Acta Pharmaceutica Turcica
XXXIX (1) 1-6 (1997)

QSAR OF SMOOTH MUSCLE RELAXATION BY 2,5 - SUBSTITUTED - BENZIMIDAZOLE DERIVATIVES

2,5 - SÜBSTİTÜE - BENZİMİDAZOL BİLEŞİKLERİNİN DÜZ KAS GEVŞETİCİ ETKİLERİNİN KANTİTATİF YAPI - ETKİ İLİŞKİLERİ

İLHAN IŞIKDAĞ*, ÜMİT UÇUCU*, ERDEM BÜYÜKBİNGÖL, YUSUF ÖZTÜRK*****

* Department of Pharmaceutical Chemistry, ***Department of Pharmacology, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey
** Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey

Quantitative structure-activity analyses were carried out for in vitro relaxant actions of a series of 2,5-substituted-benzimidazoles in rat duodenal smooth muscle. Analyses of simple and multiple linear regression models revealed modest to good correlations between the relaxant activity and structural properties of the benzimidazole derivatives such as steric, hydrophobic and electronic parameters. In the present study, it was observed that, as a collective property, parachor log(Par)+partition coefficient log(P) gives good results using either apparent affinity constant (pD_2) or intrinsic activity (α^E) as the predicted values. In this case, it was supposed that both the lipophilicity and the surface tension of molecules are responsible for the activity observed.

Bu araştırma, bir seri 2,5-sübstitüe benzimidazol bileşiğinin sıçan duedonumundaki düz kas gevşetici etkisinin in vitro denenmesi ve kantitatif yapı-etki ilişkilerini içermektedir. Basit ve çoklu regresyon analizlerinin sonuçları, düz kas gevşetici etki ile sterik, hidrofobik ve elekronik parametreler arasında orta derecede bir ilişkinin varlığını göstermiştir. Çalışmada ortak bir özellik olarak, log Par + log P kombinasyonlarının hem pD_2 hem de α^E değerleri ile iyi sonuçlar verdiği, bu durumda, aktiviteden birinci derecede moleküllerin yağda çözünürlüklerinin ve yüzey gerilimlerinin sorumlu olduğu gözlenmiştir.

Keywords: *Benzimidazoles; Smooth muscle relaxation; Quantitative structure-activity relationships (QSAR)*

Anahtar Kelimeler:*Benzimidazoller; Düz kas gevşetici etki; Kantitatif yapı-etki ilişkileri (QSAR)*

Introduction

Some 2-(substituted-aryl) and 5-nitro-2-(substituted-aryl) benzimidazole derivatives from our previous studies (1,2) were tested for their relaxant activities on isolated rat duodenum and relationships between the physicochemical parameters of the variable substituents and the relaxant activity were examined by applying simple and multiple linear regression analyses.

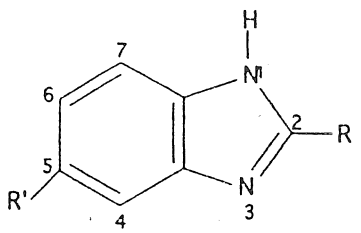

Fig.1. 2,5-substituted-benzimidazoles

Materials

All the reagents and solvents were of analytical grade (Merck, Aldrich and Sigma).

Methods

Chemistry

Compounds listed in Table 1 were synthesized according to; **1-4**; aldehyde method (3), **5-15** and **19-22**; polyphosphoric acid (PPA) method (4), **16-18**, **23** and **24**; polyphosphate ester (PPE) method (5,6).

Pharmacology

It is known that 2-benzylbenzimidazoles have a relaxant action on smooth muscles (7). Molecules having benzimidazole ring possess, in general, hypotensive and sympatholytic activities (8). They also may relax smooth muscles of the uterus, uretra and gastrointestinal tract. Precise mechanisms for the relaxant activities of benzimidazoles were not elucidated. There are, however, some experimental evidences that these

compounds may cause smooth muscle relaxant activity via cyclic nucleotide phosphodiesterase inhibition (9). The present study is based on a series of tests previously performed by Horton using the isolated rat duodenum (10).

Smooth muscle preparation

The proximal 2 cm of duodenum from rats weighing 150 to 200 g was removed after killing and kept in atropinized (0.143 mM) Krebs' solution at 6°C for two hours. In this way , spontaneous movements of duodenum were diminished. Then, the duodenum was suspended in a 10 ml organ bath containing the atropinized Krebs' solutions at 31°C and gassed with a mixture of 5% CO_2 in oxygen. The relaxations of the rat duodenum were then recorded with an isotonic transducer(Ugo Basile, No.7006) connected to a recording microdynamometer

Table 1. Parameters used to derive the QSAR equations for smooth muscle relaxation by 2,5-substituted benzimidazole derivatives.

Com-pound	R	R'	Mv (log)	MR (log)	Mw (log)	V_w	MCI (log)	Par (log)	log P	π	σ	μR	pD_2	αE
1	Ph	H	2.416	1.774	2.288	1.718	0.693	2.534	3.097	1.96	-0.01	0	4.795	1.168
2	o-OH-Ph	H	2.420	1.785	2.323	1.958	0.705	2.535	2.554	1.29	-0.37	1.63	5.295	1.106
3	m-OH-Ph	H	2.428	1.785	2.323	1.958	0.705	2.535	2.554	1.29	0.11	1.55	5.010	1.170
4	p-OH-Ph	H	2.428	1.785	2.323	1.958	0.705	2.535	2.554	1.29	-0.38	1.34	5.075	1.080
5	2-Py	H	2.411	1.760	2.291	1.668	0.681	2.525	1.719	0.32	0.16	1.94	5.448	1.424
6	3-Py	H	2.411	1.760	2.291	1.668	0.700	2.525	1.719	0.32	0.16	2.28	4.993	1.453
7	4-Py	H	2.411	1.760	2.291	1.668	0.680	2.525	1.719	0.32	0.16	2.57	5.915	0.923
8	2-Thienyl	H	2.379	1.768	2.302	1.663	0.707	2.513	2.738	1.61	0.10	0.81	4.299	1.806
9	Ph	NO_2	2.463	1.818	2.379	1.916	0.703	2.584	2.819	2.20	0.77	6.09	5.036	1.059
10	2-Py	NO_2	2.459	1.805	2.381	1.866	0.691	2.576	1.459	0.72	0.95	7.59	5.256	1.000
11	3- Py	NO_2	2.459	1.805	2.381	1.866	0.691	2.576	1.459	0.72	0.95	7.41	4.219	0.766
12	4-Py	NO_2	2.459	1.805	2.381	1.866	0.691	2.576	1.459	0.72	0.95	5.32	4.994	0.966
13	o-Cl-Ph	NO_2	2.469	1.848	2.418	2.091	0.746	2.607	3.559	2.91	1.00	7.89	4.744	1.147
14	m-Cl-Ph	NO_2	2.469	1.848	2.418	2.091	0.746	2.607	3.559	2.96	1.14	7.10	4.891	0.967
15	p-Cl-Ph	NO_2	2.469	1.848	2.418	2.091	0.745	2.607	3.559	2.91	1.00	5.34	4.707	1.158
16	p-CH_3-Ph	NO_2	2.495	1.847	2.404	2.067	0.737	2.586	3.339	2.72	0.60	5.98	4.639	1.211
17	o-OCH_3-Ph	NO_2	2.505	1.858	2.404	2.151	0.746	2.598	2.206	2.16	0.50	6.34	5.142	1.177
18	p-OCH_3-Ph	NO_2	2.505	1.858	2.404	2.151	0.746	2.598	2.206	2.16	0.50	7.03	5.229	0.824
19	2-Furyl	NO_2	2.416	1.768	2.360	0.543	0.657	2.529	1.935	1.46	0.74	5.73	5.109	0.978
20	3-Furyl	NO_2	2.416	1.768	2.360	0.543	0.619	2.529	1.935	1.46	0.74	5.72	5.058	0.947
21	2-Br-5-Furyl	NO_2	2.454	1.812	2.489	1.859	0.734	2.576	2.884	2.32	0.86	4.17	4.859	1.211
22	2-Thienyl	NO_2	2.430	1.812	2.390	1.762	0.716	2.566	2.478	1.85	0.88	4.77	4.782	1.034
23	o-OH-Ph	NO_2	2.474	1.828	2.407	1.984	0.715	2.585	2.294	1.53	0.41	6.07	4.737	0.937
24	p-OH-Ph	NO_2	2.474	1.828	2.407	1.984	0.715	2.585	2.294	1.53	0.40	6.35	4.213	0.867

(Ugo Basile, No.7050). The load on the tissue was 1.0 g and the relaxations elicited by relaxant agents were magnified 8-folds. The suspended duodenum was allowed to equilibrate for 60 minutes. During this period, the tissue was washed out in every 15 minutes. After this initial incubation, dose-response relationships were obtained for benzimidazoles using two individual dose-response procedures in all experiments. In each experiment, merely two related derivatives were tested as agonists on the same tissue. The dose cycle was 5 minutes with 60 seconds of contact time. All dilutions were prepared with 10^{-3} M HCl adjusting the pH to 5.0. Then, apparent affinity constant (pD_2) and intrinsic activity (α^E) values were calculated (11).

Calculation of molecular parameters

Steric; molar volume (Mv) (12), molar refractivity (MR) (13), molecular weight (Mw), Van der Waals volume (V_w) (14), molecular connectivity index (MCI) (15, 16).

Hydrophobic; parachor (Par) (17), partition coefficients (P) (18-20), hydrophobic substituent constant (π) values (21,22).

Electronic; dipole moment (μR) (23), electronic substituent constant (σ) (24,25) were calculated theoretically.

Synthesized compounds, pharmacological data and calculated molecular parameters are given in Table 1.

Correlation analyses

The squared correlation coefficient matrix calculated by these parameters is shown in Table 2. Single and multiple linear regression analyses were performed on a Macintosh-Classic computer running StatView (512^+) statistical programme package. In the equations, the numbers in parentheses are the 95% confidence invervals, n is the number of observations, r is the correlation coefficient, s is the standart deviation from regression, F and p are the statistical significances calculated by Fisher test and t-test, respectively.

Table 2. Squared correlation matrix between the parameters given in Table 1.

	Mv (log)	MR (log)	Mw (log)	V_w	MCI (log)	Par (log)	log P	π	σ	μR	pD_2	α^E
Mv (log)	1											
MR (log)	0.927	1										
Mw (log)	0.754	0.798	1									
V_w	0.561	0.626	0.281	1								
MCI (log)	0.645	0.799	0.533	0.949	1							
Par (log)	0.914	0.958	0.840	0.585	0.712	1						
log P	0.233	0.493	0.348	0.374	0.619	0.403	1					
π	0.293	0.461	0.446	0.061	0.307	0.439	0.515	1				
σ	0.504	0.538	0.742	–0.22	0.176	0.690	0.101	0.461	1			
μR	0.748	0.702	0.753	0.091	0.243	0.792	0.001	0.293	0.834	1		
pD_2	-0.12	-0.28	-0.313	-0.14	-0.265	-0.261	-0.369	-0.26	-0.30	-0.165	1	
α^E	-0.46	-0.31	-0.367	-0.04	0.11	-0.41	0.225	-0.03	-0.37	-0.37	-0.21	1

Results and Discussion

QSAR with empirical parameters

Initially, the compounds under investigation were divided into two sets; compounds **1-8** having no $-NO_2$ and compounds **9-24** having a $-NO_2$ function, on which regression analyses were performed separately.

Subsequently, same analyses were carried out on the collective set of compounds **1-24**. Correlation analyses with the calculated physicochemical parameters of compounds were performed both with the α^E value and the pD_2 value.

*QSAR for the first set (compounds **1-8**) with pD_2 and α^E values*

$pD_2 = 15.38\ (\pm 5.96)\ Mv - 32.17$ (1)
n=8 r=0.725 s=0.724 F=6.65 p<0.05

$pD_2 = 34.44\ (\pm 10.35)\ \log Par - 82.15$ (2)
n=8 r=0.805 s=0.183 F=11.06 p<0.05

$pD_2 = 36.29\ (\pm 5.70)\ \log Par - 0.33\ (\pm 0.008)\ \log P - 86.07$ (3)
n=8 r=0.955 s=0.342 F=25.82 p<0.005

Good correlations were observed with biological activity (affinity) in the 2nd and 3rd equations which include hydrophobic (log P) and steric (log Par) parameters (r >0.8).

$\alpha^E = -13.71\ (\pm 4.26)\ Mv + 34.74$ (4)
n=8 r=0.795 s=0.517 F=10.33 p<0.05

$\alpha^E = 21.69\ (\pm 7.55)\ MCI - 1.87\ (\pm 0.57)\ V_W - 10.51$ (5)
n=8 r=0.841 s=0.506 F=6.04 p<0.05

$\alpha^E = -14.01\ (\pm 3.95)\ Mv + 8.20\ (\pm 5.77)\ MCI + 29.38$ (6)
n=8 r=0.859 s=0.477 F=7.05 p<0.05

On the basis of the equations 4,5 and 6 the correlation (0.8) between α^E values and steric parameters emphasized the importance of these parameters, particularly MCI.

Selection of the retained equations was decided according to the t-test results in this study ($p<0.005$ or $p<0.05$). Equations in which satisfactory results could not be obtained with t-test were not considered, even though they displayed high correlation coefficients.

*QSAR for the second set (compounds **9-24**) with pD_2 and α^E values*

Since in this set no good correlation was observed with pD_2, only the equations which included α^E were retained. The biological activities of the compounds containing the 5-nitro substituent were weekly correlated with the electronic parameters as shown in equation 8. The equation was not improved by the addition of the hydrophobic parameter (π) as indicated in equation 9.

$\alpha^E = -0.87\ (\pm 1.51)\ \log Par + 0.12\ (\pm 0.40)\ \log P + 2.95$ (7)
n=16 r=0.620 s=0.460 F=4.05 p<0.05

$\alpha^E = 0.10(\pm 0.03)\ \pi + 0.81$ (8)
n=16 r=0.610 s=0.448 F=8.30 p<0.05

$\alpha^E = 0.05(\pm 0.13)\sigma + 0.10\ (\pm 0.03)\pi + 0.77$ (9)
n=16 r=0.616 s=0.464 F=3.97 p<0.05

*QSAR for the whole set (compounds **1-24**) with pD_2 and α^E values*

There was no satisfactory result when the simple regression was applied on the whole set. There were two significant equations for α^E values in this set.

$\alpha^E = 0.15\ (\pm 0.60)\ \log P - 4.30\ (\pm 1.33)\ \log Par + 11.75$ (10)
n=24 r=0.604 s=0.921 F=6.02 p<0.05

$\alpha^E = -4.06\ (\pm 3.45)\ Mv + 7.79\ (\pm 3.37)\ MCI - 0.13\ (\pm 0.17)\ V_W - 3.09\ (\pm 4.30)\ MR$ (11)
n=24 r=0.738 s=0.818 F=5.69 p<0.005

Interactions with the active site

The main structure of the benzimidazole is not an absolutely extraneous molecule for the living organisms. It creates a nuclei for current drugs of today such as ethonitazene, thiabendazole, clemizole, omeprazole, benomyl, etc. Due to its physicochemical characteristics, like stereochemical, electronic and hydrophobic properties, the benzimidazole structure is in different conformations and exhibits diverse behaviours in various sites of biophase in living organisms. Having a convenient molecular size, it causes many diverse effects by occupying various sites of action. Substituents contained are also very important for the activity of benzimidazole structure. Especially, substituent modifications done at the first, second and fifth positions of the molecule cause not only an increase in its known effects but also a development of other type of effects. While electron-drawing and electron-donating groups at these (first, second and fifth) positions affect the active site electronically, these groups may facilitate the interaction by their positive contribution to the molecular dimensions. Aromatic and heterocyclic groups at the same position establish new electronic and steric conditions in the main structure, so these groups cause certain changes in their molecular behaviours related to the site of action. In heterocyclic ring-containing derivatives which also have electron-donating groups like -OH and -OCH_3 at the second position, for example, a positively charged area in the site of action is thought to be present for the interaction with unpaired electron doublet of heteroelement. In the derivatives having aromatic ring at the same position as shown in Table 1, however, minimum activity was observed at meta position in the case of presence of electron-donating groups at one of the ortho, meta and para positions of the ring. In this case, stereochemical changes caused by a pseudoring formation in ortho substituted derivatives are supposed to elicit favourable changes in the relaxant effects. In coincidence to this opinion, especially the increase of correlation coefficient over 0.8 by the contribution of MCI parameter in the equation emphasises the importance of topographic structure of the molecule for the interaction with the site of action (Fig.2).

Observation of good or mild correlations with the hydrophobic parameters nearly in all sets also suggests the presence of a hydrophobic zone in the active site by which especially -CH_3 and -OCH_3 groups are affected.

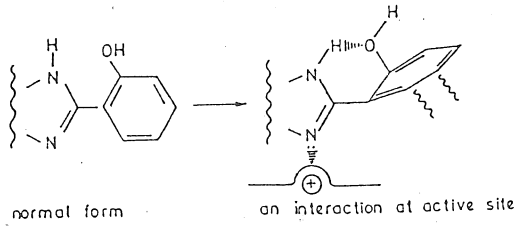

Fig. 2. The formation of pseudoring

On the basis of literature data (26,27), it is understood that the pK_a value of the compounds having a -NO_2 function at fifth position is lower than in other derivatives (Examples; Ph; 5.23 without -NO_2, 2.95 with -NO_2, 2-thienyl; 4.57 without -NO_2, 2.36 with -NO_2). This reduction which is proportional with the electron-drawing potency of the -NO_2 groups displays some variations due to the influence of a group at second position. As a result of delocalization phenomenon with electron-drawing from the main structure, the nitrogen atom is negatively charged at third position and it is, therefore, thought to be the presence of positively charged area in the corresponding site of action. Acidic test medium causes only a mild interaction, since the nitrogen atom in pyridine type is readily

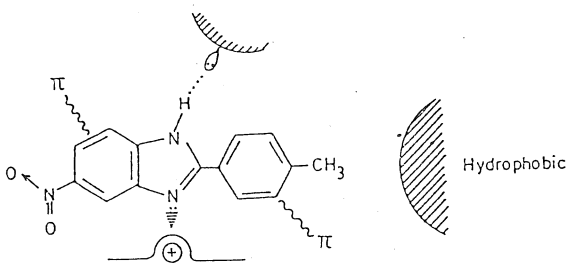

Fig.3. Possible interactions of 2,5-substituted - benzimidazoles in the active site of relative enzyme

protonated to produce a salt form. Due to the salt formation, increased water solubility of the compounds causes a difficulty in the penetration of the molecule across cellular membrane having a lipoid structure. Almost excellent correlations were obtained in the combinations of both pD_2 and α^E values withlog Par+log P. Mild correlations were observed by the combinations of π and $\pi + \sigma$ in the eight and ninth equations. A putative interaction model for 2,5-substituted-benzimidazoles are given in Fig.3.

References

1. Büyükbingöl, E., Noyanalpan, N.: J. Fac. Pharm. Gazi Univ. 2, 81(1985)
2. Işıkdağ, İ., Uçucu, Ü., Demirayak, Ş.: Doğa Bil. Der. 1, 33 (1986)
3. Weindenhagen, R.: Ber. 69, 2263 (1936)
4. Popp, F.D., Mc Ewen, W.E.: Chem. Rev. 58, 321 (1958)
5. Kanaoka, Y., Yonemitsu, O., Tanizawa, K.: Chem. Pharm. Bull. 12, 773 (1964)
6. Kanaoka, Y., Hamada, T., Yonemitsu, O.: Chem. Pharm. Bull. 18, 587 (1970)
7. Bowman, W.C., Rand, M.J.(Eds.): Textbook of Pharmacology, pp. 64-68 Blackwell Scientific Publications, London 1980
8. Lyubimov, B.I.: Bull. Exp. Biol. Med. USSR 45, 585 (1958); C.A. 53, 12491 d (1959)
9. Öztürk, Y., Büyükbingöl, E., Noyanalpan, N.: J. Fac. Pharm. Gazi Univ. 3, 153 (1986)
10. Horton, E.: Br. J. Pharmacol. 14, 125 (1959)
11. Ariens, E.J., Van Rossum, J.M.: Arch. Int. Pharmacodyn. 110, 275 (1957)
12. Berkem, A.R., Baykut, S. (Eds.): Fizikokimya, İstanbul Üniversitesi Yayınları, Sayı 2735, No 42 s. 335, 356 Fatih Yayınevi, İstanbul 1980
13. Weast, R.C. (Ed.): Handbook of Chemistry and Physics, 55th Edition, CRC Press, Ohio, E-222, 1974-75
14. Moriguchi, I., Kanada, Y., Komatsu, K.: Chem. Pharm. Bull. 24, 1799 (1976)
15. Hall, L.H., Kier, L.B.: J.Pharm.Sci. 65, 1506 (1976)
16. Hall, L.H., Kier, L.B.: J.Pharm.Sci. 66, 642 (1977)
17. Ahmad, P., Fyeed, C.A., Mellors, A.: Biochem. Pharmac. 24, 1103 (1975)
18. Iwasa, J., Fujita, T., Hansch, C.: J.Med.Chem. 8, 150 (1965)
19. Nys, G.G., Rekker, R.F.: Chim. Ther. 8, 521 (1973)
20. Currie, J.D., Lough, E.C., Silver, F.R., Holmes, L.H.: Can. J. Chem. 44, 1035 (1966)
21. Hansch, C., Leo, A., Unger, S.H., Kim, K.H., Nikaitani, D., Lien, E.J.: J.Med.Chem. 16, 1207 (1973)
22. Fujita, T., Iwasa, J., Hansch, C.: J. Am.Chem.Soc. 86, 5175 (1964)
23. Young, R.C., Durant, G.J., Emmet, J.C., Ganellin, C.R., Graham, M.J., Mitchell, R.C., Prain, H.D., Roantree, M.L.: J. Med.Chem. 29, 44 (1986)
24. Purcell, P.W., Bass, E.G., Clayton, M.J. (Eds.): Strategy of Drug Design: A Guide to Biological Activity,pp. 66- 68 Wiley-Interscience, New York 1973
25. Murray, M., Ryan, A.J., Little, P.J.: J.Med.Chem. 25, 887 (1982)
26. Albert, A., Serjant, E.P.(Eds.): The Determination of Ionization Constants, Chapman and Hall, London 1971
27. Öğretir, C., Demirayak, Ş.: Doğa-Turk. J. Chem. 10, 118 (1986)

Accepted: 03.01.1996