Carbamyl Analogues of Potent Nicotinic Agonists: Pharmacology and Computer-Assisted Molecular Modeling Study

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To investigate how the substitution of NH_2 for CH_3 affects the activity of three, potent, semirigid nicotinic agonists, carbamyl analogues were synthesized. The carbamyl agonists were 1-methyl-4-carbamyl-1,2,3,6-tetrahydropyridine methiodide (1), 1-methyl-4-carbamylpiperidine methiodide (2), and 1-methyl-4-carbamylpiperazine methiodide (3). Their potencies (reciprocals of the equipotent molar ratios) at the frog neuromuscular junction with reference to carbamylcholine were 0.77, 0.052, and 0.15, respectively. The acetyl analogues were more potent by factors of 65, 175, and 17, respectively. Explanations for this variable reduction in activity were sought by using computer-assisted molecular mechanics and calculations of electrostatic potential contours. Bioactive conformations of 1–3 were assigned on the basis of a well-supported pharmacophore and the ground-state conformation of the highly potent (50 times that of carbamylcholine) prototype, isoarecolone methiodide (4). Agonist 3 and its acetyl analogue superimposed closely in their ground-state, bioactive conformations, and the differences in their electrostatic potential contours were the least among the three pairs. Accordingly, their potencies differed the least. Agonists 1 and 2 both showed greater differences (with respect to their acetyl analogues) in their electrostatic potential contours and greater differences in potency. Agonist 2, in addition, could achieve the bioactive conformation only at the expense of 2.8 kcal mol^{-1}, and, correspondingly, its activity relative to its acetyl analogue was lowest of all.

The notion of a pharmacophore, the three-dimensional configuration of essential atoms in space that induces a pharmacological response, is of major influence in rational medicinal chemistry. Knowledge of a pharmacophore provides one with a model for constructing useful analogues and may also yield some insight into the mechanism whereby the drug initiates its response.

In 1970 Beers and Reich[1] proposed a pharmacophore for agents active at the nicotinic acetylcholine receptor. Their model consists of a cationic center (usually a quaternary ammonium group) and a hydrogen bond acceptor (usually a carbonyl oxygen). The distance between these groups (5.9 Å from the N to the van der Waals extension of the O) and (they implied) the angle between the nitrogen atom and the carbonyl bond constitute the pharmacophore. Recently Venkataraghavan et al.,[2] employing an ensemble approach to the distance geometry method, independently derived a pharmacophore with similar distances between the three points, N, C(=O), and O(=C). All nicotinic agonists (active at the neuromuscular junction of voluntary muscle) that bear a hydrogen bond acceptor, such as acetylcholine (in its most stable, gauche conformation[3,4]), fit the pharmacophore (reviewed in ref 3). Furthermore, the pharmacophore has proven useful in predicting new, active agonists, such as isoarecolone methiodide (4). Agonist 4 is one of the most potent nicotinic agonists tested at the frog neuromuscular junction, and its ground-state conformations (two of equal energy) conform to the Beers–Reich pharmacophore.[5,6]

Beers and Reich suggested that, "... the role of the carbon skeleton is simply to provide proper stereochemical localization of the functional groups which determine specificity".[1] We described[6] seven analogues of 4, all of which could easily reach a conformation corresponding to the pharmacophore with ambient thermal energy, but whose activities spanned a range of almost 10^4. Clearly, this pharmacophore, while probably necessary, is insufficient. Computer-assisted molecular modeling suggested explanations for the ranking of potency. The factors included small departures from optimal conformation, deviations in electrostatic potentials about the cationic head and in the vicinity of the carbonyl group, and the presence of a group (methyl or trifluoromethyl) α to the carbonyl group.[6]

In this paper we describe three more analogues of 4 in which an amino group substitutes for the methyl group α to the carbonyl carbon (Figure 1). Each of these carbamyl agonists is then compared to its acetyl analogue. The NH_2 substitution has the advantages of confining the modifications chiefly to the vicinity of the carbonyl group and of being nearly isosteric with the methyl group it replaces. This complication that arises with this substitution, however, is that the NH_2 group can donate a hydrogen bond.

Chemistry

Syntheses. The sodium borohydride reduction of quaternary pyridines to yield the corresponding 1,2,3,6-tetrahydropyridines was described by Lyle et al.[7]

Computer-Assisted Molecular Modeling. Details of the programs, parameterization, and strategy have been given previously.[5,6,8] An abbreviated version follows.

Molecular modeling studies employed an Evans and Sutherland PS330 color vector graphics terminal coupled to a VAX 11/785 computer. Minimum-energy conformations were calculated with a revised version of Allinger's[9] MM2 program, modified by T. Halgren[10] to handle

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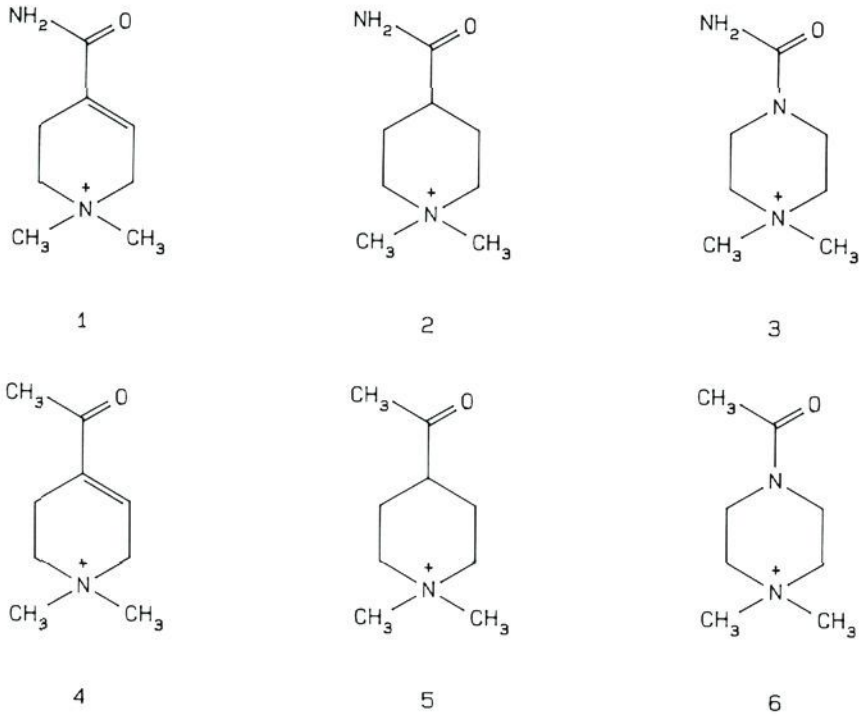

Figure 1. Structures of the nicotinic agonists.

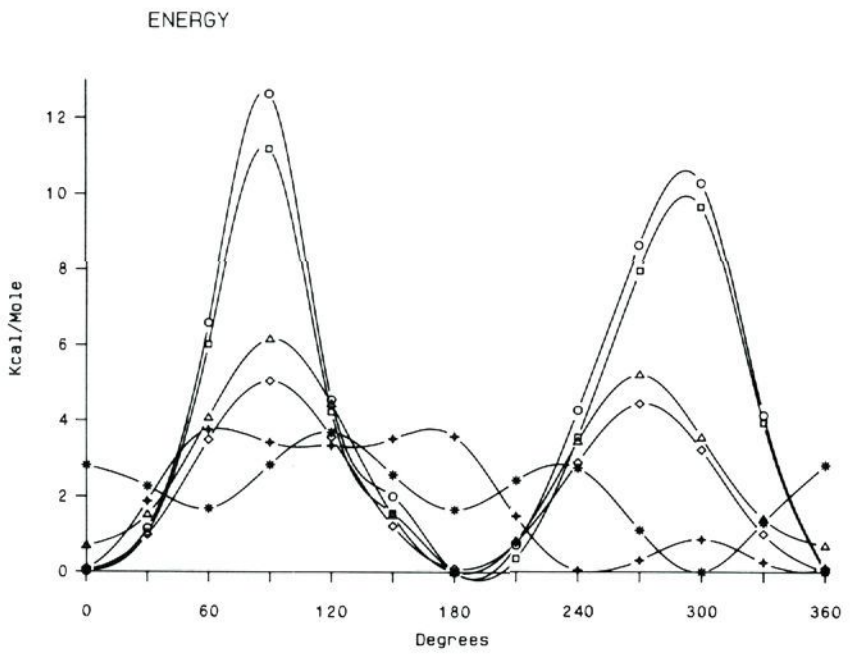

Figure 2. Energy vs dihedral angle for compounds **1** (diamond), **2** (star), **3** (square), **4** (triangle), **5** (cross), and **6** (circle). A dihedral angle of 0° is defined such that the carbonyl oxygen and position 3 of the ring are coplanar.

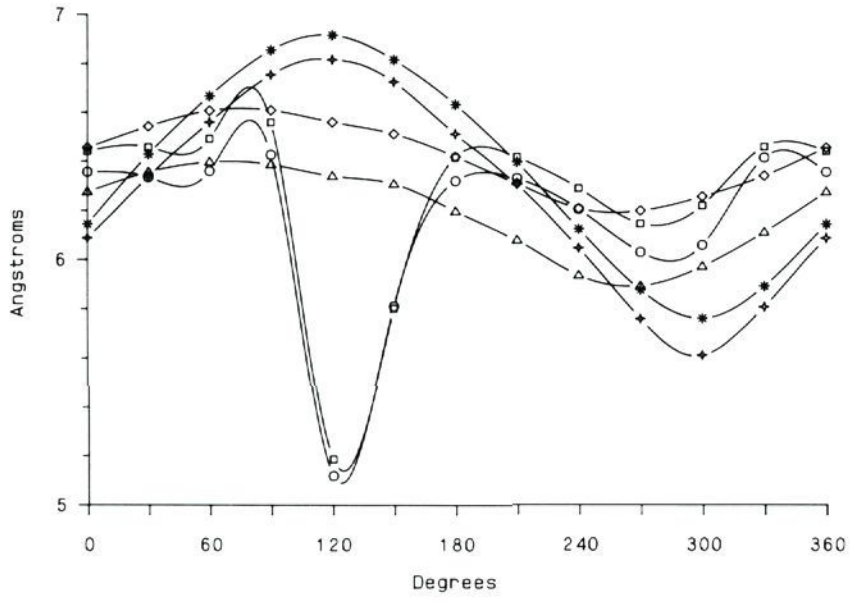

Figure 3. Distance between the nitrogen atom and the van der Waals extension of the oxygen as a function of dihedral angle for agonists **1** (diamond), **2** (star), **3** (square), **4** (triangle), **5** (cross), and **6** (circle).

formally charged molecules and parameterized for ammonium salts in collaboration with J. Snyder.[11] Amide

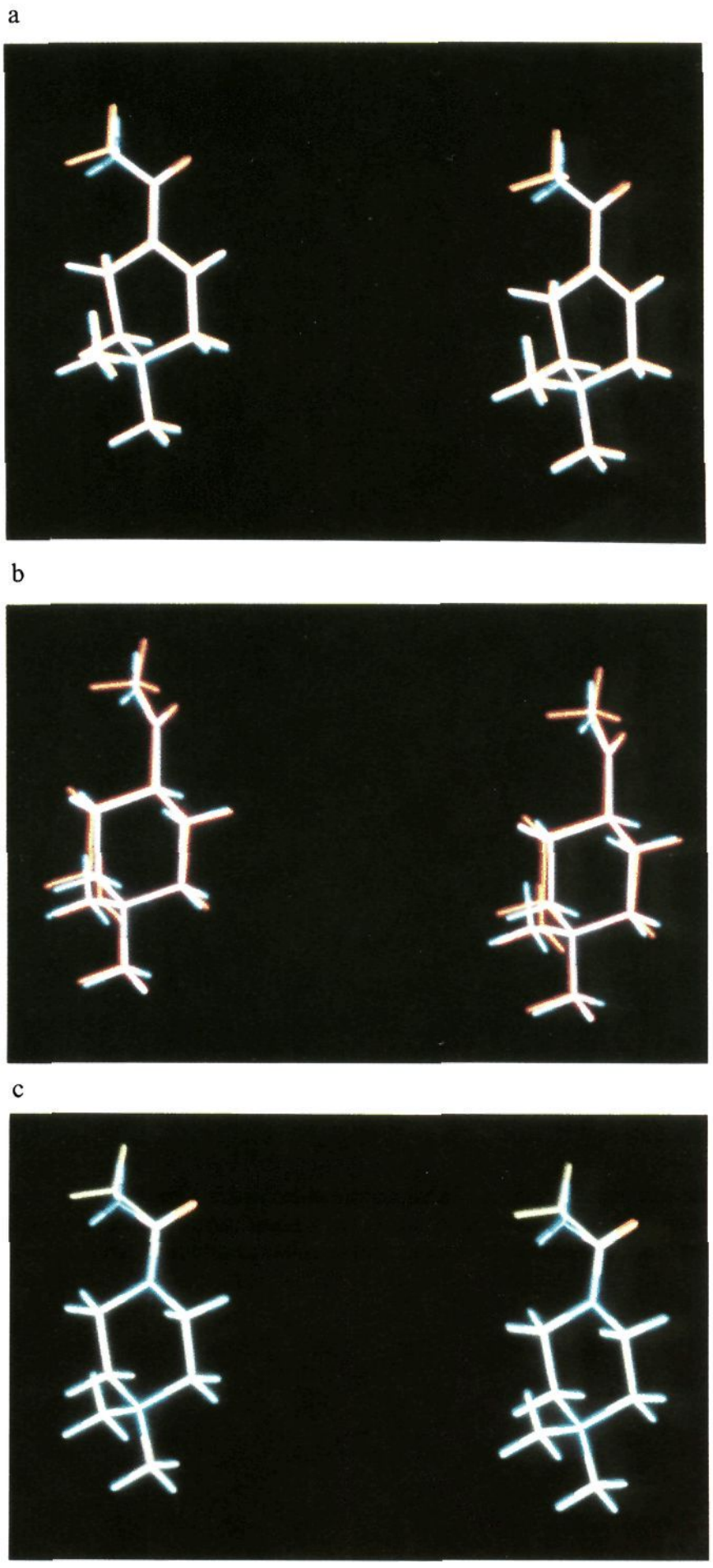

Figure 4. Stereo drawings of each carbamyl agonist superimposed on its acetyl analogue. All agonists are shown in their presumed bioactive conformations. (a) Superposition of agonist **1** and **4** (orange). (b) Superposition of agonist **2** and **5** (red). (c) Superposition of agonist **3** and **6** (yellow).

parameters were obtained from G. Marshall.[12] The dihedral driver calculations were carried out with the dihe-

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(12) Marshall, G. Washington University, St. Louis, MO, private communication.

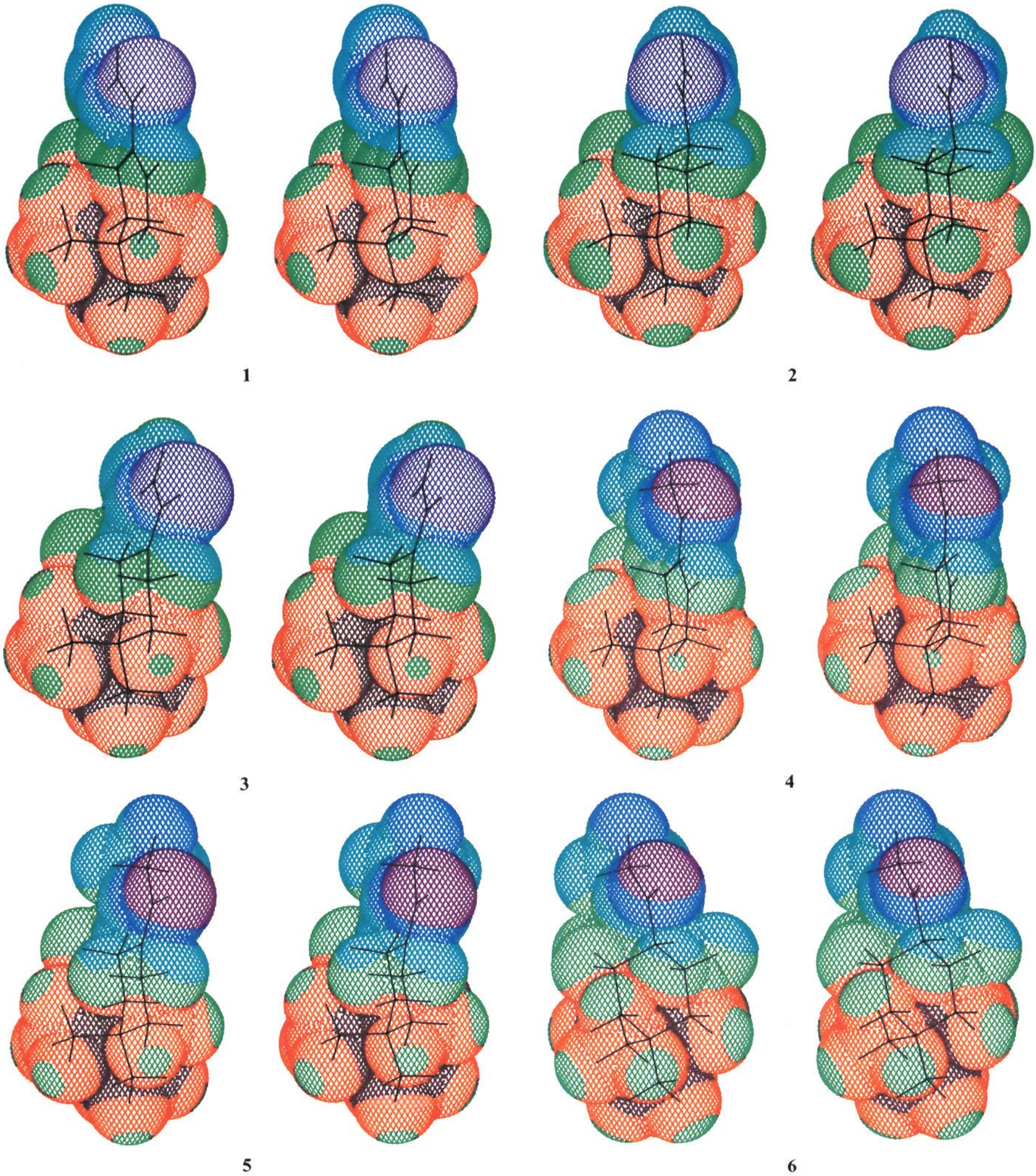

Figure 5. Stereo drawings of electrostatic potentials projected onto the van der Waals surfaces of the agonists. Color code (potentials in kcal/mol): purple (0–40), blue (40–60), turquoise (60–80), green (80–100), orange (100–120), brown (120–140), and red (140–160).

dral facility in MM2. The angle was rotated in 30-deg increments and then held constant while the remaining structure was geometry optimized. The molecules were superimposed, displayed, and manipulated by using the SYBYL[13] and ChemX[14] programs.

After bioactive conformations were assigned (see Results and Discussion), partial charges were calculated with Dewar's MNDO program from MOPAC.[15] The justification for using MNDO instead of more time demanding ab initio methods has been given.[16] The electrostatic potential (ESP) energies on the van der Waals surface were

(13) TRIPOS Associates, St. Louis, MO 63117.
(14) Chemical Design Ltd., Oxford, U.K.

(15) Dewar, M. J. S. University of Texas, Houston, TX. The MOPAC program is available from the Quantum Chemistry Program Exchange.
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Table I. Potencies of the Nicotinic Agonists

agonist	no. of frogs	potency[a]	95% confidence interval
1	5	0.77	0.70–0.85
2	6	0.052	0.041–0.063
3	6	0.15	0.13–0.17

[a] Potency is expressed as the reciprocal of the equipotent molar ratio in reference to carbamylcholine.

derived from the partial charges and a contouring program, ARCHEM.[17] The dielectric constant was set to unity. The points selected for ESP determination were drawn from a uniform distribution on the van der Waals surface. The areas of ESP ranges are determined by counting points within an energy range and multiplying by the area per point. This must be done separately for each atom type, since the area per point is slightly different for each. The areas depend on the atomic radii, which are as follows (Å): C, sp^3 = 1.70; C, sp^2 = 1.74; O = 1.50; N = 1.60; H = 1.30. Electrostatic potentials[18] were calculated in reference to an incoming positive charge. Since these agonists all bear net positive charge, the potentials are repulsive.

Results and Discussion

The potencies of the three carbamyl agonists, in reference to carbamylcholine, are given in Table I; their structures are shown in Figure 1. Each carbamyl compound in the Table corresponds to an acetyl analogue described previously[5,6] (see structures in Figure 1). The acetyl analogues (corresponding to compounds 1–3, respectively), their present compound number, and their potencies are as follows: 1-methyl-4-acetyl-1,2,3,6-tetrahydropyridine methiodide (4, isoarecolone methiodide), 50; 1-methyl-4-acetylpiperidine methiodide (5), 9.1; and 1-methyl-4-acetylpiperizine methiodide (6), 2.6. If the effect of the NH_2 group were constant throughout the series, for example by donating hydrogen bonds to water and thereby raising the energy barrier for desolvation that preceeds binding to the receptor, then the potencies of these carbamyl compounds would all be less than those of the corresponding acetyl compounds by a constant factor, and the rank orders of potencies for the two series would be the same. This expectation was not borne out. Thus, the rank order of the carbamyls was 1 > 3 > 2; that of the acetyls was 4 > 5 > 6. The potency ratios of the acetyl:carbamyl analogues was as follows: 4:1, 65;, 5:2, 175; 6:3, 17. Clearly the effect of replacing CH_3 by NH_2 was not constant. Therefore, intramolecular changes resulting from the substitution were sought by using molecular mechanics and calculations of charge distributions.

Under the assumption that the Beers–Reich pharmacophore is necessary for activity, we first determined whether the agonists could approximate this conformation and at what cost in energy. Since all of these agonists consist of carbamyl groups hinged to six-membered rings, surveying the possible conformations consisted of simply rotating the carbamyl group by using the dihedral driver from MM2. The dihedral angle is the angle between the planes defined (in 4) by C=C—C(O) and by (ring)}C—C=O. Figure 2 illustrates a plot of energy versus dihedral angle. For compounds 1 and 3 the global minima occur at those angles (0 and 180°) that maximize orbital overlap, namely those at which the carbonyl bond lies in the plane defined by the carbonyl carbon, its adjoining ring atom (C or N), and the carbon atom in either the 3- or the 5-position of the ring. Either of these conformations (though only one is likely to be the active one[3]) corresponds to the Beers–Reich pharmacophore (see below). The curves follow patterns of energy similar to those of their respective acetyl analogues (4 and 6). Compound 2, however, has its global energy minimum at 300°. By contrast, the energy minima for its analogue, 5, occurs at 0 and 240°, where its acetyl (tetrahedral) methyl group is gauche to the axial hydrogen of the C4 position of the ring. For 2 to reach the conformation of the pharmacophore (0 and 240°; see below) requires 2.8 kcal mol^{-1} to overcome the steric crowding of the amide NH_2 group by the ring methylenes (at ring positions 3 and 5). This energy penalty, not imposed on 1 or 3, contributes, we believe, to the low activity of 2 in comparison to its acetyl analogue, 5.

The Beers–Reich pharmacophore specifies a distance of 5.9 Å between the quaternary nitrogen and the van der Waals extension of the carbonyl oxygen.[1] A plot of this distance versus dihedral angle is shown in Figure 3. The paragon, 4, has energy wells at 0 and 180°, at which the corresponding Beers–Reich distances are 6.3 and 6.2 Å, respectively. We choose the 0° conformation (s-cis, by analogy to anatoxin a[19–21]) to be the bioactive one. The recently described agonist, pyrido[3,4-b]homotropane (PHT),[22] substantiates this hypothesis. Figures 2 and 3 show that the energy wells for both 1 and 3 also correspond to a favorable Beers–Reich distance (about 6.4 Å), so we assume that the rotamers at 0° are the bioactive conformations for these two agonists. The minimum-energy conformation of 2, however, (300°) corresponds to a Beers–Reich distance of 5.8 Å. Although this distance is close to the Beers and Reich proposal, the fit of this conformer onto 5 is not as good. We assume, therefore, that the bioactive conformation of 2 is the higher energy one, in which the dihedral angle is 0° and in which the Beers–Reich distance is about 6.2 Å (Figure 3). Stereo pairs of the presumed bioactive conformations of 1–3 superimposed on their acetyl analogues (4–6) are shown in Figure 4. The fit of each carbamyl agonist on its corresponding acetyl analogue is nearly perfect. The small deviations are due to a slight reduction in bond length of the new C–N amide bond.

The stereochemical findings can be summarized as follows. The bioactive conformations of the carbamyl agonists, when superimposed on the bioactive conformations of their acetyl analogues, fit nearly perfectly. Agonists 1 and 3 were in a minimum-energy conformation in this superposition, implying that their diminished potency with respect to their analogues must be sought elsewhere. Agonist 2, however, needed 2.8 kcal mol^{-1} to reach this conformation; its activity was consequently reduced with respect to its acetyl analogue.

After the bioactive conformations were derived (above), partial charges were calculated by using MNDO. Electrostatic potentials were then projected onto the van der Waals surface of the molecule by using ARCHEM[17] (Figure 5). Figure 5 shows that the change in the electrostatic picture is most apparent in the vicinity of the carbamyl group: one sees that the region of the NH_2 is distinctly

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Table II. Areas (Å^2) of Electrostatic Potentials on the van der Waals Surfaces

agonist	energy range, kcal/mol						
	0–40	40–60	60–80	80–100	100–120	120–140	140–160
1	12.50	9.38	27.51	44.52	91.52	23.46	0.67
2	12.20	7.40	25.85	60.43	90.40	19.29	0.04
3	14.18	8.42	26.58	37.65	95.10	20.49	0.54
4	7.99	19.24	33.52	45.58	88.39	22.85	0.78
5	7.46	17.14	39.16	63.69	84.52	18.37	0.00
6	11.88	15.09	39.74	44.17	88.36	23.99	0.56

more positive (less area of potential ranges 0–40 and 40–60 kcal/mol, i.e., less of the purple- and blue-coded surfaces) than the corresponding CH_3 (Figure 5 and Table II). Our previous observation, that making this area more negative (in an F_3C derivative) enhanced potency,[6] agrees with the finding that all the carbamyl agonists are weaker than their acetyl analogues. Note that, of the three pairs of agonists, the **3**, **6** pair shows the least difference in the distributions of electrostatic potentials on the carbonyl regions of their van der Waals surfaces (Figure 5 and Table II). Since the potencies of **3** and **6** also differ the least of the three pairs (Table I), this finding reinforces the suggestion that the electrostatic potential in this region influences agonist activity.

The substitution of NH_2 for CH_3 makes the carbamyl analogues more hydrophilic, since the NH_2 groups of amides can donate hydrogen bonds to water. This hydrophilicity, which would disfavor the partitioning of carbamyl agonists from the aqueous phase to the receptor phase, is probably an important, common factor that diminishes the activity of all of these agonists with respect to their less hydrophilic acetyl analogues. Quantitative estimates of this factor for *uncharged* amides compared to methyl ketones range from 1 (ref 23) to 5 (ref 24) orders of magnitude!

Our findings can be summarized as follows: All of these carbamyl compounds have reduced potencies with respect to their acetyl analogues due to a combination of factors including steric, electrostatic, and solvation. The analogues **3** and **6** superimpose in low-energy, bioactive conformations, and their electrostatic potential contours in the vicinities of the carbonyl groups are most similar of the three pairs. Correspondingly, their activities differ the least, by 17-fold. We ascribe this factor, qualitatively common to all the carbamyl agonists, mostly to the greater hydrophilicity of **3**. The bioactive conformations of the pairs **1**, **4** and **2**, **5** superimpose, so we ascribe the further diminished activity of the carbamyl agonists to the more positive electrostatic potential about the carbonyl oxygen. Agonist **2** is further reduced in activity (compared to **5**) because its bioactive conformation is of higher energy (by 2.8 kcal mol^{-1}) than its ground-state conformation.

Experimental Section

Chemistry. Melting points were taken on a Fisher Johns melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1330 spectrophotometer. Elemental microanalyses were performed by Atlantic Microlab, Inc. (Atlanta, GA), and are within 0.07% of the theoretical values. Starting materials for syntheses were purchased from Aldrich Chemical Co. (Milwaukee, WI) or from Chemical Dynamics Corp. (South Plainfield, NJ).

1-Methyl-4-carbamyl-1,2,3,6-tetrahydropyridine Methiodide (1). Nicotinamide reacted with a 2-fold excess of iodomethane in MeOH to yield sulfur yellow crystals of the methiodide salt. To 2.0 g (7.57 mmol) of this salt, stirring in 10 mL of MeOH, was added $NaBH_4$ (0.87 g, 15.2 mmol) in small portions. The solution was then brought to pH 8–9 with 1 M HCl, diluted to about 30 mL with water, saturated with NaCl, and extracted (four times) with CH_2Cl_2. The organic phase was dried ($MgSO_4$), and the solvent was removed in vacuo to yield 0.81 g (5.8 mmol) of colorless oil. The entire product was allowed to react with iodomethane in MeOH to yield 0.84 g (3.0 mmol) of white crystals, which were recrystallized twice (EtOH–H_2O): mp 221.5–222.5 °C; IR (Nujol) 3180–3455 (N–H), 1683, 1644, and 1612 (amide I, amide II, and C=C) cm^{-1}. Anal. ($C_8H_{15}N_2IO\cdot H_2O$) C, H, N.

1-Methyl-4-carbamylpiperidine Methiodide (2). Isonipecotamide (4-carbamylpiperidine, 1.0 g, 7.8 mmol) was converted directly to its *N,N*-dimethyl derivative with use of the strong base 1,2,2,6,6-pentamethylpiperidine (7.8 mmol), to scavange HI, and a 50% excess of iodomethane according to the procedure of Sommer et al.[25] The product (1.85 g, 6.5 mmol) was recrystallized (EtOH–H_2O): mp 213.5–214.8 °C (lit.[26] 213–214.5 °C).

1-Methyl-4-carbamylpiperazine Methiodide (3). To 1-methylpiperazine (2.7 g, 27 mmol) dissolved in dilute acetic acid (17%, 15 mL) was added KOCN (2.4 g, 30 mmol, dissolved in 12.5 mL of H_2O). The reaction mixture was allowed to stand at 21 °C for 4 days. The solution was brought to about pH 9 with NaOH and extracted (four times) with CH_2Cl_2. The organic phase was dried ($MgSO_4$), and the solvent was removed in vacuo to yield 1.4 g of yellow liquid. This crude product was converted to the methiodide salt with an excess of iodomethane in acetone. The white crystals were recrystallized twice: mp 218.0–220.0 °C dec. Anal ($C_7H_{16}N_3IO$) C, H, N.

Pharmacology. The frog Ringer's solution had the following composition (mM): NaCl, 116; KCl, 2.0; Na_2HPO_4, 1.3; and NaH_2PO_4, 0.7. Potencies, expressed as reciprocals of equipotent molar ratios in reference to carbamylcholine, were evaluated by isotonic contracture of the rectus abdominis muscle from the frog *Rana pipiens*. Each muscle, under 1-g load, was treated with an agonist under test for 2 min (agonists **1** and **3**) or for 5 min (agonist **2**) followed by three or more washes administered over a time period of at least 30 min. Three concentrations of carbamylcholine and three of the agonist under test were administered to each muscle for the same duration (2 or 5 min) in random order. Potency ratio and confidence intervals were calculated as described by Colquhoun.[27]

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(23) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979. This value was obtained from the fragment constants used for predicting octanol–water partition coefficients. The constants are $COCH_3$, −1.13; $CONH_2$, −2.18. The difference, 1.05, indicates that the octanol–water partition coefficient for acetyl derivatives is $10^{1.05}$ = 11 times greater than for corresponding carbamyl compounds.

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