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Quantitative Structure-Activity Relationship of Some 7-(4-Coumaryloxy) alkyl Substituted Theophyllines

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Quantitative Structure-Activity Relationship of Some 7-(4-Coumaryloxy) alkyl Substituted Theophyllines

Summary : *Some novel 7-(4-coumaryloxy)alkyl substituted theophyllines have been investigated for quantitative structure-activity relationship (QSAR). For this purpose, the in vitro bronchodilatory activities which were examined by the inhibition of acetylcholine induced contractions in tracheae isolated from guinea pigs have been correlated with various quantum chemical and physicochemical parameters.*

Keywords : *Theophylline, coumaryloxyalkyl theophyllines, quantitative structure-activity relationship.*

Bazı 7-(4-Kumariloksi) alkil Sübstitüe Teofilinlerin Kantitatif Yapı Etki İlişkileri

Özet : *Bazı yeni 7-(4-Kumariloksi)alkil Sübstitüe Teofilinler kantitatif yapı etki ilişkileri (QSAR) açısından incelenmiştir. Bu bileşiklerin in vitro bronkodilatör aktiviteleri, izole kobay trakeasında asetilkolin ile indüklenen kontraksiyonların inhibisyonunu ölçmek suretiyle incelenmiştir. Sonuçlar çeşitli kuantum kimyasal ve fizikokimyasal parametreler ile ilişkilendirilmiştir.*

Anahtar kelimeler : *Teofilin, kumariloksialkil teofilinler, kantitatif yapı aktivite ilişkisi*

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INTRODUCTION

Bronchial asthma is a chronic debilitating disease that, in its severe forms, can threaten life. At present, four classes of drugs have been employed to combat the symptoms of this disease: β-sympathomimetic agents, bronchodilators, antiallergic agents and corticosteroids. Traditionally, theophylline as a representative of bronchodilators is extensively used in the treatment of asthma[1]. However theophylline possesses cardiotonic, central nervous system (CNS) stimulatory, diuretic and other pharmacological activities in addition to the bronchodilatory activities[2]. Side effects and toxicity are often noted at blood levels considered to be within the therapeutic range[3]. Therefore theophylline has a narrow therapeutic index. Much effort has been invested to develop new theophylline derivatives without the usual CNS and cardiovascular side effects of theophylline. Most studies have modified substituents at the 7 position of theophylline though this modification has allowed to develop new compounds such as propoxyphylline[4], etophylline[5], diphylline[6], and doxophylline[7] that were reported to possess more potent activity and less toxicity than theophylline in animal models. These facts have prompted us to synthesize new compounds that involve modifications on the 7 position of theophylline. In our previous study, we reported the synthesis and bronchodilatory activity of some new 7-(2-naphthyloxy)alkyl- and 7-(4-coumaryloxy)alkyl-3,7-dihydro-1,3-dimethyl-1H-purine-2,6-diones[8]. We also reported the quantitative structure-activity relationship (QSAR) of those 7-(2-naphthyloxy)alkyl derivatives. It was found that the biological responses linearly correlated with the interfrontier molecular orbital energy difference[9]. In the present study , we consider some 7-(4-coumaryloxy)alkyl-3,7-dihydro-1,3-dimethyl-1H-purine-2,6-diones for QSAR investigation.

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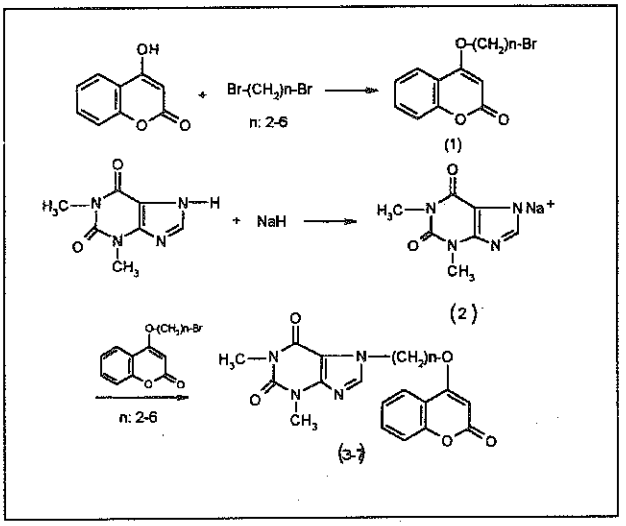
MATERIAL and METHODS

Chemistry

7-(4-coumaryloxy)alkyl-1,3-dimethyl - 3,7 - dihydro-1H-purine-2,6-diones(3-7) were synthesized by the reactions of various 4-coumaryloxyalkyl bromides (1) with theophylline sodium (2) in dimethylformamide according to the ref. 8 (see scheme 1, n:2-6).



Scheme 1

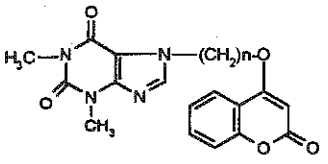
Determination of R_m values

The determination of R_m values was carried out as described in the literature[10] by using Silica Gel G (Merck, Darmstadt, Germany) plates impregnated with 5 % (v/v) of liquid paraffin (Sigma, Steinheim, Germany) in hexane. The developments of the plates was carried out in aqueous acetone. The results are shown in Table 1.

Biological methods

Pharmacological results were obtained by using tracheal strips of male and female guinea pigs (Swiss albino, Animal Care Facilities of Anadolu University, Eskişehir, Turkey) in isolated organ baths according to literature[8]. A tension of 2 g was applied and isometric recording was done using an isometric transducer (TB-651 T); Nihon-Kohlen recording system (Tokyo, Japan). All the compounds were dissolved in dimethylsulfoxide and added to the 10 mL of organ bath in volumes of 0.1 mL. The preparations were allowed to equilibriate for at least 60 min, with regular washes for every 15 min. Contractions induced by 10-4 mol/L acetylcholine were recorded after the samples were incubated with various concentrations of the compounds or aminophylline for 5 min. The results are shown in Table 2.

Table 2. The biological activity of the compounds studied.



Compd. no	n	Concentration (mole/L)	Relaxation (%)[a] Acetylcholine induced spasm
		10^{-6}	5.15±2.49
		10^{-5}	5.00±2.10
3	2	10^{-4}	7.67±2.93
		10^{-6}	0
		10^{-5}	0
4	3	10^{-4}	3.75±1.97
		10^{-6}	5.50±3.28
		10^{-5}	8.13±1.58
5	4	10^{-4}	32.60±3.38
		10^{-6}	0
		10^{-5}	0
6	5	10^{-4}	21.33±2.95
		10^{-6}	5.67±1.87
		10^{-5}	5.76±1.65
7	6	10^{-4}	12.75±2.25
		10^{-8}	0
		10^{-7}	15.50±6.44
		10^{-6}	17.00±7.49
		10^{-5}	42.15±5.22
Aminophylline		10^{-4}	100±0

The 10^{-4} mol/L concentration of aminophylline that produces a complete relaxation (100 %) of the acetylcholine induced spasm on guinea pig trachea. [a]Mean ± SD (n=6) is calculated from the mean relaxation of six experiments. n: the number of CH_2 groups in the alkyl part.

Table 1. Various quantum chemical and physicochemical parameters of the compounds studied.

Compound No	E_{HOMO}	E_{LUMO}	R_m	Log P	Refractivity	Polarizability
3	-9.1666	-.8575	-.201	.53	96.55	36.27
4	-9.0797	-.8736	-.162	.59	101.42	38.10
5	-9.0535	-.8842	-.176	1.04	106.06	39.94
6	-8.9460	-.8067	-.231	1.43	110.66	41.77
7	-8.8352	-.7503	-.087	1.83	115.26	43.61

E_{HOMO} and E_{LUMO} in eV. Refractivity and polarizability values in A^3.

Quantum chemical methods

In the present study, a semi-empirical molecular orbital calculations of AMI (Austin Model 1)[11] type at the level of restricted Hartree-Fock (RHF) approach has been carried out. All the structures were subjected to geometry optimization (a conjugate gradient optimization, Polak-Ribiere) prior to single-point molecular orbital calculations. After pre-optimization of the structures, conformational analyses were performed using the MM+ force field, followed by reoptimization of the geometries by means of AM1 method as described above. Throughout the calculations, the convergence limit and gradient values (RMS) were maintained below 10^{-4} Kcal/mol and 10^{-3} Kcal/(A° mol), respectively.

To solvate the molecules, periodic boundary conditions, involving the cubic periodic box of specific size enclosing each molecule was employed (Table 3). The minimum distance between solvent (H_2O) and the solute atoms was set to be 2.3 A°. To solvate the molecules under periodic boundary conditions, the water molecules based on Jorgensen`s Monte Carlo equilibriated box of 216 water molecules described by the TIP3P potential function were employed[12]. All these calculations and the theoretical QSAR parameters were carried out by using the Hyperchem (release 5.1) and ChemPlus 2.0 (Hypercube Inc, Florida, USA) package programs. The log P values are based on atomic parameters derived by Ghose et al[13,14]. The refractivity values are estimated by the method[14] as log P. The polarizabilities are estimated from an additivity scheme presented by Miller[15]. The solvent accessible surfaces of the molecules were obtained by using CSChem 3D Net program.

RESULTS

AM1 calculations reveal that as the CH_2 groups increase in number (n), the HOMO energy raises up (less negative values) but there is no direct effect of n is present on the LUMO energy. Most probably the LUMO energy is more dependent on the orientation of the theophylline and coumaryloxy moieties rather than the inductive effect of CH_2 groups.

The present study mainly deals with certain quantitative structure-activity relationships (QSAR) of the above mentioned compounds. Due to the limited number of the compounds considered, all the mathematical models for the biological response (BR) were based on simple independent variable in each case. Various theoretically obtained quantum chemical and physicochemical parameters, including molecular orbital energies (eigenvalues), inter-frontier energy gaps (LUMO and HOMO energy difference), molecular orbital coefficients (eigenvectors), distances between certain sites of the structures, area and volume of the molecules, log P values, refractivities and polarizabilities etc. were calculated (Table 1).

On the other hand, the inspection of the AM1 geometry optimization of the structures reveals that coumarin moiety occupies very different orientation with respect to the plane of the purine ring in each compound (Figure 1). Root-mean–square overlay error analysis of the compounds based on superimposition of the theophylline moiety present onto the free theophylline structure revealed that although the most active compound (compound 5) possesses theophylline moiety which fits the free theophylline structure best (having the minimum RMS error of 0.7114), the other structures do not exhibit a correlation between the biological response and RMS overlay error values. Whereas a direct correlation was found in between the biological response and the maximum number of water molecules (n_w) in the cubic boxes employed for the periodic boundary conditions. Also, the direct correlations of the above type were obtained for n_w/A and n_w/V where A and V stand for area (grid type calculations[16]) and volume of the molecules, respectively (see Table 3).

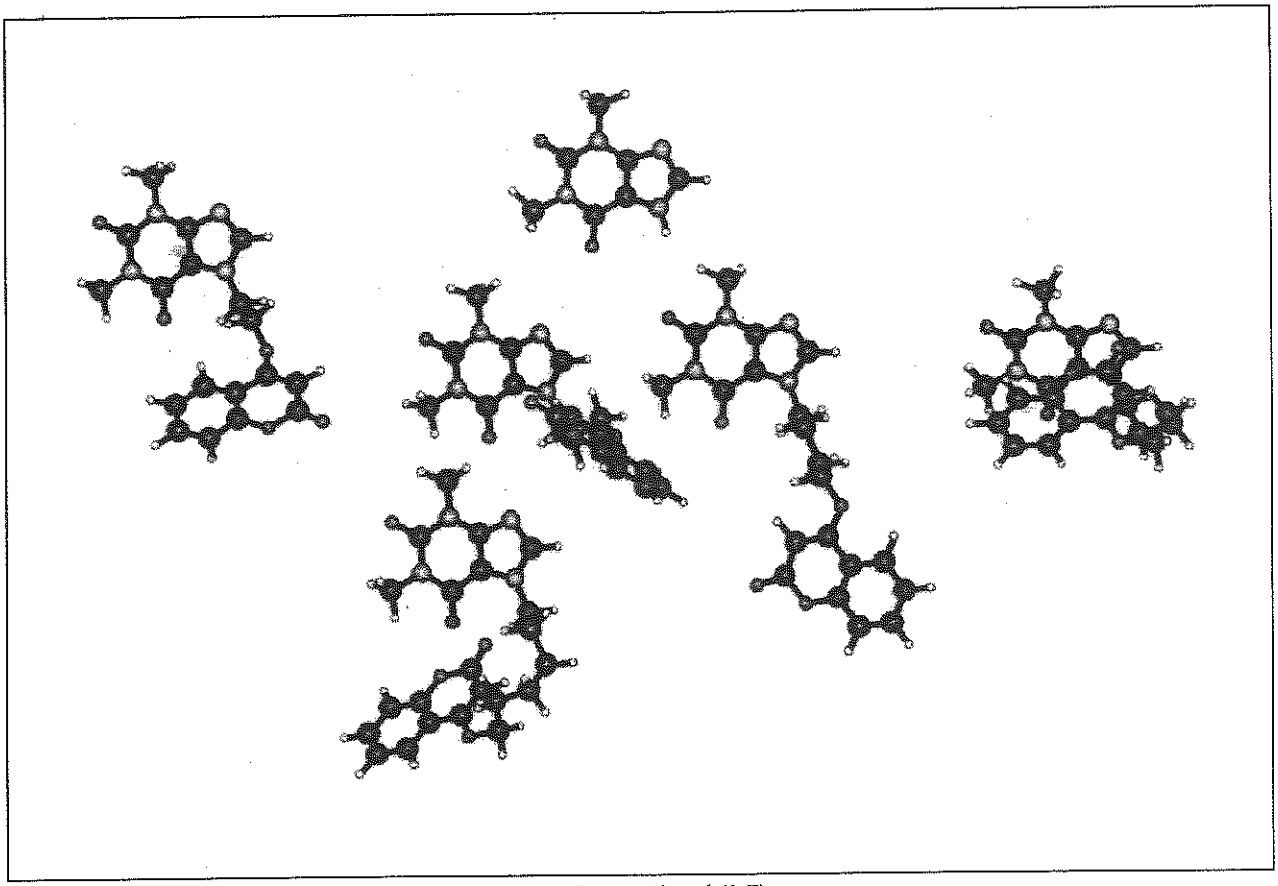


Fig.1. Geometry optimized structures of the compounds considered (3-7).
Theophylline is on the top, C:Black-big; N:Grey-big; O:Dark-small and H:The smallest balls.

Table 3. Periodic box properties for the molecules studied.

Compound No	Box size*	Max. number of H_2O molecules	Max. number of H_2O molecules/Area**	Max. number of H_2O molecules/Volume**
3	25.59	554	.98	.57
4	20.00	265	.46	.26
5	34.31	1335	2.06	1.22
6	34.50	1357	2.01	1.18
7	29.83	877	1.24	.73
Theophylline	18.70	216	.63	.41

* Edge of a cubic box in A^3. ** Molecular area and volume.

The quantitative relationship between the biological response data (relaxation %, see table 2) and the above mentioned independent variables, namely, n_w , n_w/A and n_w/V, was found to be better if the BR values were replaced with log (1/BR). Thus, the following first order linear equation of the general type was obtained where X is the independent variable and a and b are the regression coefficients. Table 4 tabulates a and b values for three different

$$\text{Log } (1/BR) = a + bX \qquad (1)$$

equations together with the regression statistics. Note that the regression equations are characterized with the sample population (N) of 5 compounds (3-7) only and degrees of freedom of 3 . The tabulated F-test and t- test values[17,18] are $F_{1,3}$ (0.01) = 34.12, $F_{1,3}(0.05)$ = 10.13 and $t_3(0.01)$ = 4.541, $t_3(0.005)$= 5.841. Note that the level of significances are in parentheses. Inspection of the regression statistics (Table 4) reveals that all

of the equations, based on the independent variable n_w, n_w/A or n_w/V are statistically significant. As the coefficients (a and b) were obtained by regression, they must maintain five or six decimal points for the best fit, however the calculated biological responses should be rounded off down to two decimal points.

Table 4. Regression characteristics of eq. 1.

	Independent variable X		
	n_w	N_w/A	N_w/V
a	-.421573	-.369322	-.380048
b	$-7.51148\ 10^{-4}$	-.527021	-.884788
R^2	.95	.96	.96
r_{YX}	-.97	-.97	-.98
F	60.944	72.308	80.059
S_Y	.092	.084	.080
S_a	.093	.918	.086
S_b	$9.621\ 10^{-5}$	.619	.098
t_s	7.806	8.503	8.947

R^2: goodness of fit, r_{YX}: simple correlation coefficient, S_Y: unexplained standard deviation, S_a and S_b : unbiased estimates of the variance of regression coefficients, t_s: testing significance of the regression coefficient b.

DISCUSSION

Figure 2 shows the solvent accessible surfaces of the molecules which are presently concerned. On the other hand, the inspection of Table 3 reveals that when the respective independent variables (n_w, n_w/A or n_w/V) for theophylline are substituted into the regression equations obtained for the set of five compounds (3-7) no correlation is found with the high biological response value for aminophylline (which is an ethylenediamine salt of theophylline[19]) although its biological activity is attributed to theophylline part. This implies that the action mechanism of the above mentioned molecules is not directly similar to that of aminophylline which acts at the cellular level after it is converted to theophylline. Theophylline acts by either inhibiting phosphodiesterase or blocking adenosine receptors in the bronchi, resulting in relaxation of the smooth muscle[20,21]. However, n_w/V values for the compounds, follow the order of 5>6>7>3>4 and which is indicative of local concentration of water molecules around the molecules, directly regress with the percent relaxation induced by the administration of the present set of compounds.

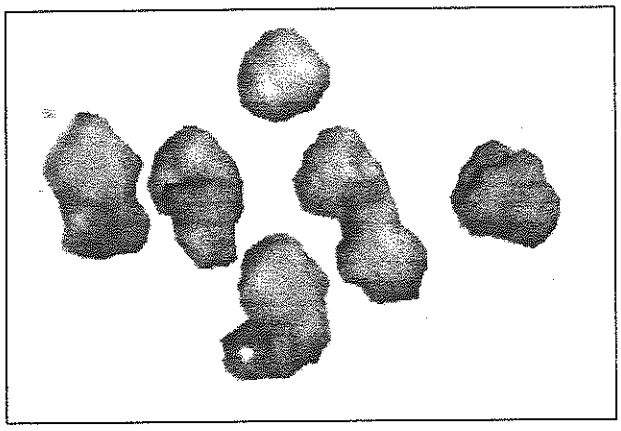


Fig.2. Solvent accessible surfaces of the molecules studied (3-7). Theophylline is on the top.

All these results and observations imply that activity of the present compounds may be mainly based on the release of theophylline molecules. As seen in Fig 1. theophylline and coumaryloxy moieties in the hydrated form in m compound 5 (n:4) are oriented in such a manner that N-dealkylation either enzymatically or by hydrolysis is sterically more probable than the others , thus leading to a high relaxation effect.

CONCLUSION

AM1 type semiempirical calculations using the periodic boundary conditions to solvate the molecules imply that relaxation caused by the presently studied 7-(4-coumaryloxy)alkyl substituted theophyllines may be due to free theophylline produced by N-dealkylation. Hence, the steric environment of the CH_2 group next to the nitrogen atom should be taken into account in the design and synthesis of more active analogous compounds.

REFERENCES

1. Persson CGA, Some pharmacological aspects of xanthines in astma, *Eur. J. Resp. Dis*, 61, 7-16, 1980.
2. Persson CGA, Andersson KE, Kjellin G, Effects of enprophylline and theophylline may show the role of adenosine, *Life Sci*, 38, 1057-1063, 1986.
3. Andersson KE, Persson CGA, Extrapulmonary effects of theophylline, *J. Resp. Dis*, 61, 17-26, 1980.
4. Pascal JC, Beranger S, Pinhas H, New antihistaminic theophylline or theobromine derivatives, *J. Med.*

Chem, 28, 647-654, 1985.

5. Fischbach VR, Haas R, Klinische und experimentelle erfahrungen mit dem nicotisäureester des oxyäthyltheophyllins, *Arzneim.-Forsch./Drug Res*, 17, 313-319, 1967.
6. Klosa J, Beitrag zur reaktionsfähigkeit der 7-stellung des theophyllins, *Arch. Pharm*, 288, 301-304, 1955.
7. Grosa G, Frazona JS, Biglino G, Metabolism of doxophylline by rat liver microsomes, *Drug Metab. Dispos*, 14, 267-270, 1986.
8. Akgün H, Balkan A, Günay S, Erol, K, Boyda S, Some new 7- aryloxyalkyl-theophyllines as bronchodilators, *Eur. J. Med. Chem*, 32, 175-179, 1997.
9. Türker L, Akgün H, Balkan A, Quantitative structure-activity relationship study of some 7-substituted theophyllines, *Arzneim.-Forsch./Drug Res*, 48(1), 39-42, 1998.
10. Boyce CBC, Milborrow BV, A simple assessment of partition data for correlating structure and biological activity using thin layer chromatography, *Nature*, 208, 537-539, 1965.
11. Dewar MJS, Zoebisch EG, Healy EF and Stewart JJP, AM1: A new general purpose quantum mechanical molecular model, *J. Am. Chem. Soc*, 107, 3902-3905, 1985.
12. Jorgensen WL, Chandrasekkar J, Madura JD, Impey RW and Klein ML, Comparison of simple potential functions for simulating liquid water, *J. Chem. Phys*, 79, 926-930, 1983.
13. Ghose AK, Crippen GM, Atomic physicochemical parameters for three dimensional structure directed quantitative structure-activity relationships III: modeling hydrophobic interactions, *J. Comp. Chem*, 9, 80-84, 1980.
14. Viswanadhan VN, Ghose AK, Revankar GN, Atomic physicochemical properties for three dimentional QSAP-parameters on applications, *J. Chem. Inf. Com. Sci*, 29, 163-165, 1983.
15. Miller KJ, Additivity methods in molecular polarizability, *J. Am. Chem. Soc*, 112, 8533-8540, 1990.
16. Bodor N, Gabanyi Z and Wong C, A new method for the estimation of partition coefficient, *J. Am. Chem. Soc*, 111, 3783-3787, 1989.
17. Draper N, Smith H, Applied regression analysis, *Addison-Wesley Reading*, 306, 1974.
18. Salvatore D, *Statistics and Econometrics*, McGraw-Hill, New York, 227, 1982.
19. Melentyeva G, Antonova L, *Pharmaceutical Chemistry*, Mir Pub, Moscow, 365, 1988.
20. Loeb S, (Ed.), *Physician's Drug Handbook*, Springhouse Co., Pensylvania, 1021, 1993.
21. Katzung BG, *Basic and Clinical Pharmacology*, Lange, Los Altos, 236, 1984.