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Design and synthesis of *N*-benzylpiperidine–purine derivatives as new dual inhibitors of acetyl- and butyrylcholinesterase

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Dedicated to Prof. Carmen Ochoa on the occasion of her 65th birthday.

Abstract—The synthesis and biological evaluation of *N*-benzyl-(piperidin or pyrrolidin)-purines are described. Compounds derived from *N*-benzylpiperidine and *N*-substituted purines showed moderate acetylcholinesterase inhibition. Preliminary structure–activity relationships and a superimposition of the best compound with the active conformation of donepezil have revealed structural features that have been used in the design of more potent *N*-benzylpiperidine inhibitors bearing an 8-substituted caffeine fragment and a methoxymethyl linker. These new compounds are interesting dual inhibitors of acetylcholinesterase and butyrylcholinesterase and have been chosen for further optimisation.

1. Introduction

Alzheimer's disease (AD), the most common dementia in elderly people, is a progressive neurodegenerative disorder characterised by three major pathological signs: β-amyloid plaques, neurofibrillary tangles and loss of central cholinergic function.[1,2] In the last few years, several rational pharmacological strategies have emerged, including cholinergic and non-cholinergic interventions.[3,4] Among the cholinergic hypothesis, the first approved drugs for the management of the disease were cholinesterase inhibitors (ChE-I) that increase neurotransmission at cholinergic synapses in the brain and thereby improve cognition.[5–7] Since recent research has revealed that several ChE-I not only facilitate cholinergic transmission but also interfere with the synthesis, deposition and aggregation of toxic amyloid-β-peptides (Aβ) slowing the neurodegenerative progression,[8,9] the current interest in these drugs has increased.[10,11]

Tacrine, donepezil, rivastigmine and galantamine are potent ChE-I. Some of these drugs selectively inhibit acetylcholinesterase (AChE), but compounds that also target butyrylcholinesterase (BuChE) may provide added benefits.[12–14] BuChE is mainly associated with glial cells and is found in much lower concentrations than AChE in the healthy brain.[15] However, over the course of AD, AChE activity progressively decreases, while BuChE activity increases, and BuChE may then act as a compensatory mechanism for ACh metabolism.[16] Consequently, as AD progresses, ACh regulation may become increasingly dependent on BuChE and dual inhibitors may provide more sustained efficacy than AChE-selective agents.[17] Moreover, a recent clinical study has shown that patients whose drug treatment also inhibited BuChE showed less cortical atrophic changes than the subgroup treated with a selective AChE inhibitor, providing an empirical evidence of an additional effect of neuroprotection using dual cholinesterase inhibitors in Alzheimer's disease.[18]

The crystal structures of human acetyl- and butyrylcholinesterase showed that the active catalytic triad is found at the bottom of a deep gorge lined mostly with aromatic residues that facilitate the recognition and guidance of the quaternary ammonium ion of the natural substrate by cation-π interactions.[19–21] Continuing with our research on different heterocyclic families of potential

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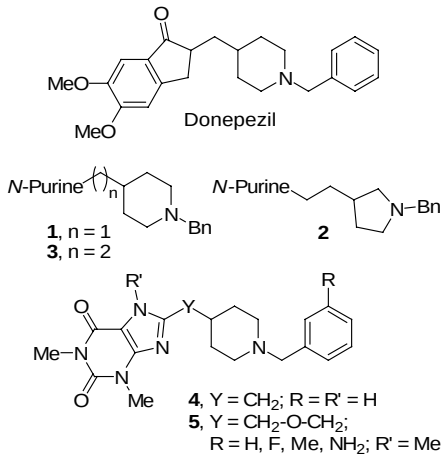


Figure 1. Purine–benzylpiperidine derivatives as new cholinesterase inhibitors.

application in Alzheimer's disease,[22–24] and taking into account that the purine ring is able to interact with aromatic aminoacid residues through π–π interactions,[25,26] we have designed new purine–benzylpiperidine derivatives **1**–**5** as potential cholinesterase inhibitors that could establish additional interactions in the gorge of the enzymes (Fig. 1).

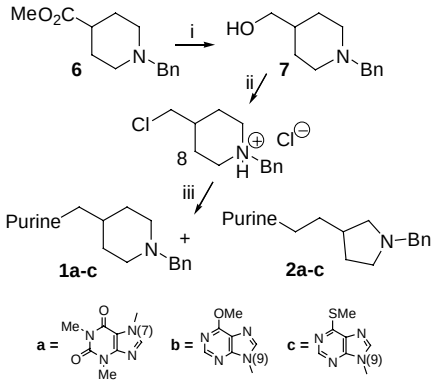
In previous papers, we described the synthesis of benzylpiperidin–purines **1**, together with the corresponding benzylpyrrolidin–purines **2**, as a result of ring contraction in the course of nucleophilic substitution.[27,28] In this work, we report on the synthesis of the *N*-substituted xanthine **3** derived from theophylline and benzylpiperidine with a dimethylene linker, the biological evaluation of series **1**–**3** and a molecular modelling study by comparing the more potent compound (**3**) with the active conformation of donepezil, known for its ability to interact along the active-site gorge of the AChE through interactions with aromatic amino acid residues.[29] On the basis of the above SAR study and molecular modelling, we designed and synthesised two new series bearing 8-substituted xanthines **4** and **5**, with the aim of increasing the potency of the previous series.

2. Results and discussion

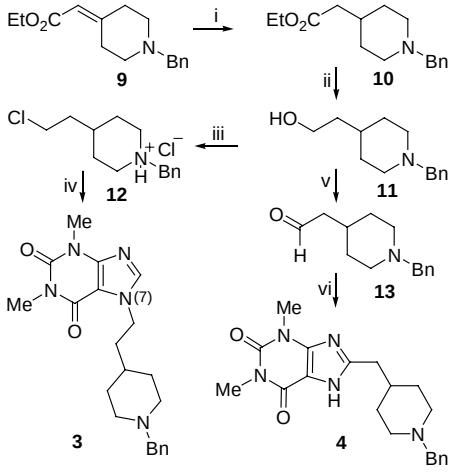
2.1. Chemistry

N-(1-Benzylpiperidin-4-ylmethyl)-purines **1a**–**c** and *N*-[2-(1-benzylpyrrolidin-3-yl)ethyl]-purines **2a**–**c** were synthesised from 1-benzyl-4-chloromethylpiperidine and commercially available purines according to Scheme 1, as previously described by us.[27,28]

New theophylline derivatives **3** and **4** were obtained, as shown in Scheme 2. Treatment of 1-benzyl-4-piperidone with triethyl phosphonoacetate in the presence of sodium hydride in dry tetrahydrofuran yielded **9** in 85% yield. In contrast with the work of Gupta et al. where both endocyclic and exocyclic olefins were formed,[30] in our case only one product was obtained, as shown by

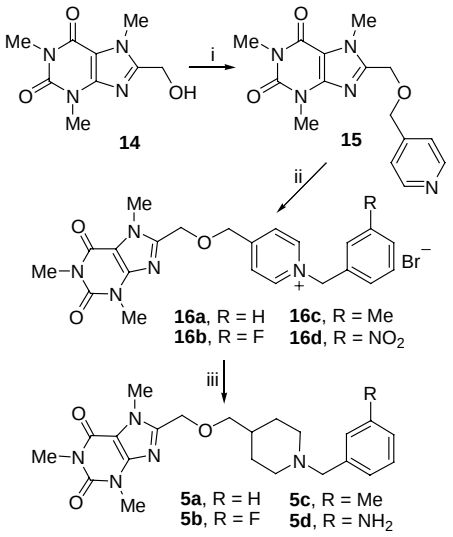


Scheme 1. Reagents and conditions: (i) $LiAlH_4$, THF, reflux, 5 h; (ii) $SOCl_2$, reflux, 3 h; (iii) purine, NaH, DME, reflux, 72 h.



Scheme 2. Reagents and conditions: (i) H_2 (35 psi), PtO_2, EtOH, rt, 5 h; (ii) $LiAlH_4$, THF, reflux, 5 h; (iii) $SOCl_2$, reflux, 3 h; (iv) theophylline, NaH, DME, reflux, 48 h; (v) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C (30 min) to rt; (vi) 5,6-diamino-1,3-dimethyl-1*H*-pyrimidine-2,4-dione, H_2O/acetic acid (3:1), reflux, 36 h.

sharp resonance signal at 5.59 ppm, attributable to the exocyclic olefinic proton, and the lack of any triplet less deshielded due to the endocyclic one. Catalytic hydrogenation of **9** over PtO_2 in ethanol at room temperature gave the aliphatic ester **10** in 81% yield. It is worth mentioning that where the above hydrogenation was carried out using Pd/C as a catalyst, a mixture of **10** and the corresponding debenzylated derivative was obtained. Reduction of **10** with lithium aluminium hydride gave the required alcohol **11** which was then treated with thionyl chloride, yielding the corresponding chloroderivative **12** in quantitative yield. Finally, the reaction of **12** and theophylline, using sodium hydride as base and dimethoxyethane as solvent, afforded compound **3**, in which the position of alkylation was determined by NMR using a combination of bidimensional ^1H and ^{13}C NMR experiments. The fact that the methylene protons bound to the purinic nitrogen showed a HMBC correlation with carbon 5 of the purine, but not with carbon 4, demonstrated that alkylation occurred at nitrogen 7.



Scheme 3. Reagents and conditions: (i) 4-chloromethylpyridine, NaH, DMF, reflux, 3 h; (ii) $RC_6H_4CH_2Br$, CH_3CN, reflux, 3 h; (iii) H_2 (20 psi), PtO_2, EtOH, rt, 6–7 h.

Moreover, Swern oxidation[31] of alcohol **11** using oxalyl chloride and dimethylsulfoxide afforded aldehyde **13**, with no carboxylic acid being produced. This aldehyde was then condensed with 5,6-diamino-1,3-dimethyl-1*H*-pyrimidine-2,4-dione affording a new 8-substituted theophylline derivative **4** in one-step cyclization, using a mixture of water:acetic acid as solvent.[32]

Finally, compounds with a methoxymethyl spacer were synthesised according to Scheme 3. Following the described methods, 8-hydroxymethylcaffeine (**14**) was obtained by condensation and ring closure reaction of 5,6-diamino-1,3-dimethyl-1*H*-pyrimidine-2,4-dione with glycolic acid,[33] followed by alkylation with methyl iodide in the presence of potassium carbonate. The reaction of **14** with sodium hydride and 4-chloromethylpyridine yielded **15**, which was then treated with several benzyl bromides without any base to yield the corresponding benzylpyridinium salt **16a**–**d**. Hydrogenation of **16a**–**c** over platinum (IV) oxide in ethanol generated the corresponding benzylpiperidine derivative **5a**–**c** in 47–49% yields. In the case of **16d**, the nitro group was also reduced to the expected amino derivative, rendering compound **5d** in 41% yield.

2.2. Biological and molecular modelling studies

The in vitro inhibition of AChE for the new synthesised compounds, as well as for intermediates, was determined by the method of Ellman et al.[34] using a commercially available AChE from bovine erythrocytes. We also used butyrylcholinesterase (BuChE) from equine serum to determine the selectivity profile of selected inhibitors. Both bovine AChE and equine BuChE show a high degree of homology (>90%) with the corresponding human enzymes.[35,36]

Initially, we tested compounds derived from *N*-substituted purines **1a**–**c**, **2a**–**c** and **3**, as well as their intermediates **6**–**12**, towards bovine AChE (Table 1). From these data, some preliminary structure–activity relationships could be derived. The *N*-benzylpiperidine derivatives (**1a**–**c** and **3**) inhibited the AChE better than the *N*-benzylpyrrolidine ones (**2a**–**c**). As regards the nature of heterocycle, xanthine derivatives were better than purine ones (compound **1a** vs **1b** and **1c**). Finally, comparing the length of the linker between theophylline and benzylpiperidine moieties, the dimethylene chain increased the enzymatic inhibition in 1 order of magnitude in relation to the methylene one, compound **3** (IC_{50} = 2 μM) being the best among these *N*-substituted purines.

Table 1. AChE inhibition activity of compounds derived from *N*-substituted purines **1a**–**c**, **2a**–**c**, **3** and intermediates **6**–**12**

Compound	IC_{50} (μM)[a]
1a	20 ± 0.1
1b	42 ± 2.1
1c	35 ± 1.5
2a	50 ± 2.6
2b	13 ± 0.1
2c	40 ± 1.9
3	2 ± 0.1
6	25 ± 1.1
7	8 ± 0.2
8	40 ± 2.6
9	100
10	30 ± 1.3
11	100
12	10 ± 0.5

[a] Data are means ± standard deviation of three independent experiments.

With regard to tested intermediates **6**–**12**, it is worth mentioning that the methanol derivative **7** showed an interesting AChE inhibition (IC_{50} = 8 μM), in contrast with the value exhibited by its homologous **11** (IC_{50} = 100 μM), derived from ethanol. This fact could be related to stabilizing interactions between the enzyme and the hydroxylic group of **7** located to one methylene group from the piperidine fragment.

With the aim of obtaining some information about the possible interactions of the new molecules with the enzyme, which, in turn, could be useful in the design of more potent inhibitors, a molecular modelling study was performed comparing **3** with donepezil, a nanomolar AChE inhibitor. Using the semi-empirical method AM1, compound **3** was optimised by rotating the torsional angles of the linear alkyl fragments and the minimum was then superimposed with the active conformation of donepezil, whose 3D coordinates were obtained from the crystal structure of the *Torpedo californica* AChE:donepezil complex[37] (PDB entry: 1EVE). As it can be seen in Figure 2, the benzylpiperidine fragments correctly superimposed in both compounds, whereas the xanthine ring was located near the indanone fragment but did not fit it exactly. From this study none can deduce that a better superimposition could be reached with 8-substituted xanthines, instead of *N*-substituted ones.

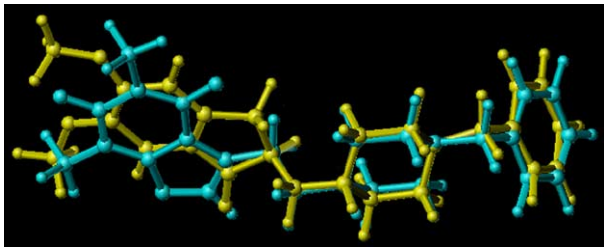


Figure 2. Superimposition of **3** (blue) and donepezil (yellow).

Combining conclusions extracted from both the structure–activity relationships and the molecular modelling, we planned to obtain new series bearing an 8-substituted xanthine, **4** and **5a–d** (see Fig. 1). Compound **4** was designed to explore the effect of substitution in the xanthine fragment, and **5a** was designed to confirm if a longer linker with an oxygen atom located to one methylene group from the piperidine fragment could be favourable for activity. Further substitutions of **5a** on the benzyl moiety at the meta position (F, CH_3 and NH_2) were chosen on the basis of their higher inhibitory activities in the donepezil series, as previously described.[38,39]

The AChE inhibition of these new compounds is given in Table 2. As the previous modelling study envisaged, compound **4** bearing an 8-substituted caffeine is more potent (IC_{50} = 2.50 μM) than its homologue derived from 7-substituted theophylline **1a** (IC_{50} = 20 μM), pointing out that the correct orientation of the xanthine improves inhibition by 1 order of magnitude. In addition, elongation of the chain and presence of an oxygen atom increased the potency of benzylpyridinium and benzylpiperidine derivatives by another order of magnitude: **16a** and **5a** showed sub-micromolar inhibition of AChE (IC_{50} = 0.10 and 0.75 μM, respectively). In contrast with the bibliographic data,[38,39] the introduction of any substituent in the position 3 of the benzyl fragment (such as fluor, methyl, nitro, or amino) did not improve the enzymatic activity (compounds **16b–d** and **5b–d** vs **16a** and **5a**, respectively). Finally, benzylpyridinium cations **16a–d** showed better inhibition than the corresponding benzylpiperidine derivatives **5a–d**, revealing that an aromatic fragment with a positive charge could establish favourable interactions with the aromatic residues from the gorge of the AChE.

Table 2. AChE and BuChE inhibition data (IC_{50}, μM)[a] of compounds derived from 8-substituted xanthines **4**, **5a–d** and **16a–d**

Compound	AChE-I	BuChE-I
4	2.50 ± 0.10	n.d.
5a	0.75 ± 0.03	2.50 ± 0.09
5b	1.00 ± 0.05	2.00 ± 0.08
5c	1.00 ± 0.04	0.40 ± 0.01
5d	1.00 ± 0.04	1.00 ± 0.05
16a	0.10 ± 0.01	2.00 ± 0.10
16b	0.30 ± 0.01	1.00 ± 0.04
16c	0.50 ± 0.02	3.00 ± 0.10
16d	0.30 ± 0.01	4.00 ± 0.10

n.d.: not determinded.

[a] Data are means ± standard deviation of three independent experiments.

The AChE/BuChE selectivity of the 8-substituted xanthines was investigated by measuring the BuChE inhibitory activity of **16a–d** and **5a–d**, which are the most potent AChE inhibitors of the series. From the IC_{50} data reported in Table 2, it becomes evident that all tested compounds did not show remarkable selectivity towards one of the two enzymes, and only the benzylpyridinium derivative **16a** showed moderate selectivity towards AChE (20-fold up compared to BuChE). The opposite selectivity was found in the 3-methylbenzylpiperidine derivative, **5c** (IC_{50} = 0.40 μM) being the best BuChE inhibitor of this work.

3. Conclusions

In summary, we have reported the synthesis and preliminary results for acetylcholinesterase and butyrylcholinesterase inhibition activity of a series of *N*-benzyl-(piperidin or pyrrolidin)-purines. Compounds derived from *N*-benzylpiperidine and *N*-substituted purines **1–3** showed only moderate AChE inhibition (IC_{50} = 20–50 μM). Preliminary structure–activity relationships and a comparison between the optimised structure of **3** and the active conformation of donepezil have envisaged that an 8-substituted caffeine substructure and a methoxymethyl spacer could increase enzymatic inhibition of these *N*-benzylpiperidine derivatives. As a result, 1-benzyl-4-[(xanthin-8-yl)-methoxymethyl]-pyridinium **16a–d** and 1-benzyl-4-[(xanthin-8-yl)-methoxymethyl]-piperidine derivatives **5a–d** exhibited better AChE inhibition (IC_{50} = 0.1–1.0 μM) by 2 orders of magnitude compared with the preceding structures. Since these derivatives also inhibited BuChE (IC_{50} = 0.4–4.0 μM), they can be considered as interesting dual inhibitors in the search of new therapies for Alzheimer's disease.

4. Experimental

4.1. Chemistry

Reagents and solvents were purchased from common commercial suppliers and were used without further purification. Tetrahydrofuran (THF) and dimethoxyethane (DME) were freshly distilled from $LiAlH_4$ prior to their use. Chromatographic separations were performed on silica gel, using flash column chromatography (Kieselgel 60 Merck of 230–400 mesh), and compounds were detected with UV light (254 nm), iodine chamber, or ninhydrin. HPLC analyses were performed on a Waters 6000 equipment, with UV detector (214–274 nm), using a Delta Pak $C_{18.5}$ μm, 300 Å (3.9 nm × 150 nm) column, eluted with mixtures of CH_3CN (solvent A) and H_2O with 0.05% H_3PO_4 and 0.04% Et_3N (solvent B), as indicated in each case, at a flow rate of 1.0 mL/min.

Melting points were determined with a Reichert–Jung Thermovar apparatus. Nuclear magnetic resonance spectra were recorded in $CDCl_3$ solutions, using Varian Unity-500, Varian Unity Inova-400 and Varian XL-300 spectrometers. Typical spectral parameters for ^1H NMR were: spectral width 10 ppm, pulse width 9 μs (57°) and

data size 32 K. The acquisition parameters in decoupled ^{13}C NMR spectra were: spectral width 16 kHz, acquisition time 0.99 s, pulse width 9 μs (57°) and data size 32 K. Chemical shifts are reported in δ values (ppm), relative to internal Me_4Si, and J values are reported in Hertz. Other experiments, such as HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation), were obtained in standard conditions. Mass spectra (MS) were obtained by electronic impact (EI) at 70 eV in a Hewlett-Packard 5973 spectrometer (with direct insertion probe) or by electron spray ionization (ESI) in positive mode using a Hewlett-Packard MSD 1100 spectrometer. Elemental analyses were carried out in a Perkin-Elmer 240C equipment in the Centro de Química Orgánica 'Manuel Lora-Tamayo' (CSIC) and the results are within ± 0.4% of the theoretical values.

N-(1-Benzylpiperidin-4-ylmethyl)-purines **1a**–**c**, *N*-[2-(1-benzylpyrrolidin-3-yl)-ethyl]-purines **2a**–**c** were obtained as previously described by us,[27,28] and intermediates **6**–**14** according to literature methods.[30,40–43]

4.2. 7-[2-(1-Benzyl-piperidin-4-yl)-ethyl]-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurine (3)

Under N_2 to a stirred suspension of NaH (53.0 mg, 1.1 mmol, oil free) in dry DME (10 mL), a solution of theophylline (43.0 mg, 0.24 mmol) in DME (10 mL) was added and the mixture was refluxed for 30 min. Then, a solution of **12** (85.0 mg, 0.31 mmol) in dry DME was slowly added and the reaction mixture was refluxed for an additional 48 h. Then, it was cooled to room temperature and then treated with a 15% aq solution of NH_4Cl until pH 8 and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (25 mL), washed with H_2O (3× 25 mL), brine (25 mL), dried (Na_2SO_4) and evaporated to dryness, yielding a syrup that was purified by flash chromatography on a silica gel column. Fractions of R_f 0.7 (CH_2Cl_2/CH_3OH, 5:1) afforded unreacted theophylline (4.5 mg, 10%). Fractions of R_f 0.5 (CH_2Cl_2/CH_3OH, 5:1) yielded compound **3** (23.8 mg, 23%) as a pure colourless syrup. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (s, 1H), 7.29 (m, 5H), 4.29 (t, 2H, J = 7.4 Hz), 3.57 (s, 3H), 3.48 (s, 2H), 3.39 (s, 3H), 2.87 (br d, 2H, J = 11.4 Hz), 1.93 (dt, 2H, J = 2.2, 11.5 Hz), 1.81 (q, 2H, J = 7.4 Hz), 1.67 (br d, 2H, J = 11.4 Hz), 1.60 (m, 1H), 1.35 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.1, 151.7, 148.9, 140.6, 138.5, 129.2, 128.2, 127.0, 106.9, 63.3, 53.5, 45.0, 37.6, 33.1, 31.9, 29.7, 28.0. MS (EI) *m*/*z* 91 (100), 397 (25, M^+). Anal. Calcd for $C_{22}H_{31}N_5O_2$ (397.51): C, 66.47; H, 7.86; N, 17.62. Found: C, 66.10; H, 7.59; N, 18.00.

4.3. 8-[(1-Benzylpiperidin-4-yl)-methyl-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (4)

A misture of 25% aq acetic acid solution and 5,6-diamino-1,3-dimethyluracil hydrate (174.5 mg, 0.99 mmol) was refluxed for 1 h. Then, a solution of **13** (215 mg, 0.99 mmol) in ethanol (4 mL) was added dropwise over 30 min and then refluxed for 48 h. After cooling to room temperature, the mixture was neutralized with 2 N sodium hydroxide solution and washed with ethyl acetate (3× 50 mL). The organic extracts were treated with water (3× 30 mL), brine (3× 30 mL), dried (Na_2SO_4) and evaporated to dryness, yielding a syrup that was purified by flash chromatography on silica gel column using CH_2Cl_2/MeOH/NH_4OH, 90:10:1 as eluent. Fractions of R_f 0.6 were evaporated to dryness, yielding **4** (66.2 mg, 18%) as a pure yellow solid (mp 203–205 °C). 1H NMR (DMSO-d_6, 400 MHz) δ 13.0 (br s, 1H), 7.25 (m, 5H), 3.37 (s, 3H), 3.35 (s, 2H), 3.17 (s, 3H), 2.72 (br d, 2H, J = 11.5 Hz), 2.56 (d, 2H, J = 7.0 Hz), 1.84 (br d, 2H, J = 11.5 Hz), 1.69 (m, 1H), 1.50 (br d, 2H, J = 11.5 Hz), 1.16 (br d, 2H, J = 11.5 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.3, 153.2, 151.5, 148.5, 138.9, 129.0, 128.4, 127.1, 106.3, 62.7, 54.0, 53.3, 35.4, 30.1, 29.0, 27.9. MS (ESI) *m*/*z* 368 $(M+H)^+$. HPLC analysis (A:B, 50:50) t_R = 3.83 min. Anal. Calcd for $C_{20}H_{25}N_5O_2$ (367.20): C, 65.37; H, 6.86; N, 19.06. Found: C, 65.35; H, 6.80; N, 19.00.

4.4. 4-[(1,3,7-Trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)-methoxymethyl]-pyridine (15)

A mixture of **14** (80.0 mg, 0.36 mmol) and NaH (17.3 mg, 0.36 mmol, oil free) in dry DME (20 mL) was refluxed for 30 min under N_2. Then, a solution of 4-chloromethylpyridine hydrochloride (59.0 mg, 0.36 mmol) in dry DME (10 mL) was slowly added and the mixture was refluxed for an additional 5 h. After cooling to room temperature, the mixture was neutralized with a 15% aq solution of NH_4Cl and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (25 mL), washed with H_2O (3× 25 mL), brine (25 mL), dried (Na_2SO_4) and evaporated to dryness, yielding a syrup that was purified by flash chromatography on a silica gel column, using CH_2Cl_2/CH_3OH, 7:1 as eluent. Fractions of R_f 0.5 afforded **15** (80 mg, 71%) as a yellow solid (mp 106–108 °C). ^1H NMR ($CDCl_3$, 400 MHz) δ 8.59 (d, 2H, J = 6.0 Hz), 7.20 (d, 2H, J = 6.0 Hz), 4.67 (s, 2H), 4.59 (s, 2H), 3.98 (s, 3H), 3.52 (s, 3H), 3.35 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 151.5, 150.1, 149.4, 147.3, 145.9, 121.7, 108.6, 70.9, 64.0, 32.2, 29.6, 27.9. MS (EI) *m*/*z* 208 (94), 315 (100, M^+). HPLC analysis (A:B, 50:50) t_R = 1.35 min. Anal. Calcd for $C_{15}H_{17}N_5O_3$ (315.33): C, 57.13; H, 5.43; N, 22.20. Found: C, 56.99; H, 5.23; N, 22.00.

4.5. General procedure for the synthesis of benzyl pyridinium salts 16 a–d

Under N_2 atmosphere, to a solution of **15** (1 equiv) in dry CH_3CN (10 mL) was slowly added a solution of the appropriate benzyl bromide derivative (2 equiv) in CH_3CN (2 mL). The mixture was stirred at room temperature for 5 h and evaporated to dryness, affording syrups that were treated with Et_2O (40 mL), yielding solids that were collected by filtration.

4.6. 1-Benzyl-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)-methoxymethyl]-pyridinium bromide (16a)

Following the general method, **15** (70 mg, 0.222 mmol) and benzyl bromide (50 μL, 0.444 mmol) afforded **16a** (107 mg, 99%) as a pure yellow solid (mp 177–179 °C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.42 (d, 2H, J = 6.5 Hz), 7.97 (d, 2H, J = 6.5 Hz), 7.62 (dd, 2H, J = 2.1 Hz, J = 4.9 Hz), 7.40–7.20 (m, 3H), 6.24 (s, 2H), 4.90 (s, 2H), 4.81 (s, 2H), 4.01 (s, 3H), 3.52 (s, 3H), 3.37 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9, 155.4, 151.5, 147.5, 147.4, 144.5, 132.7, 130.1, 129.7, 129.6, 125.1, 108.2, 69.5, 64.4, 63.8, 32.4, 29.8, 27.9. MS (ESI) m/z 406 (M^+−Br). HPLC analysis (A:B, 60:40) t_R = 4.46 min. Anal. Calcd for $C_{22}H_{24}N_5O_3Br$ (485.11): C, 54.33; H, 4.97; N, 14.40. Found: C, 54.30; H, 4.97; N, 14.37; Br, 16.42.

4.7. 1-(3-Fluorobenzyl)-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin- 8-yl)-methoxymethyl]-pyridinium bromide (16b)

According to the general method, from **15** (70 mg, 0.222 mmol) and 3-fluorobenzyl bromide (54 μL, 0.444 mmol) compound **16b** (102 mg, 91%) was obtained as a pure yellow solid (mp 185–187 °C). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.48 (d, 2H, J = 6.2 Hz), 7.95 (d, 2H, J = 6.2 Hz), 7.47 (d, 1H, $^3J_{F,H}$ = 7.7 Hz), 7.40–7.30 (m, 2H), 7.15–7.06 (m, 2H), 6.27 (s, 2H), 4.88 (s, 2H), 4.77 (s, 2H), 3.96 (s, 3H), 3.48 (s, 3H), 3.31 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.9 (d, $^1J_{C,F}$ = 249.5 Hz), 158.2, 155.3, 151.5, 147.4, 147.3, 144.6, 134.9 (d, $^3J_{C,F}$ = 8.2 Hz), 131.5 (d, $^3J_{C,F}$ = 8.2 Hz), 125.4, 125.2, 117.2 (d, $^2J_{C,F}$ = 20.6 Hz), 116.4 ($^2J_{C,F}$ = 21.9 Hz), 108.7, 69.5, 64.3, 62.6, 32.4, 29.7, 27.9. MS (ESI) m/z 424 (M^+−Br). HPLC analysis (A:B, 60:40) t_R = 4.46 min. Anal. Calcd for $C_{22}H_{23}N_5O_3BrF$ (503.10): C, 52.39; H, 4.60; N, 13.89. Found: C, 52.36; H, 4.60; N, 13.86.

4.8. 1-(3-Methylbenzyl)-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)-methoxymethyl]-pyridinium bromide (16c)

The reaction of **15** (70 mg, 0.222 mmol) and 3-methylbenzyl bromide (62 μL, 0.444 mmol), following the general method, afforded **16c** (98.8 mg, 89%) as a pure yellow solid (mp 175–177 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.29 (d, 2H, J = 6.6 Hz), 7.98 (d, 2H, J = 6.6 Hz), 7.28–7.20 (m, 4H), 6.15 (s, 2H), 4.90 (s, 2H), 4.81 (s, 2H), 4.03 (s, 3H), 3.55 (s, 3H), 3.39 (s, 3H), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.9, 155.3, 151.5, 147.4, 147.1, 144.5, 139.8, 132.4, 130.9, 130.1, 129.6, 126.6, 125.3, 108.7, 77.2, 69.7, 64.2, 32.6, 29.9, 28.0, 21.3. MS (ESI) m/z 420 (M^+−Br). Anal. Calcd for $C_{23}H_{26}N_5O_3Br$ (499.12): C, 55.21; H, 5.24; N, 14.00. Found: C, 55.20; H, 5.23; N, 14.00.

4.9. 1-(3-Nitrobenzyl)-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin -8-yl)-methoxymethyl]-pyridinium bromide (16d)

According to the general method, the reaction of **15** (70 mg, 0.222 mmol) with 3-nitrobenzyl bromide (95.9 mg, 0.444 mmol) produced compound **16d** (74.0 mg, 63%) as a pure yellow solid (mp 183–185 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 9.26 (d, 2H, J = 6.8 Hz), 8.62 (d, 1H, J = 1.2 Hz), 8.52 (dd, 1H, J = 1.2 Hz, J = 8.3 Hz), 8.31 (d, 2H, J = 6.8 Hz), 8.10 (d, 1H, J = 8.3 Hz), 7.93 (t, 1H, J = 8.3 Hz), 6.17 (s, 2H), 5.18 (s, 2H), 5.10 (s, 2H), 4.20 (s, 3H), 3.70 (s, 3H), 3.51 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 161.2, 156.8, 152.3, 150.3, 148.7, 145.8, 136.6, 136.2, 132.1, 126.9, 125.7, 125.1, 109.8, 70.7, 65.2, 63.9, 32.8, 30.1, 28.3. MS (ESI) m/z 451 (M^+−Br). HPLC analysis (A:B, 50:50) t_R = 1.41 min. Anal. Calcd for $C_{22}H_{23}N_6O_5Br$ (530.09): C, 49.73; H, 4.36; N, 15.82. Found: C, 49.72; H, 4.33; N, 15.80.

4.10. General procedure for the hydrogenation of pyridinium salts

To a solution of the corresponding pyridinium salt (1.0 mmol) and platinum dioxide (0.6 mmol) in purged ethanol (75 mL) was added triethylamine (1.3 mmol). The mixture was stirred under hydrogen atmosphere (20 psi) at room temperature for 15 min. The suspension was filtered and the solvent was removed in vacuo to give a yellow oil that was dissolved in dichloromethane (50 mL) and washed with water (3× 25 mL) and brine (3× 25 mL). The organic phase was dried and evapored under reduced pressure and the resulting syrups were purified by flash chromatography eluting with mixtures of CH_2Cl_2/MeOH (13:1).

4.11. 1-Benzyl-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)-methoxymethyl]-piperidine (5a)

Following the general method, using **16a** (150 mg, 0.31 mmol) as starting material, benzylpiperidine derivative **5a** (60 mg, 47%) was obtained as a white solid (mp 106–108 °C). R_f 0.5 (CH_2Cl_2/MeOH, 7:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (m, 5H), 4.58 (s, 2H), 3.98 (s, 3H), 3.55 (s, 3H), 3.50 (s, 2H), 3.39 (s, 3H), 3.33 (d, 2H, J = 6.4 Hz), 2.90 (d, 2H, J = 11.3 Hz), 1.97 (t, 2H, J = 11.3 Hz), 1.67 (d, 2H, J = 11.3 Hz), 1.60 (m, 1H), 1.30 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.4, 151.6, 149.4, 147.3, 129.3, 128.4, 128.2, 127.2, 108.5, 75.9, 64.7, 63.1, 53.1, 35.9, 32.2, 29.7, 28.7, 27.9. MS (ESI) m/z 412 $(M+H)^+$. HPLC analysis (A:B, 35:65) t_R = 1.88 min. Anal. Calcd for $C_{22}H_{29}N_5O_3$ (411.23): C, 64.21; H, 7.10; N, 17.02. Found: C, 63.98; H, 7.31; N, 16.87.

4.12. 1-(3-Fluorobenzyl)-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin -8-yl)-methoxymethyl]-piperidine (5b)

Following the general procedure, from **16b** (150 mg, 0.30 mmol), the corresponding piperidine derivative **5b** (62 mg, 49%) was obtained as a pure yellow solid (mp 118–120 °C). R_f 0.4 (CH_2Cl_2: MeOH, 10:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.24 (m, 1H), 7.06–7.02 (m, 2H), 6.91 (m, 1H), 4.60 (s, 2H), 4.00 (s, 3H), 3.56 (s, 3H), 3.46 (s, 2H), 3.39 (s, 3H), 3.34 (d, 2H, J = 5.8 Hz), 2.85 (d, 2H, J = 11.2 Hz), 1.95 (t, 2H, J = 11.2 Hz), 1.67 (d, 2H, J = 11.2 Hz), 1.60 (m, 1H), 1.30 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.8 ($^1J_{C,F}$ = 245.6 Hz), 155.4, 151.6, 149.4, 147.3, 141.3, 129.5 ($^3J_{C,F}$ = 8.4 Hz), 124.4 ($^3J_{C,F}$ = 3.1 Hz), 115.6 ($^2J_{C,F}$ = 21.4 Hz), 113.7 ($^2J_{C,F}$ = 21.4 Hz), 108.5, 76.0, 64.7, 62.7, 53.3, 36.1, 32.2, 29.7, 29.1, 27.9. MS (ESI) m/z 430 $(M+H)^+$. HPLC analysis (A:B, 60:40)

t_R = 1.40 min. Anal. Calcd for $C_{22}H_{28}FN_5O_3$ (429.22): C, 61.52; H, 6.57; N, 16.31. Found: C, 61.50; H, 6.47; N, 16.28.

4.13. 1-(3-Methylbenzyl)-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)-methoxymethyl]-piperidine (5c)

According to the general method, **16c** (150 mg, 0.30 mmol) was converted into **5c** (61 mg, 48%) as a white solid (mp 135–137 °C). R_f 0.5 (CH_2Cl_2/MeOH, 7:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.12 (t, 1H, J = 7.5 Hz), 7.05 (s, 1H), 7.02 (d, 1H, J = 7.5 Hz), 6.99 (d, 1H, J = 7.5), 4.53 (s, 2H), 3.93 (s, 3H), 3.50 (s, 3H), 3.38 (s, 2H), 3.34 (s, 3H), 3.27 (d, 2H, J = 6.3 Hz), 2.82 (d, 2H, J = 11.5 Hz), 2.27 (s, 3H), 1.86 (t, 2H, J = 11.5 Hz), 1.60 (d, 2H, J = 13.0 Hz), 1.54 (m, 1H), 1.22 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 151.6, 149.4, 147.3, 137.8, 129.9, 128.0, 127.7, 126.3, 108.5, 76.1, 64.7, 63.4, 53.3, 36.1, 32.2, 29.7, 27.9, 21.4. MS (ESI) m/z 426 $(M + H)^+$. HPLC analysis (A:B, 50:50) t_R = 1.43 min. Anal. Calcd for $C_{23}H_{31}N_5O_3$ (419.20): C, 64.92; H, 7.34; N, 16.46. Found: C, 64.90; H, 7.30; N, 16.50.

4.14. 1-(3-Aminobenzyl)-4-[(1,3,7-trimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)-methoxymethyl]-piperidine (5d)

Using the pyridinium salt **16d** (70 mg, 0.13 mmol), compound **5d** (25 mg, 41%) was obtained as a pure syrup. R_f 0.4 (CH_2Cl_2/MeOH, 8:1). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.33 (t, 1H, J = 8.0 Hz), 6.97 (d, 1H, J = 2.0 Hz), 6.94 (dd, 1H, J = 2.0 Hz, J = 8.0 Hz), 6.92 (d, 1H, J = 8.0 Hz), 4.85 (s, 2H), 4.23 (s, 2H), 4.18 (s, 3H), 3.69 (s, 3H), 3.64 (d, 2H, J = 5.4 Hz), 3.57 (d, 2H, J = 12.2 Hz), 3.51 (s, 3H), 3.06 (t, 2H, J = 12.2 Hz), 2.12 (d, 2H, J = 12.2 Hz), 2.09 (m, 1H), 1.75 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz) δ 147.2, 143.6, 141.8, 140.4, 139.1, 122.7, 121.3, 111.3, 108.6, 107.8, 100.1, 65.9, 55.8, 52.7, 43.7, 25.9, 23.3, 20.6, 18.7, 17.9. MS (ESI) m/z 427 $(M+H)^+$. Anal. Calcd for $C_{22}H_{30}N_6O_3$ (426.24): C, 61.95; H, 7.09; N, 19.70. Found: C, 61.85; H, 7.04; N, 19.69.

4.15. Cholinesterase inhibitory activity

Compounds were evaluated using AChE from bovine erythrocytes and BuChE from horse serum (Sigma), following the method of Ellman et al.[34] Enzymatic activities were measured in 100 mM phosphate buffer, pH 8.0, at 30 °C, using acetylthiocholine and butyrylthiocholine (0.4 mM) as substrates, respectively. In both cases, 5,5′-dithio-bis(2-nitrobenzoic) acid (DTNB, Ellman's reagent, 0.2 mM) was used and the values of IC_{50} were calculated by UV spectroscopy, from the absorbance changes at 412 nm.

4.16. Molecular modelling studies

Molecular modelling studies were performed using a SYBYL software[44] implemented in a Silicon Graphics workstation. Input geometries were taken from the standard ones within SYBYL program. Conformational analysis was performed by rotating the torsional angles of the linear alkyl fragments in 60 °C increments. Semiempirical calculations were carried out using the AM1 method[45] in MOPAC v5.0 program package[46] and full geometry optimisations were performed with the Fletcher-Power algorithm.

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References and notes

1. Terry, R. D. In *Alzheimer Disease: from Molecular Biology to Therapy*; Becker, R., Giacobini, E., Eds.; Birkhäuser: Boston, 1997; pp 19–23.
2. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.
3. (a) For recent reviews on the cholinergic hypothesis see: Wilkinson, D. G.; Francis, P. T.; Schwam, E.; Payne-Parrish, J. *Drugs Aging* **2004**, *21*, 453; (b) Johannsen, P. *CNS Drugs* **2004**, *18*, 757.
4. (a) For recent reviews on non-cholinergic strategies see: Doraiswamy, P. M. *CNS Drugs* **2002**, *16*, 811; (b) Castro, A.; Conde, S.; Rodríguez-Franco, M. I.; Martínez, A. *Mini Rev. Med. Chem.* **2002**, *2*, 37.
5. Giacobini, E. *Pharmacol. Res.* **2004**, *50*, 433.
6. Evans, J. G.; Wilcock, G.; Birks, J. *Int. J. Neuropsychopharmacol.* **2004**, *7*, 351.
7. Scarpini, E.; Scheltens, P.; Feldman, H. *Lancet Neurol.* **2003**, *2*, 539.
8. Giacobini, E. *Neurochem. Res.* **2003**, *28*, 515.
9. Castro, A.; Martínez, A. *Mini Rev. Med. Chem.* **2001**, *1*, 267.
10. Bolognesi, M. L.; Andrisano, V.; Bartolini, M.; Banzi, R.; Melchiorre, C. *J. Med. Chem.* **2005**, *48*, 24.
11. Zhang, N.; Casida, J. E. *Bioorg. Med. Chem.* **2002**, *10*, 1281.
12. Farlow, M. R.; Lilly, M. L. *BMC Geriatr.* **2005**, *5*, 3.
13. Finkel, S. I. *Clin. Ther.* **2004**, *26*, 980.
14. Darvesh, S.; McDonald, R. S.; Penwell, A.; Conrad, S.; Darvesh, K. V.; Mataija, D.; Gomez, G.; Caines, A.; Walsh, R.; Martin, E. *Bioorg. Med. Chem.* **2005**, *13*, 211.
15. Mesulam, M.-M.; Guillozet, A.; Shaw, P.; Levey, A.; Duysen, E. G.; Lockidge, O. *Neuroscience* **2002**, *110*, 627.
16. Darvesh, S.; Hopkins, D. A.; Geula, C. *Nature Rev.* **2003**, *4*, 131.
17. Mesulam, M.; Guillozet, A.; Shaw, P.; Quinn, B. *Neurobiol. Dis.* **2002**, *9*, 88.
18. Venneri, A.; McGeown, W. J.; Shanks, M. F. *Neuroreport* **2005**, *16*, 107.
19. Kryger, G.; Harel, M.; Giles, K.; Toker, L.; Velan, B.; Lazar, A.; Kronman, C.; Barak, D.; Ariel, N.; Shafferman, A.; Silman, I.; Sussman, J. L. *Acta Crystallogr. Sect., D* **2000**, *56*, 1385.
20. Nicolet, Y.; Lockridge, O.; Masson, P.; Fontecilla-Camps, J. C.; Nachon, F. *J. Biol. Chem.* **2003**, *278*, 41141.
21. Koellner, G.; Steiner, T.; Millard, C. B.; Silman, I.; Sussman, J. L. *J. Mol. Biol.* **2002**, *320*, 721.
22. Rodríguez-Franco, M. I.; Dorronsoro, I.; Castro, A.; Martínez, A.; Badía, A.; Baños, J. E. *Bioorg. Med. Chem.* **2003**, *11*, 2263.
23. Rodríguez-Franco, M. I.; Dorronsoro, I.; Badía, A.; Baños, J. E. *Arch. Pharm., Pharm. Med. Chem.* **2002**, *7*, 339.

24. Martínez, A.; Fernández, E.; Castro, A.; Conde, S.; Rodríguez-Franco, M. I.; Baños, J. E.; Badía, A. *Eur. J. Med. Chem.* **2000**, *35*, 913.
25. Nishijo, J.; Yonetani, I.; Iwamoto, E.; Tokura, S.; Tagahara, K.; Sugiura, M. *J. Pharm. Sci.* **1990**, *79*, 14.
26. Nishijo, J.; Iwamoto, E.; Yonetani, I.; Tokura, S.; Sugiura, M. *Chem. Pharm. Bull.* **1988**, *36*, 2735.
27. Rodríguez-Franco, M. I.; Fernández-Bachiller, M. I. *Synthesis* **2002**, *7*, 911.
28. Rodríguez-Franco, M. I.; Fernández-Bachiller, M. I. *Magn. Reson. Chem.* **2002**, *40*, 549.
29. Kryger, G.; Silman, I.; Sussman, J. L. *Structure* **1999**, *7*, 297.
30. Gupta, K. A.; Saxena, A. K.; Jain, P. C.; Anand, N. *Arch. Pharm. (Weinheim)* **1984**, *317*, 1010.
31. Mancuso, A.; Swern, D. *Synthesis* **1981**, *3*, 165.
32. Herrero, A.; Ochoa, C.; Páez, J. A.; Martínez-Ripoll, M.; Foces-Foces, C. *Heterocycles* **1987**, *26*, 3123.
33. Bredereck, H.; Siegel, E.; Föhlisch, B. *Chem. Ber.* **1962**, *95*, 403.
34. Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Feather-Stone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
35. Cygler, M.; Schrag, J. D.; Sussman, J. L.; Harel, M.; Silman, I.; Gentry, M. K.; Doctor, B. P. *Protein Sci.* **1993**, *2*, 366.
36. Kovarik, Z.; Bosak, A.; Sinko, G.; Latas, T. *Croat. Chem. Acta* **2003**, *76*, 63.
37. Sussman, J. L.; Harel, M.; Farlow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
38. Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1995**, *38*, 4821.
39. Kawakami, Y.; Inoue, A.; Kawai, T.; Wakita, M.; Sugimoto, H.; Hopfinger, A. J. *Bioorg. Med. Chem.* **1996**, *4*, 1429.
40. Alfar-López, J.; Okayama, Y.; Hosohata, K.; Davis, P.; Porrera, F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1999**, *42*, 5359.
41. Gilligan, P. J.; Cain, A. G.; Christos, T. E.; Cook, L.; Drummond, S.; Johson, A. L.; Kergaye, A. A.; McElroy, J. F.; Rohrbach, K. W.; Schmidt, W. K.; Tam, S. W. *J. Med. Chem.* **1992**, *35*, 4344.
42. Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Araki, S.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1992**, *35*, 4542.
43. Müller, C. E.; Sauer, R.; Geis, U.; Frobenius, W.; Talik, P.; Pawlowski, M. *Arch. Pharm., Pharm. Med. Chem.* **1997**, *330*, 181.
44. SYBYL 6.3 molecular modelling program. Tripos Associates.
45. Deward, M. J. S.; Zoebisch, E. G.; Hearly, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
46. Stewart, J. J. P. *QCPE Bull.* **1989**, *9*, 10, QCPE program no. 455.