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Quantitative Structure–Activity Relationships of Nicotine Analogues as Neuronal Nicotinic Acetylcholine Receptor Ligands

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Abstract—Quantitative structure–activity relationships of 34 pyrrolidine-modified nicotine agonists are investigated for their binding affinity toward neuronal nicotinic acetylcholine receptor. The results indicate that a large substituent at the R_1, R_2, and R_3 position is detrimental to the binding affinity. Likewise, a large substituent at the $R_{2\gamma}$ or $R_{3\gamma}$ position as well as a hydrogen bond accepting substituent at the $R_{2\beta}$ position are not beneficial to the binding. Copyright © 1996 Elsevier Science Ltd

Introduction

Alzheimer's disease (AD) is the most common cause of the dementia syndrome that afflicts mainly the elderly.[1] Although many hypotheses have been advanced regarding the etiology and pathophysiology of AD, there is no definite etiology or cure for AD. Neurochemical studies of brain tissue from autopsies have shown that AD is accompanied by multiple changes in numerous brain transmitter systems.[2–5] Although there are a number of neurotransmitter systems affected by AD, the observed decline in the cholinergic system, especially a severe depletion of cholinergic neurons, is one hallmark feature of the disease.[6] Specifically, substantial reduction (30–50%) in nicotinic cholinergic receptors has been consistently reported in the brains of patients with AD, whereas changes in muscarinic acetylcholine receptors are less remarkable and more dependent on receptor subtype.[7,8] Accumulating evidence suggests that compounds that activate neuronal nicotine acetylcholine receptors (nAChR) may have potential benefit in improving the impairment in memory and cognition function.[9] It was hypothesized that a diversity of neuronal nAChR subtypes exist and are distributed widely in the brain region. Each subtype may be involved in mediating specific behavioral function and may be selectively targeted for a defined pharmacology.[10] Nicotine, the classical nAChR agonist, has been shown to produce a significant and marked improvement in attention, information processing and short-term memory in patients with dementia of the Alzheimer type.[11] However, nicotine, which binds to the acetylcholine binding site on the α-subunit, produces cardiovascular and other CNS side effects. Recent advances in the pharmacology of neuronal nAChR suggested that cholinergic channel activators (ChCA), which include nAChR agonists as well as allosteric modulators, could enhance the central neuronal nAChR mediated transmission without having side effect liabilities by selective interaction with the central nAChR subtype. Therefore, it might be possible to modify nicotine to furnish a nicotinic receptor agonist which has beneficial effects on learning and memory but, unlike nicotine, does not have cardiovascular liability.

In a previous report,[12] the syntheses and biological potencies of 25 3′-, 4′-, and 5′-substituted nicotine analogues as ligands of the neuronal nicotinic acetylcholine receptor were presented. Since then, a number of additional compounds have been synthesized, and their biological potencies determined. In this report, the quantitative structure–activity relationships (QSAR) from these nicotine analogues are described.

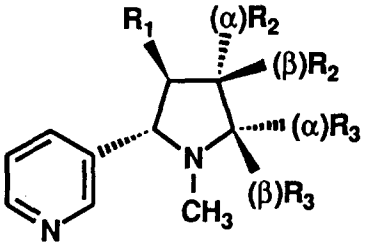
Results and Discussion

Compounds included in this study are listed in Table 1. The list includes 36 nicotine analogues (**I**) including seven R_1 derivatives, 17 R_2 derivatives, six R_3 derivatives, and five miscellaneous derivatives. The 17 R_2 compounds include three α-mono-substituents and 14 β-mono-substituents, and the six R_3 compounds consist of three α-substituents and three β-substituents. The biological data studied is the binding affinity of these compounds expressed in nanomole to the neuronal nicotinic acetylcholine receptor. These compounds have binding affinity ranging from 1.15 nM to 1000 μM. The standard errors of the binding affinity values are often within 10% of the reported values. In this study, pK_i values were used. Thus, a larger pK_i value indicates that the compound binds more tightly to the receptor. The syntheses of most of these compounds and the binding experiments have been described previously.[12] The QSAR analyses were first performed with subsets, and then the final equation was derived from the entire set of compounds.

R_1 derivatives

Equation 1 was obtained from the eight R_1 derivatives including the parent compound. They are the H (**1**), Me (**2**), CH_2F (**3**), CH_2OH (**4**), CH_2OMe (**5**), CO_2Me (**6**), CH_2CN (**7**), and $NHCOOCH_2Ph$ (**8**) derivatives. MR_1 in eq 1 is the molar refractivity for the R_1 substituents and roughly measures the size of the substituent. Equation 1 shows there is an inverse parabolic relationship between the binding affinity and the size of the R_1 substituent.. The negative coefficient of MR_1 in the equation indicates that the binding

Table 1. Observed and calculated pK_i values and physicochemical parameters of nicotine analogues using eq (4)



No	Substituent			K_i (nM)	pK_i (nM)			Physicochemical parameters					
	R_1	R_2	R_3		Obs	Calc	Dev	MR_1	$MR_{2\beta}$	$MR_{3\beta}$	$HA_{2\beta}$	$I_{2\alpha}$	$I_{3\alpha}$
1	H	H	H	1.15 (±0.04)	8.94	8.54	0.40	0.103	0.103	0.103	0	0	0
2	(±)-Me	H	H	24.9 (±1.7)	7.60	7.21	0.39	0.565	0.103	0.103	0	0	0
3	(±)-CH_2F	H	H	98.5 (±9.7)	7.01	7.27	−0.26	0.543	0.103	0.103	0	0	0
4	(±)-CH_2OH	H	H	619.2 (±12.4)	6.21	6.77	−0.56	0.719	0.103	0.103	0	0	0
5	(±)-CH_2OMe	H	H	2032 (±32)	5.69	5.36	0.32	1.207	0.103	0.103	0	0	0
6	(±)-CO_2Me	H	H	4470 (±153)	5.35	5.13	0.22	1.287	0.103	0.103	0	0	0
7	(±)-CH_2CN	H	H	1490 (±181)	5.83	5.93	−0.10	1.011	0.103	0.103	0	0	0
8	(±)-$NHCOOCH_2Ph$	H	H	6470 (±156)	5.19	5.09	0.10	4.089[d]	0.103	0.103	0	0	0
9	H	β-Me	H	4.23 (±0.28)	8.37	8.06	0.31	0.103	0.565	0.103	0	0	0
10[a]	H	β-CH_2F	H	11.1 (±50.2)	7.95	8.08	−0.13	0.103	0.543	0.103	0	0	0
11	H	β-OH	H	27.6 (±0.8)	7.56	7.72	−0.16	0.103	0.255	0.103	1	0	0
12[a]	H	β-OMe	H	36.6 (±0.8)	7.44	7.22	0.22	0.103	0.733	0.103	1	0	0
13	H	β-Et	H	50.2 (±1.1)	7.30	7.57	−0.27	0.103	1.030	0.103	0	0	0
14	H	β-CH_2CN	H	52.0 (±2.9)	7.28	6.93	0.35	0.103	1.011	0.103	1	0	0
15	H	β-OCOMe	H	102.9 (±16.7)	6.99	6.74	0.25	0.103	1.185	0.103	1	0	0
16	H	β-CH_2OH	H	157.8 (±7.4)	6.80	7.23	−0.43	0.103	0.719	0.103	1	0	0
17	H	β-CH_2Ph	H	119.4 (±18.5)	6.92	6.71	0.21	0.103	3.001[d]	0.103	0	0	0
18	H	β-OSO_2Me	H	363.6 (±17.9)	6.44	6.21	0.23	0.103	1.699	0.103	1	0	0
19	H	β-CH_2SMe	H	492.8 (±19.2)	6.31	6.72	−0.41	0.103	1.842	0.103	0	0	0
20	H	β-CH_2OMe	H	510.0 (±46.6)	6.29	6.72	−0.43	0.103	1.207	0.103	1	0	0
21[a,b]	H	β-F	H	382.0 (±43)	6.42	8.55	−2.13[c]	0.103	0.092	0.103	0	0	0
22	H	β-CH_2F	H	11.1 (±1.9)	7.95	8.08	−0.13	0.103	0.543	0.103	0	0	0
23	H	α-F	H	77.1 (±30.6)	7.11	6.83	−0.28	0.103	0.103	0.103	0	1	0
24	H	α-CH_2OH	H	294.0 (±11)	6.53	6.83	−0.30	0.103	0.103	0.103	0	1	0
25	H	α-CN	H	82.0 (±2.0)	7.09	6.83	0.26	0.103	0.103	0.103	0	1	0
26	H	H	β-Me	34.9 (±1.9)	7.46	8.06	−0.60	0.103	0.103	0.103	0	0	0
27	H	H	β-Bu	125.2 (±4.7)	6.90	6.61	0.29	0.103	0.103	1.961	0	0	0
28	H	H	β-Ph	1242.3 (±12.4)	5.91	6.01	−0.10	0.103	0.103	2.536	0	0	0
29	H	H	α-Me	1205.3 (±34.6)	5.92	5.75	0.17	0.103	0.103	0.103	0	0	1
30	H	H	α-Bu	1381.4 (±209)	5.86	5.75	0.11	0.103	0.103	0.103	0	0	1
31	H	H	α-Ph	3353.5 (±196.9)	5.47	5.75	−0.28	0.103	0.103	0.103	0	0	1
32	H	Me_2	H	268.0 (±8)	6.57	6.35	0.22	0.103	0.565	0.103	0	1	0
33	(±)-Me	(±)-β-Me	H	96.6 (±6.5)	7.02	6.73	0.29	0.565	0.565	0.103	0	0	0
34	H	α-Me, β-OH	H	1060 (±18)	5.97	6.01	−0.04	0.103	0.255	0.103	1	1	0
35	(±)-CO_2Me	(±)-α-CO_2Me	H	1000000[f]	3.00	3.42	−0.42	1.287	0.103	0.103	0	1	0
36[b,c]	H	α/β-F	H	91.1 (±30.8)	7.04	7.69	−0.65	0.103	0.092	0.103	0	0	0

[a]HCl salt.
[b]Not used in the equations.
[c]Compound **36** is a mixture of α/β-F derivatives and has binding affinity of 7.04. Based on other compounds such as **16** (observed pK_i = 6.80) and **24** (observed pK_i = 6.53), it was expected that compound **21** (β-F) would have a higher binding affinity than compound **23** (α-F) as predicted by eq 4. The calculated pK_i of compound **21** is 8.55, whereas the observed pK$_i$ value is 6.42. See the text for further discussion.
[d]MR_1 value is set to 1.30 in eqs 1b, 4, 5–9, and $MR_{2\beta}$ value is set to 1.85 in eq 2b, 4, 5–9 as discussed in the text.
[e]The calculated value of this compound was assigned as the average value of the calculated values of α-F (6.83) and β-F (8.55) derivatives.
[f]Single determination.

affinity of the compound decreases as the size of the R_1 substituent becomes larger. Although the addition of MR_1^2 term is statistically supported (RMSE = 1.050, $r^2 = 0.433$ for the corresponding single variable equation), the meaning of MR_1^2 cannot be well defined with the present set of compounds since only one compound has an MR_1 value larger than the minimum MR_1 value (3.68) in this inverse parabolic correlation. $NHCOOCH_2Ph$ (**8**; $MR_1 = 4.09$) is the largest substituent and shows the weakest binding affinity. Depending on the binding affinity of other compounds, the ascending curve of the inverse parabolar may be flattened considerably or even stay in plateau. In the inverse parabola, the positive coefficient of the term MR_1^2 indicates that an even larger substituent provides additional binding. However, if the ascending curve stays in plateau, it indicates that some part of a large substituent is exposed to the solvent. In order to describe the latter situation, a bilinear model is usually employed.[13] In the present case, a bilinear model cannot be applied because it requires one more variable than the corresponding parabolic model. The number of compounds per variable in a bilinear model will be even smaller than the parabolic model, and this increases statistical uncertainty of the model with only eight compounds available. Additional compounds with larger MR_1 value are required to clarify the relationship. Without additional data, however, different approaches can be employed. One approach is to omit the compound until further compounds are available. Omitting compound **8** ($NHCOOCH_2Ph$) resulted in eq 1a. In this case, however, eq 1a cannot be used to calculate the biological potency of compound **8**, and this compound has to be treated as an outlier. Another approach is to truncate the substituent if the substituent is larger than a specific size.[14,15] In this approach, it is assumed that the portion of the substituent which exceeds the specific size does not contribute toward the binding affinity. When the maximum MR_1 value is set to 1.30 (which is a little larger than the largest substituent except compound **8** in this set), eq 1b results. The coefficient of MR_1, the intercept, and overall quality of eq 1b are very similar to those of eq 1a. The maximum MR_1 value provides an insight into the size of the R1 substituent influencing the binding affinity . Overall, it is clear from eq 1, 1a or 1b that a large substituent at the R_1 position is detrimental to the binding affinity. The RMSE values of all equations are reasonable, and the correlation coefficients are excellent.

$$pK_i = -4.02(\pm 0.47)MR_1 + 0.74(\pm 0.10)MR_1^2 + 9.22(\pm 0.32) \quad (1)$$

$n = 8$, RMSE = 0.338, $r^2 = 0.951$, $F = 48.4$, $p = 0.0005$, $RMSE_{cv} = 3.290$

$$pK_i = -2.91(\pm 0.40)MR_1 + 8.92(\pm 0.34) \quad (1a)$$

$n = 7$, RMSE = 0.407, $r^2 = 0.915$, $F = 53.7$, $p = 0.0007$, $RMSE_{cv} = 0.492$

$$pK_i = -2.89(\pm 0.33)MR_1 + 8.91(\pm 0.31) \quad (1b)$$

$n = 8$, RMSE = 0.372, $r^2 = 0.929$, $F = 78.0$, $p = 0.0001$, $RMSE_{cv} = 0.443$

R_2 derivatives

There are 17 compounds with a substituent at the R_2 position; they are β-Me (**9**), β-CH_2F (**10**), β-OH (**11**), β-OMe (**12**), β-Et (**13**), β-CH_2CN (**14**), β-OCOMe (**15**), β-CH_2OH (**16**), β-CH_2Ph (**17**), β-OSO_2Me (**18**), β-CH_2SMe (**19**), β-CH_2OMe (**20**), β-F (**21**), β-CH_2F (**22**), α-F (**23**), α-CH_2OH (**24**), and α-CN (**25**). Eq 2 was derived from these compounds, plus the parent compound. Compound **21** was not included (see the discussion below). $MR_{2\beta}$ is the molar refractivity value of the $R_{2\beta}$ substituent, and again roughly measures the size of the substituent. $HA_{2\beta}$ is an indicator variable for the hydrogen bond accepting substituent at the $R_{2\beta}$ position. $I_{2\alpha}$ is indicator variable for the α isomer at the $R_{2\alpha}$ position.

As in eq 1 or 1a, the negative coefficient of $MR_{2\beta}$ in the equation indicates that a large substituent at the $R_{2\beta}$ position is detrimental to the binding affinity of the compound. Although the substituents at the $R_{2\alpha}$ position are not included in $MR_{2\beta}$, it cannot be ruled out that there is no size limit around the $R_{2\alpha}$ position because all the compounds included in the current data set is small. An indication about such a size limit around the $R_{2\alpha}$ position is provided by the negative coefficient of $I_{2\alpha}$; the negative coefficient of $I_{2\alpha}$ indicates that any substituent at the $R_{2\alpha}$ position is detrimental to the binding affinity. Parameterization of $R_{2\alpha}$ substituents with MR instead of $I_{2\alpha}$ did not provide a better correlation.

Inclusion of the $MR_{2\beta}^2$ variable in eq 2 is similar to the situation for MR_1^2 in eq 1. The addition of the MR_2^2 term is statistically supported. However, there is only one data point for the $MR_{2\beta}$ parameter which is far greater than others among the compounds included; β-CH_2Ph ($MR_{2\beta} = 3.00$) is the largest substituent. Omitting this compound (β-CH_2Ph) resulted in eq 2a. When the maximum $MR_{2\beta}$ value is set to 1.85 (which is a little larger than the largest substituent except compound **17** in this set), eq 2b resulted. The coefficients of $MR_{2\beta}$, $I_{2\alpha}$, and $HA_{2\beta}$, the intercept, and overall quality of eq 2b are very similar to eq 2a. Equation 2b suggests that the maximum negative size influence of the $R_{2\beta}$ substituent may be about 1.85 in MR scale. Equations 2, 2a or 2b clearly show that a large substituent at the R_2 position is detrimental to the binding affinity of the compound. The RMSE values of all three equations are comparable to those of eq 1, 1a and 1b, and their correlation coefficients are reasonable.

$$pK_i = -2.03(\pm 0.45)MR_{2\beta} + 0.44(\pm 0.15)MR_{2\beta}^2 - 1.87(\pm 0.30)I_{2\alpha} - 0.54(\pm 0.20)HA_{2\beta} + 8.99(\pm 0.26) \quad (2)$$

$n = 17$, RMSE = 0.346, $r^2 = 0.834$, $F = 15.0$, $p = 0.0001$, $RMSE_{cv} = 0.511$

$$pK_i = -1.20(\pm 0.19)MR_{2\beta} - 1.70(\pm 0.27)I_{2\alpha} - 0.59(\pm 0.19)HA_{2\beta} + 8.73(\pm 0.20) \quad (2a)$$

$n = 16$, RMSE = 0.336, $r^2 = 0.841$, $F = 21.2$, $p = 0.0001$, $RMSE_{cv} = 0.392$

$$pK_i = -1.11(\pm 0.17)MR_{2\beta} - 1.68(\pm 0.27)I_{2\alpha} - 0.65(\pm 0.18)HA_{2\beta} + 8.70(\pm 0.20) \quad (2b)$$

$n = 17$, RMSE = 0.336, $r^2 = 0.830$, $F = 21.2$, $p = 0.0001$, $RMSE_{cv} = 0.389$

The negative coefficient of $HA_{2\beta}$ term in eq 2, 2a or 2b indicates that a hydrogen bond accepting substituent at the $R_{2\beta}$ position decreases the binding affinity of the compound.

R_3 derivatives

From the six R_3 derivatives (α- or β-Me, Bu, and Ph) and the parent compound, eq 3 was derived. $MR_{3\beta}$ is the molar refractivity value of the $R_{3\beta}$ substituent roughly measuring the size of the substituent. $I_{3\alpha}$ is an indicator variable for the α derivative at the $R_{3\alpha}$ position. The negative coefficient of $MR_{3\beta}$ in eq 3 indicates that the binding affinity of the compound decreases as the size of the $R_{3\beta}$ substituent becomes larger. As in eqs 2 or 2a, the substituent at the $R_{3\alpha}$ position is not included, even though exactly the same kind of substituents as $R_{3\beta}$ are present at this position. The negative coefficient of $I_{3\alpha}$ indicates that any substituent at the $R_{3\alpha}$ position is detrimental to the binding affinity. It is not surprising that both $I_{2\alpha}$ and $I_{3\alpha}$ have negative coefficients since they are next to each other. Since the coefficient of $I_{3\alpha}$ is more negative than the coefficient for $I_{2\alpha}$, this suggests that the space around the $R_{3\alpha}$ position may be more limited than that of the $R_{2\alpha}$ position. As in $I_{2\alpha}$, parameterization of $R_{3\alpha}$ substituents with *MR* instead of $I_{3\alpha}$ did not provide a better correlation.

$$pK_i = -1.03(\pm 0.22)MR_{3\beta} - 2.78(\pm 0.42)I_{3\alpha} + 8.63(\pm 0.35) \quad (3)$$

$n = 7$, RMSE = 0.428, $r^2 = 0.919$, $F = 22.7$, $p = 0.007$, $RMSE_{cv} = 0.609$

The number of compounds per variable for eq 3 is not as high as one would like to have. However, the RMSE values and the correlation coefficients are comparable to the correlations from other sets of compounds.

Combining all derivatives

The results of eqs 1b, 2b and 3 show that the intercepts are essentially identical considering their standard errors. This indicates that there is no significant difference in the intrinsic affinity associated with each subset. Thus, it is reasonable to combine all compounds. Equation 4 is the result; it was developed from 34 compounds including four multi-substituted compounds (compounds **32–35** in Table 1). In a preliminary investigation, the binding affinity of these four compounds was predicted well by the correlation derived from the above 30 compounds (equation is not shown). Therefore, no special treatment was necessary for these miscellaneous compounds in order to include them in the equation. The reasonable fit of these miscellaneous compounds can be seen in eq 4 as well as in Table 1; the calculated and observed pK_i values are well fit within the standard error of estimation of the correlation.

$$pK_i = -2.88(\pm 0.18)MR_1 - 1.71(\pm 0.17)I_{2\alpha} - 2.79(\pm 0.24)I_{3\alpha} - 1.05(\pm 0.14)MR_{2\beta} - 1.04(\pm 0.13)MR_{3\beta} - 0.66(\pm 0.16)HA_{2\beta} + 9.05(\pm 0.17) \quad (4)$$

$n = 34$, RMSE = 0.338, $r^2 = 0.921$, $F = 52.5$, $p = 0.0001$, $RMSE_{cv} = 0.378$

Equation 4 summarizes the QSAR from all the compounds. A large substituent at the R_1, R_2, and/or R_3 position is detrimental to the binding affinity. Any substituents at the $R_{2\alpha}$ and/or $R_{3\alpha}$ position decrease the binding affinity of these compounds, indicating that the size of the receptor pocket around these positions is limited. A hydrogen bond accepting group at the $R_{2\beta}$ position is also deleterious to the binding. There are some indications that larger substituents at the R_1 and R_2 position may be exposed to the solvent.

Although compound **35** fits the correlation very well, it was noted that this compound has significantly lower binding affinity than all other compounds in the set. In order to examine whether this one extreme point influences the correlation, this compound was deleted. Equation 4a was obtained without compound **35**. Comparison of eqs 4 and 4a reveals that there is no significant difference in either the number of variables in the correlation or their coefficients. The statistics of the two equations are also very similar; while one has a higher r^2 value, the other has a smaller RMSE value.

$$pK_i = -2.69(\pm 0.21)MR_1 - 1.54(\pm 0.19)I_{2\alpha} - 2.70(\pm 0.24)I_{3\alpha} - 0.64(\pm 0.15)MR_{2\beta} - 0.99(\pm 0.13)MR_{3\beta} - 0.64(\pm 0.15)HA_{2\beta} + 8.93(\pm 0.18) \quad (4a)$$

$n = 33$, RMSE = 0.328, $r^2 = 0.890$, $F = 35.1$, $p = 0.0001$, $RMSE_{cv} = 0.361$

Compound **36** is a mixture of α/β-F derivatives and thus not included in the analysis. On the basis of other compounds, such as **16** (β-CH_2OH; observed $pK_i = 6.80$) and **24** (α-CH_2OH; observed $pK_i = 6.53$) and three sets of R_3 substituents (**26–31**), it was expected that compound **21** (β-F) would have a higher binding affinity than compound **23** (α-F). It is difficult to

understand the observed binding affinity of compound **21**, and this compound was also not included in the analysis. The calculated pK_i value of compound **21** by eq 4 is 8.55, whereas the observed pK_i value is 6.42. Attempts to improve the correlation including compound **21** with various electronic parameters were not successful. The negative coefficient of $HA_{2\beta}$ term in eq 4a indicates that a hydrogen bond accepting substituent at the $R_{2\beta}$ position decreases the binding affinity of the compound. Based on the classification of Hansch and Leo,[16] F was treated as a non-hydrogen bonding atom. However, since F is a strong electronegative atom and can participate in a hydrogen bond,[17,18] the presence of this atom at β-position in this molecule might be why the binding affinity of this compound decreased much more than expected from eq 4. On the other hand, it is interesting to note that the observed potency of α/β-F derivatives (**36**) is not very different from the average value (7.69) of the calculated values of α-F (6.83) and β-F (8.55) using eq 4.

As in eqs 1a and 2a, omission of compounds **8** and **17** and the relevant variables MR_1^2 and $MR_{2\beta}^2$ do not influence the results (eq 4b).

$$pK_i = -2.91(\pm 0.20)MR_1 - 1.72(\pm 0.18)I_{2\alpha} - 2.80(\pm 0.25)I_{3\alpha} - 1.11(\pm 0.17)MR_{2\beta} - 1.04(\pm 0.13)MR_{3\beta} - 0.63(\pm 0.17)HA_{2\beta} + 9.07(\pm 0.18) \quad (4b)$$

$n = 32$, RMSE $= 0.347$, $r^2 = 0.918$, $F = 46.8$, $p = 0.0001$, $RMSE_{cv} = 0.393$

The calculated pK_i values of all the compounds using eq 4 along with the physicochemical parameter values used in this study are shown in Table 1. Figure 1 is the plot between the observed and calculated pK_i values

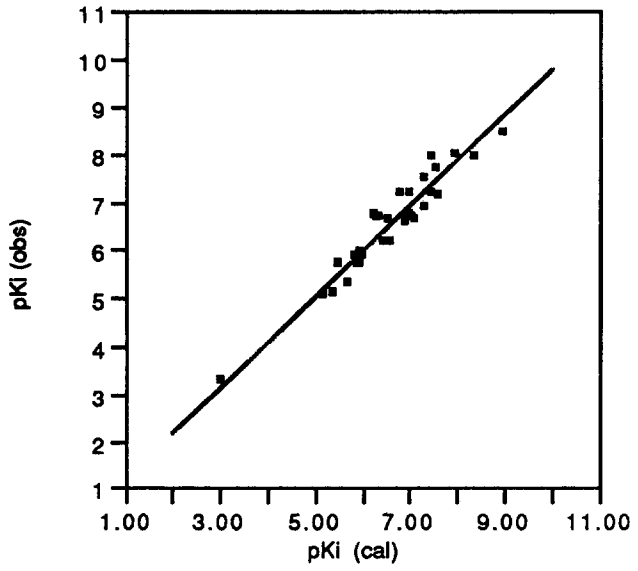


Figure 1. Observed and calculated pK_i values of nicotine analogues using eq 4.

using eq 4. Equations 5–9 show the stepwise development of eq 4. The mean, standard deviation, Pearson correlation coefficients, and other statistics of the physicochemical parameters used in this study are given in Table 2.

$$pK_i = -1.60(\pm 0.37)MR_1 + 7.17(\pm 0.19) \quad (5)$$

$n = 34$, RMSE $= 0.882$, $r^2 = 0.365$, $F = 18.4$, $p = 0.0002$, $RMSE_{cv} = 0.925$

$$pK_i = -1.77(\pm 0.34)MR_1 - 1.42(\pm 0.49)I_{2\alpha} + 7.35(\pm 0.18) \quad (6)$$

$n = 34$, RMSE $= 0.793$, $r^2 = 0.502$, $F = 15.6$, $p = 0.0001$, $RMSE_{cv} = 0.831$

$$pK_i = -1.82(\pm 0.30)MR_1 - 0.97(\pm 0.32)I_{2\alpha} - 1.62(\pm 0.44)I_{3\alpha} + 7.56(\pm 0.18) \quad (7)$$

$n = 34$, RMSE $= 0.707$, $r^2 = 0.617$, $F = 16.1$, $p = 0.0001$, $RMSE_{cv} = 0.751$

$$pK_i = -2.23(\pm 0.30)MR_1 - 1.28(\pm 0.30)I_{2\alpha} - 2.09(\pm 0.42)I_{3\alpha} - 0.74(\pm 0.24)MR_{2\beta} + 8.14(\pm 0.25) \quad (8)$$

$n = 34$, RMSE $= 0.625$, $r^2 = 0.710$, $F = 17.8$, $p = 0.0001$, $RMSE_{cv} = 0.676$

$$pK_i = -2.68(\pm 0.22)MR_1 - 1.65(\pm 0.22)I_{2\alpha} - 2.59(\pm 0.30)I_{3\alpha} - 1.16(\pm 0.18)MR_{2\beta} - 0.94(\pm 0.16)MR_{3\beta} + 8.83(\pm 0.21) \quad (9)$$

$n = 34$, RMSE $= 0.428$, $r^2 = 0.869$, $F = 37.1$, $p = 0.0001$, $RMSE_{cv} = 0.469$

In a preliminary study during the course of this research, QSAR analyses were performed with the 22 compounds (described in the previous report[12]) which were a subset of the compounds listed in Table 1. These compounds included nicotine, four R_1 derivatives, 12 R_2 derivatives, and six R_3 derivatives. From these compounds, eq 10 was derived. Since then, a number of additional compounds were synthesized, and additional binding affinity data were obtained for some of the compounds.

$$pK_i = -2.53(\pm 0.37)MR_1 - 2.63(\pm 0.29)I_{3\alpha} - 1.31(\pm 0.23)MR_2 - 1.19(\pm 0.15)MR_3 - 0.61(\pm 0.30)HA - 0.61(\pm 0.30)HD + 9.13(\pm 0.24) \quad (10)$$

$n = 22$, RMSE $= 0.371$, $r^2 = 0.885$, $F = 19.3$, $p = 0.0001$,

Table 2. Mean, standard deviation, Pearson correlation coefficients and other statistics of the physicochemical parameters used in eq 4

Variable	N	Mean	Std dev	Sum	Minimum	Maximum
MR_1	34	0.33	0.41	11.06	0.10	1.30
$MR_{2\beta}$	34	0.48	0.54	16.42	0.10	1.85
$HA_{2\beta}$	34	0.24	0.43	8.00	0.00	1.00
$I_{2\alpha}$	34	0.18	0.39	6.00	0.00	1.00
$MR_{3\beta}$	34	0.24	0.52	8.26	0.10	2.54
$I_{3\alpha}$	34	0.09	0.29	3.00	0.00	1.00

Pearson correlation coefficients

	MR_1	$MR_{2\beta}$	$HA_{2\beta}$	$I_{2\alpha}$	$MR_{3\beta}$	$I_{3\alpha}$
MR_1	1.00	−0.36	−0.30	−0.03	−0.15	−0.17
$MR_{2\beta}$		1.00	0.42	−0.24	−0.19	−0.22
$HA_{2\beta}$			1.00	−0.07	−0.15	−0.17
$I_{2\alpha}$				1.00	−0.13	−0.14
$MR_{3\beta}$					1.00	−0.08
$I_{3\alpha}$						1.00

$$RMSE_{cv} = 0.495$$

In eq 10, *HD* is an indicator variable for the hydrogen donor ability of the substituents, and the least important variable in the correlation. This variable was found to be unimportant in the final correlation. One compound (**8**, $NHCOOCH_2Ph$) was not included in deriving eq 10 because of its poor fit. On the basis of the QSAR, it was expected that the most potent compound would be the parent compound; any substituent at the R_1, R_2, and/or R_3 position would be detrimental to the binding affinity. Substituents that can participate in hydrogen bonds were expected to reduce the affinity dramatically. Equation 10 predicted well the biological potency of the newly synthesized derivatives. All the new compounds showed lower binding affinity than the parent compound, nicotine. With additional 12 compounds described in this study, it was possible to show that the preliminary QSAR had reasonable predictability and to refine the QSAR. All the terms in eq 4 that were present in eq 10 are better defined based on the standard error of the coefficients. The results clearly suggest that it would be more beneficial to do the structural modifications at a position other than R_1, R_2 and/or R_3 or to work on different ring structures.

Conclusions

Quantitative structure–activity relationships of 34 pyrrolidine-modified nicotine agonists for their binding affinity toward the neuronal nicotinic acetylcholine receptor have been investigated. A large substituent at the R_1, R_2, and/or R_3 position is detrimental to the binding affinity. Substituents at the $R_{2\alpha}$ and/or $R_{3\alpha}$ position decrease the binding affinity of these compounds, indicating that the size of the receptor pocket around these positions is limited. A hydrogen bond accepting group at the $R_{2\beta}$ position is also deleterious to the binding.

Experimental

QSAR

Multiple regression analysis techniques were employed in this study. The regression analyses were performed using the SAS program on a VAX computer. The 'best' equation was selected based on the results of subsets and all possible equations. In the correlation equations, n is the number of compounds used in the analysis, RMSE is the root mean square error, r^2 is the squared multiple correlation coefficient, $RMSE_{cv}$ is the root mean square error of the corresponding leave-one-out cross-validation test, and F and p are the F statistics and significance probability, respectively. MR_1, $MR_{2\beta}$, and $MR_{3\beta}$ are the molar refractivity values of the R_1, $R_{2\beta}$, and $R_{3\beta}$ substituents, respectively. *MR* values used were taken from the literature[16] and scaled by 10 before being used in the correlation. $HA_{2\beta}$ is an indicator variable for the hydrogen bond acceptor for the substituent at $R_{2\beta}$ position. $I_{2\alpha}$ and $I_{3\alpha}$ are indicator variables for the a isomers at the $R_{2\alpha}$ and $R_{3\alpha}$ position, respectively. Besides these physicochemical parameters, hydrophobic effects of the substituents on the binding affinity were also examined using literature π values, but were found to have negligible influence.

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