Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 481–486

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

COMPARATIVE MOLECULAR FIELD ANALYSIS (COMFA) MODELS OF PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE (PNMT) AND THE α_2-ADRENOCEPTOR: THE DEVELOPMENT OF NEW, HIGHLY SELECTIVE INHIBITORS OF PNMT

Gary L. Grunewald,* Timothy M. Caldwell, Vilas H. Dahanukar, Ravi K. Jalluri, and Kevin R. Criscione

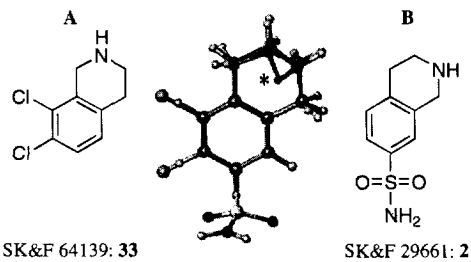
Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045, U.S.A.

Received 10 November 1998; accepted 22 December 1998

Abstract: As a guide to the development of new and more selective inhibitors of phenylethanolamine *N*-methyltransferase (PNMT) vs the α_2-adrenoceptor, we have performed a comparative molecular field analysis (CoMFA) on a series of 80 benzylamine analogues. Using the models obtained, we have proposed a series of 3-trifluoromethyl-1,2,3,4-tetrahydroisoquinolines and predicted the activity of other analogues.

Keywords: Enzyme inhibitor; Antihypertension; Molecular modelling/mechanics; Receptors

As part of our studies to determine the role of epinephrine (Epi) in the central nervous system, we have targeted phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28),[1] the enzyme that catalyzes the final step in the biosynthesis of Epi, for inhibition. 1,2,3,4-Tetrahydroisoquinolines (THIQs) are potent inhibitors of PNMT. However, previous studies have shown that most inhibitors of PNMT, including THIQs, display affinity for the α_2-adrenoceptor.[2] To elucidate the factors that determine potency and selectivity of THIQs for PNMT vs the α_2-adrenoceptor, we previously performed a comparative molecular field analysis (CoMFA),[3] a type of 3-D QSAR, on thirty 7-substituted-THIQs at both PNMT and the α_2-adrenoceptor.[4] This study found that a predictive model for PNMT and the α_2-adrenoceptor could only be obtained when the compounds in the training set were aligned in either of two orientations, based on the lipophilicity of the 7-substituent (Figure 1). In this study we have expanded our previous CoMFA models with the addition of 50 benzylamine analogues (1,2,3,4-tetrahydroisoquinolines, 2,3,4,5-tetrahydro-1*H*-2-benzazepines, benzylamines, 1,2,3,4,5,6-hexahydro-2-benzazocines, 2,3,4,5-tetrahydro-5*H*-1,4-benzodiazepine, 2,3,4,5-tetrahydro-5*H*-1,4-benzoxazepine, 2,3,4,5-tetrahydro-5*H*-1,4-benzothiazepine, and 2,3-dihydroisoindoline) and have used these updated models to predict the activity of compounds not included in the training set.



SK&F 64139: **33** SK&F 29661: **2**

Figure 1. Proposed binding orientations of 1,2,3,4-tetrahydroisoquinolines at PNMT and the α_2-adrenoceptor showing SK&F 64139 (Lipophilic: Alignment A) and SK&F 29661 (Hydrophilic: Alignment B) and the SYBYL generated overlay of the two compounds. The asterisk indicates the area in space where the lone pairs of SK&F 64139 and SK&F 29661 can overlap.

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PII: S0960-894X(99)00022-0

Comparative Molecular Field Analysis (CoMFA)

Eighty benzylamine analogues with a variety of substituents were included in this investigation (Table 1). Structures of the compounds used in this study were constructed using the SYBYL 6.4 software package[5] and the minimum energy conformations were calculated with electrostatics using the Tripos force field and charges calculated by the AM1 method in MOPAC (SYBYL 6.4 implementation). The conformation of compounds containing side-chains were calculated by the "systematic search" option in SYBYL to locate the global minimum energy conformation. Side chains were aligned so that they occupied the same region of space, even though this sometimes resulted in the use of a local minimum energy conformation (within 2 kcal/mole of the corresponding global minimum).

The molecules in this study were aligned according to the alignment rules used in the previous CoMFA study for 7-substituted-THIQs.[4] Compounds were aligned in either lipophilic orientation A or hydrophilic orientation B (Figure 1). The molecules were fit using three points: the two ends of a normal (2 Å long) passing through the centroid of the aromatic ring of the ligand and the end of the axial lone pair (2.4 Å long) on the benzylamine nitrogen. The CoMFA procedure was implemented by placing the aligned molecules in a three-dimensional box containing a grid with a spacing of 2 Å. This region extended the shape of each molecule by at least 4 Å. The steric (van der Waals) and electrostatic (coulombic) interaction energies were sampled using an sp^3 hybridized carbon (charge +1) as a probe atom at each intersection point of a three-dimensional lattice, except at those points where the steric interaction energy indicated that the probe atom was "inside" the aligned molecules (i.e., energy greater than 30 kcal/mol).

In order to evaluate the optimal number of components and the predictive power of the resulting models, initial partial least squares (PLS) runs with cross-validation were calculated with up to 8 components using both steric and electrostatic CoMFA fields. Any grid point for which the standard deviation of the energies was less than 2.0 kcal/mol was discarded to decrease the background noise and the number of CoMFA columns. Once the optimal number of components was determined by examination of the standard error, a non-validated PLS run was used to yield the final model. The statistical results are shown in Table 2.

Results and Discussion

The results of the non-validated PLS runs were examined graphically (Figures 2 and 3) and by prediction of the activity of compounds not included in the training set (Table 3). The resulting steric and electrostatic maps indicate that there are two areas that can be exploited to increase selectivity for.PNMT vs the α_2-adrenoceptor. First, the steric maps show that both sites are very similar in their areas of steric bulk tolerance and intolerance. However, one area of difference is found in the α_2-adrenoceptor map (Figure 3a) at the 3-position of THIQs aligned in hydrophilic orientation B, where there is an area of steric bulk intolerance (yellow contours). The second area is found in the electrostatic maps, where the contours indicate that there are major differences between these sites in their electrostatic preferences. The PNMT map (Figure 2b) reveals an area encompassing the 7-position of THIQs aligned in hydrophilic orientation B that prefers electron density (magenta contours), whereas the same area in the α_2-adrenoceptor map (Figure 3b) disfavors electron density

Table 1. Structures used for calculating the PNMT and the α_2-adrenoceptor CoMFA models (80 compounds).

	Compound	Aln.[a]	PNMT		α_2		Ref
			pK_i[b]	ΔpK_i[c]	pK_i[b]	ΔpK_i[c]	
1	1,2,3,4-Tetrahydro-isoquinoline (THIQ)	B	5.01	0.35	6.45	0.58	4
2	THIQ-7-SO_2NH_2	B	6.26	-0.12	4.00	-0.16	9
3	THIQ-7-CH_3	A	5.57	-0.41	6.44	0.00	4
4	THIQ-7-SO_2CH_3	B	5.87	0.47	3.79	-0.24	4
5	THIQ-7-CH_2OH	B	4.97	0.84	5.64	0.23	4
6	THIQ-7-$NHCO_2CH_3$	B	3.73	0.11	5.04	-0.07	9
7	THIQ-7-OCH_3	B	4.70	-0.35	6.33	0.44	4
8	THIQ-7-$COCF_3$	B	5.24	-0.28	4.49	-0.15	4
9	THIQ-7-$COCH_3$	B	5.31	0.16	5.21	0.43	4
10	THIQ-7-NH_2	B	4.57	0.50	5.51	-0.27	4
11	THIQ-7-$SO_2CH_2CH{=}CH_2$	B	5.01	-0.01	4.02	0.10	4
12	THIQ-7-SO_2Ph	A	4.85	-0.03	4.67	0.28	4
13	THIQ-7-Br	A	6.53	0.11	6.64	0.05	4
14	THIQ-7-$CONH_2$	B	4.19	-0.37	4.64	-0.19	4
15	THIQ-7-I	A	6.43	0.08	6.66	0.00	4
16	THIQ-7-SO_2CCl_3	A	5.90	0.01	4.47	-0.02	4
17	THIQ-7-CF_3	A	6.74	0.03	5.89	-0.44	4
18	THIQ-7-NO_2	B	6.38	0.10	5.37	0.63	4
19	THIQ-7-SCH_3	A	6.21	-0.02	6.39	-0.11	4
20	THIQ-7-CN	B	5.87	0.19	5.14	-0.23	4
21	THIQ-7-CH_2NH_2	B	2.72	-0.12	4.92	-0.05	4
22	THIQ-7-CO_2H	B	3.33	-1.15	3.33	-1.17	4
23	THIQ-7-$NHSO_2CH_3$	B	4.32	0.01	4.96	-0.18	9
24	THIQ-7-$N(SO_2CH_3)_2$	B	2.35	-0.05	3.72	-0.17	9
25	THIQ-7-$CH_2NHSO_2CH_3$	B	3.48	-0.07	4.74	0.26	9
26	THIQ-7-SO_2NH(Ph-p-Cl)	A	5.58	-0.09	5.20	0.03	9
27	THIQ-7-OH	B	5.58	0.36	5.65	-0.21	9
28	THIQ-7-CO_2CH_3	B	5.17	0.06	5.34	0.43	4
29	THIQ-7-CH_2Ph	A	4.21	-0.07	5.33	0.23	4
30	THIQ-7-COPh	A	5.54	0.12	4.57	-0.30	4
31	THIQ-7-SO_2NHCH_3	B	5.33	0.00	3.88	-0.08	9
32	THIQ-8-OH	B	3.22	-0.35	6.14	0.47	7
33	THIQ-7,8-$(Cl)_2$	A	6.69	0.34	7.66	0.91	6
34	THIQ-6,7-$(OH)_2$	A	5.41	-0.29	6.32	-0.05	7
35	THIQ-5-OCH_3	A	3.26	0.26	6.89	0.51	7
36	THIQ-6-OCH_3	A	3.96	-0.08	5.85	-0.13	7
37	THIQ-8-OCH_3	A	5.43	0.16	6.52	-0.11	7
38	THIQ-*N*-CH_3	B	4.30	-0.07	6.62	0.56	7
39	THIQ-5,8-$(OCH_3)_2$	B	3.00	-0.35	6.25	-0.21	7
40	THIQ-5,7-$(NO_2)_2$	B	4.24	0.07	4.11	-0.30	7
41	THIQ-5-OH	A	4.04	0.25	6.11	0.07	7
42	THIQ-6-OH	A	5.24	-0.23	6.00	-0.25	7
43	THIQ-*R*-3-CH_2OH-7-NO_2	B	6.62	-0.23	4.42	0.19	7
44	THIQ-*S*-3-CH_2OH-7-NO_2	B	6.05	0.04	4.87	0.62	7
45	THIQ-*R*-3-CH_2OH	B	6.25	0.28	5.36	-0.42	7
46	THIQ-*S*-3-CH_2OH	B	4.83	0.31	4.77	-0.63	7
47	THIQ-*R*-3-CH_3-7-NO_2	B	5.89	-0.21	4.28	-0.22	7
48	THIQ-*S*-3-CH_3-7-NO_2	B	6.60	0.06	4.72	0.41	7
49	THIQ-*R*-3-CH_3	B	4.42	-0.17	5.24	-0.42	10
50	THIQ-*S*-3-CH_3	B	6.00	0.34	6.31	0.46	10
51	THIQ-*R*-3-CH_3-7,8-$(Cl)_2$	A	6.45	-0.24	6.66	-0.13	7
52	THIQ-*S*-3-CH_3-7,8-$(Cl)_2$	A	6.04	0.03	6.37	-0.31	7
53	1,2,3,4-Tetrahydrobenz-[h]isoquinoline-8-OH	A	6.01	0.17	7.10	0.04	9
54	1,2,3,4-Tetrahydrobenz-[h]isoquinoline-8-OCH_3	B	4.85	-0.10	6.08	-0.77	9
55	2,3,4,5-Tetrahydro-1*H*-2-benzazepine (THBA)	B	5.48	0.34	4.97	-0.08	10
56	THBA-8,9-$(Cl)_2$	A	6.58	0.53	5.39	0.07	10
57	THBA-8-SCH_3	A	5.98	-0.35	4.92	-0.17	7
58	THBA-8-NO_2	B	6.41	0.13	4.18	0.40	7
59	THBA-8-Br	A	6.54	0.16	5.05	-0.10	7
60	THBA-8-NH_2	B	4.62	0.08	3.96	-1.08	7
61	THBA-8-SO_2CH_3	B	5.11	-0.29	3.82	-0.14	7
62	THBA-6,8-$(NO_2)_2$	B	4.44	-0.08	4.60	0.33	7
63	THBA-*R*-3-CH_3-8,9-$(Cl)_2$	A	6.38	-0.34	5.56	-0.03	7
64	THBA-*S*-3-CH_3-8,9-$(Cl)_2$	A	5.24	-0.46	5.36	-0.21	7
65	THBA-8-SO_2NH_2	B	5.78	0.37	3.82	-0.16	7
66	Napthalene-1-CH_2NH_2	A	5.33	0.04	5.79	0.19	9
67	Napthalene-6-OH-1-CH_2NH_2	A	5.08	-0.25	5.68	0.24	9
68	Napthalene-6-OCH_3-1-CH_2NH_2	A	4.82	0.24	5.34	-0.02	9
69	Benzylamine (BA)	B	3.75	-0.18	4.70	0.19	7
70	BA-*R*-α-CH_3	B	2.79	-0.08	3.54	-0.27	7
71	BA-*S*-α-CH_3	B	3.84	-0.02	3.92	-0.69	7
72	BA-*R*-α-CH_3-3,4-$(Cl)_2$	A	4.49	-0.09	4.39	0.46	7
73	BA-*S*-α-CH_3-3,4-$(Cl)_2$	A	5.30	0.13	4.44	-0.15	7
74	1,2,3,4,5,6-Hexahydro-2-benzazocine (HHBA)	B	4.67	0.70	4.81	-0.14	10
75	HHBA-*N*-CH_3	B	2.94	-0.18	5.39	0.40	7
76	HHBA-9-NO_2	B	5.34	0.13	4.08	-0.55	7
77	2,3,4,5-Tetrahydro-5H-1,4-benzothiazepine	B	5.39	0.37	5.34	0.27	10
78	2,3,4,5-Tetrahydro-5H-1,4-benzooxazepine	B	4.67	-0.37	5.39	0.24	10
79	2,3,4,5-Tetrahydro-5H-1,4-benzodiazepine	B	4.55	-0.30	5.58	0.52	10
80	2,3-Dihydroisoindoline	B	3.64	-0.91	6.09	0.29	10

[a] Alignment as shown in Figure 1. [b] $pK_i = -\log K_i$. [c] $\Delta pK_i = -\log$ (actual K_i) + log (predicted K_i).

(cyan contours). Therefore, these two models indicate that in order for a THIQ-type inhibitor to be selective for PNMT, it should contain a 3-substituent and a hydrophilic electron-withdrawing 7-substituent. Previous QSAR studies of the THIQ nucleus from our laboratory have shown that the area of steric bulk tolerance around the 3-position of THIQ at PNMT is limited to either a 3-methyl or a 3-hydroxymethyl moiety.[6] Substitution of larger groups such as 3-ethyl or 3-methoxymethyl[7] resulted in reduced potency at PNMT. Therefore, the 3-substituent must be limited in size.

Using these updated CoMFA models, the activities of eight compounds (**81–88**) not included in the data set were predicted, including a unique series of 3-trifluoromethyl-THIQs (**84–88**), which were proposed and synthesized based on these CoMFA models.[8] The predicted and actual activities of these compounds are shown in Table 3. Overall, our CoMFA models did an excellent job of predicting the activity of these compounds at PNMT and a reasonable job for the α_2-adrenoceptor. Compounds **81** and **82** were predicted and found to be nonselective inhibitors of PNMT. Compound **83** takes advantage of the two areas shown in our CoMFA models and was predicted and found to be the most selective inhibitor in this series of compounds.

For the 3-trifluoromethyl-THIQs (**84–88**), the PNMT CoMFA model was able to predict the relative order of potency of these compounds. However, the α_2-adrenoceptor CoMFA model under-predicted these compounds by an average of over 300-fold. Compounds **85–87** were predicted and found to be selective inhibitors of PNMT. However, these compounds displayed much lower affinities for the α_2-adrenoceptor than predicted by our model. Compounds **84** and **88** were predicted to have good affinity for the α_2-adrenoceptor, but were found to have almost no affinity (Table 3). This may be an indication that some other factor, not included in our CoMFA study, is influencing the activity of this series of 3-trifluoromethyl-THIQs at the α_2-adrenoceptor. This factor may be the decreased pK_a of the THIQ amine or the increased lipophilicity caused by the addition of the 3-trifluoromethyl moiety or both. This series of 3-trifluoromethyl THIQs are some of the most selective inhibitors of PNMT known.

Conclusion

We generated two 3-D QSAR models that not only have the ability to explain the structure–activity relationships of previously evaluated compounds, but also have the ability to predict the biological activity of new compounds. These models could be refined by increasing the structural diversity in the data set (i.e., inclusion of the 3-trifluoromethyl-THIQs **84–88**) or by the addition of other parameters (such as the pK_a of the respective amines or a hydrophobic parameter).

Table 2. Statistics for the CoMFA approach for the Compounds of Table 1.

Model	Compounds	Components	Cross-val. r^2	Press s	Non-val. r^2	s	F_{calc}[a]	F_{table}[d]
PNMT	80	7	0.656	0.670	0.919	0.326	116.08[b]	2.91[e]
α_2	80	4	0.633	0.592	0.845	0.385	102.41[c]	3.58[f]

[a] Calculated F-test value. [b] $F_{(7, 72)}$. [c] $F_{(4, 75)}$. [d] F-test distribution table value at the 99% confidence limit. A model may be considered to be statistically significant if the calculated F-value is greater than the table F-value. [e] $F_{(7, 72, 0.01)}$. [f] $F_{(4, 75, 0.01)}$.

Figure 2a.

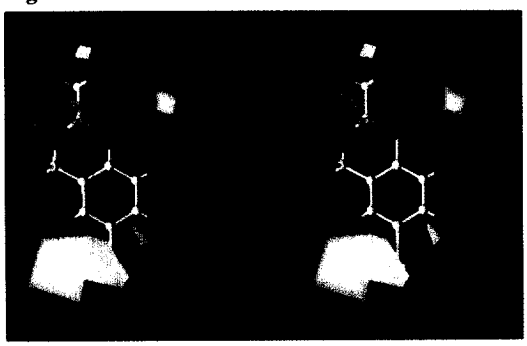

Figure 2b.

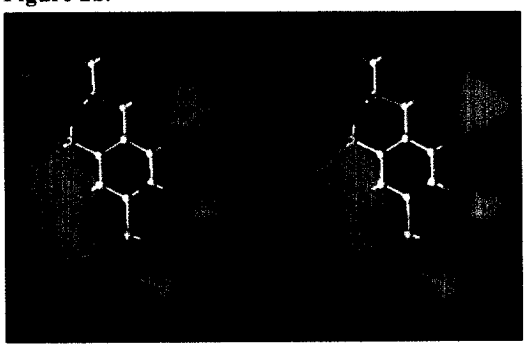

Figure 2. Contour maps for the PNMT CoMFA. *S*-**83** in alignment B is shown as the reference molecule. (a) Steric: The contours where steric bulk is positively correlated with activity are shown in green, while the contours where steric bulk is negatively correlated with activity are shown in yellow. The contours are drawn at the contribution levels of 75:25. (b) Electrostatic: The contours where electron deficiency is positively correlated with activity are shown in cyan, while the contours where electron density is positively correlated with activity are shown in magenta. The contours are drawn at the contribution levels of 80:30.

Figure 3a.

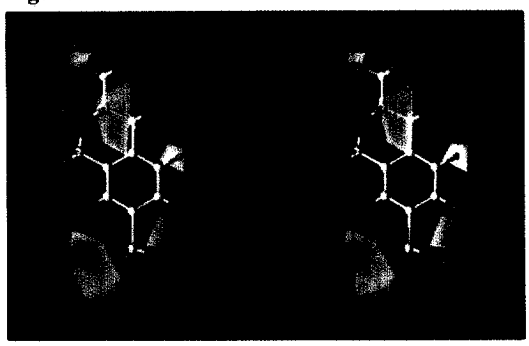

Figure 3b.

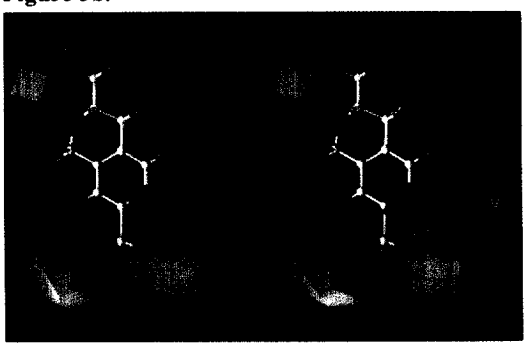

Figure 3. Contour maps for the α_2-adrenoceptor CoMFA. *S*-**83** in alignment B is shown as the reference molecule. (a) Steric: The contours where steric bulk is positively correlated with activity are shown in green, while the contours where steric bulk is negatively correlated with activity are shown in yellow. The contours are drawn at the contribution levels of 80:30. (b) Electrostatic: The contours where electron deficiency is positively correlated with activity are shown in cyan, while the contours where electron density is positively correlated with activity are shown in magenta. The contours are drawn at the contribution levels of 75:25.

Table 3. Predicted and actual biological activity of compounds **81–88**.

Compound		PNMT (K_i μM)		α_2-Adrenoceptor (K_i μM)		Selectivity	Ref
		Predicted	Actual	Predicted	Actual	α_2 K_i /PNMT K_i	
81	(±)		0.38		0.15	0.39	6
	R	0.78		0.25		0.32	
	S	0.12		0.25		2.1	
82 SK&F 7698	(±)		0.45		0.20	0.44	11
	R	0.27		0.34		1.3	
	S	0.80		0.062		0.080	
83	(±)		0.34		1400	4100	7
	R	0.19		56		290	
	S	3.3		200		61	
84	(±)		15		400	26	8
	R	4.0		2.0		0.50	
	S	44		2.1		0.050	
85	(±)		36		3900	110	8
	R	0.93		180		190	
	S	8.1		180		22	
86	(±)		2.0		1500	750	8
	R	0.11		26		240	
	S	1.3		32		25	
87	(±)		13		2900	220	8
	R	0.40		5.8		15	
	S	4.8		6.8		1.4	
88	(±)		0.52		>1000[a]	>1900	8
	R	0.46		0.38		0.83	
	S	0.18		0.46		2.6	

[a] Precipitate was noted at 1000 μM in the α_2-adrenoceptor assay.

Acknowledgements: This research was supported by NIH grant HL 34193 and NIH predoctoral training grant GM 07775.

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11. PNMT and α_2-adrenoceptor K_i's for literature compounds were determined in our laboratory for consistent internal comparison.