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Report

Quantitative Correlations and Reexamination of the Importance of Hydrophobic and Steric Factors in Anticholinergic Drug Receptor Interactions

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The pA_2 (or log K) values at the muscarinic cholinergic receptor of the guinea pig ileum for a series of 1,3-dioxolanes have been correlated with the hydrophobic constant π and π^2. A correlation coefficient of 0.97 and standard deviation of 0.30 were obtained with 16 of the 18 compounds ($P < 0.05$). A series of 17 analogues of amino esters of α-methyl tropic acid and α-substituted phenyl acetic acids, which were tested in the isolated rat ileal muscle, was analyzed, with a correlation coefficient of 0.92 and a standard deviation of 0.25 ($P < 0.05$). Based on these results, the role of hydrophobic forces in the drug receptor interactions of muscarinic cholinergic receptors was analyzed. Optimum values for the physicochemical parameters were determined and their use in enhancing potency was suggested.

KEY WORDS: acetylcholine; hydrophobic interactions; quantitative structure activity relationships (QSAR); muscarinic receptor; substituted phenyl acetic acid; substituted methyl tropic acid; 1,3-dioxolanes; log molecular weight; steric interactions; anticholinergics.

INTRODUCTION

Anticholinergic drugs are useful in the treatment of several diseases and organophosphate intoxication. However, there are few quantitative structure activity relationships (QSAR) studies on this class of therapeutic agents (1,2). In the absence of an easily accessible and abundant natural source for the muscarinic receptor, it had not been possible in the past to study directly the importance of different intermolecular forces involved in drug binding to this receptor. The availability of different cell lines containing the cloned muscarinic receptor has however changed that picture drastically (3). Such systems have been used to study the pharmacology and binding properties of the muscarinic receptor subtypes (4).

Van Rossum and Ariëns (5) recognized in 1957 the importance of hydrophobic forces in cholinergic drug receptor interactions. They showed that by taking a five-carbon atom acetylcholine agonist and introducing four straight-chain methylene groups into its structure (thereby increasing its hydrophobicity by a factor of 100), it was possible to obtain a potent antagonist. On the other hand, by introducing two methylene groups, the resulting compound which had only a 10-fold greater hydrophobic character than its parent compound and was partially agonistic and partially antagonistic. It has since been established that agonist–antagonist transitions take place by progressive incorporation of hydrophobic groups as demonstrated for neurotransmitters in general (6). This effect can be explained by the fact that alkyl groups provide additional hydrophobic interaction of the antagonist with the receptor and thus increases its binding. Hansch and Lien (7) further showed that, instead of having a linear relationship, hydrophobicity and biological activity involving a specific receptor had a parabolic dependence for several classes of drugs (8,14).

While increased hydrophobicity (below the optimum value) enhances the affinity of an agent to bind to one class of receptors, it also decreases its affinity to another receptor class with a resultant change in the overall pharmacological effect (8). Therefore, the topography and the role hydrophobic groups play at different acetylcholine receptors in the body may vary considerably.

The main purpose of this report is to correlate quantitatively the biological activities of a series of amino esters of α-substituted phenyl acetic acid (9) and α-methyl tropic acid (10), both of which have been reported by the same group of authors as probes for the muscarinic receptor. Esters of amino alcohols which are bulkier than acetylcholine were found to block the muscarinic receptor and were investigated as possible anticholinergic agents. Although esters of phenyl acetic acid has shown little activity, amino esters of substituted phenyl acetic and substituted tropic acid are promising. Along with these compounds, the activities of a series of 1,3-dioxolanes, QSAR studies on which have already been reported in the literature (2), were reexamined and improvements over the original report were made. Suitable modifications for enhancing activity are suggested, and reasons for deleting some outliers are discussed in both cases.

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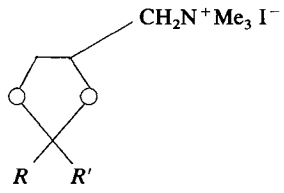
MATERIALS AND METHODS

The compounds and their corresponding biological activities, shown in Tables I and II, are from the work of Chang *et al.* (2) and Lu *et al.* (9,10), respectively. The pA_2 values shown in Table I were obtained by testing the affinity of the compounds for the postganglionic (muscarine-sensitive) acetylcholine receptors of the guinea pig ileum. The stereochemistry of the compounds was not considered because only a few compounds in the data set showed a difference in absolute configuration and the resultant difference in biological activity was not enough for the compounds to be considered stereoselective. Since a racemic mixture contains 50% of the more active isomer (entomer) and 50% of the less active isomer (distomer), the more active isomer will have twice the activity of the racemic mixture. After logarithmic transformation, the difference between them will then become only 0.3 (log 2 = 0.3). In Table II, the pA_2 values for the compounds were calculated from the reported ID_{50} data (9,10) against the isolated guinea pig ileum, except for compounds 12, 15, 16, and 17, where the rat ileum was used instead. Although using another assay system may have introduced an unknown variable into the QSAR analysis, this difference may not be significant, and only four compounds were tested in this fashion. The biological activity of the racemic mixture was considered wherever the compound showed optical isomerism. A compound was ignored if biological data for the racemic mixture were not available or if it showed geometrical isomerism.

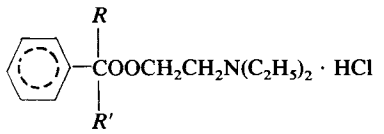
Table I. pA_2 Values and Physicochemical Data for Antagonism of Muscarinic Receptors by 1,3-Dioxolanes[a]



No.	R	R'	pA_2[b]	π (R,R')	log MW[c]
1	C_6H_5	H	4.61	1.89	2.54
2	H	C_6H_5	4.51	1.89	2.54
3	C_6H_5	4-HOC_6H_4	5.52	3.17	2.65
4	C_6H_5	C_6H_5(RS)	7.66	3.78	2.63
5	C_6H_5	C_6H_5	7.55	3.78	2.63
6	C_6H_5	C_6H_5(D)	7.62	3.78	2.63
7	C_6H_5	2-MeC_6H_4	7.74	4.44	2.64
8	C_6H_5	3-MeC_6H_4	7.28	4.29	2.64
9	C_6H_5	4-MeC_6H_4	7.39	4.30	2.64
10	C_6H_{11}	C_6H_5(RS)	7.88	4.40	2.64
11	C_6H_5	C_6H_{11}(RS)	8.12	4.40	2.64
12	C_6H_{11}	C_6H_5(2S,4S)	8.03	4.40	2.63
13	C_6H_{11}	C_6H_5(2R,4R)	7.73	4.40	2.63
14	C_6H_5	C_6H_{11}(2R,4S)	7.83	4.40	2.63
15	C_6H_5	C_6H_{11}(2S,4R)	8.26	4.40	2.63
16	C_6H_{11}	C_6H_{11}	6.44	5.02	2.63
17	$CH_3(CH_2)_3$	$CH_3(CH)_3$	6.59	3.52	2.58
18	$CH_3(CH_2)_2$	$CH_3(CH)_2$	6.00	2.86	2.55

[a] Adapted from Ref. 2.
[b] As measured against the muscarinic receptors of the guinea pig ileal longitudinal muscle.
[c] Calculated from the molecular formula.

Table II. Antispasmodic Activity and Physicochemical Data for Amino Esters of α-Substituted Phenyl Acetic Acid, α-Methyl Tropic Acid, and Related Compounds[a]



No.	R	R'	pA_2	π^b (R,R')
1	C_6H_5	H	6.13	1.96
2	C_6H_5	Me	7.28	2.49
3	C_6H_5	Et	6.48	2.98
4	C_6H_5	nPr	5.92	3.46
5	C_6H_5	iPr	5.88	3.26
6	C_6H_5	nBu	5.59	3.94
7	C_6H_5	iBu	5.92	3.74
8	H	CH_2OH	7.15	0.31
9	H	$(CH_2)_2OH$	5.96	1.81
10	CH_3	CH_2OAc	6.41	0.47
11	CH_3	CH_2OH	6.32[c]	0.81
12	CH_3	CH_2OTs	7.41	1.84
13	CH_3	CH_2F	6.39	0.51
14	CH_3	CH_2Cl	5.93	0.17
15	CH_3	CH_2Br	6.90[c]	1.19
16	CH_3	CH_2I	7.41[c]	1.95
17		$=CH_2$	5.86[c]	0.30

[a] Adapted from Refs. 10 and 11.
[b] The Hansch–Fujita π constant calculated by the additive principle of Lien *et al.* (12,13).
[c] The pA_2 values shown were measured against the isolated guinea pig ileum except for compounds 12, 15, 16, and 17, where the isolated rat ileum was used.

The Hansch–Fujita π constant was used as an index of the lipophilic character of the substituent. While the π values in Table I were reported by the authors for the 1,3-dioxolanes, those in Table II were calculated by the additive principle from the reports of Lien *et al.* (12,13). The molecular weight was calculated from the chemical formulas of these compounds.

The equations shown in Tables III and IV were arrived at by using the BMDP 1R (multiple linear regressional) program based on the least-squares method. A SAECO 286 computer was used in the data processing and computation.

RESULTS AND DISCUSSION

The equations obtained by correlating the biological activity with the different physicochemical parameters for the 1,3-dioxolane derivatives (Table I) are shown in Table III, and the *F* ratio is indicated in each case. It should be noted that the biological data in Table I span a range of 3.75 log units, indicating that there is an almost 6000-fold difference in activity between the most and the least potent compounds.

Equation (1) was derived using only π as the independent variable. Addition of a log molecular weight did not improve the correlations Eq. (2). However, addition of the π^2 term while increasing the correlation coefficient de-

TABLE III. Equations Correlating Biological Activity with Physicochemical Data for the 1,3-Dioxolanes

Equation	n	r	s	F ratio
(1) $pA_2 = 1.13\,\pi + 2.71$	18	0.85	0.64	40.94
(2) $pA_2 = 0.94\,\pi + 5.39 \log MW - 10.68$	18	0.86	0.65	20.03
(3) $pA_2 = 4.13\,\pi - 0.45\,\pi^2 - 1.88$	18	0.90	0.55	31.58
(4) $pA_2 = -0.11\,\pi^2 + 10.59 \log MW - 22.3$	18	0.83	0.70	16.10
(5) $pA_2 = 4.43\,\pi - 0.48\,\pi^2 - 2.67 \log MW + 4.43$	18	0.90	0.56	19.87
(6) $pA_2 = -16.60\,\pi + 5.92\,\pi^2 - 0.62\,\pi^3 + 18.98$	18	0.96	0.36	55.58
(7) $pA_2 = -12.21\,\pi + 4.65\,\pi^2 - 0.50\,\pi^3 + 14.45$	17	0.97	0.31	67.55
(8) $pA_2 = 2.76\,\pi - 0.23\,\pi^2 + 0.13$	16	0.97	0.30	104.20

creased the standard deviation [Eq. (3)], and it was found that the addition of a log MW term [Eq. (5)] to this equation was not statistically significant. Using a π^3 term [Eq. (6)] the results improved significantly, and after deleting an outlier (compound 3), an even better fit was obtained as shown in [Eq. (7)]. However, after deleting compound 16, the π^3 term was found to be insignificant for the remaining compounds and, hence, using only the π and π^2 terms [Eq. (8)] was obtained.

The ideal π_0 value appears to lie between 4.40 and 4.44 (Fig. 1). Compound 16, with the highest π value among the compounds in Table I, has a relatively low biological activity and behaves as an outlier (the deviation is greater than three times the standard deviation), possibly because of the bulk of two cyclohexyl groups exceeding the bulk tolerance of the binding site. Compound 3, on the other hand, appears to take part in hydrogen bonding on account of the presence of an –OH group in the R' substituent, which results in a lower lipophilicity and decreased affinity for the nonpolar area of the receptor. The high lipophilicity of compound 16 probably renders the π^3 term insignificant when it is deleted because of the narrower range in π. The observed and calculated [based on Equation (8)] pA_2 values are shown in Table V, along with the correlation matrix of the parameters used.

The correlations obtained here represent a significant improvement over that reported by the original authors (2). Using nine compounds (1,3,4,7–9,15,17,18), the correlation coefficient they obtained was 0.942. Subsequently with only five of those compounds (1,4,15,17,18), they improved their correlation coefficient to 0.971. These results, however, cannot be considered reliable since over 75% of the original compounds were deleted, and the use of only five data points in any QSAR study enhances the probability of a chance correlation.

It can be hypothesized that for maximum cholinergic potency the R and R' groups in the 1,3-dioxolanes can be either phenyl or cyclohexyl. However, when both R and R' are cyclohexyl groups, the potency decreases because the π value far exceeds the optimum. When R or R' is substituted by less lipophilic groups (2), like H, hydroxyl cholinergic agonists result.

It has been shown by Lien *et al.* (1) that the $CH_3N^+(C_2H_5)_2$ has been associated with maximum anticholinergic potency. Replacement of the $-N^+(CH_3)_3$ head by this group may therefore produce more potent compounds.

The different equations that have been generated for the substituted phenylacetic acid and tropic acid esters in Table II are shown in Table IV along with the relevant F ratios. Table VI shows the observed and calculated values for the biological activity based on Eq. (5) in Table IV and also the correlation matrix.

Equation (1) was derived by correlating all the data points with π values. Equation (2) resulted from the addition of a second term, π^2, to Eq. (1). Subsequent addition of a π^3 term [Eq. (3)] proved to be insignificant, as did the inclusion of a log MW term [Eq. (4)]. It was observed that compound 8 had a very high biological activity in spite of its low π value, probably because it is able to take part in some sort of hydrogen bonding, which decreases as the alkyl chain length is increased as shown with compound 9. Compound 1, on the other hand, had a low activity in spite of the hydrophobic nature of its substituent groups. Compounds 1 and 8 were deleted as possible outliers and Eq. (5) resulted when the remaining 15 data points were correlated with π and π^2. The correlation coefficient increased significantly and the standard deviation also decreased after the deletion of the outliers. Equation (5) is considered to be the best equation with a parabolic dependence on π. It can be concluded, therefore, that hydrophobic interactions play a significant role in the binding of these compounds to the cholinergic receptor.

Although it has been shown (5) that the minimum structure necessary for pure antagonistic activity is $C_6H_5CHR'COOCH_2CH_2N(Me)_2$, the hydrogen in the R' position of compound 1 cannot interact well with the hydrophobic areas of the receptor and thereby affects the affinity of the compound for the receptor; hence the compound was

Table IV. Equations Correlating Biological Activity with the Physicochemical Parameters for the α-Methyl Tropic Acid and α-Substituted Phenyl Acetic Acid Derivatives

Equation	n	r	s	F ratio
(1) $pA_2 = 0.85\,\pi + 6.55$	17	0.19	0.61	0.562
(2) $pA_2 = 1.220\,\pi - 0.336\,\pi^2 + 5.88$	17	0.73	0.44	8.014
(3) $pA_2 = 0.66\,\pi - 0.52\,\pi^2 - 0.05\,\pi^3 + 6.05$	17	0.72	0.45	7.459
(4) $pA_2 = 1.120\,\pi - 0.003 \log MW - 0.29\,\pi^2 + 6.72$	17	0.73	0.46	2.590
(5) $pA_2 = 1.718\,\pi - 0.448\,\pi^2 + 5.54$	15	0.92	0.25	34.131

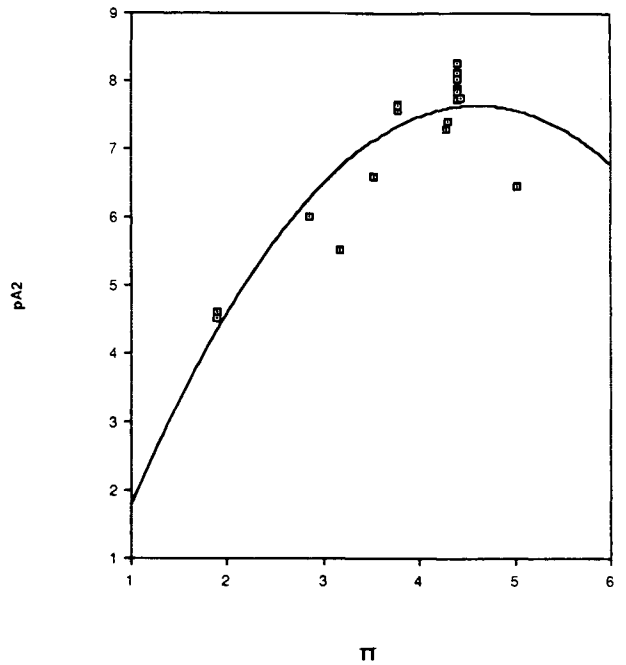


Fig. 1. Parabolic dependence between the observed biological activity and π for the compounds shown in Table I. The optimum π value is about 4.40.

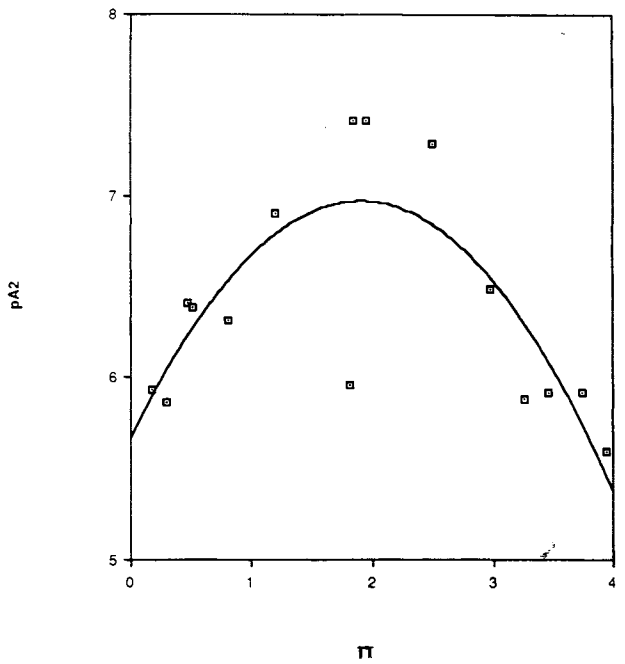


Fig. 2. Parabolic relationship between the observed biological activity and π for the compounds shown in Table II. The optimum π value is about 2.

deleted. More lipophilic groups in the R' position enhance binding as shown when hydrogen was successively replaced by methyl, ethyl, propyl, and butyl. Activity decreases with the propyl or butyl groups, probably because of steric interactions and the nonuniform nature of the hydrophobic sites in the muscarinic receptor. The methyl substituent (in compound 2) with a π (R, R') value of about 2.49 marks the optimum hydrophobic limit. Any increase above that limit decreases activity.

Table V. Predicted and Observed Values for Table I Using the Best-Fit Equation

No.	Predicted	Observed	Residual
1	4.326	4.610	0.284
2	4.326	4.510	0.184
3	6.710	5.520	−1.190
4	7.328	7.660	0.332
5	7.328	7.550	0.222
6	7.328	7.620	0.292
7	7.621	7.740	0.119
8	7.589	7.280	−0.309
9	7.591	7.390	−0.201
10	7.614	7.880	0.266
11	7.614	8.120	0.506
12	7.614	8.030	0.416
13	7.614	7.730	0.116
14	7.614	7.830	0.216
15	7.614	8.260	0.646
16	7.555	6.440	−1.115
17	7.105	6.590	−0.515
18	6.268	6.000	−0.268

Correlation matrix

	pA_2	π	log MW	π^2
pA_2	1			
π	0.8479	1		
log MW	0.7597	0.8362	1	
π^2	0.8015	0.9914	0.7978	1

Table VI. Predicted and Observed Values for Table II Using the Best-Fit Equation

No.	Predicted	Observed	Residual
1	6.980	6.130	−0.850
2	6.835	7.280	+0.445
3	6.532	6.480	−0.052
4	6.080	5.920	−0.160
5	6.287	5.880	−0.407
6	5.473	5.590	+0.117
7	5.744	5.920	+0.176
8	6.225	7.150	+0.925
9	6.225	5.960	−0.265
10	6.379	6.410	−0.031
11	6.647	6.320	−0.327
12	6.987	7.410	+0.423
13	6.414	6.390	−0.024
14	6.077	5.930	−0.147
15	6.856	6.900	+0.044
16	6.981	7.410	+0.429
17	6.215	5.860	−0.355

Correlation matrix

	pA_2	π	π^2	π^3
pA_2	1.0000			
π	−0.1900	1.0000		
π^2	−0.3500	0.9722	1.0000	
π^3	−0.4396	0.9246	0.9872	1.0000

Incorporation of a $-CH_2OH$ in the R' position (compound 8) markedly enhances activity to an extent where the compound becomes an outlier. However, when the distance between the α-carbon atom of the acyl group and the hydroxyl group is increased (compound 9), activity falls sharply. Other workers have reported that optical isomers of tropic acid have different potencies. Hence there is a small isolated hydrogen bonding site in the structure of the receptor which is, nonetheless, important for drug binding activities as described earlier (1).

The replacement of the hydrogen atom by the methyl group in the R' position (compounds 10–16) was thought to cause complete loss of any hydrogen bonding that may have been involved. As a result the less bulky phenyl group can be redirected to a site that prefers the aromatic ring, leaving the bulky R' groups to interact with the less restrictive hydrophobic regions of the molecule. This orientation could account for the activities of compounds 12, 15, and 16.

A striking feature of this group of compounds is the absence of the quarternized nitrogen head that is common to most anticholinergic drugs. In spite of the nitrogen atom not being completely charged, these compounds can exert their antagonistic action since at the physiological pH, the amino group is largely protonated and carries a positive charge that interacts with the anionic site of the receptor.

In conclusion, compounds belonging to the class of amino esters of substituted phenyl acetic acid or tropic acid can serve as leads to derive more potent antagonists. Already several variations of the basic structure have evolved into clinically useful drugs and it is hoped that future QSAR studies will reveal a greater understanding of the electronic forces at work that have been largely ignored in studies reported thus far.

ACKNOWLEDGMENTS

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