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Synthesis, Absolute Configuration, Conformational Analysis and Binding Affinity Properties of Enantiomeric Forms of DAU 5750, a Novel M1–M3 Muscarinic Receptor Antagonist

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Abstract—Both the enantiomeric forms of DAU 5750, a novel muscarinic receptor antagonist, have been synthesized in order to assess the relevance of configurational/conformational features for high affinity binding to muscarinic receptor subtypes. The attribution of absolute stereochemistry and conformational analysis by means of molecular modelling and NMR techniques are also reported.

Introduction

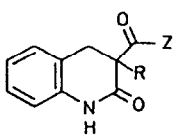
Genomic DNA cloning techniques have revealed the existence of at least five muscarinic receptor subtypes, four of which have been functionally characterized in selected tissues.[1] The therapeutic potential associated with antagonist compounds selective for each receptor subtype has been thoroughly investigated in the last decade and some results have been obtained with peripherally acting, selective M1 antagonists employed as antisecretory antiulcer drugs[2] and M2 selective antagonists that are under clinical investigation for the treatment of bradycardia.[3]

At present, compounds also showing a mixed profile of affinity and efficacy at different receptor subtypes are receiving considerable attention. For example, SDZ-ENS 163[4] an M1 postsynaptic agonist and M_2 presynaptic antagonist, is under study for the treatment of Alzheimer's disease and mixed M1–M3 antagonists have been indicated as effective bronchospasmolytics possibly free of anticholinergic side effects[5] such as mydriasis and dry mouth. Therefore, the assessment of the efficacy/affinity profiles of new ligands towards the various muscarinic receptor subtypes may suggest a potential usefulness in the above therapeutic indications.

We have synthesized a series of 2-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid esters of general formula (I)[6] (Fig. 1) and characterized it as a novel class of muscarinic receptor antagonists[7] endowed with a selectivity binding profile in the order M1 > M3 >> M2. Some of these compounds seem to be very interesting for their high affinity binding values toward M1 and M3 muscarinic receptors.

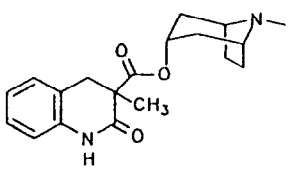
As the stereochemical and conformational properties of a molecule may have a great influence on the biological activity,[8] we selected the compound bearing *endo*-3-tropanol, DAU 5750, as representative of our class of compounds to study these aspects closely.

The present work reports the synthesis of both the enantiomeric forms of DAU 5750, the assignment of their absolute configuration, their affinity profiles and a conformational study of the most active compound made with NMR and molecular modelling techniques.



R = H, Me, Et, Ph
Z = Azacyclic or Azabicyclic alcohol

I



DAU 5750

Figure 1.

Results

Synthesis of enantiomers

The synthesis of the enantiomeric forms of DAU 5750 is shown in Scheme I.

Commercially available 2-nitrobenzylchloride (**1**) was reacted with diethyl methylmalonate sodium salt to give **2** in quantitative yield. Treatment of the nitroderivative **2** with Fe and AcOH at 80 °C gave directly the product of reduction and cyclization (±)-**3** as a racemate.

The ester (±)-**3** was hydrolysed to the corresponding acid (±)-**4** which was then treated with a chiral amine and resolved by crystallization of the resulting diastereoisomeric salts. The best resolving agent proved to be α-methylbenzylamine which after only one recrystallization and recovery of the free acid gave compounds (–)-**4** and (+)-**4** with enantiomeric excess >92 % (determined by chiral HPLC on the esters (–)-**7** and (+)-**7** see Experimental Section).

Reaction of the carboxylic acids (–)-**4** and (+)-**4** with thionyl chloride followed by esters formation with *endo*-8-methyl-8-azabicyclo[3.2.1]octanol (*endo*-tropanol) gave the desired tropylesters (–)-**7** and (+)-**7**.

Biological activity and absolute configuration

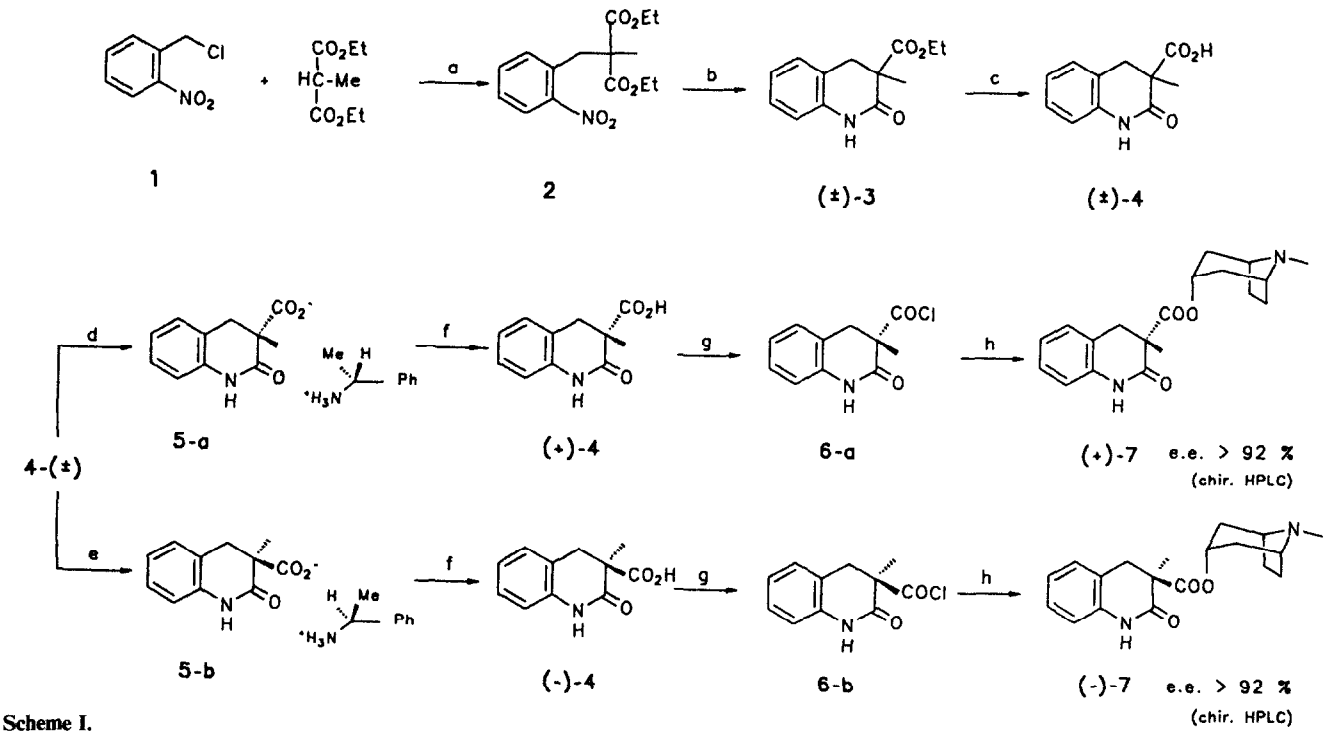
The affinity profiles of the two enantiomers were determined on the three pharmacologically defined muscarinic receptors, M1, M2 and M3. The results shown in Table 1 clearly indicate a certain degree of stereoselectivity in the binding process with the receptors studied. The affinity ratio between the distomer, the less active isomer (–)-**7**, and the eutomer, the most active (+)-**7**, is about 28.

The absolute configuration of the tetrahydroquinolinone ring stereocenter C–3 was determined by chemical correlation, converting the acid (–)-**4** to the homochiral amine (+)-**8** which was also obtained with a stereospecific synthesis (Scheme II). The acid (–)-**4** was converted, via the acyl chloride **6-b**, to the corresponding

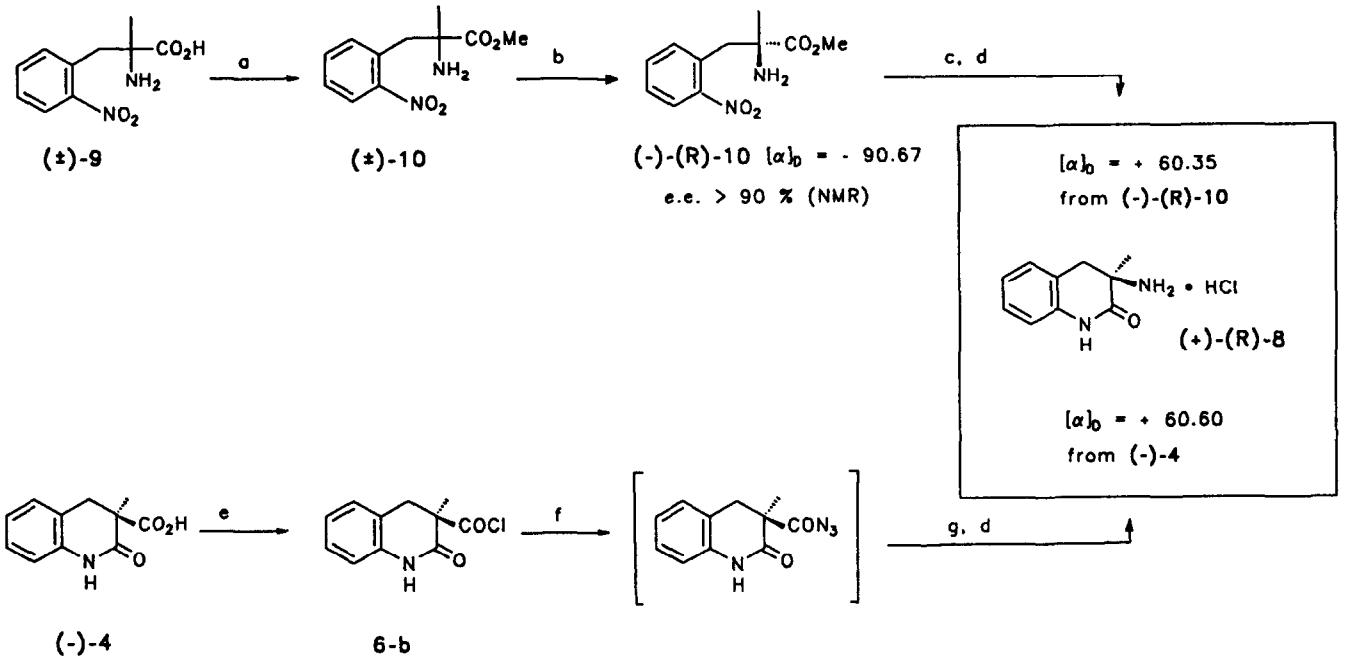
Table 1. Muscarinic receptor binding affinities in rat tissues

Compd.	Binding affinity[a,b] (Kd, nM)			Ratios		
	M1	M2	M3	M2/M1	M3/M1	M2/M3
DAU 5750 (Racem.)	26 (22-31)	1066 (766-1382)	140 (80-205)	41.0	5.4	7.6
(-)-7	530 (400-730)	20000 (12500-28200)	2100 (1300-2752)	37.7	3.9	9.5
(+)-7	19 (11-27)	670 (450-880)	80 (50-120)	35.3	4.2	8 4

[a]Displacement of [^{3}H]-PZ in cerebral cortex (M1). [^{3}H]-NMS in heart (M2) and [^{3}H]-NMS in submandibular glands.
[b]The values represent the geometrical mean of three determinations; numbers in parentheses represent 95 % confidence limits.



Scheme I.



Scheme II.

azide which, after heating in toluene and treatment with HCl, gave (+)-**8** through the Curtius rearrangement, known to be configuration retentive.[9]

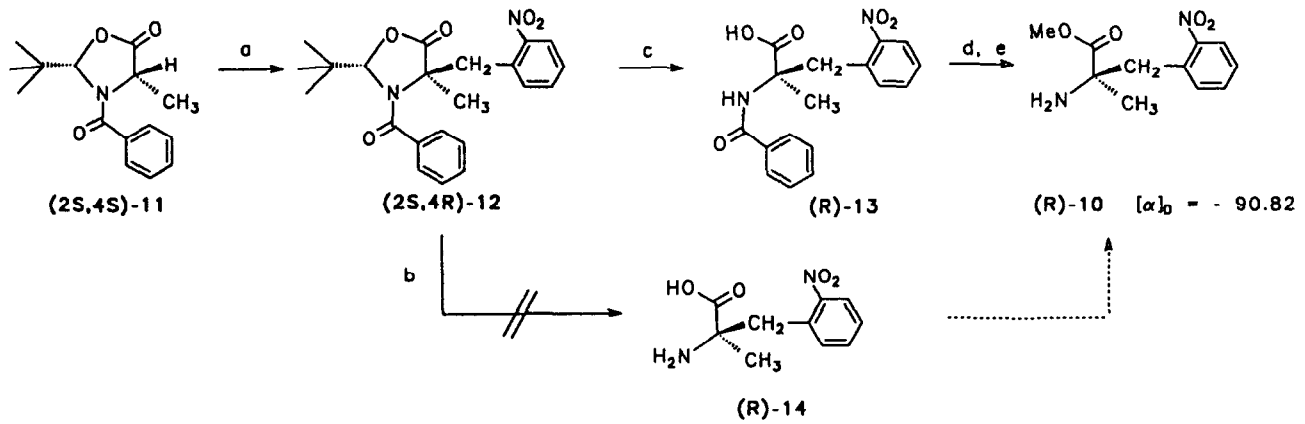
The same amine was prepared also from α-methyl(2-nitrophenyl)alanine, taking advantage of the high stereospecificity of α-chymotrypsin, as follows: treatment of the racemic α-methyl(2-nitrophenyl)alanine (±)-**9**[10] with MeOH and gaseous HCl gave the ester (±)-**10** which was submitted to enzymatic resolution with α-chymotrypsin. This resulted in a highly enantioselective hydrolysis of the racemic mixture that permitted the recovery of the unaffected amino ester (–)-**10** in a good yield. The enantiomeric excess of the ester (–)-**10** was greater than 90 % as determined by NMR analysis of the same compound in the presence of the Schift reagent $Eu(hfc)_3$.

Hydrogenation of (–)-**10** with H_2 on 10 % Pd/C in EtOH 95 % gave, upon reduction of the nitro group, the product of cyclization (+)-**8** which showed the same specific rotation as the isomer synthesized from the acid (–)-**4**.

In analogy with the reported (*S*) stereospecificity of the hydrolytic action of α-chymotrypsin towards α-aminoesters[11–13] and α-methyl-α-aminoesters,[14,15] we assumed that the unaffected aminoester (–)-**10** and, as a consequence, compounds (+)-**8** and (–)-**4**, had the absolute configuration *R*. Given the homochiral relation between the tetrahydroquinolinone ring of compound (–)-**4** and compound (–)-**7**, the *R* stereochemistry at ring stereocenter C-3 had to be assigned for (–)-**7**, i.e. *S* for (+)-**7**.

In order to check our α-chymotrypsin stereospecificity assumption, we prepared (*R*)-α-methyl(2-nitrophenyl) alanine methyl ester (*R*)-**10** with the method indicated by Seebach[16] for the synthesis of similar compounds (Scheme III).

Compound (2*S*,4*S*)-**11**[16] was converted to the corre-



Scheme III.

sponding lithium enolate with lithium diethylamide (LDEA) and rapidly alkylated with 2-nitrobenzyl bromide. The reaction gave a mixture of *N*,*N*-diethyl-(2-nitrobenzyl)amine, compound (2*S*,4*R*)-**12** and a trace of the diastereoisomer with opposite stereochemistry at the C-4. Column chromatography and recrystallization from Et_2O/cyclohexane afforded pure compound (2*S*,4*R*)-**12**.

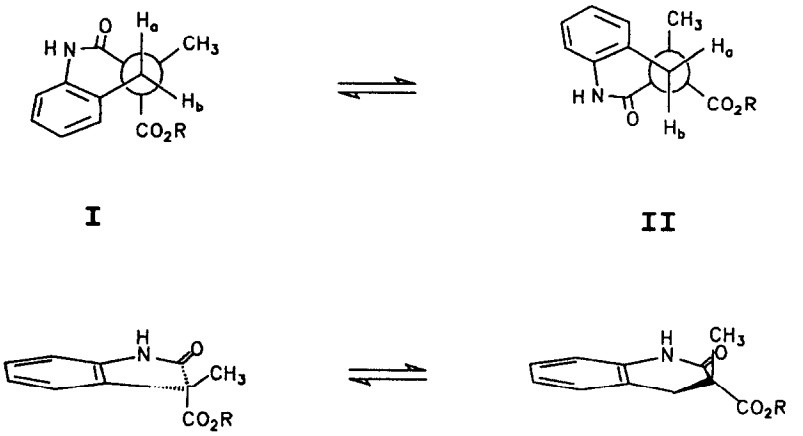
The NMR spectrum of (2*S*,4*R*)-**12** shows some very broad signals at room temperature owing to the hindered rotation of the benzyl moiety around the amide bond. These signals sharpen dramatically when the spectrum is performed in C_6D_6 at ca 75 °C, allowing the detection of the intramolecular NOEs. Thus, irradiation of the *t*-Bu group enhances the methyl signal (2.6 %) while irradiation of the benzylic protons causes an enhancement of the methine signal (1.5 %). These experiments establish unequivocally the *cis* arrangement of *t*-Bu and CH_3 groups for compound (2*S*,4*R*)-**12**.

Unexpectedly, the mild hydrolysis with $FeCl_3/SiO_2$ reagent utilized by Seebach did not work at all on our substrate (2*S*,4*R*)-**12**. Nevertheless, we opened the oxazolidinone ring in mild conditions using MeO^-/MeOH and cleaved the benzamide group with HCl. The resulting amino acid was reacted with MeOH in acidic conditions to obtain the desired aminoester (*R*)-**10** that has the same specific rotation as the isomer coming from α-chymotrypsin resolution, i.e. the ester (–)-**10** has *R* absolute configuration as we assumed.

Conformational analysis

The conformations of DAU 5750 (**7**) and of the two model compounds **3** and **4** were investigated via NMR spectroscopy and molecular modelling calculations. The δ-lactamic ring of these molecules is essentially planar apart from carbon C-3 which may lie above or below the plane, as a consequence substituents on that carbon atom can be pseudoaxially or pseudoequatorially oriented[17] (Fig. 2). Energy calculations were carried out on compounds **4**, **3** and **7** for both conformations. In all cases the energies of the conformers with equatorial Me-3 were lower than those of the corresponding conformers with axial Me-3. The energy differences E_{ax}–E_{eq} were, respectively, 0.14, 0.67 and 0.38 kcal mol^{-1}. According to these data, the Boltzmann populations at room temperature of conformers with equatorial Me-3 for compounds **4**, **3** and **7** amounted to 56, 76 and 68 %. A more accurate conformational search was carried out for compound **7** (see Experimental Section). Its overall energy and, therefore its conformational preferences, turned out to be mainly dictated by the isoquinolonic ring puckering and the torsion angles α and β (respectively, Me_3-C_3-CO-O and CO-O-C_4'-C_5', see Experimental Section). The structure with equatorially oriented Me-3 corresponding to the energy minimum was characterized by the lactamic ring in a slightly distorted half-chair conformation and the torsion around the C_3-CO bond (dihedral α) of –131°. The corresponding minimized structure with Me-3 in axial position showed a similar half-chair six-membered ring and a value of α of +13°. In both structures the tropanic moiety is not free to rotate around the O–C linkage and the equilibrium value for torsion angle β is –79 °, irrespective of the axial or equatorial position of Me-3. This shows that the two torsional degrees of freedom described by the dihedral angles α and β are not correlated one upon the other.

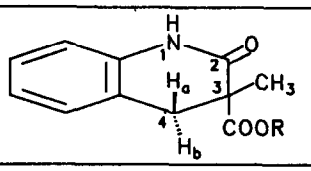
The conformation in solution of **3**, **4** and **7** has been



R =	3	4	7
	Et	H	Tropane

Figure 2.

Table 2. Selected chemical shifts and coupling constants of compounds **3**, **4** and **7**[a]



R = 3: Et; 4: H; 7: Tropane

	3	4	7
	δ_H	δ_H	δ_H
CH_3	1.51	1.46	1.50
H_a	2.89	2.83	3.08
H_b	3.43	3.24	3.34
NH	9.29	10.23	--
	δ_C	δ_C	δ_C
C-2	171.3	169.7	175.4
C-3	48.5	48.3	51.8
C-4	37.0	36.2	38.5
COO-3	172.0	175.5	174.5
CH_3	20.0	20.1	22.0

	3	4	7
	J(H,C)	J(H,C)	J(H,C)
^2J(NH,CO-2)	2.0	1.4	--
^2J(H_a,C-3)	4.4	4.4	4.4
^2J(H_b,C-3)	4.4	4.4	4.4
^2J(CH_3,C-3)	4.4	4.4	4.4
^3J(NH,C-3)	4.4	4.4	--
^3J(H_a,CH_3)	3.5	3.2	3.2
^3J(H_b,CH_3)	3.5	3.2	2.0
^3J(H_a,CO-2)	4.4	3.8	3.4
^3J(H_b,CO-2)	6.4	6.8	7.4
^3J(H_a,COO-3)	7.4	8.0	9.4
^3J(H_b,COO-3)	3.6	3.2	3.4

[a]Solvents: **3** ($CDCl_3$), **4** (DMSO-d_6), **7** (D2O).

investigated by NMR spectroscopy with the aim of experimentally supporting the conclusion of the molecular modelling calculations. The lack of vicinal H–H coupling constants of the δ-lactamic ring prompted us to focus our attention on the exploitation of the long range heteronuclear CH coupling constants as a suitable tool to elucidate the conformational preferences of the flexible part of the tetrahydroquinolonic ring. The dependence of ^3J(H,C) from the dihedral angles is well established. In a theoretical study[18] carried out on the propane molecule, it was found that ^3J(H,C) is 2 Hz for the *gauche* and 8.8 Hz for the *anti* orientation of the two nuclei. In the case of amino acids, it was shown[19] that ^3J(H,COO^-) ranges from 0.4 to 2.0 Hz for the *gauche* and from 9.8 to 11.9 Hz for the *anti* orientation. In the present case, the coupling constants of interest are those between the methylene hydrogens at C-4 and the carbon atoms of the Me-3 and COO-3 groups. The hydrogen and carbon NMR data relative to the $N_1C_2C_3C_4$ fragment for **3**, **4** and **7** are reported in Table 2.

The spectral assignment of the geminal hydrogens H_a and H_b and of the two carbonyl carbons CO-2 and COO-3 is crucial for the interpretation of the measured ^3J(H,C). The assignment of H_a vs H_b was deduced from the NOEs obtained by selective irradiation of Me-3; signal enhancements by 3–5 and 1–2 % were observed for the two methylene hydrogens, the major effect being assigned to the hydrogen *cis* to Me-3 (H_a). The carbon signals CO-2 and COO-3 of **3** and **4** have been distinguished from the coupling constant with the amide hydrogen displayed by carbon CO-2 (2.0 and 1.4 Hz, respectively). This coupling disappears for **7** since it was dissolved in deuterated water and in this case COO-3 was recognized from the coupling constant with the hydrogen H-4' of the tropanic moiety (3.4 Hz).

The values of ^3J(H,C) quoted in Table 2 show significant differences. J(H_a,COO-3) varies from 7.4 to 9.4 Hz and J(H_b,COO-3) ranges from 2.0 to 3.5 Hz suggesting that H_a and COO-3 are preferentially in an *anti* orientation. J(H_a,Me) and J(H_b,Me) show values in the range 2.0–3.5 Hz in agreement with a pseudoequatorial orientation of the methyl group for all compounds. Finally the endocyclic carbonyl group CO-2 shows couplings with H_a and H_b of 3.0–4.4 Hz and 6.4–8.2 Hz, respectively, suggesting a pseudoequatorial orientation of H_b.

Thus, from the values of vicinal J(H,C) it clearly emerges that the tetrahydroquinolinone ring exists preferentially in a conformation with equatorially oriented Me-3 in agreement with the molecular modelling findings. On the other hand, the experimental coupling constants are significantly different from that expected for pure *gauche* or *anti* orientations of the interacting nuclei, indicating that most probably an equilibrium exists in solution between two conformations as suggested by the modelled structures. The equilibrium populations of each conformer can be calculated from the experimantal vicinal coupling constants through the time-average equation $J_{av} = P_1J_1 + P_2J_2$, where P_1J_1 and P_2J_2 are, respectively, the populations and the vicinal coupling constants of the molecule in the two limiting conformational states. Thus populations of the

conformer with pseudoequatorial methyl group for compounds **4**, **3** and **7**, are approximately 70, 65 and 80 %. The discrepancies with respect to the populations deduced from the energy differences of the modelled structures should reasonably be ascribed to solvent effects. (Table 2, Fig. 2)

Discussion

In the present work, we described the synthesis of both the enantiomeric forms of DAU 5750, the most potent compound out of a novel class of muscarinic receptor antagonists. These novel compounds are made of the unusual 2-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid esterified with an azacyclic- or azabicyclic-alkanol group which, in the case of DAU 5750, corresponds to α-tropanol. The presence of the stereocenter C-3 in the tetrahydroquinolinone ring makes this compound to be a racemic mixture and the single enantiomers could be expected to have different biological properties.

In our binding studies (Table 1), the isomer (+)-**7** showed about 30-fold higher affinity than that of (–)-**7** for the three muscarinic receptor subtypes M1, M2 and M3. Therefore, it can be concluded that the stereochemistry of the tetrahydroquinolinone ring is crucial for high affinity binding of DAU 5750 and, probably, of the whole class to the three muscarinic receptor subtypes.

Interestingly, the selectivity profiles of both the eutomer and the distomer as measured by the affinity ratios for the M1, M2 and M3 subtypes were almost identical, the absolute values being uniformly affected.

The synthesis of the enantiomeric forms of DAU 5750 involved a separation of the racemic acids **3**-(±) by means of the classical crystallization of the diastereoisomeric salts with (+) and (–) α-methylbenzylamine; meanwhile, the determination of their absolute configurations was accomplished by chemical correlation. Amine (+)-**8**, derived from acid (–)-**4** by means of Curtius rearrangement, was compared with a pure sample of amine (+)-(*R*)-**8** obtained by a facile synthesis which employs enzymatic resolution of α-methyl-(2-nitrophenyl)alanine methyl ester by α-chymotrypsin. Accordingly, the eutomer (+)-**7** was given the absolute configuration (*S*), and the distomer (–)-**7** the configuration (*R*).

The assumption that the above enzyme stereospecifically cleaves (*S*) α-methyl-α-aminoesters was confirmed by the independent and stereospecific synthesis of (–)-(*R*)-**10**, namely methyl-(2-nitrophenyl)alanine methyl ester, which was left unreacted by α-chymotrypsin. Notably, such stereospecificity is maintained also on *o*.NO_2 substituted α-methyl-phenylalanine methyl ester.

According to NMR and molecular modelling results, a preferred conformation of the isoquinolinone ring should exist; the conformer with the pseudoequatorial methyl group accounting for 70–80 % of the population in aqueous solution at room temperature.

Unfortunately, this feature is of too low relevance to acquire more precise structure–activity relationships, as the energetic barrier for the interconversion of the two conformers is probably low with respect to the energy usually involved in the binding process.[20]

Since in functional studies this class of compounds behaved as muscarinic antagonists,[7] they are under investigation as potential bronchospasmolytic drugs for the treatment of chronic obstructive lung diseases.

Experimental

Melting points were determined on a Büchi 530 capillary apparatus and are uncorrected. Thin layer chromatography was carried out on silica gel 60 F 254 precoated glass plates (Merck, 0.25 mm). Column chromatography was performed on silica gel 60 (Merck, 70–230 mesh). Chiral HPLC analysis were performed on an Enantiopak (LKB) using phosphate buffer: iPrOH, 92:8, as mobile phase. Optical rotations were determined at 22 °C on a Perkin Elmer 241 polarimeter.

Molecular modelling was done with the CVFF[21] force field as implemented in Biosym's INSIGHT/DISCOVER software[22] on a Silicon Graphics IRIS 4D-35 personal workstation. Molecules **4**, **3** and **7** were built using the SKETCH routine within the Bulder module of Insight II. A reasonable *in vacuo* starting geometry for compounds **4**, **3** and **7** was obtained after approximately 1000 iterations using steepest descent minimization algorithm. In this first run cross-terms (coupling between deformations of internal coordinates) were not included and armonic potentials for bond-stretching terms were used. This allowed us to obtain physically meaningful structures starting from distorted geometries as those generated from scratch. In all cases, the maximum energy derivative was ≤0.05 kcalÅ^{-1}. Subsequently all the structures previously generated were reminimized until a maximum energy derivative ≤0.001 kcalÅ^{-1} was obtained using the quasi-Newton–Raphson minimization algorithm, including in the calculation cross-terms and using Morse potentials for bond stretching.

A more systematic conformational search for compound **7** was achieved exploring the potential energy surface by rotating the torsion angles α and β defined respectively as Me-3–C-3–CO–O and CO–OC-4'–C-5' (where the prime symbol refers to the tropanic ring system). The dihedral α and β were rotated 360° in 36° steps, computing the total energy for each step. The structure corresponding to the deepest minimum of the energy grid generated in this way was, in turn, reminimized till the maximum energy derivative was ≤0.001 kcalÅ^{-1}.

^{1}H and ^{13}C NMR spectra were recorded on Varian-CFT-20, -VXR-200. Bruker-CPX-300 and -AC-250. Chemical shift values are expressed as δ (ppm) with respect to TMS used as internal standard. The long range H,C coupling constants were extracted from the undecoupled carbon spectra. Each coupling constant was assigned by performing several ^{13}C spectra where

the appropriate proton signal was selectively decoupled. The CW irradiation was performed with the decoupler in the low power mode using an attenuation of 33–38 dB. For the ^1H NOE difference spectra, an experiment was performed with the decoupler on-resonance and then subtracted from a control spectrum with the decoupler off-resonance. The irradiation time was 4 s and the relaxation delay 8 s.

Ethyl 3-(2-nitrophenyl)-2-methyl-2-carbethoxypropionate (**2**)

A solution of diethyl methyl malonate (50.7 g, 0.29 mol) in THF (50 mL) was added dropwise to a stirred suspension of NaH (8.7 g of 80 %, 0.29 mol) in THF (300 mL). The solution was stirred for 30 min at room temperature until evolution of H_2 ceased. A solution of 2-nitrobenzyl chloride (50 g, 0.29 mol) in THF (100 mL) was added dropwise and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was poured into aqueous NH_4Cl and the product was extracted with AcOEt. The combined organic layers were washed with H_2O, dried ($MgSO_4$) and concentrated to give an oil. Distillation under vacuum gave the title compound as a yellow oil (86 g): bp 144–146 °C at 0.2 mmHg. ^1H NMR (80 MHz; $CDCl_3$) :δ 7.88 (1H, m, arom. *o*-nitro), 7.2–7.6 (3H, m, arom.), 4.17 (4H, q, $C\underline{H}_2CH_3$), 3.67 (2H, s, CH_2), 1.32 (3H, s, CH_3), 1.20 (6H, t, $CH_2C\underline{H}_3$).

(±)-3-Carbethoxy-3-methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline [(±)-3]

A stirred suspension of **2** (86 g, 0.278 mol) and Fe powder (46.6 g, 0.834 mol) in AcOH was heated at 80 °C for 13 h. After cooling, the mixture was poured into H_2O and extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was triturated with petroleum ether to give the title compound as an ivory solid (55 g), mp 110–111 °C. ^1H NMR (250 MHz; $CDCl_3$) : δ 9.38 (1H, s, NH), 6.81–7.25 (4H, m, arom.), 4.11 (2H, m, $OC\underline{H}_2CH_3$), 3.24 (1H, d, H_b), 2.89 (1H, d, H_a, $J(H_a,H_b)$ = 17.0 Hz), 1.51 (3H, s, CH_3), 1.09 (3H, t, $CH_2C\underline{H}_3$, $J(CH_2,CH_3)$ = 6.7 Hz). ^{13}C NMR (250 MHz; $CDCl_3$) :δ 172.0 (COO-3), 171.32 (CO-2), 136.69, 128.00, 127.07, 115.37 (arom.), 61.55 ($OC\underline{H}_2CH_3$), 49.47 (C-3), 37.04 (C-4), 20.09 (CH_3), 13.57 ($CH_2C\underline{H}_3$).

(±)-3-Methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline-3-carboxylic acid [(±)-4]

A solution of (±)-**3** (13.5 g, 0.058 mol) and KOH (7.6 g of 85 %, 0.115 mol) in EtOH (95 %, 130 mol) was stirred at room temperature for 1 h. The solid was filtered, washed with absolute EtOH and dissolved in H_2O. The insoluble in H_2O was filtered off, the solution cooled in an ice bath and treated carefully with 20 % HCl. The precipitated solid was filtered, washed with H_2O and dried under vacuum at 50 °C to afford the title compound as a white solid (10 g). As free acid: mp 164–165 °C. ^1H NMR (80 MHz; $CDCl_3$) : δ 10.03 (1H, br NH), 6.6–7.1 (4H, m, arom.) 2.79, 3.25 (2H, d, CH_2), 1.34 (3H, s, CH_3).

3-Methyl-2-oxo-1H-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (R)-*(+)-α-methylbenzylamine salt (**5-a**)*

(±)-**4** (3.2 g, 0.016 mol) was added to a stirred solution of (*R*)-(+)-α-methylbenzylamine (1.9 g, 0.016 mol) in EtOH (95 %, 550 mL). With a brief heating was obtained a clear solution that was left at room temperature for 2 days. The precipitated solid was filtered, washed with EtOH 95 % and dried under vacuum at 50 °C to afford the title compound as a white solid (1.4 g): mp 175–176 °C.

3-Methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline-3-carboxylic acid (S)-*(−)-α-methylbenzylamine salt (**5-b**)*

(±)-**4** (3.2 g, 0.016 mol) was added to a stirred solution of (*S*)-(−)-α-methylbenzylamine (1.9 g, 0.016 mol) in EtOH (95 %, 550 mL). With a brief heating a clear solution was obtained and left at room temperature for 2 days. The precipitated solid was recovered by filtration, washed with EtOH 95 % and dried under vacuum at 50 °C to afford the title compound as a white solid (1.5 g), mp 173–174 °C.

(+)-3-Methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline-3-carboxylic acid [(+)-4]

Compound **5-a** (3 g, 0.0092 mol) was dissolved in H_2O (150 mL) and the solution acidified (pH 2.0) with HCl 10 %. The precipitated solid was filtered and dried under vacuum at 40 °C to afford the title compound (1.3 g) as a white solid. As free acid: mp 139–140 °C. $[\alpha]_D^{22}$ + 37.19° (*c* 2; EtOH). ^1H NMR (250 MHz; DMSO-d_6) : δ 12.5 (1H, br, COOH), 10.30 (1H, s, NH), 6.80–7.20 (4H, m, arom.), 3.24 (1H, d, H_b), 2.84 (1H, d, $J(H_a,H_b)$ = 16.0 Hz), 1.36 (3H, s, CH_3-3). ^{13}C NMR (250 MHz; DMSO-d_6) : δ 175.50 (COOH), 169.68 (CO-2), 114.68, 122.0, 122.09, 127.24, 127.77, 137.62 (arom), 48.38 (C-3), 36.25 (C-4), 20.05 (CH_3-3).

(−)-3-Methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline-3-carboxylic acid [(−)-4]

Compound **5-b** (3 g, 0.0092 mol) was dissolved in H_2O (150 mL) and the solution acidified (pH 2.0) with 10 % HCl. The solid precipitated was filtered and dried under vacuum at 40 °C to afford the title compound (1.4 g) as a white solid. As free acid: mp 140–141 °C. $[\alpha]_D^{22}$ −38.98° (*c* 2, EtOH) NMR data as compound (+)-**4**.

*3-Methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline-3-carbonyl chloride (**6-a**)*

A suspension of compound (+)-**4** (1.54 g, 0.0075 mol) in $SOCl_2$ (15 mL) was heated at 50 °C for 1 h. The solution obtained was concentrated and stripped several times with toluene to give the title compound as an ivory solid (1.64 g), mp 101–103 °C.

*3-Methyl-2-oxo-1H-1,2,3,4-tetrahydroquinolin-2-one-3-carbonyl chloride (**6-b**)*

A suspension of compound (–)-**4** (1.54 g, 0.0075 mol) in $SOCl_2$ (15 mL) was heated at 50 °C for 1 h. The solution obtained was concentrated and stripped several times with toluene to give the title compound as an ivory solid (1.55 g), mp 102–103 °C.

*(+)-*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 3-methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline-3-carboxylate [(+)-7]*

To a stirred solution of *endo*-8-methyl-8-azabicyclo-[3.2.1]octan-3-ol (tropanol) (1.11 g, 0.0079 mol) and NEt_3 (0.94 g, 0.0093 mol) in CH_3CN (40 mL) was added dropwise a solution of **6-a** (1.6 g, 0.0071 mol) in CH_3CN (30 mL). After stirring for 6 h at room temperature the solvent was removed under vacuum and the residue treated with 5 % HCl. The acidic solution was washed with AcOEt, basified with 10 % NaOH and extracted with AcOEt. The combined organic extracts were washed with NaCl-saturated water, dried ($MgSO_4$) and concentrated to give the title compound (0.37 g,) as a yellow oil. Chiral HPLC analysis indicated that this product was greater than 95.5 % enantiomerically pure. As hydrochloride salt: mp 223–230 °C, $[\alpha]_D^{22}$ + 21.29° (*c* 1.5, EtOH 95 %). ^1H NMR; (200 MHz; $CDCl_3$) : δ 9.30 (1H, s, NH), 7.28–6.85 (4H, m, arom.), 4.95 (1H, t, CHOCO), 3.42, 2.90 (2H *gem*, d, CH_2 tetrahydroquinolone), 3.03 (2H, br, CH), 2.23 (3H, s, NCH_3), 2.15–1.37 (8H, m, CH_2 tropane), 1.54 (3H, s, CH_3).

*(–)-*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 3-methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline-3-carboxylate [(–)-7]*

To a stirred solution of *endo*-8-methyl-8-azabicyclo-[3.2.1]-octan-3-ol (tropanol) (1.04 g, 0.0074 mol) and NEt_3 (0.88 g, 0.0087 mol) in CH_3CN (40 mL) was added dropwise a solution of **6-b** (1.5 g, 0.0067 mol) in CH_3CN (30 mL). After stirring for 6 h at room temperature, the solvent was removed under vacuum and the residue treated with 5 % HCl. The acidic solution was washed with AcOEt, basified with 10 % NaOH and extracted with AcOEt. The combined organic extracts were washed with NaCl saturated water, dried ($MgSO_4$) and concentrated to give the title compound (0.37 g) as a white solid. Chiral HPLC analysis indicated that this product was greater than 95.5 % enantiomerically pure. As hydrochloride salt: mp 228–238 °C, $[\alpha]_D^{22}$ – 22.76° (*c* 1.5; EtOH 95 %) ^1H NMR (250 MHz; D_2O) : δ 7.32–6.95 (4H, m, arom.), 4.97 (1H, t, CHOCO), 3.85, 3.75 (2H, m, N-CH; distinct resonances are due to the stereocenter C-3), 3.34 (1H, d, H-4b), 3.08 (1H, d, H-4a, $J(H_a,H_b)$ = 16.5 Hz), 2.72 (3H, s, NCH_3), 1.63–2.38 (8H, m, 4 CH_2), 1.50 (3H, s, CH_3-3). ^{13}NMR (250 MHz; D_2O) : δ 175.4 (COO-3), 174.5 (CO-2), 138.3, 130.7, 130.68, 126.6, 124.9, 118.2 (arom.), 69.0 (CH-O), 64.5, 64.4 (2 N-CH), 51.8 (C-3), 41.0 (NCH_3), 38.5 (CH_2-4), 36.9, 36.8 (2 CH_2), 25.8, 25.7 (2 CH_2), 22.0 (CH_3-3).

*Methyl(2-nitrophenyl)alanine methyl ester [(±)-**10**]*

Gaseous HCl was bubbled into a solution of **9** (16.5 g, 0.054 mol) in MeOH (250 mL) while keeping the temperature below 35 °C. The solution was heated under reflux for 5 h. After cooling, the reaction mixture was concentrated, treated with H_2O and washed with Et_2O. The aqueous solution was basified with solid Na_2CO_3 and extracted with AcOEt. The combined organic extracts were dried ($MgSO_4$) and concentrated to give as a brown oil. The oil was converted into the corresponding hydrochloride that was recrystallized from EtOH/Et_2O to give the desired product (11.7 g). As hydrochloride salt: ^1H NMR (80 MHz; $CDCl_3$) : δ 8.89 (3H, br, NH_3^+), 8.00 (1H, m, arom. *o*-nitro), 7.35–7.75 (3H, m, arom.), 3.63 (3H, s, CO_2CH_3), 3.42, 3.79 (2H *gem*, d, CH_2), 1.50 (3H, s, CH_3).

*Methyl(2-nitrophenyl)alanine methyl ester (R)-(–)-**10** from (±)-**10***

A solution of **(±)-10** hydrochloride (14 g, 0.051 mol) in H_2O (700 mL) at 37 °C was brought to pH 5 with 0.5 N LiOH (3 mL). α-Chymotrypsin was added and the mixture left 50 h under stirring at 37 °C keeping the pH at 5 with 0.5 N LiOH. The mixture was filtered on Celite, the filtrate basified to pH 10 and extracted with AcOEt. The organic phase was dried ($MgSO_4$) and evaporated to give the desired product (4.3 g). As free base: ^1H NMR (200 MHz; $CDCl_3$) : δ 7.86 (1H, m, arom. *o*-nitro), 7.30–7.60 (3H, m, arom.), 3.61 (3H, s, CO_2CH_3), 3.56, 3.27 (2H *gem*, d, CH_2), 1.57 (2H, br, NH_2), 1.32 (3H, s, CH_3). As hydrochloride salt: mp 90–93 °C, $[\alpha]_D^{22}$ – 90.67° (*c* 2; H_2O)

*(+)-3-Amino-3-methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline (+)-**8** from **6-b***

A solution of **6-b** (4 g, 0.018 mol) in acetone (10 mL) was added dropwise to a stirred solution of NaN_3 (1.32 g, 0.021 mol) in H_2O (25 mL). After 1 h the reaction mixture was extracted with toluene. The combined organic extracts were washed with H_2O, dried ($MgSO_4$) and heated at 70 °C for 1 h then 37 % HCl (2 mL) was added and heating was continued for 30 min more. After cooling, the solid was filtered and recrystallized (*i*PrOH/EtOH) to afford as a white solid (1.12 g). As hydrochloride salt: $[\alpha]_D^{22}$ + 60.60° (*c* 2; H_2O). ^1H NMR (200 MHz; DMSO) : δ 10.82 (1H, s, NH), 8.85 (3H, b, NH_3^+), 7.2–7.3 (2H, arom.), 6.9–7.1 (2H, arom.), 3.36, 3.09 (2H *gem*, d, CH_2), 1.29 (3H, s, CH_3).

*(+)-3-Amino-3-methyl-2-oxo-1H-1,2,3,4-tetrahydroquinoline (+)-**8** from (–)-(R)-**10***

A mixture of compound (–)-(*R*)-**10** (3.6 g, 0.015 mol), 10 % Pd/C (0.200 g) in 95 % EtOH was hydrogenated for 5 h. The reaction mixture was filtered from the catalyst and the filtrate evaporated. The solid residue was purified by column chromatography and treated with MeOH/HCl gas to give the desired compound (1.7 g). As hydrochloride salt: $[\alpha]_D^{22}$ + 60.35° (*c* 2; H_2O). ^1H NMR (200 MHz; DMSO) : δ 10.82 (1H, s, NH), 8.85

(3H, b, NH_3^+), 7.2-7.3 (2H, m, arom.), 6.9–7.1 (2H, m, arom.), 3.36, 3.09 (2H *gem*, d, CH_2), 1.29 (3H, s, CH_3).

(2S,4R)-3-Benzoyl-4-(2-nitrobenzyl)-2-(tert-butyl)-4-methyl-1,3-oxazolidin-5-one [(2S,4R)-12]

A freshly made solution of LDEA [($HNEt_2$ (0.74 g, 0.010 mol), THF (21 mL) and BuLi 1.6 M in hexane (7.5 mL, 0.012 mol)] was added dropwise to a stirred solution of compound (2*S*,4*S*)-**11** (2.2 g, 0.0084 mol) in THF (20 mL) cooled at –78 °C. After stirring for 40 min, a solution of 2-nitrobenzyl bromide (3.6 g, 0.0168 mol) in THF (20 mL) was added dropwise and the mixture was allowed to warm to room temperature over 2 h. After stirring overnight, the reaction mixture was poured into a cold half-saturated NH_4Cl solution and extracted with Et_2O. The organic phase was washed with H_2O, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography using hexane:AcOEt, 8:2, as eluant and the resulting solid was recrystallized from Et_2O/cyclohexane to give the title product (0.5 g). ^1H NMR (200 MHz; C_6D_6; 75 °C) : δ 6.65–7.55 (9H, m, arom.), 0.75 (9H, s, $C(CH_3)_3$), 6.12 (1H, s, CH), 3.58 and 3.44 (2H, d, benzylic hydrogens, J_{gem} = 14.2 Hz), 1.59 (3H, s, CH_3).

(R)-α-*Methyl-N-benzoyl-(2-nitrophenyl)alanine [(R)-13]*

A solution of compound (2*S*,4*R*)-**12** (0.500 g, 0.0013 mol), MeONa (0.14 g, 0.0025 mol) in MeOH (20 mL) was stirred at room temperature. After 20 h the reaction mixture was poured in H_2O and the alcohol evaporated under reduced pressure. The aqueous phase was washed with AcOEt, made acidic with 10 % HCl and extracted with AcOEt. The combined organic extracts were dried ($MgSO_4$) and concentrated to give the title compound (0.42 g) as an oil. As free acid: ^1H NMR (200 MHz; $CDCl_3$) :δ 7.60–7.80 (3H, arom.), 7.20–7.50 (6H, arom.), 6.87 (2H, br, CO_2H and NH), 3.79, 3.73 (2H, 2d, CH_2), 1.63 (3H, s, CH_3)

(R)-α-*Methyl-(2-nitrophenyl)alanine methylester hydrochloride [(R)-10]*

A solution of compound (*R*)-**13** (0.10 g, 0.09 mol) in HCl 37 % (5 mL) was refluxed for 3 h. After cooling to room temperature, the solution was washed with Et_2O and the aqueous solution concentrated under reduced pressure. The residue was taken over with MeOH/HCl and refluxed for 10 h. After cooling the solution was concentrated, taken over with H_2O and washed with Et_2O. The aqueous phase was basified with Na_2CO_3 and extracted with AcOEt. The combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was dissolved with MeOH:HCl and concentrated again to give (0.045 g) of the title compound. As hydrochloride salt: $[\alpha]_D^{22}$ – 90.82 (*c* 2; H_2O). NMR data as reported above for the compound obtained from (±)-**10**

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