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Conformation-activity relationships of opiate analgesics

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SUMMARY

Extensive conformational calculations were performed on the potent opiate analgesics etorphine, PET, R30490 and etonitazene to determine all of their many low-energy conformations. The results were used to characterize four possible models for binding of a simple pharmacophore, comprising two phenyl rings plus a protonated nitrogen, to opiate analgesic receptors. These four models may define the necessary three-dimensional features leading to particular opiate actions. The model favoured for μ receptor activity can accommodate a protonated nitrogen, an aromatic ring (which may be substituted with an electronegative group) and a second lipophilic group. These structural features must be presented in a precise three-dimensional arrangement. It appears likely that a hydrophilic substituent in a certain region of the analgesic pharmacophore may also interact with the receptor as a secondary binding group.

INTRODUCTION

Recent studies in our laboratory have led to the observation that many different classes of central nervous system (CNS) active drugs share a common characteristic three-dimensional arrangement of two functional groups, a phenyl ring and a nitrogen atom, and that the specific activities of individual CNS drug classes may be due to the presence of secondary binding groups in specific locations relative to this common pharmacophore [1, 2]. Subsequent studies have identified the location and nature of the secondary binding groups in antidepressants [3] and outlined the use of the common model in CNS drug design [4]. In this paper we set out to identify the features responsible for the specific activities of the opiate analgesics.

Since the original structure-activity relationship proposed by Beckett and Casy [5], many structural and conformational models have been advanced to explain opiate analgesic activity [6–14]. In general, these fall into two classes: (a) those based on similarities with the structure of mor-

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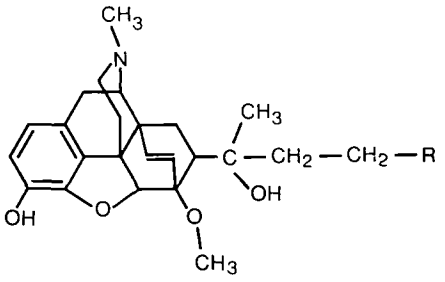
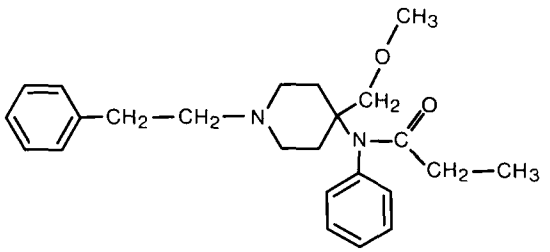
phine [5–9] and (b) those based on the structures of the enkephalins [10–14]. We have taken a slightly different approach, and have based our modelling on four compounds that share the characteristics of being extremely potent analgesics (1000 times the activity of morphine) and extremely tight binders at opiate μ-receptors (50 times tighter than the binding of morphine).

The compounds chosen were the potent oripavine derivatives etorphine (1) and PET (2), the fentanyl analogue R30490 (3), and the relatively novel analgesic etonitazene (4). As is evident from their structures, each of these analgesics contains several additional structural features over and above those present in either the common model or morphine (5), as well as a large number of significant conformational variables. The purpose of our work has been to identify the common receptor-binding characteristics and corresponding biologically active conformations of these molecules by carrying out the following steps: (a) identification of common structural features likely to be responsible for specific activity; (b) comparison of all energetically accessible conformations of the molecules to determine whether common arrangements of these groups, or the corresponding receptor groups, is possible; and (c) testing of the models derived for match to other potent opiate analgesics.



1 R = CH_3

2 R = 



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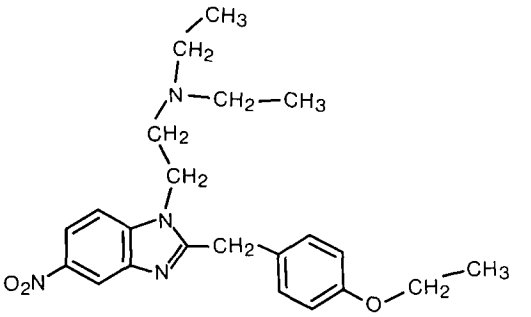

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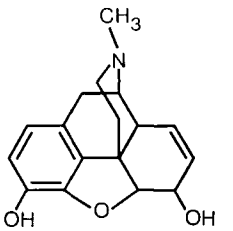

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METHODS

Conformational Analysis

A very simple classical potential energy calculation procedure, CONES, was used to locate energy minima. CONES is a further development of COMOL [15] that allows rapid energy calcula-

tions on large molecules as a function of up to three torsion angles, while holding bond geometries constant. The objective, to locate all possible low energy conformations available to the molecules, is performed rapidly using CONES, although energy differences and barriers between energy minima are somewhat overestimated by the program. Supplementary calculations for the complete geometry optimisation of key conformations were therefore performed using the standard molecular mechanics programs MMI/MMPI [16] and MM2 [17].

Torsion angles specified here as τ (ABCD) are defined as the clockwise rotation of atom A required to eclipse atom D while looking along the B–C bond from atom B to atom C [18]. Potential energy contour maps were generated from CONES data using a modified version of the subroutine KONTOR [19]; the difference in energy between adjacent lines is 2.5 kcal/mol and only the first 20 contour lines are shown.

Computer Graphics and Receptor Modelling

The MORPHEUS computer graphics package developed at the Victorian College of Pharmacy Ltd. was used for the display, manipulation and superimposition of molecules. Molecular geometries for input into the various programs available were obtained from crystal structure coordinates or, in their absence, standard bond distances and angles [20].

To simulate the opiate receptor surface, dummy atoms were built onto selected key features of the opiate analgesics prior to matching the structures. The dummy atoms or receptor points of all three molecules should be able to be matched if the chosen binding features are relevant to opiate analgesic activity. In order to best approximate the receptor surface these receptor points were built using known geometries for interactions between binding substituents and binding sites. Thus a perpendicular point at a distance of a 3.5Å from the centre of the ring was used for a phenyl ring receptor point [21] while a hydrogen bond distance of 2.8Å [22, 23] and the geometry of an acidic proton was used for the interaction between a protonated nitrogen and its binding site.

The receptor modelling was performed by firstly determining all possible low-energy conformations of the potent opiate analgesics. The accessible conformations of one of the molecules, PET, then served as templates against which the other potent opiates were matched in low-energy conformations. Dreiding molecular models were used for initial matching of R30490 and etonitazene to the PET templates and refinement of these proposed binding conformations was achieved using a combination of programs. The first of these programs (HAYSTK) performs a multidimensional grid search while another two perform stepwise searches over surfaces to locate best fit conformations (MAPGID and FIT). Although these subroutines are inadequate for locating all possible matches between two flexible molecules, they are extremely useful for rapid optimisation of crude matches determined from molecular models.

RESULTS AND DISCUSSION

Because of the large number of conformational variables associated with each of these analgesics the results of the conformational analysis will initially be discussed individually, and then drawn together in the final section on receptor modelling.

R30490

The compound R30490 (3) is a potent analgesic of the fentanyl class and has eight rotatable

bonds (excluding terminal methyl rotations), an amide bond that may be either *cis* or *trans*, and a piperidine ring that might exist in at least four different conformations. Thus there are at least ten degrees of conformational freedom in this molecule.

The basic geometry of R30490 was obtained from the crystal structure [24] and was subsequently used for most of the conformational work. Initially, to minimise the number of variables, energy calculations were performed on fragments of the molecule, as indicated in Fig. 1. The molecule was then treated as a whole for calculations designed to determine the effects on energy of various piperidine ring conformations. All conformations within 10 kcal/mol as calculated by CONES or 5 kcal/mol for MM1 and MM2 calculations, were assumed to be potentially biologically active.

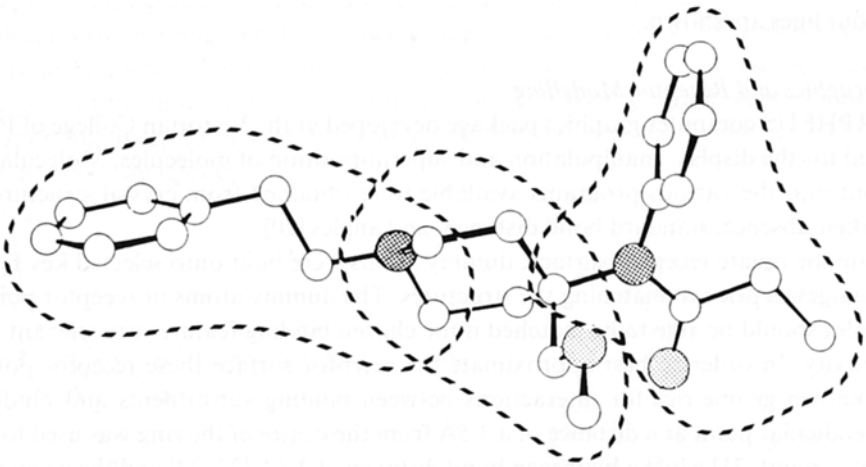
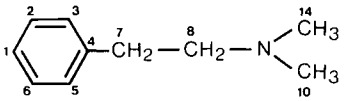


Fig. 1. Crystal structure of R30490 with portions of the molecule encircled to indicate the fragments used in the initial conformational calculations. Nitrogen atoms are shown in dark shading and oxygen atoms in light shading.

Fragment calculations. The phenethylamine component of R30490 comprises three torsion angles (Fig. 2a). The two methyl groups bonded to the nitrogen in this fragment form part of the piperidine ring in R30490 so were maintained at their crystal structure torsion angle values in these calculations.

The rotation of the phenyl ring, τ_1, is not restricted though it prefers a perpendicular conformation (90°) with respect to the rest of the sidechain. If τ_3 only is considered, it is possible to distinguish three discrete conformations, two of these place the phenethyl sidechain below what would be the plane of the piperidine ring in the R30490 molecule, the other conformation places it above the ring. This last conformer is always 5 kcal/mol or more higher in energy than the other two low-energy conformations of τ_3. For the lower energy τ_3 conformations (80° and 150°), τ_2 can assume two different conformations, one an extended chain and the other a skewed conformation. At the higher energy τ_3 conformation (300°), τ_2 can only assume the extended conformation (Fig. 2b). Thus there are five broad minima for this fragment, and hence the phenyl ring, while rotating freely around its axis, can be placed in any of five regions with respect to the piperidine ring of R30490.

The second fragment investigated was the piperidine ring with a 4-methoxymethyl substituent shown in Fig. 3a. Only the chair conformation of the piperidine ring with an axial methoxymethyl



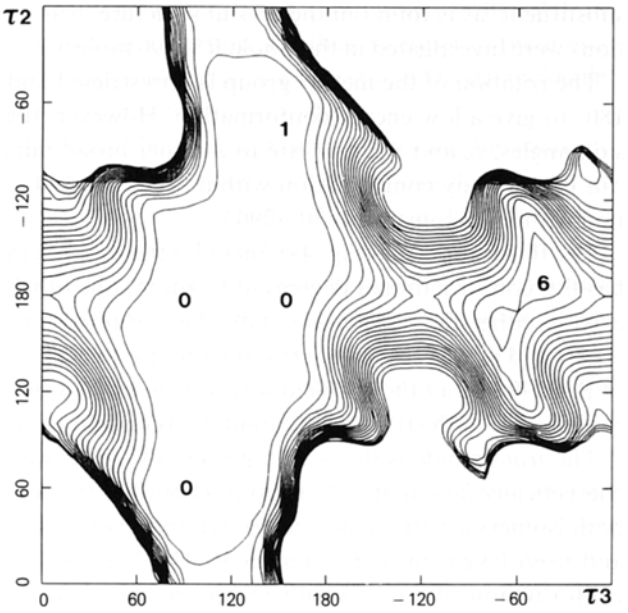
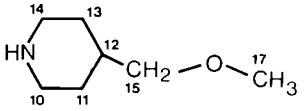


Fig. 2. (a) Fragment 1 of R30490. The three flexible bonds are τ_1: C3-C4-C7-C8, τ_2: C4-C7-C8-N and τ_3: C7-C8-N-C14. The contour map (b) shows the low energy regions for this fragment for the concurrent rotations of τ_2 and τ_3 with τ_1 set at 80°.



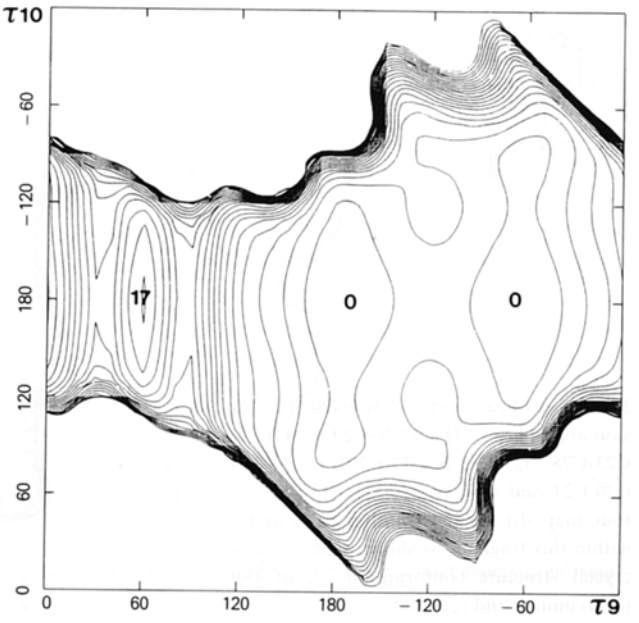


Fig. 3. (a) Fragment 2 of R30490. The torsion angles are τ_9: C13-C12-C15-O, τ_{10}: C12-C15-O-C17 and τ_{11}: C15-O-C17-H. The contour map (b) shows the energies associated with rotation of τ_9 and τ_{10} within this fragment with τ_{11} set at 180°, the piperidine ring in the chair form and the substituent group axial.

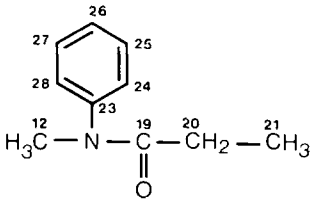
substituent, as is found in the crystal structure, was treated as a fragment. Other ring conformations were investigated in the whole R30490 molecule.

The rotation of the methyl group is unrestricted and so this torsion angle was kept constant at 180° to give a low energy conformation. However, the combined rotations of the other two torsion angles, τ_9 and τ_{10}, give rise to a rather broad minimum shown in Fig. 3b, indicating that τ_9 can take up any conformation within the range 120°–360° while τ_{10} may assume any conformation within the constraints 70°–290°.

The third fragment (Fig. 4a), includes the 4-N-phenylpropanamide substituent of R30490, and has five conformational degrees of freedom, one of which is an amide bond that can presumably exist in either the *cis* or *trans* state. The terminal methyl was set to a low energy conformation (180°) and τ_4 was set to the crystal structure torsion angle to simulate the piperidine ring portion of R30490 so that the problem was condensed to a three variable one; the concurrent rotations of τ_5 and τ_7 for both *cis* and *trans* amide (τ_6) configurations.

The *trans* amide is the isomer present in the crystal structure and might be expected to be the energetically favoured of the two possibilities. In fact the calculated global minimum energies of both isomers are the same and the relative energies and position of secondary minima for the *cis* and *trans* fragment 3 are equivalent. The N-phenyl rotation, τ_5, is restricted to a perpendicular conformation (90° ± 30°) with respect to the amide, and the ethyl portion of the substituent is quite flexible as τ_7 can take up any conformation in the range 70°–280° (Fig. 4b).

Whole molecule calculations. Calculations on the complete R30490 molecule showed no significant change in the conformational preference of τ_1, τ_2, τ_3, τ_5, τ_7, τ_8 or τ_{10} from those found for the fragment calculations. However the movement of groups associated with τ_9 and τ_{10} is curtailed in



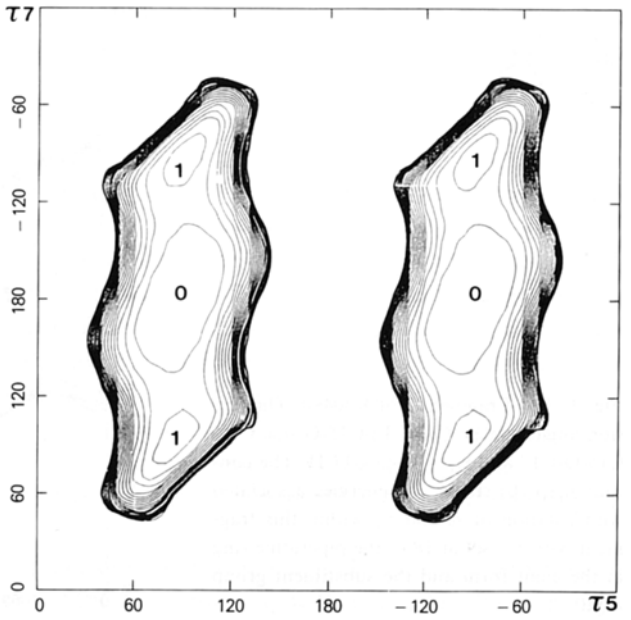


Fig. 4. (a) Fragment 3 of R30490. The torsion angles are τ_4: H-C12-N-C23, τ_5: C12-N-C23-C28, τ_6: C12-N-C19-C20, τ_7: N-C19-C20-C21 and τ_8: C19-C20-C21-H. The contour map (b) for the rotation of τ_5 and τ_7 within this fragment is shown with τ_4, set at crystal structure conformation, τ_6 at 180° (*trans* amide) and τ_8 at 180°.

the whole molecule. Thus τ_9 is restricted to two conformations (i) 130°–160° and (ii) 240°–300° in the molecule by the presence of the N-phenylpropanamide group. Further, at either of these two conformations the other variable torsion angle of fragment 2, τ_{10}, is also restricted in the molecule to 130°–220° for the former and 100°–240° for the latter conformation of τ_9.

An important variable that could not be assessed in the fragment calculations was the rotation of τ_4 and its dependence on the conformation of τ_9. There appear to be three possible low-energy conformations available to τ_4, these being roughly at 0°, 120° and 240° (Fig. 5). At the 0° and 240° minima the bond between the amide nitrogen and the phenyl ring is in the eclipsed conformation with respect to the piperidine ring, and at the 120° minimum the N-phenyl bond is in the eclipsed conformation with respect to the 4-methoxymethyl substituent. However these minima are all dependent on the conformation of τ_9 since for various values of τ_9 each of the three τ_4 minima can be abolished and/or the relative energies of the minima altered. This is a result of unfavourable steric interactions between the amide oxygen or phenyl ring of the N-phenylpropanamide substituent and the ether oxygen or methyl of the methoxymethyl substituent.

Interestingly, the amide bond favours the *trans* isomer in the whole molecule, by 30 kcal/mol or more, while in the fragment calculations there seemed to be little difference between *cis* and *trans*. This preference for the *trans* isomer in the whole molecule seems to occur because of an unfavourable steric interaction between the methylene of the N-phenylpropanamide and the 4-methoxymethyl substituent in the *cis* isomer.

Piperidine ring conformations. A minimum of four piperidine ring conformations might exist, these being the chair ring in which both the 1-phenethyl and 4-N-phenylpropanamide substituents are equatorially oriented (CHEQ), the chair conformation in which both substituents are oriented

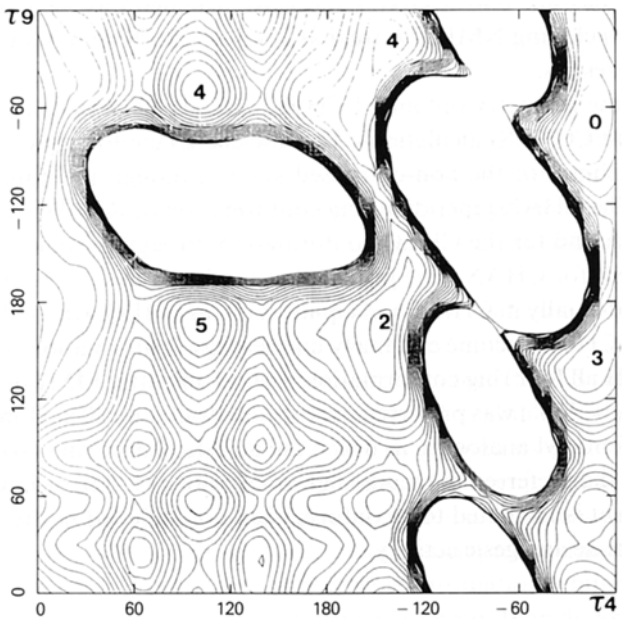


Fig. 5. Contour map for the rotation of τ_4 and τ_9 in R30490. All other torsion angles are set to lowest energy conformations.

TABLE 1
ENERGIES OF GLOBAL MINIMUM CONFORMATIONS OF FOUR PIPERIDINE RING CONFORMATIONS OF R30490 (CONES) AND OF THESE SAME RING SPECIES AFTER GEOMETRY OPTIMISATION (MM2). ENERGIES CALCULATED IN CONES ARE BASED ON VAN DER WAALS' INTERACTIONS BETWEEN NON-BONDED ATOMS WHILE ENERGIES CALCULATED IN MM2 INCLUDE OTHER STERIC CONTRIBUTIONS

R30490 Ring conformation	Relative energy (kcal/mol)	
	CONES	MM2
CHEQ	0	0
CHAX	17	3
BOEQ	13	5
BOAX	>100	8

axially (CHAX) and the corresponding boat ring conformations (BOEQ and BOAX). These four extreme ring systems were built into the crystal structure conformation of R30490 in order to assess their energetic feasibility.

The global minimum energies calculated by CONES for these four ring species (Table 1) indicate that the crystal structure conformation of the ring, CHEQ, is probably also the biologically active species. Geometry optimisation of these global minimum conformations, using the standard molecular mechanics program MM2 and revised parameters for phenyl rings [25] and amides [26], fully support the result that the lowest energy ring conformation is CHEQ, though the energy of the CHAX conformation is decreased substantially after geometry optimisation and it too may be biologically accessible. The boat conformations however can be ignored as insignificantly populated species on the basis of both CONES and MM2 calculations. Data on various fentanyl-type opiate analgesics including NMR [27] rigid analogues [28, 29] and X-ray diffraction analysis [30] also support these results.

The coordinates of the geometry optimised CHEQ and CHAX ring conformations of R30490 were used in subsequent CONES calculations. The low-energy conformations thus observed were generally the same as those for the non-optimised systems though the minima became broader and lower in energy. The CHAX piperidine ring conformation of R30490 exhibits low-energy regions similar to those found for the CHEQ conformation, though in some cases local minima of CHEQ are not apparent for CHAX. The principal examples of this are τ_3 and τ_4, corresponding to substituents oriented axially in CHAX and equatorially in CHEQ, where the slightly higher energy conformations of CHEQ become extremely high-energy conformations in CHAX.

In all cases, that is for all four ring conformations before and after MM2 optimisation, the *trans* isomer of the amide ($\tau_6 = 180°$) was preferred over the *cis* isomer by 30 kcal/mol or more.

Interestingly, rigid fentanyl analogues in which the anilide phenyl ring (τ_5) is restricted to conformations other than the preferred perpendicular orientation [31, 32], or in which the N-phenylpropanamide substituent is restricted to a conformation not included in the τ_4 minima described here [33], possess no opiate analgesic activity.

Etorphine and PET

There are eleven degrees of conformational freedom in etorphine and PET, these being the ten flexible bonds (definitions given in Fig. 6) and the nitrogen axial-equatorial isomerism.

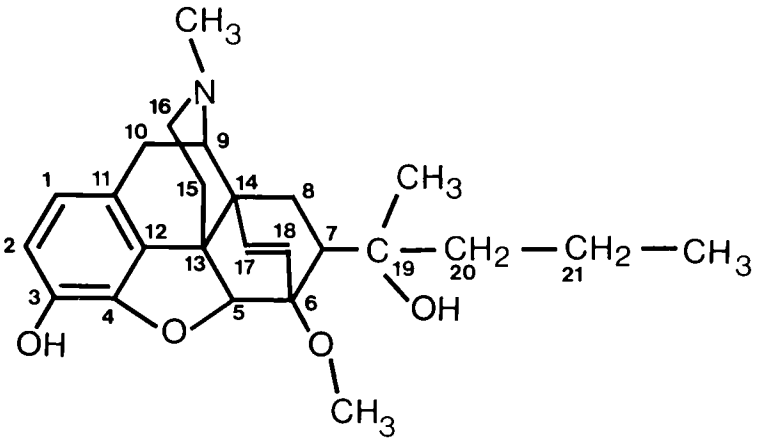


Fig. 6. Structure of etorphine. The flexible bonds are τ_1: C2-C3-O-H, τ_2: C5-C6-O-C, τ_3: C6-O-C-H, τ_4: C6-C7-C19-C20, τ_5: C7-C19-O-H, τ_6: C7-C19-C-H, τ_7: C7-C19-C20-C21, τ_8: C19-C20-C21-C, τ_9: C20-C21-C-H and τ_{10}: C16-N-C-H.

Results of conformational calculations, using a geometry based on the crystal structure of the 3-methoxy derivative [34], showed that the rotations of τ_1, τ_3, τ_5, τ_6, τ_9 and τ_{10} are virtually unhindered. Since these rotations correspond to the movement of hydrogen atoms, except in the case of τ_9 of PET which describes the rotation of a phenyl ring, the respective torsion angles were set to minimum energy values to reduce the number of variables to four rotatable bonds for each of the axially and equatorially substituted nitrogen isomers of etorphine and PET. The low-energy conformations calculated for etorphine were also those calculated for PET, except of course for τ_9. Thus, although reference is made exclusively to etorphine, the discussion and results are equally applicable to PET.

Nitrogen inversion. Firstly the effect of axially and equatorially oriented nitrogen substituents was explored. The difference in energy of these two isomers in morphine, based on the crystal structure [35] was calculated to be about 9 kcal/mol in favour of the equatorially substituted molecule. This same calculation for etorphine also favoured the equatorially positioned substituent, but by 95 kcal/mol.

However, full geometry optimisation of the ring *nucleus* in each case, performed using MMPI, indicated that the high energy difference between axially and equatorially substituted etorphine is exaggerated by maintaining rigid bond lengths and angles in the CONES calculations.

After optimisation the morphine axial isomer is within 3 kcal/mol of the equatorial form and the axial isomer of etorphine is 7 kcal/mol higher in energy than its equatorial counterpart. This greater preference for the equatorial isomer in etorphine when compared with morphine may conceivably be a contributing factor to the much enhanced analgesic potency of the former over the latter. In particular, equatorially substituted molecules, which presumably would include all etorphine but only a proportion of morphine accessible conformations, might allow a more facile binding interaction between the nitrogen and the opiate receptor than do axially substituted molecules. However, although these results indicate that the axial form comprises only a small proportion of etorphine molecules, it was decided not to eliminate it as a conformational possibility at

this point because at least one opiate pharmacophore model hinges on this axial-equatorial isomerism [5].

Full geometry optimisation on the whole molecule, rather than the nucleus only, for the axially and equatorially substituted etorphine was also carried out, this time using MM2 and the parameter set described previously for the MM2 calculations on R30490. The MM2 results served a dual purpose in that they firstly verified the results of the MMPI calculations (etorphine axial isomer calculated to be 5 kcal/mol higher in energy than the equatorial isomer) using a newer force field and the whole structure of the molecule and secondly the resultant minimised atomic coordinates were then available for use in subsequent CONES calculations. These CONES calculations were employed to characterise all possible low-energy conformations of both the axially and equatorially substituted isomers.

Low energy conformations. As mentioned previously, the six terminal bond rotations were maintained at their minimum energy torsion angles since their rotation had little bearing on the preferential or accessible conformations of more important torsion angles and also since the number of variables was reduced to a more manageable number. Of the four remaining torsion angles, τ_2 and τ_4 are quite tightly defined since there are only two allowable conformations for τ_2 and three possibilities for τ_4 (Fig. 7a). The two τ_2 minima at 80° and 320° roughly correspond to two gauche conformations, the first placing the attached methyl group in the plane of the cage ring system and pointing towards the phenyl ring and the second with the methyl group perpendicular to the nucleus on the piperidine ring side. The τ_4 minima at 50°, 180° and 280° place the large side chain under the nucleus and towards the piperidine ring, extended out at right angles from the nucleus, and under the nucleus but directed away from the piperidine ring, respectively. The other variables, τ_7 and τ_8, are less restricted since the former can assume any conformation in the range 30°–300° and the latter 60°–300° (Fig. 7b), though τ_8 for PET is restricted to the range 100°–270° due to the effect of the second phenyl ring. These results are independent of the N-substituent orientation.

It is interesting to compare these results to those obtained using a semi-empirical technique on a series of compounds related to etorphine [36], which indicated that the minimum energy conformations were invariably those in which a hydrogen bond existed between the C19-hydroxy group and the C6-methoxy substituent, giving a τ_4 torsion angle of around 185° (using the definition employed here). It was supposed that this stabilised structure, made semi-rigid by the hydrogen bond, might provide the pharmacophoric conformation of the molecule. However, more recent studies in which either the C6-methoxy [37] or the C19-hydroxy substituent [38] was removed from the etorphine nucleus, thus abolishing any possible hydrogen bonding, indicated retention of potent activity. The formation of such a bond therefore does not provide conformational motivation for a pharmacophoric interaction with the receptor, and all three low-energy conformations of τ_4 reported here, i.e. 50°, 180° and 280° need to be considered.

Etonitazene

Thirteen rotatable bonds were investigated in etonitazene (Fig. 8). The geometry used in the calculations was based on the crystal structure of etonitazene [39], though the phenyl of the phenethoxy sidechain was altered to give a symmetrical geometry.

The terminal methyl groups were found to rotate freely, without influencing the position of possible binding substituents. They were therefore fixed in their low-energy (staggered) conforma-

tions. The nitro substituent was also kept at its lowest energy conformation. Thus this problem is reduced to a nine dimensional one. The structure was then treated in subdivisions, similar to the fragment technique used for R30490, though in this case the whole molecular structure was considered at all times even if the variables only described movement within one of these subdivisions.

The torsion angles τ_8, τ_9 and τ_{10} describe the conformations of the ethoxy sidechain. The rota-

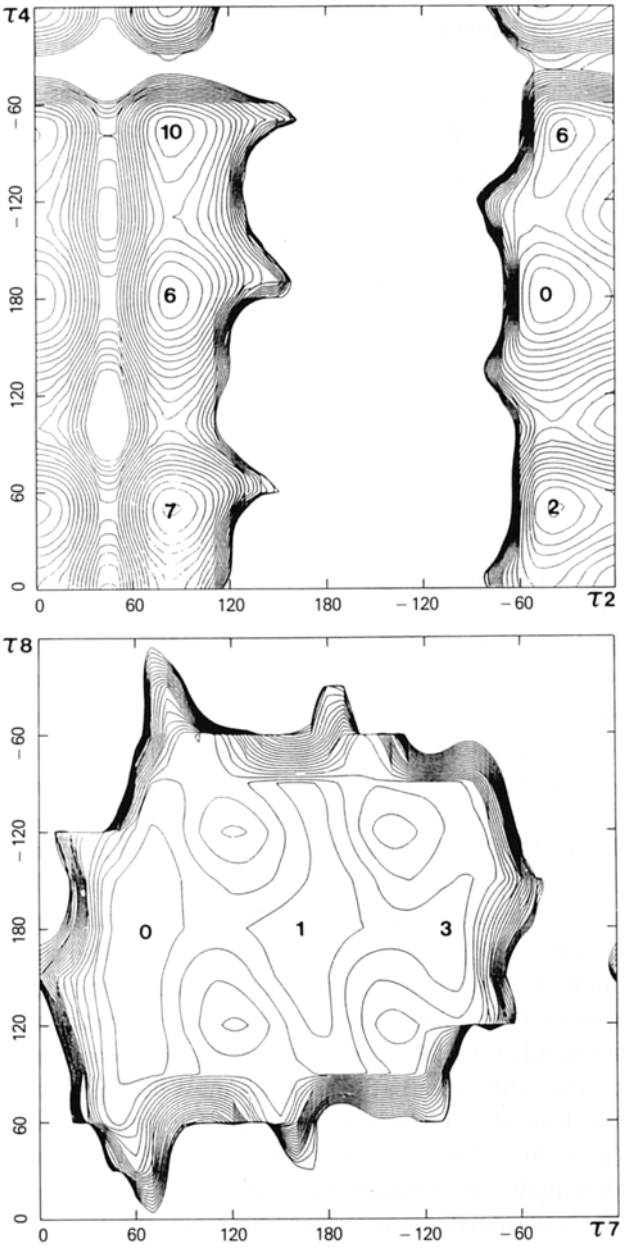


Fig. 7. Contour maps for the rotation of (a) τ_2 and τ_4 and (b) τ_7 and τ_8 in etorphine. Other torsion angles were set to low-energy conformations.

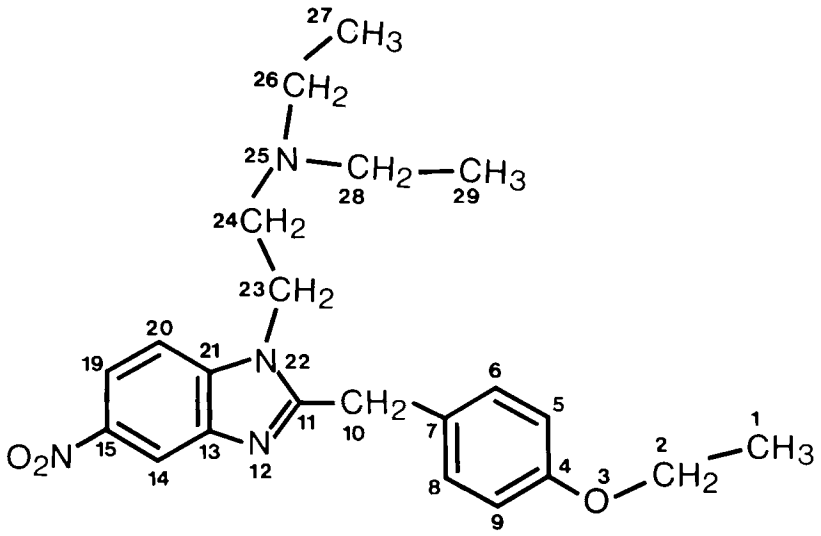


Fig. 8. Structure of etonitazene. The definitions of the flexible bonds are τ_1: C21-N22-C23-C24, τ_2: N22-C23-C24-N25, τ_3: C23-C24-N25-C26, τ_4: C24-N25-C26-C27, τ_{4a}: C24-N25-C28-C29, τ_5: N25-C26-C27-H, τ_{5a}: N25-C28-C29-H, τ_6: N22-C11-C10-C7, τ_7: C11-C10-C7-C6, τ_8: C5-C4-O3-C2, τ_9: C4-O3-C2-C1, τ_{10}: O3-C2-C1-H, and τ_{11}: C14-C15-N-O.

tion of τ_{10}, the terminal methyl group, is of course unhindered but as mentioned above it was set to the low-energy (staggered) conformation to lower the number of variables. The potential energy contour surface illustrated in Fig. 9a shows that the rotation of τ_8 is virtually unhindered as long as τ_9 is in the range 150°–210° and the movement of τ_9 is unrestricted if the conformation of τ_8 is set to 90° (or the equivalent 270°). For the most part however, the torsion angle τ_9 can take up any conformation in the range 60°–300° while τ_8 can access conformations between 30°–150° (or the equivalent 210°–330° range).

The methylene group connecting the benzimidazole ring system with the p-ethoxyphenyl group imparts two degrees of conformational freedom to the molecule, namely τ_6 and τ_7. Conformations of τ_6 which place the phenyl substituent in the vicinity of the N22 sidechain, i.e. in which τ_6 is close to 0°, are not allowed because of the steric crowding that would occur. This disallowed region of τ_6 is increased if τ_7 also skews the sidechain unfavourably – for instance a τ_6 torsion angle of 60° is abolished if τ_7 is set to 110°–170° (Fig. 9b). Further calculations also showed that the rotations about τ_6 and τ_7 are not influenced by rotations about τ_8 and τ_9 in the p-ethoxy sidechain.

Two of the τ_6 conformers (70° and 290°) represent species in which the phenyl ring is positioned either side of the benzimidazole ring. These two minima should theoretically be equivalent, however the presence of the large substituent at N22 which must lie on one or other side of the ring too (τ_1) creates nonequivalence due to steric interactions. The other low-energy conformation of the methylene bridge ($\tau_6 = 90° - 270°$, $\tau_7 = 90° \pm 50°$ and equivalent $\tau_7 = 270° \pm 50°$) is slightly higher in energy though it allows the *p*-ethoxyphenyl group to swing from either side of the benzimidazole nucleus (on the N12 side) if it is in a perpendicular orientation with respect to the C11 – C10 bond. Thus this region of the molecule is quite restricted, and also dependent on the placement of the 2-diethylaminoethyl substituent at N22.

The large substituent at N22 possesses seven degrees of conformational freedom, however the rotations of the terminal methyl groups are unhindered so they were set to low-energy (staggered) conformations leaving five variables to be considered. The rotation of τ_1 is limited to two conformational regions, $30° - 140°$ and $210° - 320°$, which places the side chain on either side of the benzimidazole nucleus. These regions of accessibility can be restricted further by altering the confor-

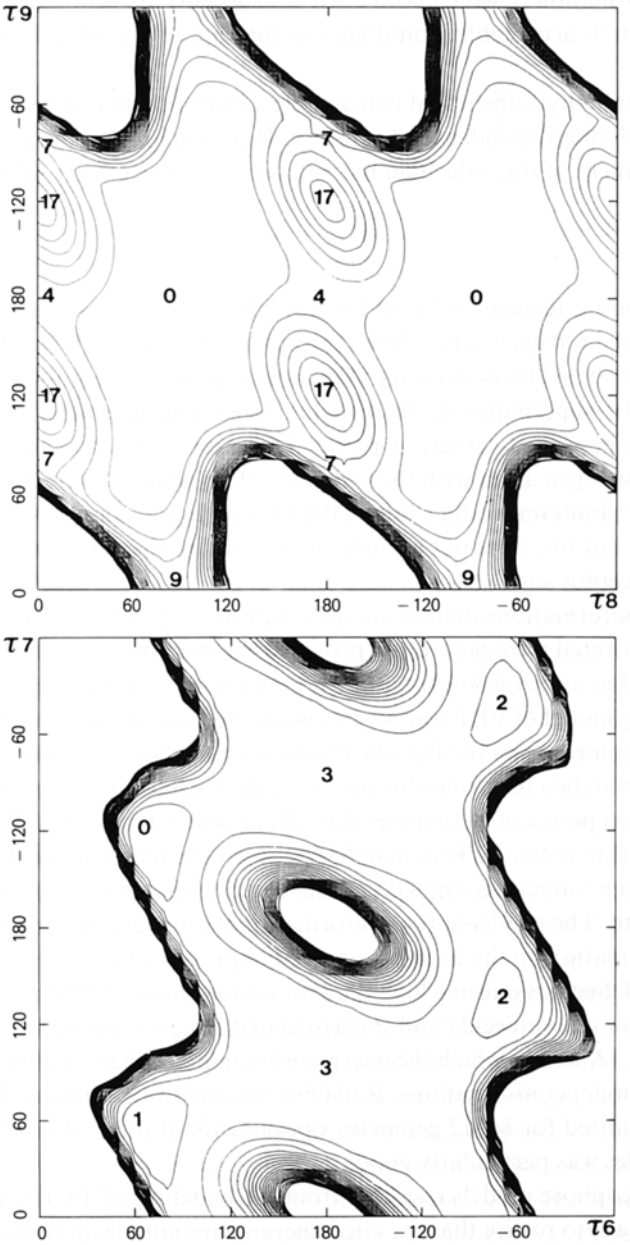


Fig. 9. Contour map for the rotation of (a) τ_8 and τ_9 and (b) τ_6 and τ_7 in etonitazene. Other torsion angles were set to low-energy conformations.

mation at τ_2 and at τ_3. The allowable values of τ_2 are dependent on both τ_1 and τ_3 rotation, the largest range being $60° - 320°$.

Rotation about τ_3 results in the movement of the ethyl substituents connected to the nitrogen atom. The allowable values of τ_3 are $60° - 210°$ and $270° - 300°$ which correspond to conformations in which neither N-ethyl substituent is in an eclipsed conformation i.e. τ_3 is never 0° or 240°. However, the conformation in which both ethyl substituents are eclipsed with hydrogen atoms of C24, that is $\tau_3 = 120°$ is acceptable. Simultaneous rotation of τ_4 and τ_{4a} is associated with four regions of low energy.

This 2-diethylaminoethyl substituent can assume a large number of low-energy conformations, though there is much interdependence of rotation. Rotation around the methylene bridge (τ_6 and τ_7) affects the movement of this sidechain but rotations in the *p*-ethoxy substituent (τ_8, τ_9 and τ_{10}) do not.

Receptor Modelling

The common binding features selected for modelling the potent analgesics were two phenyl rings and a protonated nitrogen, since these features are present in PET, R30490 and etonitazene. The presence of two phenyl rings in each of the molecules means that there are two possible ways in which they can be superimposed; both match types were examined in this work. Receptor points were built as described earlier, at points 3.5Å above and below the centre of the phenyl rings and onto the nitrogen atom with the geometry of an acidic proton but at a distance of 2.8Å. Receptor points were built onto either side of the phenyl rings so that both the position and orientation were defined, but this does not exclude the possibility that only one side of the phenyl ring interacts with the receptor site.

Six low-energy conformations defined by the rotations of τ_4 and τ_7 of PET in either the axially or equatorially substituted nitrogen isomer provided the starting point for the modelling. Molecular models were used to match R30490 to the PET conformers according to the following method. Firstly, the Dreiding model of PET was set to one of the low-energy conformations for either the axial or equatorial isomer. Then the R30490 model, either in the CHEQ or CHAX piperidine ring conformation, was matched to this conformer using the receptor points of the phenyl rings and nitrogen as the common points of both molecules. All flexible bonds of R30490, and two of PET (τ_8 and τ_9), were allowed to rotate. Where matches between the receptor points of the two molecules occurred, torsion angle values were noted and then used to match and optimise the fit on the computer graphics system. The results of the conformational calculations were sufficient in most cases to discount a conformation on the basis of energetic improbability.

A good match is defined as one in which the root mean sqaure (RMS) distance between the chosen receptor points of one molecule and the associated receptor points of the second molecule is less than or equal to 1Å and in which the energy of both molecules is within 10 kcal/mol of the respective global minimum conformations. R30490 conformations within 20 kcal/mol of the global minimum were submitted for MM2 geometry optimisation if the RMS and the structural match between the molecules was particularly good.

The four pharmacophore models resulting from the matching of PET and R30490 are given in Table 2. It is important to realise that the cited energies are not meant to be used for comparative purposes or to indicate that one match is better than another. As stated earlier the program used to calculate the energies can reliably locate minima but tends to overestimate energies and energy

TABLE 2
FOUR PROPOSED BINDING CONFORMATIONS OF PET AND THE MATCHING BINDING CONFORMATION OF R30490. TORSION ANGLE DEFINITIONS ARE AS GIVEN PREVIOUSLY, THOSE TORSION ANGLES NOT LISTED ARE SET TO LOW-ENERGY VALUES. ENERGIES ARE GIVEN IN KCAL/MOL RELATIVE TO THE GLOBAL MINIMUM

	Model A			Model B		Model C		Model D		
	PET		CHEQ	PET	CHEQ	PET	CHEQ	PET	CHEQ i	ii
τ_4	162°	τ_1	126°	177°	104°	−180°	−108°	178°	81°	119°
τ_7	156°	τ_2	−170°	150°	−178°	−87°	−166°	129°	177°	−169°
τ_8	179°	τ_3	56°	−103°	64°	−135°	133°	94°	178°	74°
τ_9	80°	τ_4	−127°	−50	3°	−57°	20°	62°	38°	94°
		τ_5	95°		−79°		103°		97°	106°
RMS(Å)			0.5		0.3		0.9		0.7	0.6
Energy	2.2		7.9	3.3	6.8	4.9	5.7	5.4	4.1	4.3

barriers. Thus the stated values are not for quantitative comparison but are given merely to indicate that the particular conformation is probably accessible to the molecule.

Some important points to note are that all the proposed binding modes of PET are equatorially substituted isomers and all matching conformations of R30490 have a CHEQ piperidine ring. Also there are two possible binding modes of R30490 to one of the conformations of PET (Di and Dii).

Etonitazene, like PET and R30490, was initially matched using the two phenyl rings and protonated nitrogen binding features. However no matches could be found between etonitazene and the four proposed models. Dreiding molecular models were then used to see if *any* matches might occur between etonitazene and PET by allowing all variables of both molecules to rotate. The resulting best fit between the two molecules (RMS 0.8Å) is unacceptable because the calculated energy of PET is about 150 kcal/mol above the global minimum. The best match between the two molecules in which the energies are acceptable has an RMS of 1.3Å.

The conclusion drawn from further Dreiding modelling of the two compounds was that the relative distances between the two phenyl rings of PET and etonitazene cannot be matched. For all conformations of etonitazene, the two phenyl rings are spaced about 6 – 7Å apart, while for PET this distance ranges between 8 – 11Å. Manipulation of the flexible bonds of PET does give conformations in which the phenyl rings are within the etonitazene spatial requirements, but this cannot be done without an enormous energy increase. The initial selected binding requirements appear then not to apply to etonitazene. Thus the presence of two phenyl rings and a nitrogen within the etonitazene structure does not account for its potent analgesic activity. Indeed it should not come as a great surprise that two phenyl rings are not a necessary requirement for potent activity since etorphine, which is as potent as PET, possesses an alkyl chain in place of the second phenyl ring of PET. The corresponding lipophilic group of etonitazene appears more likely on the basis of Dreiding molecular modelling to be the ethyl portion of the *p*-ethoxy sidechain.

The terminal methyl group of the etonitazene ethoxy substituent was therefore matched to the centre of the second aromatic ring of PET. The results for the matching of etonitazene using the modified matching system are given in Table 3. The conformations of etonitazene that match the

TABLE 3
CONFORMATIONS OF ETONITAZENE THAT MATCH THE PROPOSED MODELS FOR POTENT OPIATE ANALGESIC ACTIVITY. ENERGIES ARE GIVEN IN KCAL/MOL ABOVE THE GLOBAL MINIMUM. TORSION ANGLES ARE AS DEFINED PREVIOUSLY AND THOSE NOT LISTED ARE SET TO LOW-ENERGY VALUES

Torsion angle	Model A		Model B		Model C		Model D	
	i	ii	i	ii	i	ii	i	ii
τ_1	104°	58°	115°	64°	100°	56°	80°	70°
τ_2	119°	153°	121	175°	120°	155°	135°	149°
τ_3	104°	126°	89°	142°	105°	127°	120°	171°
τ_6	−90°	−43°	−108°	−36°	−85°	−55°	−90°	−57°
τ_7	−132°	−46°	−114°	−44°	−140°	−57°	−135°	−42°
τ_8	−99°	−108°	−103°	−107°	−105°	−115°	−105°	−110°
τ_9	78°	−112	63°	−106°	80°	−127°	90°	−139°
RMS(Å)	0.7	0.3	0.9	0.4	0.4	0.3	0.4	0.4
Energy	7.2	4.2	8.2	7.0	6.7	3.3	3.0	4.6

models can be divided into two groups differing mainly in the conformation around the methylene bridge connecting the benzimidazole and phenyl rings. These are given as match type i (where τ_6 is −80° to −90°, and τ_7 is −135°) and match type ii (where τ_6 is −45° and τ_7 is −45°) in Table 3.

Apart from the three classes of potent analgesics already seen, there is probably only one other class of compounds that exhibits very potent opiate analgesic activity, this being the 4-amino-4-aryl-cyclohexanols [40] of which 4-(*p*-bromophenyl)-4-(dimethylamino)-1-phenethylcyclohexanol (BDPC, Table 4) is an example. This compound is stated to be about 10000 times more potent than morphine in analgesic tests and has 30 times the receptor affinity of morphine [41] and so is a good test molecule for the proposed models.

The crystal structure of BDPC was not available so matching was done using a molecule built from standard geometries [20] and the conformations optimised using MM2 and the parameter set described for use with R30490. The *trans* amino alcohol is the active species [41], thus the chair cyclohexane with equatorial *p*-bromophenyl and phenethyl substituents was deduced to be the energetically favoured conformation.

This cyclohexane ring conformation was used to match BDPC to the proposed models though all other conformational variables were allowed to alter and both possible ring matches tested. Refinement of the matched conformations of BDPC was performed where necessary using MM2. The results of the matching of BDPC to the four postulated models are shown in Table 4. As for the other potent opiate analgesics, this compound is able to fit to all four proposed models.

CONCLUSION

The four potent analgesics superimposed in the four proposed binding conformations are given in Fig. 10. Where there were two possible matches to PET the match which gave the best spatial

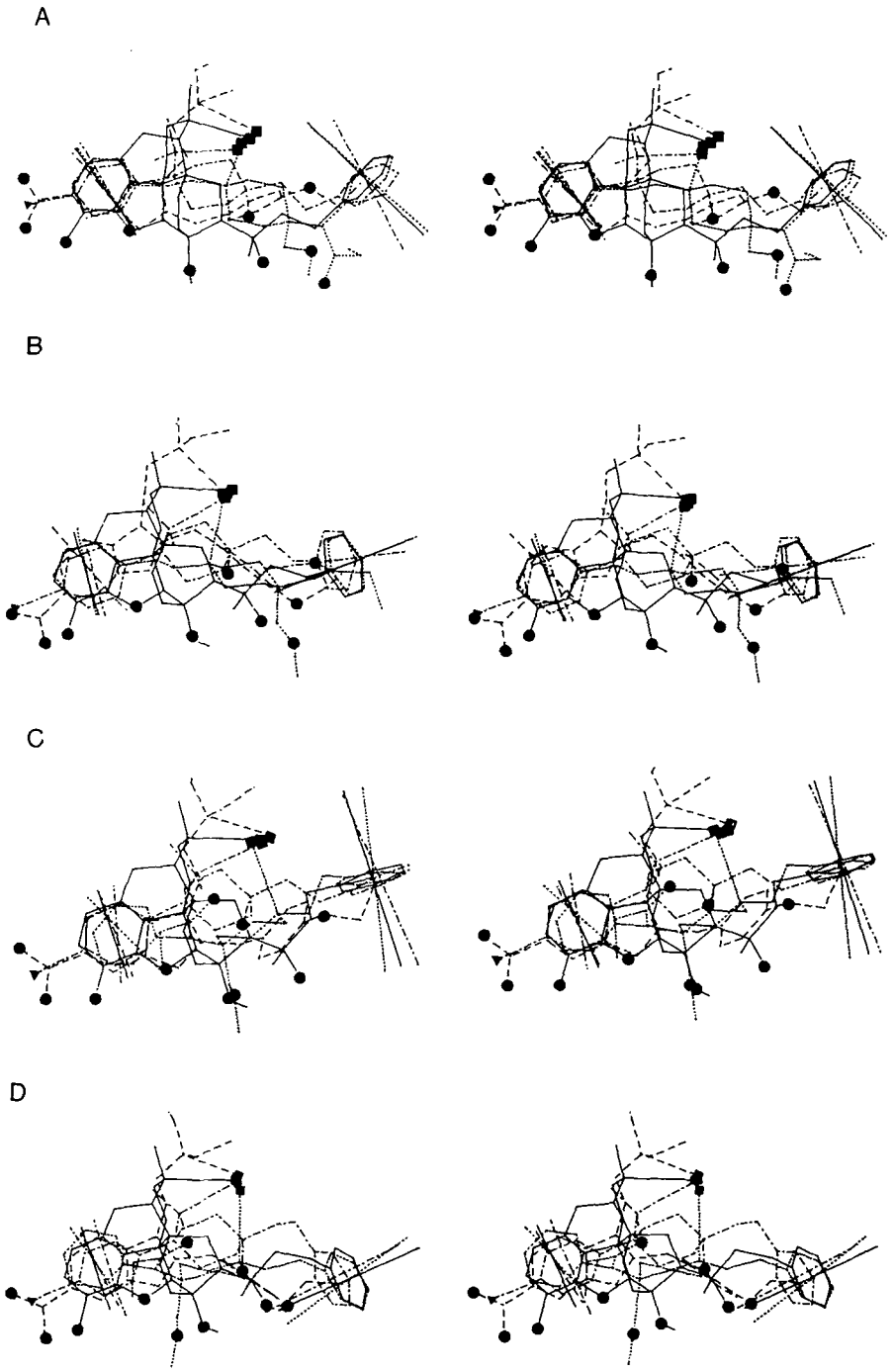
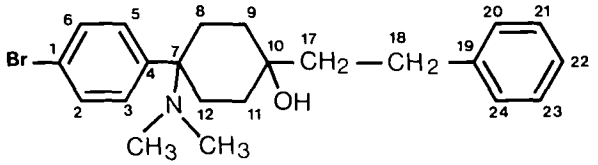


Fig. 10. Stereo diagrams of the superimposition of four potent opiate analgesics PET (—), R30490 (...), etonitazene (- -) and BDPC (-.-) in four proposed pharmacophore conformations (A, B, C, D). Oxygen atoms are indicated by solid circles, nitrogen receptor points by solid squares and bromine atoms by solid triangles.

TABLE 4
CONFORMATIONS OF BDPC THAT MATCH THE PROPOSED MODELS FOR POTENT OPIATE ANALGESIC ACTIVITY. ENERGIES ARE GIVEN IN KCAL/MOL RELATIVE TO THE GLOBAL MINIMUM. THE DIAGRAM BELOW INDICATES THE NUMBERING USED IN THE DEFINITIONS OF THE FLEXIBLE BONDS OF BDPC THAT ALTER THE POSITIONS OF THE RECEPTOR POINTS



Torsion angle	Model A	Model B	Model C	Model D	
				i	ii
C9-C10-C17-C18	−148°	−170°	160°	−57°	−85°
C10-C17-C18-C19	146°	−119°	160°	−112°	−89°
C17-C18-C19-C20	−123°	142°	−64°	141°	−178°
C5-C4-C7-N	−99°	−97°	−95°	−78°	−96°
C4-C7-N-C	−71°	−50°	−60°	−77°	−50°
RMS (Å)	0.9	0.7	0.4	0.8	0.7
Energy	6.8	6.9	2.9	7.0	9.4

overlap was chosen as the more likely binding conformation and used in these superimpositions. Table 5 lists the energies and RMS distances of each of the molecules in these final four models.

From the figures one can see that models A and B are quite well-defined compared to models C and D. Thus models A and B are preferred candidates for the potent opiate analgesic pharmacophore. Note though that in each of the models it is the nitrogen receptor points rather than the nitrogen atoms of the individual molecules that superimpose. Other authors have also come to the conclusion that the opiate protonated nitrogen may interact with the receptor site in a variety of

TABLE 5
ENERGIES (IN KCAL/MOL RELATIVE TO THE GLOBAL MINIMUM) AND RMS (IN Å) OF THE POTENT OPIATE ANALGESICS FOR THE FOUR POSTULATED POTENT OPIATE PHARMACOPHORES. THE PARTICULAR MATCH TYPE CHOSEN IS INDICATED IF MORE THAN ONE BINDING MODE WAS POSSIBLE

		Model A	Model B	Model C	Model D
PET	energy	2.2	3.3	4.9	5.4
R30490	energy	7.9	6.5	5.7	4.1 (i)
	RMS	0.5	0.3	0.9	0.7
Etonitazene	energy	4.2 (ii)	7.0 (ii)	3.3 (ii)	3.0 (i)
	RMS	0.3	0.4	0.3	0.4
BDPC	energy	6.8	6.9	2.9	9.4 (ii)
	RMS	0.9	0.7	0.4	0.7

geometries [9, 42]. Interestingly, the two compounds that have a low safety margin, PET and etonitazene, both interact with the nitrogen receptor site from one region of space. The other two compounds, R30490, renowned for its high safety margin [43] and BDPC, safety uncited to date, attack the nitrogen receptor site from a completely different region. Thus although the nitrogen receptor binding site can accommodate a variety of interaction geometries, some of these may be associated with side effects. Of course this may simply result from the presence of *any* atoms in the upper region of the models rather than the specific geometry of interaction of the protonated nitrogen to its receptor site. We are currently testing this hypothesis by designing, synthesising and testing PET and etonitazene anologues with nitrogen positioned in the 'safe' region of space.

The other important point to note here is that the determination of four models that can accommodate the potent analgesics is entirely consistent with the biochemical observation of a number of opiate binding sites. These models may thus correspond to different opiate receptor types. However only one of them, presumably the best one, should be the model for the opiate analgesic μ receptor. Models A and B appear to be the best possibilities since the common binding features match up very tightly for all four molecules, and in both models there is also a region where oxygen atoms of the individual molecules are in close proximity (around C19 hydroxyl of PET) suggesting that they may interact with a single site on the surface of the receptor.

Selection between models A and B is difficult, although some discrimination is provided by recent conformational studies on fentanyl derivatives [44]. They imply that a τ_4 conformation of 240° for R26800 — a close analogue of R30490 — is required for binding to the analgesic receptor. We therefore favour model A as the analgesic μ receptor model, since it is the only one of the four in which R30490 has this τ_4 conformation. Note that for model A, as in all four models, the phenolic hydroxyl of PET, the bromine of BDPC and the nitro group of etonitazene are reasonably closely aligned suggesting that these substituents may share a common binding site on the receptor surface.

In conclusion, the required binding features for potent opiate analgesic activity have been defined as being (1) a protonated nitrogen; (2) an aromatic ring; and (3) a second lipophilic group, not necessarily a phenyl ring. Secondary binding requirements may include an electronegative substituent on the aromatic ring and a hydrophilic group. Of particular interest is the observation that the compounds which exhibit a low safety margin may have certain receptor-interaction features in common. Some portions of the structures of the potent analgesics appear not to be involved directly in a receptor interaction though these may provide the skeletal framework for positioning the substituents that do bind, or for optimising other properties necessary for drug activity. The question of how the opioid peptides fit into the proposed models has not been addressed though it is hoped this work will be taken up in the future. If the peptides could be shown to match one of the four postulated models then that particular model may well define the requirements for binding of the δ opiate receptor subtypes.

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