1-(Substituted-benzyl)imidazole-2(3*H*)-thione Inhibitors of Dopamine β-Hydroxylase

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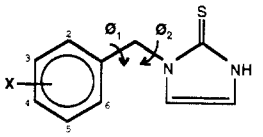
Molecular shape and quantitative structure–activity relationship (QSAR) analyses of 52 1-(substituted-benzyl)-imidazole-2(3*H*)-thione inhibitors of dopamine β-hydroxylase were carried out. QSARs were developed for sets of 45 and sets of 47 analogues. Molecular shape, as represented by common overlap steric volume and the composite charge density on carbons 3, 4, and 5 of the substituted-benzyl ring are the major inhibition-potency descriptors. Five of the 52 compounds were eliminated prior to analyses on the basis of difficulties in characterizing shape and charge state. Two compounds were outliers. The active conformation deduced in the analyses is a low-energy conformer for both active and inactive inhibitors. This suggests that the intrinsic shape of the molecule due to the selection of X is more important than torsion-angle selection for the bonds between the two rings. The QSARs found in this study have only general similarities to one put forth by Kruse et al. using linear free energy descriptors.

Kruse et al.[1] have carried out a quantitative structure–activity relationship (QSAR) analysis of a set of 1-(substituted-benzyl)imidazole-2(3*H*)-thiones, I, as multisubstrate inhibitors of dopamine β-hydroxylase (DβH). These



I

inhibitors effectively reduce blood pressure in adult male Okamoto–Aoki spontaneously hypertensive rats using oral or intraperitoneal dosing. DβH is a copper-containing, mixed-function oxidase that catalyzes the conversion of dopamine to norepinephrine. As such, this DβH-inhibitor system represents an intervention endpoint for treatment of cardiovascular disorders related to hypertension.

Linear free energy descriptors were used by Kruse et al.[1] to construct their QSARs. The optimal QSAR derived in their study is

$$-\log IC_{50} = 1.28\ (\pm 0.22) I_{(4\text{-OH})} + 0.65\ (\pm 0.16)\pi_{345} - 0.14\ (\pm 0.02)MR_{345} + 1.42\ (\pm 0.33)F_{345} - 1.26$$
$$N = 25,\ R = 0.91,\ F = 22.9,\ S = 0.44 \qquad (1)$$

where IC_{50} is the micromolar concentration needed to inhibit the activity of DβH by 50%, $I_{(4\text{-OH})}$ is an indicator variable to note the presence of a hydroxyl group at the 4-position of the phenyl ring, and π_{345} is the sum of the π constants summed over the 3-, 4-, and 5-positions of the phenyl ring. Likewise, MR_{345} is the sum of the molar refractivity values over those same positions and F_{345} is the sum of the inductive terms as described by Swain and Lupton[2] over those positions.

An analysis of the structure–activity relationship (SAR) of the DβH inhibitors[1] suggested to us that conformational behavior might play a role in specifying inhibition potency. Diortho-substituted compounds are observed to be quite inactive, monoortho-substituted analogues are moderately active, and the nonortho substituted compounds can be quite active. Ortho substitution could be expected to alter conformational profiles with respect to ϕ_1 and ϕ_2 (see I). Thus, we felt that a QSAR study based upon molecular shape analysis (MSA)[3,4] should be carried out on these DβH inhibitors.

Methods

(1) Biological Activity. Kruse et al.[1] reported the DβH inhibitory activities of 52 thione analogues (I). Two different measurements of DβH inhibition were made on these compounds. The less active analogues (compounds 1–18 of Table I) have inhibition reported in terms of percent inhibition at a fixed inhibitor concentration of 1.0×10^{-4} M. The inhibition potencies of the rest of the analogues are given as actual IC_{50} values. We have attempted to put these two inhibition measurements on a common scale so that all 52 compounds could be considered in our MSA–QSAR analyses.

The following relationship was assumed in order to combine the two activity scales:

$$\text{activity} = -\log IC_{50} = \log\left(\frac{X \times 10^4\ \text{M}}{50\%\,\text{inhibition}}\right) \qquad (2)$$

where X is the percent inhibition reported at 1.0×10^{-4} M. The set of inhibition potencies, based upon the IC_{50} measure, for all 52 compounds is given in Table I.

(2) Building the Molecules. The compounds selected for the SAR database were built using standard bond lengths and angles with the CHEMLAB-II molecular modeling package.[5] The geometries were optimized by free valence molecular mechanics using the MMFF option in CHEMLAB-II; this is a version of Allinger's MM2 program[6] with extended parameterization and force field function generalization.

(3) Molecular Shape Analysis. There are seven operations involved in the current formulation of MSA. The seven operations are listed in Figure 1 and are described in this section. The final selection of the requirements for each operation, for example, choice of the shape reference compound, is based upon optimizing the QSAR in terms of statistical significance. That is, the set of choices available for each operation are employed to generate trial QSARs. That QSAR which corresponds to the best fit between observed activities and computed molecular de-

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(6) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127.

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Table I. General SAR Table for Some X-Substituted 1-Aralkylimidazole-2(3*H*)-thiones

no.[b]	X	V_o[d]	$Q_{3,4,5}$	Q_6	π_4	π_0^2	obs −log IC_{50}	pred[a] −log IC_{50}	diff (obs − pred) Δ−log IC_{50}
1	4-CO_2H								
2*	2,6-Me_2	0.816	−0.03	0.04	0.00	19.86	3.00	3.12	−0.12
3	4-CH_2OH								
4*	2,6-Cl_2	0.842	0.06	0.07	0.00	21.01	3.15	3.31	−0.16
5	3-SO_2NH_2, 4-OMe								
6	2,6-$(OMe)_2$	0.748	−0.03	0.19	0.00	10.02	3.30	3.62	−0.32
7*	2-Cl	0.908	0.04	0.00	0.00	14.98	3.45	3.65	−0.20
8	2-Me	0.896	0.05	0.02	0.00	14.49	3.47	3.56	−0.09
9	3,4-$(OMe)_2$	0.824	0.28	0.01	−0.02	6.79	3.47	3.83	−0.36
10	4-CF_3	0.894	0.07	0.01	0.88	16.33	3.70	3.58	0.12
11	3-CF_3, 4-OMe	0.855	0.10	0.04	−0.02	17.68	3.76	3.43	0.33
12	2,6-Cl_2, 4-OMe	0.763	0.17	0.10	−0.02	22.21	3.81	3.91	−0.10
13	4-CH_3	0.944	0.07	0.02	0.00	14.49	3.83	4.18	−0.35
14	4-Br	0.917	0.16	0.01	0.86	16.17	3.94	4.03	−0.09
15	3-Br, 4-OMe	0.876	0.30	0.00	−0.02	14.44	4.08	3.99	0.09
16*	3-F, 4-OMe	0.897	0.31	−0.01	−0.02	11.29	4.13	4.18	−0.05
17	2-OMe	0.883	0.01	0.03	0.00	9.47	4.13	3.36	0.77
18	3-Me, 4-OMe	0.885	0.16	0.02	−0.02	13.88	4.16	3.73	0.43
19	2-OH	0.947	0.01	0.03	0.00	6.21	3.24	4.10	−0.86
20*	3-NO_2, 4-OMe	0.883	0.19	0.01	−0.02	5.21	3.45	3.78	−0.33
21*	4-OMe	0.898	0.10	0.01	−0.02	7.45	3.69	3.69	0.00
22	3-OMe	0.900	0.20	−0.01	0.00	9.47	3.80	3.95	−0.15
23	3-OH	0.986	0.20	0.00	0.00	6.21	3.83		
24	3-CF_3, 4-OH	0.920	0.11	0.04	−0.67	15.16	3.92	3.94	−0.02
25	2,4,6-Cl_3	0.798	0.17	0.08	0.71	28.06	3.99	3.65	0.34
26*	2,5-Cl_2	0.908	0.12	0.00	0.00	21.01	4.01	3.83	0.18
27	4-Cl	0.948	0.16	0.01	0.71	14.98	4.02	4.46	−0.44
28	2,6-Cl_2, 4-OH	0.827	0.17	0.10	−0.67	19.03	4.12	3.57	0.55
29	2,3,5,6-F_4, 4-OH	0.951	0.42	0.19	−0.67	9.36	4.21	5.11	−0.90
30	4-NO_2	0.942	0.13	0.02	−0.28	8.59	4.28	4.30	−0.02
31*	2,3-Cl_2	0.904	0.12	0.00	0.00	21.01	4.28	3.80	0.48
32	3-Me, 4-OH	0.964	0.16	0.02	−0.67	9.86	4.31	4.73	−0.42
33	4-F	0.989	0.17	0.03	0.14	10.90	4.33	5.26	−0.93
34	3,5-Cl_2, 4-OMe	0.902	0.30	0.04	−0.02	14.46	4.33	4.19	0.14
35	3,5-F_2, 4-OMe	0.900	0.50	−0.06	−0.02	12.77	4.44	4.64	−0.20
36*	H	0.948	0.02	0.00	0.00	17.98	4.48	4.14	0.34
37	3-NO_2, 4-OH	0.952	0.22	0.03	−0.67	5.13	4.51	4.66	−0.15
38*	3,4-Cl_2	0.951	0.19	0.00	0.71	21.01	4.55	4.57	−0.02
39*	2,4-Cl_2	0.874	0.14	0.00	0.71	36.77[c]	4.77		
40	3-Br, 4-OH	0.959	0.02	0.00	−0.67	11.36	4.92	4.32	0.60
41	3-Cl	0.986	0.15	0.02	0.00	14.98	4.92	5.16	−0.24
42	3-F	0.991	0.25	−0.01	0.00	10.90	5.25	5.48	−0.28
43#	3,5-F_2								
44	4-OH	0.989	0.13	0.03	−0.67	6.21	5.59	5.17	0.42
45*	3,5-Cl_2	0.993	0.19	0.01	0.00	21.01	5.62	5.40	0.22
46*	3,4-$(OH)_2$	0.983	0.28	0.01	−0.67	3.33	5.66	5.39	0.27
47#	3,5-Cl_2								
48*	3-Cl, 4-OH	0.999	0.19	0.02	−0.67	10.37	5.70	5.53	0.17
49*	3-F, 4-OH	1.000	0.30	−0.01	−0.67	7.03	5.82	5.81	0.01
50	3,5-F_2	0.991	0.43	−0.07	0.00	11.86	5.92	5.90	0.02
51*	3,5-Cl_2, 4-OH	1.000	0.27	0.03	−0.67	14.46	6.17	5.74	0.43
52*	3,5-F_2, 4-OH	1.000	0.50	−0.06	−0.67	7.09	7.13	6.28	0.85

[a] Values of −log (IC_{50}) predicted by eq 6. [b] An asterisk indicates compounds used to develop trial QSARs. A pound sign indicates rings connected by three methylene units, not congeneric in series. [c] The measured π_0 value (ref 1) was used in place of the calculated value to construct eq 7. A value of 1.5 units to account for the missing fragment value in the CLOGP calculations (see the text) was subtracted from the measured π_0 value and the resulting value was squared to arrive at a value analogous to others in the list. [d] See ref 3. Integration step = 0.5 Å.

scriptors defines the specific requirements for each MSA operation.

There are two points that must be kept in mind when optimizing the QSAR. Firstly, this is a multidimensional optimization process and there may be multiple maxima. One cannot be sure that the largest maximum in statistical fit has been achieved, nor how many unique QSARs exist which correspond to relative maxima in statistical fit. Secondly, the measured biological activities totally govern the MSA operations. Shortcomings in the quantity and/or quality of the activity measures can prevent a MSA application or result in a misleading QSAR.

A. Conformational Analysis. The first operation in MSA is the conformational analysis of the analogues under study. In this case, the CHEMLAB-II modeling package option SCAN was used to perform a fixed valence conformational energy scan at 10-deg increments of ϕ_1 and ϕ_2. The reference conformational for $\phi_1 = \phi_2 = 0°$ corresponds to the coplanar ring conformation defined by (I). Flexible side chains were also scanned at 10-deg increments about the principal bonds. The side chains with rotational flexibility are OCH_3, OH, NO_2, and CF_3. A molecular mechanics force field composed of dispersion/steric, electrostatic, and, where applicable, hydrogen-bonding contributions were used to estimate the conformational energy. The nonbonded steric MMFF parameters from CHEMLAB-II, which are extended MM2 parameters of Allinger,[6] were used to compute the dispersion/steric interactions. A second set of nonbonded steric parameters, a "soft" set developed by Hopfinger,[7] were used to assess

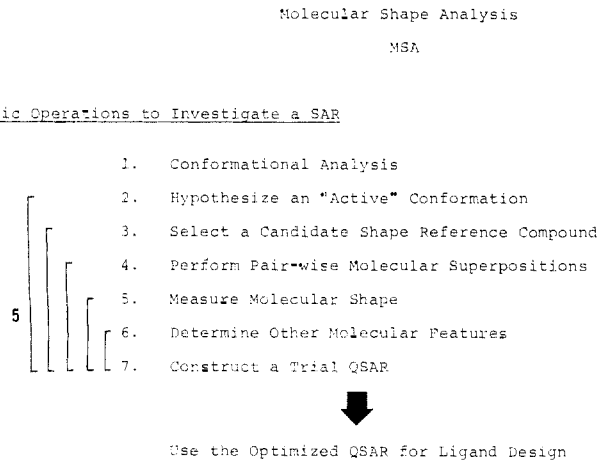

Figure 1. The basic operations involved in the molecular shape analysis formalism.

the role of the force field on conformational behavior and, ultimately, the QSAR (see the Results Section). The electrostatic interactions were calculated by using a Coulombic representation with the dielectric constant equal to 3.5 and atomic charges calculated by the CNDO/2 method.[8] When hydrogen-bonding atoms were available for bonding, the hydrogen-bonding potential developed by Hopfinger[7] was used.

The global conformational energy minimum was used to define the relative stability of each conformational state sampled, i.e., the relative conformational stability of a compound is defined as the difference in energy between a particular conformation and the global conformational energy minimum.

B. Selection of Individual QSAR Conformations. The QSAR conformation of an analogue refers to that conformation whose shape measurement, relative to that of the shape reference compound, yields the most significant QSAR. Conformations within ΔE^*_u kcal/mol of the global energy minimum for each analogue u were considered as candidates for the QSAR conformation. Three separate ΔE^*_u values (1, 3, and 6 kcal/mol) were considered in this study. The actual set of candidate QSAR conformations for each analogue was generated by constructing 5°-resolution grids in (ϕ_1,ϕ_2) space centered at each minimum energy conformation satisfying the ΔE^*_u constraint. In turn, selected representative conformations corresponding to intersection grid points within ΔE^*_u were QSAR conformation candidates.

C. Selection of the Shape Reference Compound and Active Conformation. The shape reference compound is the compound to which all others in the analogue series are compared. Each analogue in the data set is evaluated as possibly being the shape reference compound. Selection of the shape reference compound is, as mentioned earlier, ultimately dictated by maximizing the statistical significance of the resulting QSAR. However, the choice for the shape reference compound also depends upon the conformations assigned to a candidate reference compound. In this investigation, conformations within $\Delta E^*_v = 1.0$ kcal/mol of the global energy minimum for analogue v were selected as possible conformers for v as the shape reference compound. The grid-locator approach, used to generate active-conformation candidates, was also employed to select possible shape reference conformations for each thione analogue.

In many previous MSA studies[3,4,9] the active conformation was postulated by observing which conformational energy minimum common to active analogues was not energetically available to inactive analogues. The postulated active conformation, or the minimum-energy conformation nearest to the active conformation in torsion-angle space, was assigned to candidate shape reference compounds. This constraint is not imposed in the approach used in this study and described above. However, the $\Delta E^*_v = 1.0$ kcal/mol constraint limits the selection of conformations for the shape reference compound to be energetically close to the global minimum energy conformation of each analogue. Still, the active conformation can be stable for both active and inactive analogues.

Perhaps the use of the term "active conformation" is misleading in this particular study. It might be better to term the specific conformation used to construct the MSA–QSAR as the biologically relevant conformation. That is, the conformation used to construct the MSA–QSAR might not mechanistically differentiate active from inactive analogues.

D. Molecular Superposition. The geometric criterium for pairwise analogue molecular superposition was to place the N–C–N of the thione rings of each pair of molecules identically upon one another. Each analogue v from the initial set (see the asterisked entries of Table I) was considered as the shape reference compound and assigned a conformation from the set of conformations satisfying $\Delta E^*_v = 1.0$ kcal/mol. Each analogue u in the data set was then assigned the conformations consistent with the ΔE^*_u constraint and compared to v. The criterium for selecting a unique conformation for u in a trial QSAR was to maximize the shape similarity measure (see below) between u and v in terms of the conformation of u. This process was, in turn, repeated for each conformation available to reference compound v. To put this procedure in perspective, suppose there are 50 analogues to analyze such that 15 are chosen to be shape reference candidates and 10 conformations are available to each analogue as possible shape reference conformations ($\Delta E^*_v = 1.0$ kcal/mol) and 30 candidate active conformations are available to each compound ($\Delta E^*_u = 3.0$ kcal/mol). In this case, 150 (= 15 × 10) reference conformations are each compared with the set of all possible conformations for all analogues including itself (= 50 × 30) for a total of 225 000 (150 × 1500) pairwise molecular superposition shape comparisons.

E. Quantitative Measures of Molecular Shape. Two descriptors of relative shape similarity were considered. The common overlap steric volume, V_o, between each analogue u in the data set and the reference compound v was determined as

$$V_o = V_u \cap V_v \tag{3}$$

where V_x represents the spatial occupancy of compound x.[3,4,9] V_o was computed by using a numerical integration scheme in CHEMLAB-II[5] and is a measure of how similar in shape the analogues are to the reference compound.

The other shape similarity descriptor considered in this investigation is given by

$$f_o = \frac{V_u \cap V_v}{V_u} \tag{4}$$

where f_o is a relative measure of how similar in shape the reference compound is to each analogue in the data set.[4]

(7) Hopfinger, A. J. *Conformational Properties of Macromolecules*; Academic Press: New York, 1973.
(8) Pople, J. A.; Beveridge, D. C. *Approximate Molecular Orbital Theory*; McGraw-Hill: New York, 1970.
(9) Hopfinger, A. J. *J. Am. Chem. Soc.* **1980**, *120*, 7196.

Table II. Importance of the Shape Reference Compound v on the Correlation of Common Overlap Volume with -log IC_{50} by R^2 Comparison[a]

compd	R^2	compd	R^2	compd	R^2
2	0.00	20	0.18	36	0.10
4	0.06	21	0.11	37	0.25
6	0.00	22	0.14	38	0.29
7	0.03	23	0.19	39	0.04
8	0.07	24	0.24	40	0.30
9	0.11	25	0.00	41	0.23
10	0.11	26	0.06	42	0.15
11	0.16	27	0.12	44	0.18
12	0.00	28	0.01	45	0.36
13	0.13	29	0.18	46	0.25
14	0.11	30	0.13	48	0.34
15	0.20	31	0.11	49	0.25
16	0.15	32	0.27	50	0.18
17	0.03	33	0.13	51	0.47
18	0.18	34	0.31	52	0.30
19	0.06	35	0.19		

[a] Terms are significant at the 0.05 level except for those compounds which have an $R^2 < 0.06$. These results are linear with respect to shape; a quadratic expression for compound 51 gives an $R^2 = 0.62$.

V_o and f_o do not contain information regarding the intramolecular stability of each conformation of the pair of compounds from which they are derived. Loss in conformational stability in order to realize particular measures in V_o and/or f_o was taken into account by defining the shape commonality index, I_c

$$I_c = S(u,v,w) - \left\{ \frac{\Delta E_u}{[\Delta E^*_u(\Delta E^*_u + \Delta E^*_v) + \epsilon]^{1/2}} + \frac{\Delta E_v}{[\Delta E^*_v(\Delta E^*_u + \Delta E^*_v) + \epsilon]^{1/2}} \right\} \quad (5)$$

where $S(u,v,w)$ is wV_o or wf_o, and ΔE_x is the difference in conformational energy between the global minimum and the conformation used to compute V_o or f_o, for analogue x = u and/or v.[10] The parameter w is a weighting factor between shape similarity and loss in intramolecular conformational stability. The MSA–QSAR is optimized as a function of w. If w is small, conformational stability is important to the QSAR. If w is large, intramolecular conformational energetics should have minimal effects on biological activity.

As already reported, an upper limit on ΔE_v, $\Delta E^*_v = 1.0$ kcal/mol, was imposed for the shape reference compound v. Upper limits on ΔE_u of $\Delta E^*_u = 1$, 3, and 6 kcal/mol were placed on the test analogue u in three separate studies.

F. Other Molecular Descriptors. In addition to molecular shape, other molecular properties were considered in the construction of the QSAR. The descriptors used in trial QSARs are described below.

Lipophilicity. The lipophilicities of the compounds were determined by the Medicinal Chemistry Project (Pomona College) program CLOGP.[11] The descriptor π_0 is the lipophilicity of the whole molecule, and π_4 is the water/octanol fragment constant of the substituent in the 4-position of the phenyl ring. A consistent fragment value for the heterocyclic ring was not available in the CLOGP program library and is not included in the reported lipophilicity values. The actual π_0 are lower than the values given in Table II by approximately 1.5 units as determined by comparing the measured values by Kruse et al.[1] (for six compounds) to those calculated by CLOGP.

Partial Atomic Charges. The partial atomic charges were computed by using the CNDO/2 method.[8] Combinations of atomic charges were considered in the trial MSA-QSARs. In particular, the descriptor $Q_{3,4,5}$, representing the sum of the charges on C_3, C_4, and C_5, the meta and para phenyl carbons, was of special interest. The sum of the charges, $Q_{3,4,5}$, correlates with F_{345} in eq 1 such that $R^2 = 0.5$. The charge descriptor Q_6 is the charge on the ortho carbon at position 6 on the phenyl ring. The values of $Q_{3,4,5}$ are given in Table I.

Dipole Descriptors. The dipoles of the molecules were calculated by the CNDO/2 method.[8] If the molecules are aligned with respect to a common frame of reference as in the MSA, then the dipoles will be different at every conformation of (ϕ_1,ϕ_2) and can be dependent upon the rotation of the flexible side chains. The dipoles were compared among the molecules in two ways. The dipole can be parsed into **x**, **y**, **z** vectors and each of these used as separate molecular descriptors. Alternatively, the most active compound can be assumed to have the optimum dipole moment. Other dipoles can be compared to this dipole by describing the angle between the two dipoles and/or their relative magnitude. Those compounds with a rotatable OH moiety had the dipole calculated for rotations between 0° and 90° at 30° increments, where 0° is in the plane of the phenyl ring and 90° is perpendicular to that plane. Each of these dipole representations was used as a separate molecular descriptor.

Entropy Descriptor. The conformational entropy, S, was calculated from the conformational partition function, Q, of each molecule as given by the CHEMLAB-II option INTRADAT (population selection)[6] at 298 K and used as a descriptor.

G. Construction of Trial QSARs. Trial QSARs were generated by using the multidimensional linear regression analysis facilities in a SAS software package.[12] In each case the cross-correlation descriptor matrix was examined to eliminate trial QSARs in which pairs of descriptors had cross-correlation coefficients greater than 0.50. Analogues were considered outliers and removed from the regression analysis when the difference in predicted and observed activities exceeded 2.5 standard deviations from the mean.

The methods described above were first applied to a subset of the 52 compounds reported by Kruse et al.[1] Only 17 compounds comprised this subset. However, these compounds were carefully selected to include very active, inactive, and moderately active inhibitors which, as often as possible, had the same substituent groups, but at different positions, on the benzyl ring. The reason for constructing and first analyzing this analogue subset was to streamline the MSA. Conformational analyses and molecular shape comparisons are labor intensive. Thus, we took the view that it would be most efficient to first develop a trial MSA–QSAR on a subset of compounds which spans the entire range in inhibition potency and then extend it to the remainder of the analogues in the data set.

Results

(1) SAR Database. Forty-seven of the 52 compounds from the Kruse et al. database were selected for the QSAR analysis. Five were not included in the study for the following reasons: two had longer chains than all of the others, two had substituents with high conformational

(10) Hopfinger, A. J.; Burke, B. J. In *Quantitative Structure–Activity Relationships in Drug Design*; Fauchere, J. L., Ed.; Alan R. Liss: New York, 1989; p 151.

(11) Medicinal Chemistry Software, *MedChem Software Manual*, Release 3.51; Pomona College: Claremont, CA, April, 1987.

(12) SAS Institute, Inc. *SAS User's Guide: Basics*, SAS Release 5.18; SAS Institute Inc.: Cary, NC, 1986.

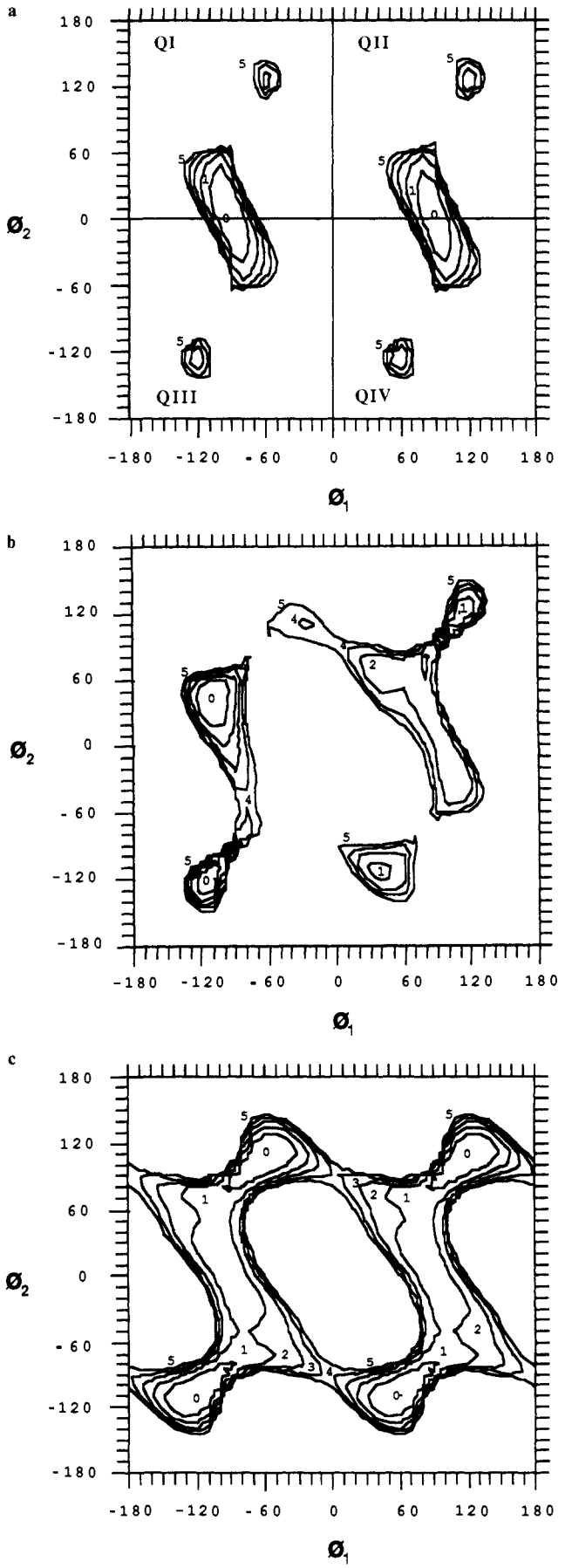

Figure 2. Conformational energy maps of three DβH inhibitors with MMFF potentials: (a) diortho-substituted analogue, X = 2,6-Cl_2, -log IC_{50} = 3.15; (b) monoortho-substituted analogue, X = 2,5-Cl_2, -log IC_{50} = 4.01; and (c) nonortho-substituted analogue, X = 3,5-Cl_2, -log IC_{50} = 5.62. Quadrants are listed in part a.

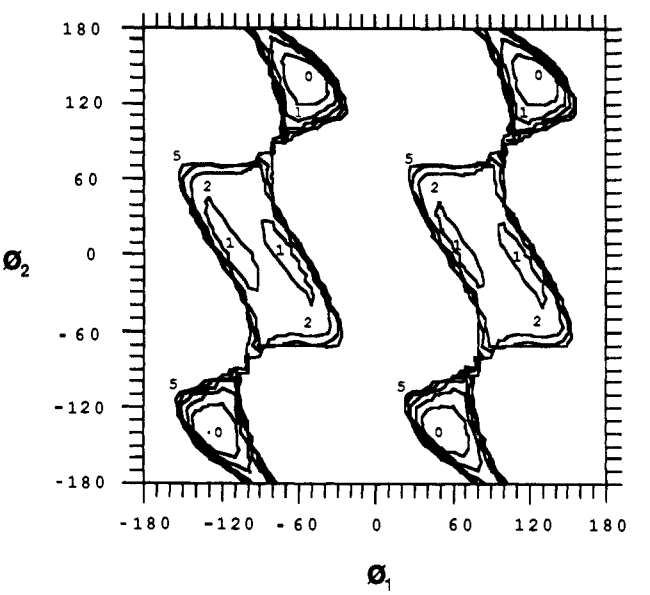

Figure 3. Conformational energy map of diortho-substituted analogue (X = 2,6-Cl_2) generated with Hopfinger potential set instead of MMFF potential set (see Figure 2a).

flexibility, and one was potentially a charged species. Each of these classes of substituents represent possible complications to the application of MSA. Since over 90% of the original database could be analyzed, we opted to delete these five potentially problematic analogues. The 47 selected compounds cover a range of activity of 4.13 log ($1/IC_{50}$) units or 13 490 molar concentration units.

(2) Conformational Analyses. The conformational profiles of the 47 analogues can be characterized by three conformational (ϕ_1,ϕ_2) energy maps shown in Figure 2. The three maps represent analogues having diortho substituents (2,6-Cl_2), Figure 2a, monoortho substitution (2-Cl), Figure 2b, and nonortho substituents (4-Cl), Figure 2c. The three maps of Figure 2 were generated with the MM2 nonbonded potentials. To facilitate the presentation of the results, we divide the maps into four quadrants as defined in Figure 2a. All three maps have common conformational energy minima in quadrants II and III at energies within one kcal/mol of the global minimum. Quadrant IV contains a relatively small space where minima are found in all three maps. However, the minima of the mono- and diortho compounds are relatively high energy conformers (about 3 kcal/mol above the global minimum). Locations of conformational energy minima in quadrant I vary from map to map.

The sensitivity of conformational stability and, ultimately, fidelity of the corresponding QSAR to force field representation was also explored. The nonbonded potential set of Hopfinger,[7] which is a "soft" set of potentials[13] relative to the MM2 potentials, was also used in the conformational analyses. Figure 3 is the energy map for the 2,6-Cl_2 analogue generated with Hopfinger potentials, so that this map is the comparison equivalent to the map in Figure 2a. These two conformational energy maps are quite similar with the two major differences being (1) small shifts in the precise location of minima and (2) greater allowed conformational flexibility for the map generated with the Hopfinger set of potentials.

(3) Shape Reference Compound and Active Conformation. The compounds noted by an asterisk in Table I were considered as candidates for the shape reference

(13) Crawford, R. J.; Pearlstein, R. A.; Mabilia, M.; Hopfinger, A. J. *Tetrahedron Comput. Methodol.* **1989**, *1*, 185.

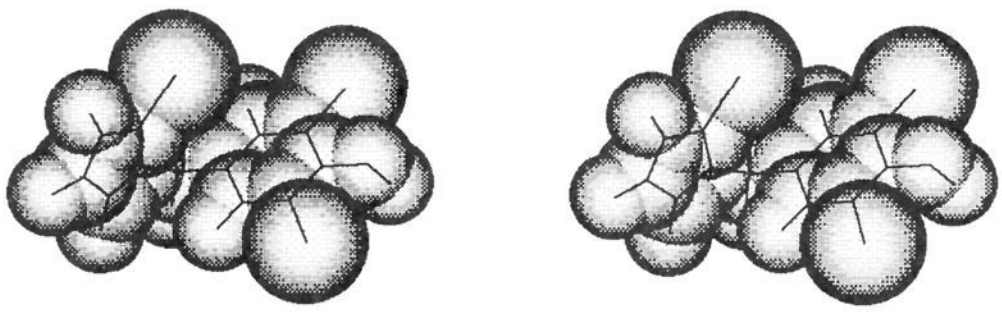

Figure 4. A space-filling stereo representation of compound **51** (X = 3,5-Cl_2, 4-OH) for (ϕ_1,ϕ_2) = (−120°,−120°).

compound v. The optimum QSAR was found with compound **51**, the X = 3,5-Cl_2, 4-OH analogue. The shape reference compound from past investigations has usually been either the most active or largest analogue in the data set. Compound **51** is the second most active analogue and one of the largest. The conformation of compound **51** as the shape reference compound corresponds to (ϕ_1,ϕ_2) = (−120°,−120°), which is shown in Figure 4. This conformation is close to (0.3 kcal/mol) the global energy minimum for compound **51**. It must be stressed that (ϕ_1,ϕ_2) = (−120°,−120°) is not necessarily the "active" conformation since inactive analogues can adopt this conformation as a stable minimum-energy state. Rather, the shape reference compound conformational state corresponds to the conformer that optimizes the QSAR from the set of conformations available to the shape reference compound.

The results of directly regressing common overlap volume, V_o, *against* −log IC_{50} *using different shape reference* candidates for the analogues in Table I are reported in Table II. It is clear from Table II that choice in the shape reference compound can markedly alter the common overlap volume–inhibition potency correlation.

(4) QSARs. The optimum QSAR in terms of the *F*-statistic measure is

$$-\log IC_{50} = -117.4\ (\pm 21.9)V_o + 70.4\ (\pm 12.3)V_o^2 + 2.33\ (\pm 0.51)Q_{3,4,5} + 52.12 \quad (6)$$

$N = 45$, $R = 0.90$, $F = 57.4$, $S = 0.41$, $\Delta E^*_u = 6$ kcal/mol

shape reference compound = **51** *of Table I*

where V_o is defined by eq 4 and $Q_{3,4,5}$ is the sum of the charge densities on C_3, C_4, and C_5 (see I). Equation 6 states that inhibition potency can be increased by analogues which have the steric shape of compound **51**, as shown in Figure 4, resulting from substituents which make −(−C_3–C_4–C_5–)− a positive-charge domain. There are two outliers for eq 6. Compound **39** is an outlier unless lipophilicity is included in the QSAR. Compound **23** is the second outlier. Predicted and residual (Δ −log IC_{50}) inhibition potencies are given in Table I along with V_o and $Q_{3,4,5}$ values. A plot of observed versus predicted −log IC_{50} values, using eq 6, are shown in Figure 5.

The stability of eq 6 with respect to selection of ΔE^*_u and shape reference compound conformation can be gleaned from Table III. Correlation coefficients, *R*, for QSARs of the form of eq 6 are reported for various choices in (ϕ_1,ϕ_2) for compound **51** and for ΔE^*_u = 1, 3, and 6 kcal/mol. It is seen that the variation in the *R* is quite minimal for changes in (ϕ_1,ϕ_2) and for choice of ΔE^*_u. This, in turn, suggests that eq 6 is quite reliable with respect to these variables.

We also considered the effect of loss in intramolecular stability in order to achieve a particular measure of V_o using eq 5. A surprise finding is that any attempt to penalize the shape fit, V_o, *for loss in intramolecular stability decreases* the significance of the corresponding QSAR relative to eq 6. Possible implications of these findings are presented in the Discussion Section.

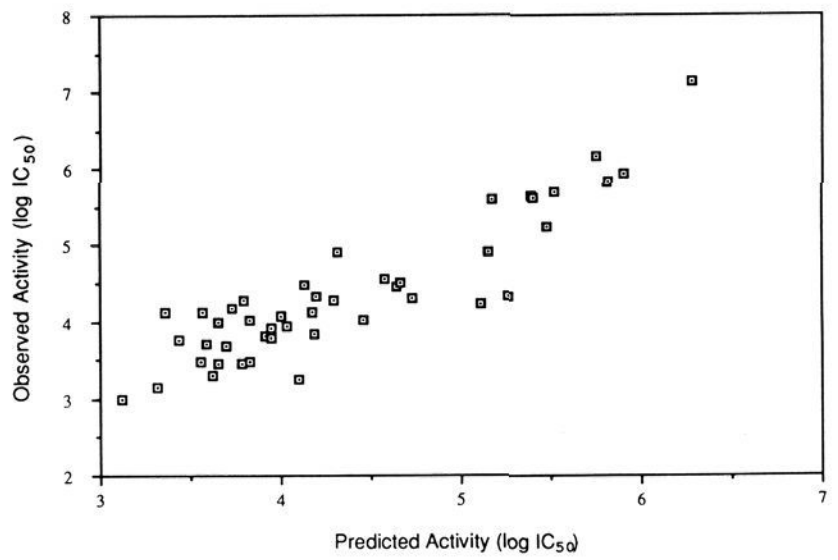

Figure 5. A plot of observed activity (−log IC_{50}) vs predicted activity (−log IC_{50}) as predicted by eq 6.

Table III. Importance of Conformation of Shape Reference Compound v on the Correlation of Common Overlap Volume with −log IC_{50} by R^2 Comparison[a]

conformation (ϕ_1,ϕ_2)	R^2 ΔE = 6 kcal/mol	ΔE = 3 kcal/mol	ΔE = 1 kcal/mol
(50,−120)	0.963	0.961	0.950
(60,−110)	0.968	0.964	0.957
(70,−100)	0.965	0.948	0.930
(80,−90)	0.961	0.950	0.924
(40,−110)	0.949	0.941	0.904
(50,−100)	0.964	0.925	0.908
(60,−120)	0.964	0.965	0.968
(−120,−120)	0.963	0.964	0.967

[a] Compound **39** removed for this calculation, n = 16. All individual terms significant at 0.05 level or better.

Stability of the QSARs of the form of eq 6 are also relatively independent of choice of force field used in the conformational analyses. The most notable effect of force field is on location in (ϕ_1,ϕ_2) space of the conformations used for the u and v analogues. Generally, changes in conformation ($\Delta\phi_1$,$\Delta\phi_2$) as a result of change in force field are small and conserved (constant) over this set of thione inhibitors.

In an attempt to assess the role of lipophilicity on DβH inhibition, we considered lipophilicity terms in the regression fits. The resulting optimum QSAR for 47 compounds with respect to the correlation coefficient *R* is

$$-\log IC_{50} = -119.6\ (\pm 22.8)V_o + 70.6\ (\pm 12.6)V_o^2 + 2.09\ (\pm 0.51)Q_{3,4,5} - 4.63\ (\pm 1.59)Q_6 + 0.0460\ (\pm 0.0108)\pi_0^2 - 0.595\ (\pm 0.168)\pi_4 + 53.38 \quad (7)$$

$N = 47$, $R = 0.91$, $F = 33.6$, $S = 0.39$, $\Delta E^*_u = 6$ kcal/mol

shape reference compound = **51** of Table I

In eq 7, Q_6 is the partial charge on C_6, π_0 is the sum of the π constants for the aromatic ring and substituents,[11] including hydrogens, and π_4 is the π constant for C_4 sub-

stituents.[14] The three additional terms in eq 7, although statistically significant, only marginally enhance the overall quality of the QSAR. Compound **39** (2,5-Cl_2) is no longer an outlier in the model, but it is replaced by compound **17** (2-OMe) as an outlier, while compound **23** (3-OH) also remains an outlier. If we delete compounds **17** and **23**, we get an equation virtually identical with 7 with the following statistics of fit: $N = 45$, $R = 0.94$, $F = 52.1$, $S = 0.32$, and $\Delta E^*_u = 6$ kcal/mol with **51** as the shape reference compound. There are no obvious reasons why any of these compounds should be outliers within the framework of the MSA–QSAR analyses described here.

Discussion

A comparison of eq 1 and 6 demonstrates that the use of V_o reduces the number of independent descriptors needed to quantitatively describe the SAR. Equation 6 includes only V_o and $Q_{3,4,5}$, two less descriptors than eq 1, has a similar R value, better F statistic, and a smaller standard deviation of fit than eq 1. In addition, eq 6 is a QSAR for 45 analogues, while eq 1 spans only 25 compounds.

V_o is a general descriptor with respect to type and location of substituents on the phenyl ring. This is in contrast to the descriptors of eq 1, which are limited to substituents on the 3-, 4-, and 5-positions. Thus, the QSAR expressed by eq 1 does not provide information about ortho- and diortho-substituted analogues. The use of V_o in construction of a QSAR for the DβH inhibitors of Table I eliminates the need for an indicator variable and provides physicochemical information about the ligand–receptor interaction.

Application of MSA, as represented by the QSAR of eq 6, was successful in accounting for the inhibition potency of X = 4-CF_3, which is an outlier to eq 1, and in accurately explaining the activity of low-potency 3,4,5-substituted analogues, many of which are problematic when using eq 1.

Equation 7, which can be viewed as an extended MSA–QSAR to eq 6, includes a lipophilicity term which brings it closer in form to eq 1. However, the contribution of lipophilicity to the MSA–QSAR is less important (partial $R^2 \leq 0.08$) than for eq 1 (partial $R^2 = 0.21$).

Perhaps the most interesting finding in this study, as it relates to the MSA formalism, is the "active" conformation. In previous MSA studies the hypothesized active conformation corresponded to that conformer state for the analogues whose intramolecular stability decreases as biological activity decreases. However, for the 1-(substituted-benzyl)imiazole-2(3*H*)-thione DβH inhibitors, we conclude the arrangement of the substituents on the phenyl ring, that is the shape of the molecule due to its *configuration*, overrides conformational effects due to torsion angles ϕ_1 and ϕ_2 in specifying activity. Of course, conformation is important in that only a limited set of low-energy conformational states are available to a particular configuration. Still, both active and inactive thione analogues can energetically adopt the postulated "active" conformation.

The possible active conformations were arrived at by determining which set of V_o, as a function of (ϕ_1,ϕ_2) and ΔE^*_u, yields the optimum MSA–QSAR. Low-energy conformers in quadrants II and III, see Figure 2a, are the best active-conformation candidates. Quadrants II and III comprise two sets of conformers which are enantiomeric and are not distinguishable in MSA. We arbitrarily decided to use conformers in quadrant III in this investigation. There is a range in (ϕ_1,ϕ_2) conformer states which satisfy the ΔE^*_u constraints yet yield very significant QSARs. This is demonstrated by the data in Table III. Overall, we cannot postulate the active conformation with respect to (ϕ_1,ϕ_2) with confidence.

In distinguishing between quadrants III and IV, we prefer conformations in quadrant III. The main difference between the conformations in quadrants III and IV is that, in order to adopt the maximum shape similarity, some medium active compounds must adopt conformations which must be destabilized by 2 to 3 kcal/mol in quadrant IV. The same common overlap volume can be adopted for those compounds at much lower corresponding destabilization energies in quadrant III [compare quadrants III and IV in Figure 2b]. In particular, $(\phi_1,\phi_2) = (-120°,-120°)$ is selected for the active conformation because the destabilization energies are minimized for the compounds and, additionally, the shape reference compound **51** satisfies the $\Delta E^*_v = 1$ kcal/mol constraint. Since compound **51** is very active, it is reasonable to postulate it will express this activity in a conformation close to its intramolecular global minimum energy state.

The use of an alternate set of nonbonded potentials, namely the soft set of Hopfinger,[7] does not alter the form or the quality of the MSA–QSARs. Said another way, the MSA–QSARs are not dependent upon force field parameterization. The two sets of low-energy conformational states for the two potentials fall within the range of plausible active conformation candidates. Thus, neither set of potentials can resolve selection of the active conformational state.

Table III also indicates that the MSA–QSARs are not particularly sensitive to ΔE^*_u. Taken at face value, this finding suggests that inhibition potency is not too dependent upon the conformational stability of the ligand. Even when the maximum allowed destabilization, ΔE^*_u, is 6 kcals/mol, the contribution of the energy penalty function of eq 5 appears to have a minimal effect on the QSAR fitting. A close analysis indicates that this observation may, at least in part, be a consequence of the compounds in the database and the use of regression analysis. The ligands most sensitive to ΔE_u are the very inactive *diortho* analogues, which are relatively few in number. For these compounds, an increase in S(u,v,w) is offset by the energy pentalty function to maintain a constant I_c value. At the same time, the remainder of the analogues in the SAR can adopt conformations that maximize S(u,v,w) at little expenditure of ΔE_u. Moreover, the S(u,v,w) of these analogues are relatively constant over a range of low-energy (ϕ_1,ϕ_2) states. Thus, I_c is essentially independent of ΔE_u for the medium and highly active inhibitors. Overall, this gives the appearance of the QSAR being independent of ΔE_u.

Interestingly, in contrast to this investigation, in a previous study of 27 2,4-diaminotriazine inhibitors of dihydrofolate reductase,[10] the selection ΔE^*_u was critical to the significance of the QSAR.

Overall, the shape commonality index, I_c, can provide a means of determining the balance between ligand molecular shape and ligand conformational stability on inhibition potency. In so far as ligand molecular shape can be construed as a representation of ligand-receptor binding, I_c can be viewed as a composite measure of ligand stability and ligand binding potential. The inclusion of I_c into MSA overcomes many of the restrictions and limitations of comparing the shape similarity among *flexible* molecules.

(14) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.

A major thrust in future research will be to further explore and define the shape commonality index.

Acknowledgment. We thank Kathy Rowberg of our laboratory for her help with the statistical package from SAS. B.J.B. was a University Fellow at the University of Illinois at Chicago for 1987-1988 academic year and is a Fellow of the American Foundation of Pharmaceutical Education (AFPE). Funds from the Laboratory of Computer-Aided Molecular Modeling and Design here at UIC were used in support of this work. Molecular graphics were by the Molecular Viewer program created by John Nicholas.

Registry No. 1, 105763-92-6; 2, 105763-93-7; 3, 105763-94-8; 4, 95333-69-0; 5, 105763-95-9; 6, 105763-96-0; 7, 95333-70-3; 8, 105763-97-1; 9, 105763-98-2; 10, 105763-99-3; 11, 95333-91-8; 12, 95333-90-7; 13, 105764-00-9; 14, 105764-01-0; 15, 95333-88-3; 16, 105764-02-1; 17, 95333-53-2; 18, 105764-03-2; 19, 95333-51-0; 20, 101913-05-7; 21, 95460-09-6; 22, 95333-54-3; 23, 95333-50-9; 24, 95333-61-2; 25, 95333-79-2; 26, 95333-71-4; 27, 95333-72-5; 28, 95333-59-8; 29, 95333-62-3; 30, 105764-04-3; 31, 95333-73-6; 32, 95333-52-1; 33, 95333-74-7; 34, 105764-05-4; 35, 104197-16-2; 36, 23269-10-5; 37, 95333-58-7; 38, 95333-75-0; 39, 95333-76-9; 40, 95333-48-5; 41, 95333-77-0; 42, 95333-80-5; 43, 95333-81-6; 44, 95333-64-5; 45, 95333-78-1; 46, 95333-66-7; 47, 95333-78-1; 48, 95333-68-9; 49, 95333-49-6; 50, 985333-81-6; 51, 95333-57-6; 52, 95333-60-1; dopamine β-hydroxylase, 9013-38-1.

Tertiary 2-Haloethylamine Derivatives of the Muscarinic Agent McN-A-343, [4-[[*N*-(3-Chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium Chloride

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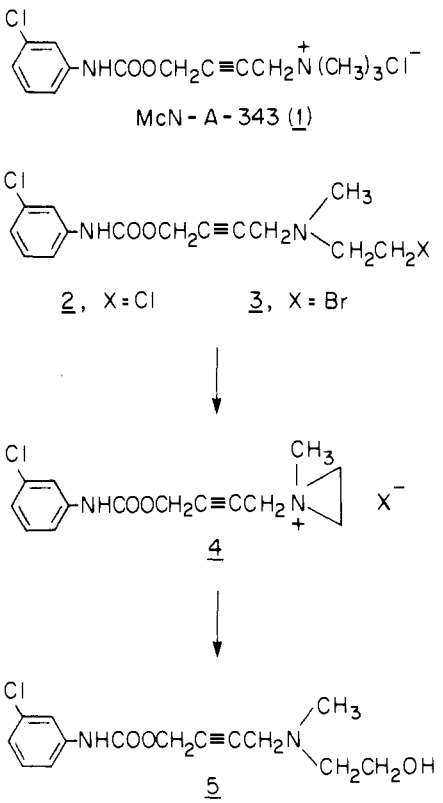
4-[(2-Chloroethyl)methylamino]-2-butynyl *N*-(3-chlorophenyl)carbamate (**2**) and 4-[(2-bromoethyl)methylamino]-2-butynyl *N*-(3-chlorophenyl)carbamate (**3**) were synthesized. Compounds **2** and **3** cyclized at neutral pH to an aziridinium ion (**4**). The rate constants for the cyclization of **2** and **3** at 37 °C were about 0.01 and 0.4 min^{-1}, respectively, as measured by titrimetric analysis and by ^1H NMR spectroscopy. The aziridinium ion had $^1/_4$ the potency of McN-A-343 (**1**) as a ganglionic muscarinic stimulant in the anesthetized, pentolinium-treated rat but showed no muscarinic effects on the isolated guinea pig ileum. It caused alkylation of muscarinic receptors in homogenates of the rat cerebral cortex. An irreversible blockade of central muscarinic receptors was also observed after intravenous administration of **3** to mice. Because of its selectivity, irreversible actions, and ability to pass into the central nervous system, **3** should become a valuable tool in studies of muscarinic receptors.

McN-A-343, 1 (Scheme I), was first described by Roszkowski.[1] Like many other muscarinic agents, such as muscarine and oxotremorine, 1 is potent in stimulating muscarinic receptors in autonomic ganglia. The unique feature of 1 is its relatively weak muscarinic actions outside ganglia, as for example in the isolated heart and on intestinal smooth muscle.[1,2] Many analogues of 1 have been synthesized, but only a few of these possess selectivity and potency similar to that of 1.[3-7] In particular, no close analogues of 1 capable of passing into the central nervous system are presently known. Since ganglionic muscarinic receptors appear to resemble certain central muscarinic receptors (M1 receptors) in their structural specificity,[8] such analogues have been suggested as potential therapeutic agents in conditions associated with deficits in central cholinergic function, e.g. Alzheimer's disease.[9]

We describe here an *N*-methyl-*N*-(2-chloroethyl)amino derivative (**2**, BR 383) and an *N*-methyl-*N*-(2-bromoethyl)amino derivative (**3**, BR 384) which are capable of cyclizing to an aziridinium ion (**4**) closely resembling 1. This aziridinium ion maintains the selectivity shown by 1 for ganglionic muscarinic receptors. We also show that **3** enters into the central nervous system after systemic administration and that **4** interacts covalently with central muscarinic receptors.

Scheme I



Results

Synthesis of 2 and 3. Amino alcohol **5** was obtained in a Mannich reaction from 2-propynyl *N*-(3-chlorophenyl)carbamate, paraformaldehyde, and *N*-methyl-*N*-(2-hydroxyethyl)amine. 2-Chloroethylamine **2** was pre-

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0022-2623/90/1833-0281$02.50/0