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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Structure–activity relationship of phenytoinergic antiepileptic drugs related to ameltolide

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Abstract Ameltolide shares with phenytoin and carbamazepine a common mode of action involving interaction with central voltage-dependent sodium channels. Ameltolide and structurally related benzanilides were subjected to molecular modeling studies using both molecular mechanics (MM2, Amber96, and OPLS) and semiempirical quantum mechanics (AM1, PM3, and PM3 Cosmo) to resolve a paradox: while compounds with a phenytoin-like pharmacological profile possess a CO-NH moiety in a *cis*-configuration, ameltolide was found via X-ray crystallography to exist in the *trans*-configuration. Results obtained both by molecular mechanics and semiempirical methods indicate that for ameltolide, the *cis* and *trans* forms have similar energy content. Additional *ab initio* calculations performed at 6–31G** gave a ΔE ($Z - E$) on the order of 3 kcal/mol. In view of this small energy difference between the *cis* and *trans* forms, it is conceivable that these benzanilides bind to their biological target in their *cis* configuration, therefore assuming a common structure–activity relationship with classical antiepileptic agents.

Keywords *Ab initio* calculations · Ameltolide · Benzanilides · Molecular Modeling · Structure–activity relationship

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Introduction

Centered on the 4-aminobenzoylamino pharmacophore, Clark et al. established through a series of successive works the anticonvulsant potential in animal epilepsy models for the amino-substituted benzamides derived from alkyl-, arylalkyl-, and arylamines (Clark et al., 1984, 1985, 1986; Clark and Davenport, 1987). While 4-aminobenzamide per se is inactive in the maximal electroshock seizure (MES) test, *N*-alkylation and *N*-arylation on the nitrogen of the amide result in active compounds. For the benzamide phenyl ring, an amino substituent is required for optimal MES potency with potency following the order *para* > *meta* > *ortho*. This ranking suggests that the amino group represents a strong signal in the molecular recognition process. On the 4-amino-*N*-phenylbenzamide framework, implementation of a methyl on the *N*-phenyl moiety strongly increases MES activity. Addition of a second *ortho*-methyl group reinforces this activity, which leads to the highly potent ameltolide **(1),** a compound developed by Eli Lilly (LY 201116), which exhibits a phenytoin-like profile. Ameltolide is very potent in the MES test, while it is inactive in the subcutaneous pentylenetetrazol (scPTZ) test (Clark, 1988). On a molar basis, **1** was found three times more active in the MES test than phenytoin **(2)** and carbamazepine **(3),** two well established standard antiepileptic drugs. The 4-aminophthalimides, from which structure is the prominent representative, are structurally related to **1** and have given rise to a class of antiepileptic agents also endowed with a phenytoinergic profile (Poupaert et al., 1995) Fig. 1.

In another study using the batrachotoxin affinity assay, it was noted that the 4-amino moiety played an important role in the molecular recognition process at the level of the receptor modulating the voltage-dependent sodium channel status, and

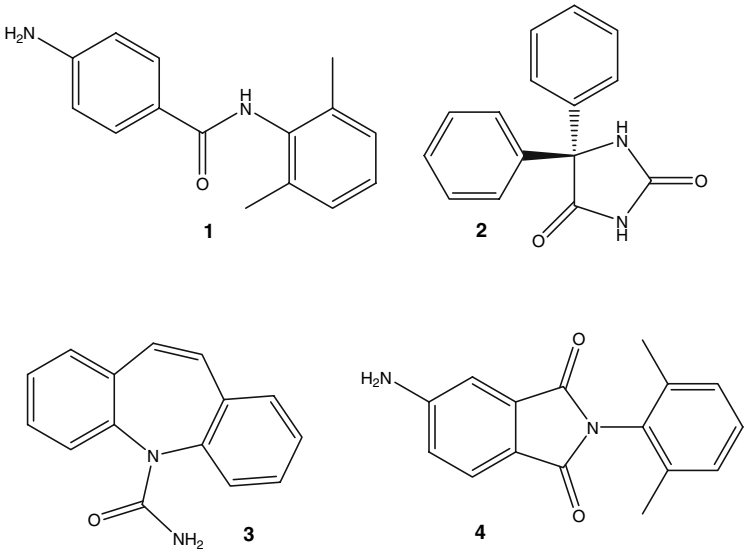
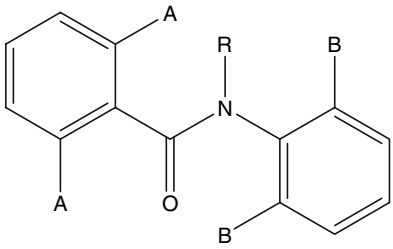


Fig. 1 Structures of compounds **1–4**



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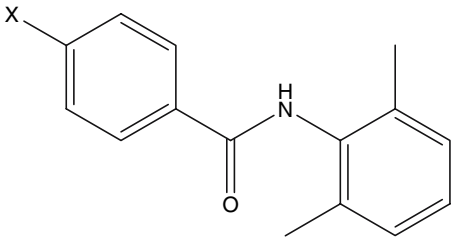

1, 11 - 15

Fig. 2 Structures of compounds **1** and **5–15.** (**5** A, B, R = H; **6** A = CH_3, B, R = H; **7** B = CH_3, A, R = H; **8** A, B = H; R = CH_3; **9** A, R = CH_3, B = H; **10** A = H, B, R = CH_3; **1** X = NH_2 (ameltolide); **11** X = OH; **12** X = OCH_3; **13** X = CN; **14** X = NO_2; **15** X = CF_3)

that both phenytoin and ameltolide bind to the same region of the voltage-dependent sodium channel (Vamecq et al., 1998). Paradoxically, however, while the HN-C=O moiety is obviously in the *cis* form in phenytoin, it is generally accepted that the amide system is in its *trans* form in benzanilides (Azumaya et al., 1994) and ameltolide was indeed found to exist in the *trans* form of the X-ray crystal structure (Poupaert et al., 1990; Duke and Codding, 1992).

In an effort to resolve the above paradox, we performed extensive studies using both molecular mechanics (MM2, Amber96, and OPLS) and semiempirical quantum mechanics (AM1, PM3, and PM3 Cosmo) as implemented in Hyperchem 7 or Chem3D 8.0 on six reference compounds as well as on ameltolide **(1)** and six structurally related benzanilides. In addition, calculations were performed for **1** in a periodic box of water (OPLS). Finally, *ab initio* quantum calculations performed at 6 – 31G** were also undertaken for all compounds Fig. 2.

Results and Discussion

The structure of each compound was drawn in 2D using ISIS/Draw (MDL Information Systems, San Ramon, CA) and was converted to 3D using HyperChem 7.0 (Molecular Mechanics and Quantum Chemical Calculations Package, Hyper-Cube, Ontario, Canada). The resulting structures were energy-minimized via Polak–Ribiere geometry optimization using MM+ as implemented in HyperCube

(Extended MM2 Molecular Mechanics method). The initial structures (either in their *cis* or *trans* configuration) so obtained were used as the starting point, subjected to a molecular dynamics trajectory using the Langevin dynamics module (100 ps at 300 K in increments of 1 fs), and conformers were collected every 2 ps. A set of 50 conformers was collected and each conformer was again energy-minimized via the Polak–Ribiere optimization procedure using Amber 96 (Cornell et al., 1995), CHARMM (Brooks et al., 1983) OPLS (for molecular mechanics; Jorgensen and Tirado-Rives, 1988), or AM1 and PM3 (for semiempirical quantum mechanics; Dewar et al., 1985). Additional calculations were performed for MM2 (Allinger, 1977) and PM3 Cosmo (Klamt and Shürmann, 1993; the latter simulates an aqueous environment) using Chem3D Ultra 8.0 (CambridgeSoft, Cambridge, MA). Energy minimizations were performed until the absolute value of the largest partial derivative of energy with respect to the coordinates was below 0.001 kcal mol^{-1} A^{-1}. In this way, an energy minimum for the various force fields, or Hamiltonians, was obtained for the corresponding *cis* and *trans* forms of compounds **5–16**. The best *cis* and *trans* conformers obtained with AM1 were finally submitted to *ab initio* energy minimization calculations performed via Gaussian 03 (Gaussian, Wallingford, CT) using the Hartree-Fock method with the 6-31G** basis set.

In an effort to verify if the 100 ps trajectory was sufficient to explore the conformational space of these benzanilides, a longer molecular dynamics trajectory was computed for 1 (1000 ps at 300 K in increments of 1 fs) both for the *cis* and *trans* forms, conformers were collected every 10 ps and energy-minimized as described in the preceding text with the OPLS force field. We initially selected reference compounds that are known by means of spectroscopic [^{1}H-nuclear magnetic resonance (NMR), infrared (IR), and fluorescence] studies to have a conformational preference either for the *trans* or the *cis* forms (Azumaya et al., 1994). This preliminary screening of computational methods was performed to select and validate the most suitable methods. In this context, benzanilide **(5),** 2,6-dimethylbenzanilide **(6)** and 2′,6′-dimethylbenzanilide **(7)** were chosen for their strong *trans* preference. Conversely, *N*-methylbenzanilide **(8),** *N*-methyl-2,6-dimethylbenzanilide **(9),** and *N*-methyl-2′,6′-dimethylbenzanilide **(10)** were selected for their strong *cis* preference (Azumaya et al., 1994).

The energy difference between *cis* and *trans* configurations (ΔE (*cis–trans*) expressed in kcal/mol) for compounds **5–10** and the various methods explored are listed in Table 1. A positive value denotes a preference for the *trans* configuration. There is a fairly good correlation between the published conformational behavior of **5–10** (Azumaya et al., 1994) and the theoretical calculations with Amber, OPLS, AM1, and HF. On the contrary, no MM2, CHARMM (data not shown), and PM3 Cosmo data were found to have any diagnostic value. For PM3, there was no clear cut difference between the set **5–7** (showing a *trans* preference) and the set **8–10** (showing a *cis* preference), although there was a trend for the *N*-methyl derivatives **8–10** to give more negative ΔE (*cis–trans*) figures. OPLS tends to exacerbate the dichotomy between the sets **5–7** and **8–10**.

Values for **1** and other *para*-substituted benzanilides structurally related to ameltolide are listed in Table 2. The substituents of compounds **1**, **11**, and **12** have a negative Hammet σ_p value, while in the set **13–15** they have a positive one.

Table 1 ΔE (*Z*–*E*) in kcal/mol for compounds 5–10

	MM2	Amber	OPLS	AM1	PM3	PM3Cosmo	HF 6–31G**
5	+2.02	+0.40	+6.35	+0.35	+0.17	−2.11	+3.91
6	+0.46	+0.92	+5.60	+0.57	−0.46	−2.54	+3.86
7	+0.03	+0.65	+6.47	+0.35	−0.06	−2.03	+3.41
8	−2.23	−0.42	−6.90	−1.76	−2.11	−3.37	−3.11
9	+0.88	−0.38	−7.76	−0.54	−2.57	−4.59	−0.49
10	−0.78	−0.92	−6.59	−0.63	−2.14	−3.18	−1.59

Table 2 ΔE (*Z*–*E*) in kcal/mol for compounds **1** and **11–15**

	MM2	Amber	OPLS	AM1	PM3	PM3Cosmo	HF6–31G**
1	+0.36	+0.38	+2.01	+0.63	−2.57	−4.59	+3.25
11	−0.02	+0.40	+0.35	+0.41	−2.11	−3.37	+3.34
12	+0.60	+0.79	+1.13	+1.79	−2.39	−4.41	+3.34
13	−0.25	+0.40	+1.43	+6.35	−0.35	−2.11	+2.97
14	+0.19	+0.02	−1.90	+7.76	−0.11	−3.37	+2.94
15	+0.02	+4.78	+9.50	+5.60	−0.14	−3.18	+3.11

Considering only Amber, OPLS, AM1, and HF (which were found to be of diagnostic value above in the training set **5**–**10**), it is difficult to rationalize the data in Table 2 on the basis of the mesomeric effect of the X substituents.

As the PM3 Cosmo values were systematically negative, we were puzzled by this result and consequently we performed additional calculations using a water periodic box containing 480 water molecules in which were inserted the *cis* and *trans* forms of **1**. Molecular dynamics trajectories (150 ps at 300 K in increments of 0.1 fs) were acquired for both forms and conformers sampled every 10 ps and subsequently energy-minimized using AM1. The resulting ΔE (*cis*–*trans*) value was found in this case equal to 2.3 kcal/mol in favor of the *trans* form of **1**.

Conclusion

In combination, the results we obtained using various molecular modeling methods, ranging from molecular mechanics to semiempirical and *ab initio* quantum mechanics, tend to indicate that the energy difference between *cis* and *trans* forms of **1**, and related structures, is relatively small. As binding to this recognition site generates considerable stabilizing energy (via H bonds, dipole–dipole, and van der Waals interactions, hydrophobic forces, etc.), it is conceivable that these benzanilides could bind to their biological target in their *cis* configuration, assuming, therefore, a common structure–activity relationship with classical antiepileptic agents such as phenytoin, phenobarbital, primidone, etc., which obviously possess *cis* amide systems.

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