.700	4.879	73.48
31	0.321	0.156	−0.450	−0.282	0.057	−0.130	−8.313	1.416	4.864	73.82
32	0.316	0.158	−0.448	−0.287	0.093	−0.130	−7.957	1.467	4.712	91.20

[a] Descriptors were not obtained for derivative **8** at 6-31G** level of calculations.

Table 4
Correlation coefficient (r^2) matrix for the descriptors used in equations

	CMR	q_S	q_{C2}	q_{N3}	q_{N4}	q_{C5}	q_{C1}	log P	R_{Mw}	log $k_{w(IAM)}$
CMR	1.000	0.009	0.024	0.010	0.003	0.042	0.012	0.879	0.121	0.601
q_S		1.000	0.000	0.000	0.002	0.289	0.242	0.011	0.041	0.044
q_{C2}			1.000	0.955	0.675	0.087	0.011	0.003	0.001	0.015
q_{N3}				1.000	0.680	0.040	0.004	0.000	0.002	0.002
q_{N4}					1.000	0.219	0.023	0.000	0.000	0.000
q_{C5}						1.000	0.267	0.068	0.005	0.139
q_{C1}							1.000	0.003	0.048	0.163
log P								1.000	0.230	0.606
R_{Mw}									1.000	0.295
log $k_{w(IAM)}$										1.000

Misfit of some analogues of the series to the general model equation is frequent [29].

2.2. T47D cell line

The simple, classic parabolic relationship was obtained between the antiproliferative activity and the lipophilicity parameter R_{Mw} (Fig. 2) described by the following equation:

$$\log 1/ID_{50} = -0.183(\pm 0.061)(R_{Mw})^2 + 1.924(\pm 0.589)R_{Mw} - 6.167(\pm 1.345) \quad n = 11,\ r = 0.812,\ r^2 = 0.659,\ s = 0.257,\ F = 4.45\ (df2, 8) \qquad (3)$$

Using log $k_{w(IAM)}$ values expressing the membrane affinity of compounds and the same mathematical model considerably worse results were obtained ($r = 0.59$) where compound **27** shows deviation from the proposed equation clearly.

The best electronic parameter correlated to the antiproliferative activity in the one-parameter analysis is the partial charge of sulfur atom (q_S): $\log 1/ID_{50} = -20.890(\pm 7.894)q_S + 5.040$ (± 2.465) with the following statistical parameters: $n = 10$, $r = -0.683$, $r^2 = 0.468$, $s = 0.322$, $F = 7.0$ (df1,8). Somewhat better results of fitting were obtained using the molar refractivity, CMR, as one variable in the second power described by the expression:

$$\log 1/ID_{50} = -0.0014(\pm 0.0009)(CMR)^2 + 0.266(\pm 0.167)CMR - 13.881(\pm 7.093),\ n = 11,\ r = 0.703,\ r^2 = 0.494,\ s = 0.313,\ F = 3.91\ (df2, 8)$$

The best two-parameter classic equation applying the lipophilicity parameter (R_{Mw}) in the second power and the partial charge of carbon atom (q_{C5}) including all analogues is described by the following expression:

$$\log 1/ID_{50} = -0.181(\pm 0.031)(R_{Mw})^2 + 1.936(\pm 0.292)R_{Mw} + 0.517(\pm 0.304)q_{C5} - 6.387(\pm 0.670) \quad n = 11,\ r = 0.967,\ r^2 = 0.938,\ s = 0.128,\ F = 29.75\ (df3, 6) \qquad (4)$$

Similar results of fit were obtained using the partial charge of nitrogen atom q_{N4} as the electronic parameter ($r = 0.961$, $r^2 = 0.921$, $s = 0.143$, $F = 23.26$ (df3,6)).

The dependence indicates existence of the optimal value of lipophilicity promoting activity for these types of compounds against the T47D cell line. Then activity increases with the largest electron gap on carbon atom of the thiadiazole ring (q_{C5}) as well as the smallest positive partial charge of sulfur atom (q_S) and negative one accumulated on atom of nitrogen (q_{N4}) in the heterocyclic ring.

The activity values calculated from Eq. (4) are presented in Table 2 showing good agreement with the observed values.

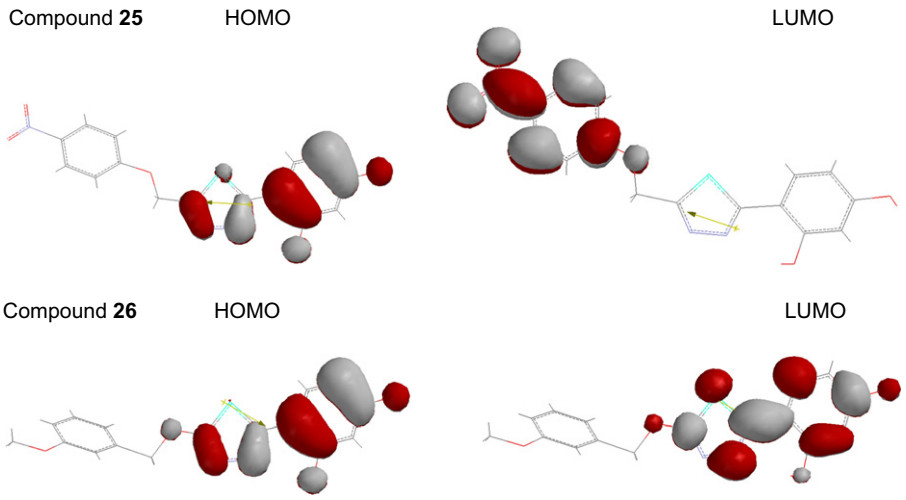


Fig. 1. HOMO and LUMO isosurfaces for compounds **25** and **26**. Different surface colours represent opposite signs of the wavefunction.

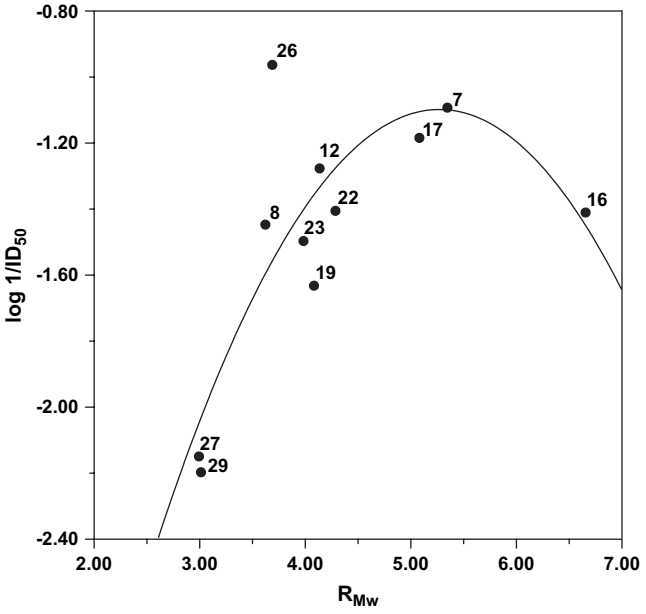


Fig. 2. Relationship between the R_{Mw} parameter from RP-8 OPLC and the log $1/ID_{50}$ values against the T47D cell line as described by Eq. (3).

2.3. SW707 cell line

As for the activity of compounds against the SW707 cell line good results in the one-parameter analyses were obtained only taking charges of thiadiazole ring atoms into consideration. The best fit was found considering the nitrogen atom charge (q_{N3}), described by the equation:

$$\log 1/ID_{50} = 47.436(\pm 14.797)q_{N3} + 20.142(\pm 6.787)$$
$$n = 10,\ r = 0.750,\ r^2 = 0.562,\ s = 0.305,$$
$$F = 10.28\ (df1, 8) \quad (5)$$

Moreover, sulfur atom charge is a very significant factor of activity ($r = -0.727$, $r^2 = 0.528$, $s = 0.317$, $F(1,8) = 8.9$). The analysis indicates preferences of compounds of small negative partial charge of N-3 atom at small positive partial charge of sulfur atom.

The best model in the two-parameter analysis was obtained using the molar refractivity (CMR) and the charge of nitrogen atom (q_{N3}) as two independent variables expressed as follows:

$$\log 1/ID_{50} = 45.009(\pm 7.906)q_{N3} + 0.025(\pm 0.005)CMR$$
$$+ 16.681(\pm 3.695) \quad n = 10,\ r = 0.944,$$
$$r^2 = 0.891,\ s = 0.163,\ F = 28.65\ (df2, 7) \quad (6)$$

The equation shows more significant contribution of electronic factor compared to the steric parameter.

The activity values calculated from Eq. (6) are presented in Table 2 and show good agreement with the observed values except for an inactive compound at a given level of concentrations (compound **29**), which was not taken into account while forming the equation. The obtained equation predicts much higher activity for this type of derivative (Table 2).

2.4. A549 cell line

As for the A549 cells good results in the one-parameter analysis were obtained only considering thiadiazole ring atom charges. The best fit was found considering nitrogen atom charges described by the equation:

$$\log 1/ID_{50} = 37.095(\pm 11.101)q_{N3} + 15.325(\pm 5.091)$$
$$n = 10,\ r = 0.763,\ r^2 = 0.583,\ s = 0.290,$$
$$F = 11.17\ (df1, 8) \quad (7)$$

The two-parameter analysis gives the best results applying the molar refractivity (CMR) and the partial charge of nitrogen atom (q_{N3}) as independent variables, described by the expression:

$$\log 1/ID_{50} = 30.998(\pm 6.521)q_{N3} + 0.018(\pm 0.005)CMR$$
$$+ 10.917(\pm 3.092) \quad n = 9,\ r = 0.936,$$
$$r^2 = 0.876,\ s = 0.131,\ F = 21.31\ (df2, 6) \quad (8)$$

The equations did not include compound **12**. The activity values calculated from Eq. (8) are presented in Table 2 and show good agreement with the observed values except for compound **29** which is inactive at a given level of concentrations, where the model predicts higher activity.

The reason for discrepancy with the model of compounds **12** and **29** can be atypical size of charge of C-5 carbon atom of 1,3,4-thiadiazole ring (Table 3). Compared to the other analogues, both derivatives are characterized by small electron gap and the charges of this carbon atom also correlate with activity and probably play an essential role in interactions with an active site. However, the best dependences are obtained for the partial charge of the third nitrogen atom.

3. Discussion

The analysis of QSAR equations for individual cell lines points to some similarities and differences of electron, steric factor and lipophilicity of analogues affecting antiproliferative activity. The T47D cell line is the best model system where both relations between the single descriptors and the two-parameter equations with the lipophilicity descriptor in the square power from the RP-8 chromatography and the partial charge of thiadiazole ring carbon atom (q_{C5}) were obtained.

The SW707 and A549 cell lines exhibit the largest similarities of factors influencing the activity. The parameter which correlates best with activity is the partial charge of nitrogen atom (q_{N3}) of 1,3,4-thiadiazole ring. The best equations were obtained in the two-parameter analysis using the same descriptors occurring in the equation of the same power (q_{N3} and CMR). Influence of lipophilicity or phospholipophilicity determined by RP-8 or IAM chromatography, respectively, on the activity of compounds is insignificant.

Electronic and steric factors determine antiproliferative activity of compounds studied against the SW707 and A549 cell lines.

Derivative **29** is inactive towards both cell lines whereby the model equation indicates higher level of activity. Changes of compounds' activity in relation to these cell lines are analogous which confirms the observation that in the case of some lines strong linear dependences between log 1/*c* values are found ($r = 0.808$, $s = 0.261$) [1,13].

Compounds' activity against the HCV29T cell line is the most difficult to describe with the applied model and assumes the form somewhat departing from the others. The two-parameter equation, not including the phase affinity factor into account and at the same time pointing to an essential effect of the steric and electronic factors proves to be the best. However, in this case the charge of nitrogen atom q_{N4} is the electron factor the best correlated to activity.

The obtained models describe best antiproliferative effect of compounds of average activity. The greatest deviations were found for the derivatives of largest activity and for the inactive derivative. This may suggest existence of activity affecting factors not considered in the proposed analysis or specific effects characteristic only of some derivatives against individual cell lines.

The obtained results show that the most effective factor of activity of these types of compounds are electron properties of 1,3,4-thiadiazole ring. Changes of charge distribution of this moiety are result of type of substitution at the fifth carbon atom. Binding affinity of 1,3,4-thiadiazoles for the human adenosine A_3 receptor subtype is also a function of Wang–Ford charges of atoms and lipophilicity [30]. However, no explicit dependences between activity and energy of frontier orbitals were obtained contrary to *N*-substituted-2-amino-1,3,4-thiadiazoles where energy of the lowest unoccupied molecular orbital (LUMO) is the factor considerably influencing the antiproliferative activity. Molar refractivity (CMR) is next parameter considerably influencing on antiproliferative effect of derivatives against cell lines studied.

Lipophilicity of compounds determined by RP-8 chromatography described activity of compounds against the T47D cell line only. Against expectations no better correlations with the activity of the parameter log $k_{w(IAM)}$ describing the membrane affinity were obtained compared to R_{Mw} applying the stationary octyl phase though these compounds possess the hydrophilic groups (–OH) whose interaction effects should influence membrane transport and are taken into account in retention on the IAM phase. Analogous studies of compounds of another group characterized by anticancer activity did not provide explicit dependences [31].

The obtained differences in the model QSAR equations can be related to some differences in the shield structure of cells of studied lines (lipophilicity parameter) or fine differences of interaction sites with the ligand (partial charges) and the steric parameter [7,32]. They indicate constitutional and steric preferences while constructing next analogues of the presented group of compound with the directional antiproliferative activity.

4. Experimental protocols

4.1. Chemistry

The studied compounds (Table 1) were prepared following the synthetic procedures previously reported [16,33].

4.2. Biological test

The compounds were tested *in vitro* against the four human cell lines: T47D (breast cancer), SW707 (rectal adenocarcinoma), A549 (non-small lung carcinoma) from the American Type Culture Collection (Rockville, Maryland, USA) and HCV29T (bladder cancer) from the Fibiger Institute, Copenhagen, Denmark, as previously described [16]. The SRB test measuring the cell proliferation inhibition in *in vitro* culture was applied [34]. The cytotoxic activity *in vitro* was expressed as ID_{50} (μM), the concentration of compound that inhibits proliferation rate of the tumour cells by 50% as compared to the control untreated cells.

4.3. OPLC

OPLC studies were performed on the precoated plates of RP-8_{254S} (10 × 20) (Merck, Darmstadt, Germany). One microlitre of samples of the solutes (0.5 mg/ml in methanol) were spotted with the Linomat 5 applicator (Camag). The chromatograms were developed over a distance of 7.0 cm in the automatic Personal OPLC BS-50 chromatograph (OPLC-NIT, Budapest, Hungary). Water–methanol systems were applied as the mobile phases in the concentration range of 0.5–0.75 v/v of methanol at 0.05 intervals. OPLC determinations were performed at 21 °C, external pressure 50 bar, volume of rapid flow 150 μl, flow rate 150 μl/min, and volume of mobile phase 800 μl. Two independent runs were carried out in all experiments. All developed plates were dried at room temperature. Compounds were detected with the Camag VideoStore 2 system (Camag, Switzerland) consisting of the Reprostar 3 UV/Vis analysis lamp with the cabinet cover or by a Shimadzu CS-9000 duel-wavelength TLC scanner (Shimadzu Europe, Duisburg, Germany).

4.4. IAM HPLC

HPLC was carried out using a liquid chromatograph (Knauer) with a dual pump, a 20 μl simple injection valve and a UV–visible detector (330 and 275 nm). Rexchrom IAM (12 μm, 100 × 4.6 mm, 300 Å) column was used as the stationary phase. The samples were prepared as the solutions in ethanol. The mobile phase consisted of different volume mixtures of acetonitrile and 20 mM phosphate buffer as the aqueous phase to give pH 7.4 (0.02 M KH_2PO_4 and 0.15 M KCl). The acetonitrile concentration ranged from 0.05 to 0.50, depending on the structure of compound, at 0.05 intervals. The flow rate was 1 ml/min at room temperature. The retention time of an unretained solute (t_0) was determined by the injection of a small amount of citric acid dissolved in water [35]. Log

$k_{w(IAM)}$ values for water as the mobile phase were determined by the extrapolation technique.

4.5. Computational methods

The compounds were built with a standard bond length and angles using the PC SPARATN Pro Ver 1.08 molecular modelling program. The energy was minimised by the molecular mechanic methods and then by the Hartree–Fock method at 6-31G** level [36]. Charge of atoms from the Mulliken population analysis was determined [37]. The molar refractivity (CMR) was obtained from the HyperChem Pro 6 program.

4.6. Statistic analysis

The coefficients in the regression equations were calculated by the multiple regression analysis Statistica Program, Version 6.0. The statistical significance of regression equation was tested by the correlation coefficient (r), the standard error of estimate (s), and the variance ratio (F) at specified degrees of freedom (df).

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