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The active analog approach applied to the pharmacophore identification of benzodiazepine receptor ligands

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SUMMARY

Applied to seven potent benzodiazepine-receptor ligands belonging to chemically different classes, the active analog approach allowed the stepwise identification of the pharmacophoric pattern associated with the recognition by the benzodiazepine receptor.

A unique pharmacophore model was derived which involves six critical zones: (a) a π-electron rich aromatic (PAR) zone; (b) two electron-rich zones δ_1 and δ_2 placed at 5.0 and 4.5 Å respectively from the reference centroid in the PAR zone; (c) a freely rotating aromatic ring (FRA) region; (d) an out-of-plane region (OPR), strongly associated with agonist properties; and (e) an additional hydrophobic region (AHR).

The model accommodates all presently known ligands of the benzodiazepine receptor, identifies sensitivity to steric hindrance close to the δ_1 zone, accounts for *R* and *S* differential affinities and distinguishes requirements for agonist versus non-agonist activity profiles.

INTRODUCTION

At present a large number of synthetic ligands for the benzodiazepine receptor are known, belonging to very different chemical classes such as benzodiazepines, cyclopyrrolones, β-carbolines, triazolopyridazines, pyrazoloquinolines and many other heterocyclic systems (Fig. 1). In several of these chemical classes a very slight change can induce a shift from agonistic (full benzodiazepine-like) properties to inverse agonistic properties (completely benzodiazepine-opposite activities) or to antagonistic properties (strong interaction with the benzodiazepine receptor but no pharmacological response, antagonism towards the two other classes). Any intermediate situation between these three profiles, which were first defined by Haefely and his group [1,2] can also

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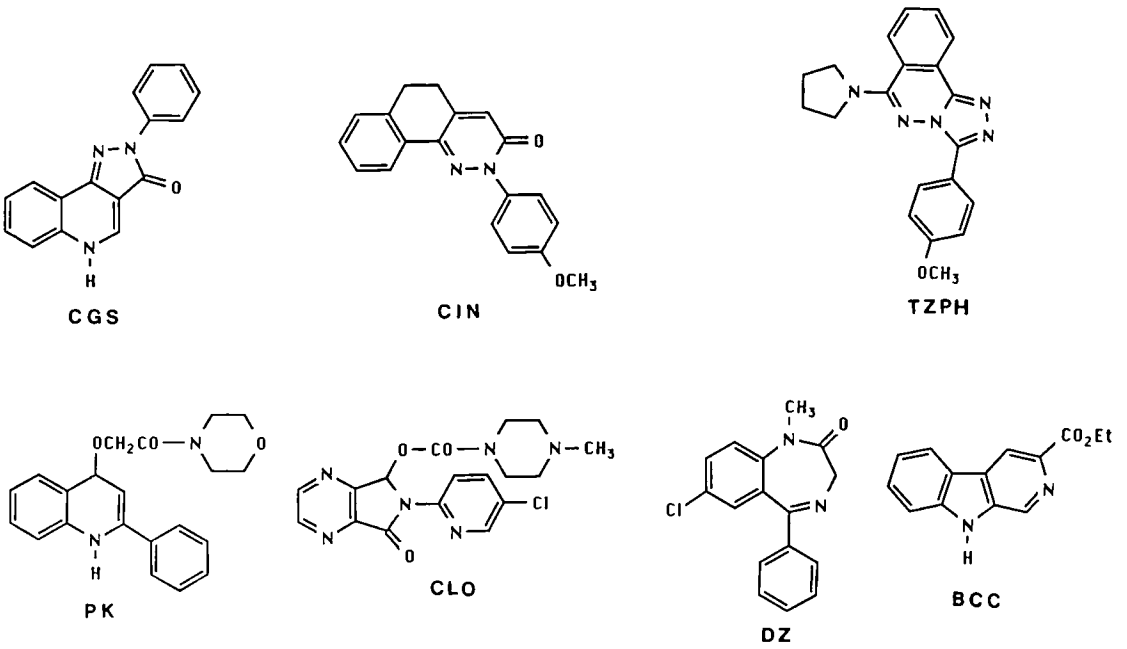


Fig. 1. Benzodiazepine-receptor ligands utilized for the pharmacophore model construction.

occur, the corresponding structures showing partial agonistic or partial inverse agonistic activity profiles.

Early structure-activity studies such as classical SAR [3], QSAR [4] but also conformational and electronic studies [5] were mainly restricted to the benzodiazepines. Later Crippen, using distance geometry analysis, compared benzodiazepines with two non-benzodiazepine classes, β-carbolines and cyclopyrrolones, and defined a model comprising 15 interaction sites for binding with the benzodiazepine (BZ)-receptor [6]. The discovery of an increasing number of new non-benzodiazepinic ligands stimulated the elaboration of models taking into account the diversity of these new chemical structures. The model presented by Codding and Muir is based on crystallographic data and points out some similarities between the non-agonists Ro 15-1788, CGS 8216 and β-CCM [7]. The model presented by Fryer [8] is an attempt to rationalize the pharmacophore of the centrally acting BZ-receptor ligands. In this model both the agonists and the non-agonists have three critical regions in common: a flat, aromatic area called A, and two regions presenting a relatively high electron density (and therefore able to insure hydrogen bonds) called π_1 and π_2. For Fryer the main difference between agonists and non-agonists resides in the greater distance between the center of A and π_1 for the non-agonists (> 6 Å) than for the agonists (< 6 Å). Very recently Borea et al. published a detailed paper on the same subject containing additional crystallographic data on zopiclone and the triazolopyridazine CL 218872, and the presentation of a general stereochemical model accounting for both binding abilities and pharmacological profiles for a large number of benzodiazepine-receptor ligands [9]. This model retains the three interactions proposed by Fryer but adds (i) a new region (AG_2) responsible for agonistic properties; (ii) a re-

gion responsible for inverse agonistic properties; and (iii) some areas sensitive to steric hindrance. In this model antagonistic structures are characterized by their ability to occupy the agonist as well as the inverse agonist region.

Following on from our interest in pyridazine derivatives [10–13], we prepared triazolopyridazines with inverse agonist activities [14, 15]. Attempts to explain this unexpected profile led us to reconsider the published pharmacophore models and to propose a new model which is consistent with all the benzodiazepine-receptor ligands described today and which may be helpful in the interpretation of active vs. inactive compounds as well as of agonists vs, antagonists or inverse agonists.

In the first part of this article we describe the pharmacophore model construction, followed by a discussion of its application to the interpretation of SAR within each chemical family of BZ ligands.

METHODS

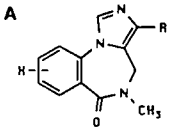
1. The working hypothesis

For our modelling studies we hypothesized that BZ agonists, antagonists and inverse agonists share a common recognition site. Several chemical and pharmacological arguments are in favor of this view:

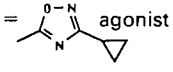
(i) In the imidazobenzodiazepine series the change from the 8-fluoro substituent of Ro 15-1788, to a 7-chloro substituent (compound Ro 15-3505), induces a shift from antagonist to inverse agonist properties (Fig. 2A). In its turn Ro 15-3505 can be converted to the agonist Ro 19-0528, by a bioisosteric replacement of the carbethoxy group by an oxadiazole equivalent [16]. It seems difficult to admit that such discrete chemical modifications can entail a totally different mode of interaction with the receptor site. Analogous conclusions result from comparisons in the pyrazoloquinoline or the β-carboline series. Thus, in the pyrazoloquinoline series, the pharmacological profile is only related to the nature of the substituent (H, Cl, OCH_3) in the para position of the phenyl ring (Fig. 2B) [17–20]. In the β-carboline series again, substituent effects generate agonistic properties from an inverse agonist (β-CCM; ZK 93423; Fig. 2C) [21–23]. Finally our own observations in the triazolopyridazine series demonstrated that in compound CL 218872 and its analogues the displacement of the phenyl group from the 6- to the 7-position causes a switch from agonist to inverse agonist properties (Fig. 2D) [15, 24]. Taken together these examples strongly suggest a common receptor site for agonists, antagonists and inverse agonists;

(ii) On the pharmacological side no strong arguments refute our hypothesis. The proposed BZ_1 and BZ_2 receptor subclasses [25] are in fact both able to recognize all the BZ-receptor ligands, some of them with similar affinities (BZ), some of them with 5–10 fold preferential affinity for BZ_1 receptors (TZHP; β-CCM) [26]. Therefore the differences between these two subclasses are probably small and, according to some authors, may reflect the existence of two conformations of only one class of receptors [27]. Similar findings were made by Skolnick and Paul on the basis of equilibrium studies with [^{3}H] β-carbolines which yielded Scatchard plots that were not clearly resolvable into multiple components [28].

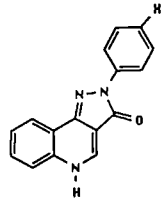
A



Ro 15-1788 : X = 8-F R = $CO_2C_2H_5$ antagonist
Ro 15-3505 : X = 7-Cl R = $CO_2C_2H_5$ inverse agonist

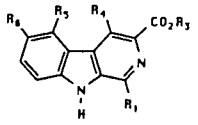
Ro 19-0528 : X = 7-Cl R =  agonist

B



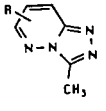
CGS 8216 : X = H partial inverse agonist
CGS 9895 : X = OCH_3 agonist, antagonist
CGS 9896 : X = Cl partial agonist

C

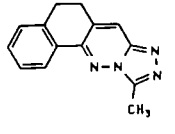


β-CCM : R_3 = Me inverse agonist
β-CCE : R_3 = Et inverse agonist
β-CCPr : R_3 = n-Pr antagonist
1-Me-βCCM : R_1 = R_3 = Me inactive
ZK 91926 : R_3 = Et R_4 = $-CH_2-OMe$
R_5 = $-O-CH_2-Ph$ partial agonist
ZK 93423 : R_3 = Et R_4 = $-CH_2-OMe$
R_6 = $-O-CH_2-Ph$ agonist

D

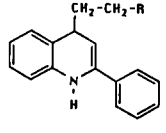


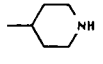
CL 218872 : R = 6-mCF_3-Ph partial agonist
SR 95195 : R = 7-Ph inverse agonist

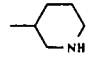


SR 95587 : antagonist

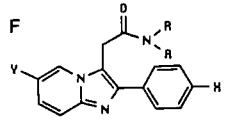
E



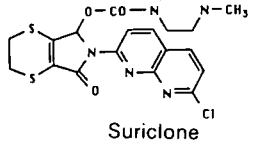
PK 8165 : R = 

PK 9084 : R = 

F

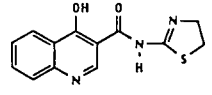


Zolpidem : X = Me Y = Me R = Me
X = Cl R = H

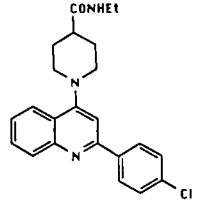


Suriclone

G



RU 40904



Ro 23-1590

Fig. 2. Various benzodiazepine-receptor ligands discussed in the present study.

2. Compound selection

The dominant criterion for compound selection was to pick up, in each family of BZ-receptor ligands, the most potent one in terms of affinity, regardless of its efficacy as agonist, inverse agonist or antagonist. The second criterion, in conformity with the active analog approach [29], was to start the model construction with the most rigid candidate structures. Whenever several candidate structures were available in a given family, we chose the structure showing the greatest resemblance and general shape to the candidate of the following family.

3. Computer graphics and receptor modelling

The SYBYL software package and the TRIGRAPH graphic station [30] were used for the display, manipulation and superposition of molecules. The host computer was a GOULD/SEL 32/77. Hard copies of structures were produced on a Hewlett-Packard 7221c plotter. Molecular geometries were obtained from crystal structure coordinates for diazepam [31] or from standard bond distances and angles. Standard fragments available on the system were further added to complete the molecules. In a next step, the geometry was optimized through a simple minimization procedure (MM2, Maximin) and, when necessary, systematic conformational search was achieved to locate the sterically allowed conformers.

RESULTS AND DISCUSSION

1. Construction of the model

The reference structure considered was the compound CGS 8216 in which the para-substituted phenyl ring is linked to a rigid pyrazoloquinoline skeleton and whose affinity for the BZ-receptor is very high (IC_{50} = 0.46 nM [17, 18]). This compound was first compared to 2-(4-methoxyphenyl)-benzo [h] 3-cinnolone ('CIN'; IC_{50} = 6.6 nM [32]). Both structures show great similarities: a freely rotating phenyl ring attached to a rigid and planar tricyclic skeleton and an identical structural sequence, O=C-N(Ar)-N=C(-R)-. Taking into account the substituent effects (para-H, Cl or OCH_3) in CGS and CIN analogues, we priviledged the recovering of the freely rotating phenyl rings.

Fig. 3 shows the superposition of both molecules, taking particular care to coincide the carbonyl dipoles (δ_1 region) and the imino dipoles (δ_2 region).

The second compound to be compared to CGS was selected from the triazolopyridazine series (Fig. 4). We retained 3-(4-methoxyphenyl)-6-pyrrolidino-triazolo [4,3-a] phthalazine ('TZPH') as candidate structure. This compound is described as a potent anxiolytic [33] and possesses high affinity for the BZ-receptor (IC_{50} = 3.9 nM) in comparison to the more extensively studied Lederle compound (CL 218872; IC_{50} = 116 nm [24]). TZPH again contains a freely rotating phenyl ring linked to a planar phthalazine structure in which two electronegative regions are present: the N(5) pyridazine nitrogen and the N(1)-N(2) region of the triazolo nitrogens. An additional feature, not found in CGS and in CIN, is the presence of the pyrrolidino ring in the 6 position. Other aliphatic amines bearing polar groups (morpholine, *N*-methyl-aminoethanol) in the same position of the

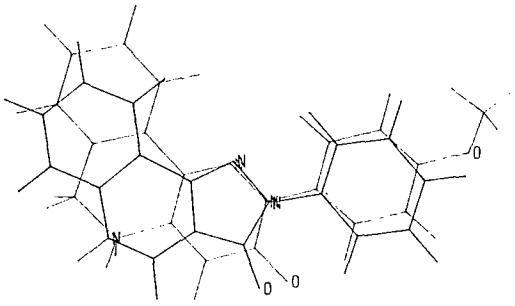


Fig. 3. Superposition of CGS and CIN.

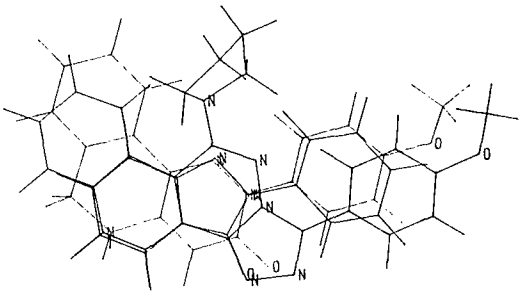


Fig. 4. Superposition of CGS, CIN and TZPH.

phthalazine, also yield potent ligands [33]. After superimposing the benzo ring of TZPH with the pyridine ring of the quinoline in CGS ('ring A') and overlapping the freely rotating phenyl rings, two possibilities of comparison remained, depending on the choice between the N(5) or the N(1)-N(2) atoms as interaction partners with the δ_1 region. Our preference (Fig. 4) went to the C-N(1) imino bond covering the carbonyl dipole region δ_1. This mode of superimposition orientates the bulky pyrrolidino ring in a region of space close to the δ_2 region and thus does not create steric hindrance near the δ_1 dipoles. As pointed out by Cain et al. [21] and by Haefely et al. [2] steric hindrance in the proximity of this site is detrimental to the interaction with the BZ-receptor.

The bioisosteric match of zopiclone (RP 27267, 'CLO'; IC_{50} = 29 nM [34]), as representative of the cyclopyrrolone class, rests on the same topological and electronic criteria as previously: (i) a freely rotating aromatic ring; (ii) a planar aromatic pyrazinopyrrolone system; and (iii) two electronegative regions susceptible to occupy the δ_1 and δ_2 areas. Again an uncertainty in the attribution of the CLO dipoles as δ_1 or δ_2 partners is found, as illustrated in Figs. 5 and 6. In both cases a good fit of the dipoles with the δ_1 and δ_2 regions is observed. However, it appears clear that the superposition mode depicted in 6 possesses the double advantage of placing the piperazine ring of

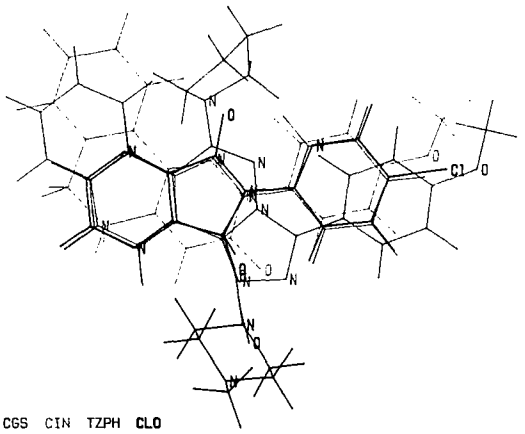


Fig. 5. Superposition of CGS, CIN, TZPH and CLO (mode a).

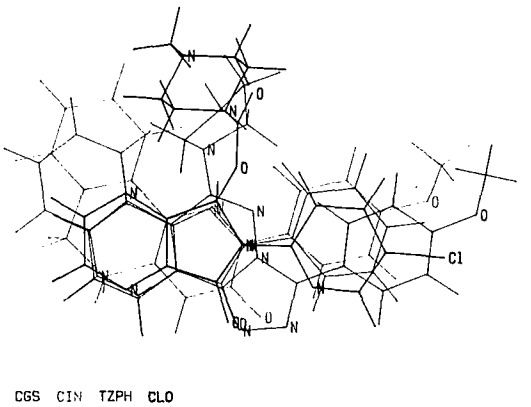


Fig. 6. Superposition of CGS, CIN, TZPH and CLO (mode b).

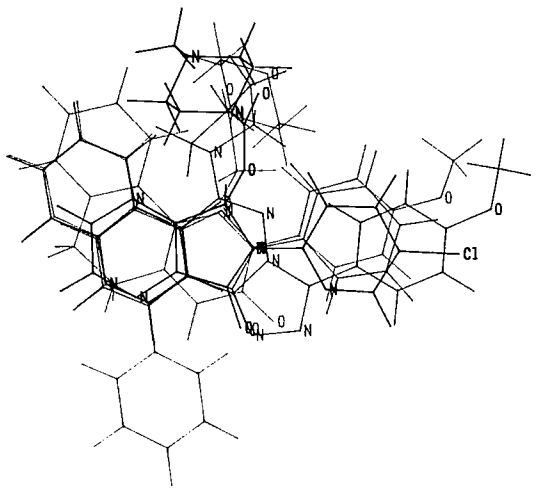


Fig. 7. Superposition of CGS, CIN, TZPH, CLO and PK.

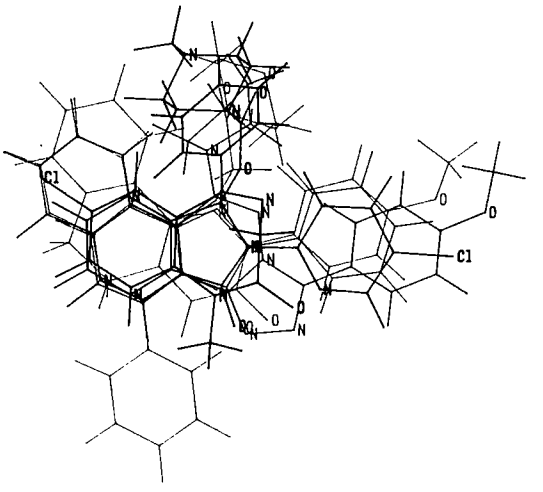


Fig. 8. Superposition of CGS, CIN, TZPH, CLO, PK and DZ.

CLO in the same region as the pyrrolidino ring of TZPH and keeping the δ_1 interaction region free from a bulky substituent.

The 2-phenylquinoline ('PK'; IC_{50} = 8.0 nM [35]) described by searchers of the Pharmuka group has a quinolinic ring system, a freely rotating aromatic ring and a morpholinoacetamide side chain. When its quinolinic cycle is superimposed on that of CGS and the exocyclic oxygen of PK overlaps the δ_2 region, we observe that the acetamide side chain lies in the same area as the pyrrolidino group of TZPH or the *N*-carbamoylpiperazine of CLO (Fig. 7). However, in spite of its high affinity for the BZ-receptor, PK lacks a functional group able to interact with the δ_1 site

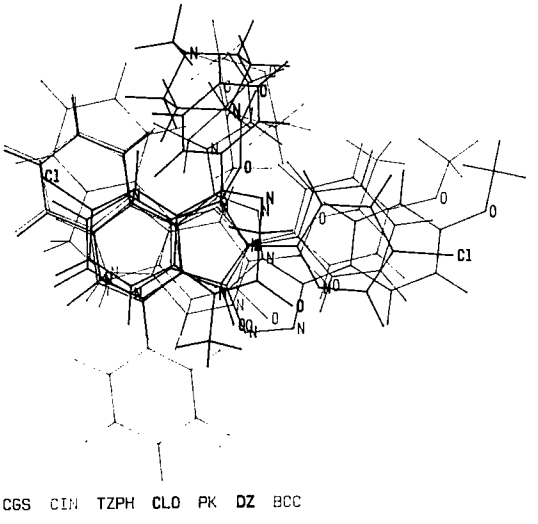


Fig. 9. Superposition of CGS, CIN, TZPH, CLO, PK, DZ and BCC.

and presents a phenyl ring oriented towards a yet undefined region in space. Two other quinoline compounds can be compared to PK (Fig. 2G): the first is Ro 23-1590 (IC_{50} = 4 nM [36]), which yields a quasi-identical superimposition as PK; and the second is the Roussel compound RU 40904 (IC_{50} = 1.2 nM [37]) in which the phenyl ring at the 2 position is deleted and which resembles CGS in its interaction abilities.

Diazepam itself ('DZ'; IC_{50} = 8.1 nM [2]) accommodates easily with our model if its benzo ring is placed slightly above ($\approx$ 0.7 Å) the pyridine ring (ring A) of CGS, the amidic carbonyl covering the δ_1 region and the imino group the δ_2 region (Fig. 8). Under these conditions the phenyl ring occupies the same portion of space as the earlier encountered bulky amino or amido substituents (pyrrolidine of TZPH, piperazine of CLO, and acetamido-morpholine of PK). An inverted mode of superimposition (DZ carbonyl in δ_2 and DZ imine in δ_1) appears rather unfavorable insofar as it would not account for the sensitivity of the δ_1 region to steric hindrance.

In contrast to the other classes of BZ-receptor ligands, the β-carbolines constitute completely flat molecules. A typical representative is β-CCM ('BCC'; IC_{50} = 8.0 nM [38]). Since it has been shown that the imino function is essential for activity in this compound [21], and that introducing steric hindrance in its vicinity is detrimental (1-CH_3-β-CCM; IC_{50} = 4900 nM [38]), we chose a comparison mode in which the C(1)-N(2) imino dipole of BCC was placed in the δ_1 region (Fig. 9). The indolic pyrrole ring was then brought to coincide with the A ring of our aromatic system; consequently the ester function becomes located in the region occupied until now by the freely rotating phenyl rings.

2. Description of the model

The general model resulting from our stepwise construction is depicted in Fig. 10. It possesses the following characteristics:

(a) Presence of a π-electron rich aromatic zone (PAR zone). This zone is often occupied by a fused bicyclic heterocycle: isoquinoline (CGS), quinoline (PK), indole (BCC), and was therefore divided in an A part and an R part, the A part corresponding to the heterocyclic moiety of the above bicyclic systems. All BZ-receptor ligands possess at least one aromatic ring in the PAR zone. The relative importance of the A and R cycles for receptor affinity remains still unclear insofar as potent compounds such as benzodiazepines and TZPH only possess the A cycle.

Conversely the potent CIN possesses the aromatic R cycle but cannot be considered as fully aromatic for the A site. The centroid of the A ring was taken as reference for distance measurements.

(b) Presence of two electron rich zones δ_1 and δ_2 corresponding roughly to Fryer's π_1 and π_2 zones. They contain oxygen and nitrogen lone pairs, generally delocalized over three (or more) atoms in the δ_1 region and over two atoms in the δ_2 region. Both δ_1 and δ_2 are directly linked to the A cycle, the most electronegative atom of the δ_1 region being at a distance of 5.0 $\pm$ 0.5 Å from the A ring centroid and the δ_2 region being slightly closer (4.5 $\pm$ 0.5 Å). It is noteworthy that, with the major exception of the δ_1 region of the benzodiazepines which is slightly out of the plane (h = 0.7 Å), all other δ_1 and δ_2 regions are coplanar with the PAR region. Therefore, their electronic distribution can be modulated by mesomeric effects exerted on the A ring. The distance between the electronegative atoms of δ_1 and δ_2 is approximately 3.2 Å.

(c) Existence of steric sensitivity in the surrounding of the δ_1 region. As mentioned earlier, steric

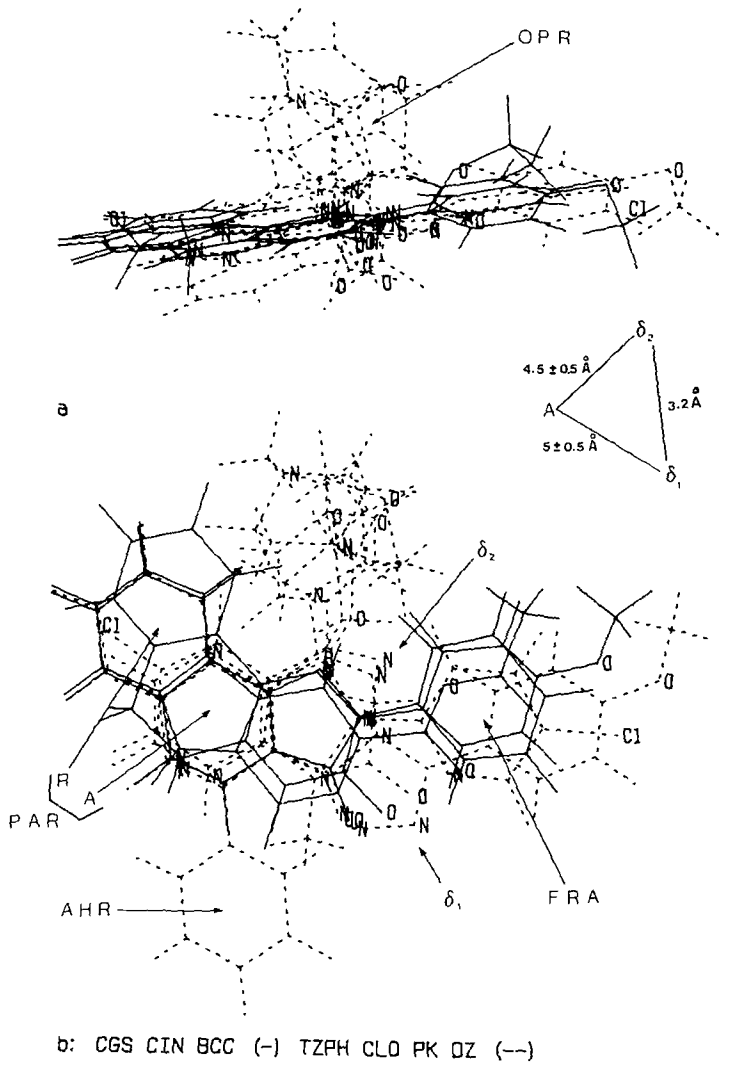


Fig. 10. The pharmacophore model: profile (a), front view (b) and critical distances. PAR: Planar aromatic region; δ_1 and δ_2: electronegative zones; FRA: freely rotating aromatic ring; OPR: out-of-plane region. AHR: additional hydrophobic region.

hindrance in the proximity of the benzodiazepine carbonyl or the β-CCM imino function rapidly leads to inactive substances. These two classes of compounds superimpose their sterically sensitive groups in our model. The necessity for free access to the δ_1 negative atom suggests a hydrogen bond or a dipole-dipole interaction with the receptor which is located in the mean plane of the molecule whereas the δ_2 region might be considered as an extensioin of the π-aromatic PAR system, rather involved in a sandwich π acceptor-donor interaction.

(d) Presence of a freely rotating aromatic ring (FRA) region. The freely rotating rings of CGS, CIN, TZPH and CLO define the FRA zone in which is also found the β-carbethoxy side chain of

BCC. This region is not occupied by DZ and PK. It can be compared to the AG_I zone defined by Borea et al. [9] and increasing lipophilic substitution on its constituents shifts the properties in the agonist direction (CGS 8216 -CGS 9896; CL 218872 – TZPH; CLO – SUR) or at least attenuates the inverse agonist to antagonist properties (β–CCM – β–PrCC). The FRA ring (or the carbalcoxy function) can be coplanar with the PAR zone and thus extend the flat zone of the pharmacophore.

(e) Existence of an out-of-plane region (OPR) which can be occupied by bulky polar moieties (pyrrolidine of CIN, piperazine of CLO, acetamidomorpholine of PK) but which also accommodates the phenyl rings of the benzodiazepines. The agonistic character of BZ-receptor ligands is strongly associated with the presence of a substituent in the OPR region. Thus compound Ro 05-3663, which differs from diazepam only by changing the phenyl to a methyl group, presents convulsant properties [2] (Fig. 11a,c). OPR groups are also absent from the potent antagonist Ro 15-1788 and its analogues. It should be mentioned that introducing sufficient lipophilic substitution in the FRA zone can compensate the missing OPR occupation and restore agonistic properties to a non-agonist. This is illustrated by the change from the antagonistic Ro 15-1788 to the agonistic Ro 19-0528 [16] (Fig. 11b, d). Conversely the inverse agonist properties of the β-carbolines can be counterbalanced by adequate substituents in the OPR zone. An example of this shift is found in the partial agonist ZK 91926 or in the weak agonist ZK 93423 (Fig. 12c,d) in which a 4-methoxymethylene and a 5-, respectively 6-benzyloxy group occupy the OPR region.

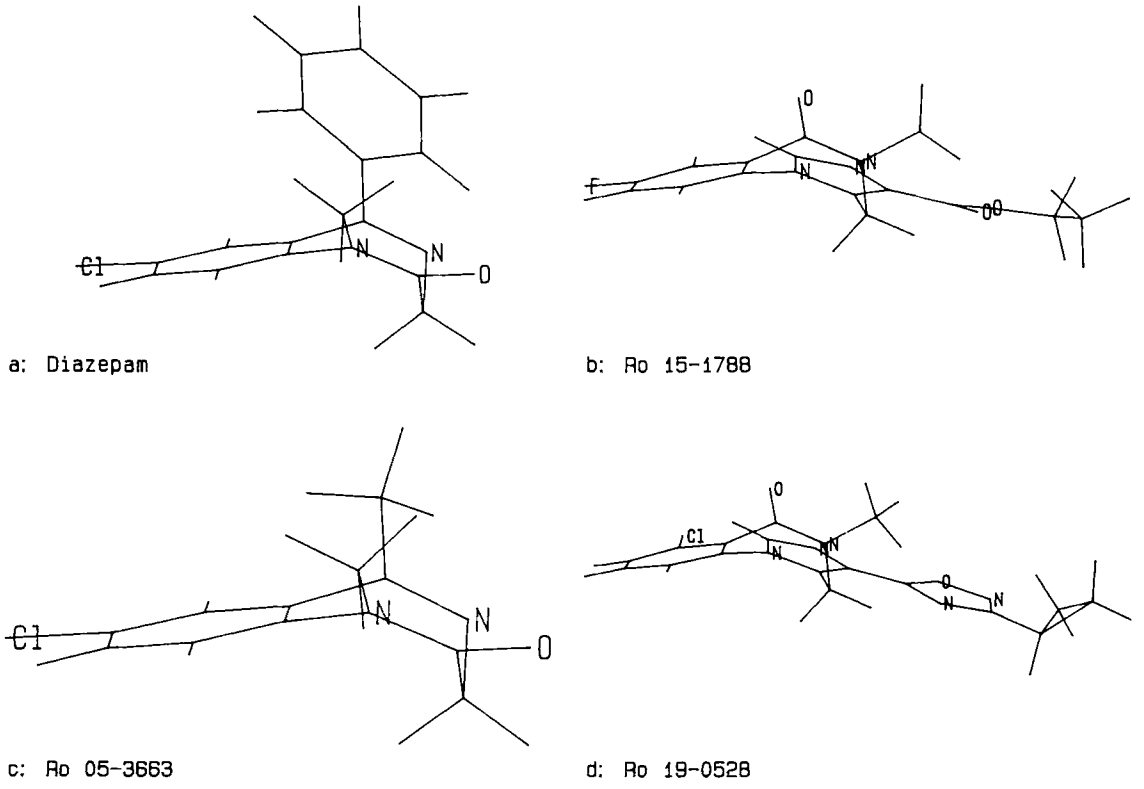


Fig. 11. Perspective views of diazepam (a); Ro 15-1788 (b); Ro 05-3663 (c) and Ro 19-0528 (d).

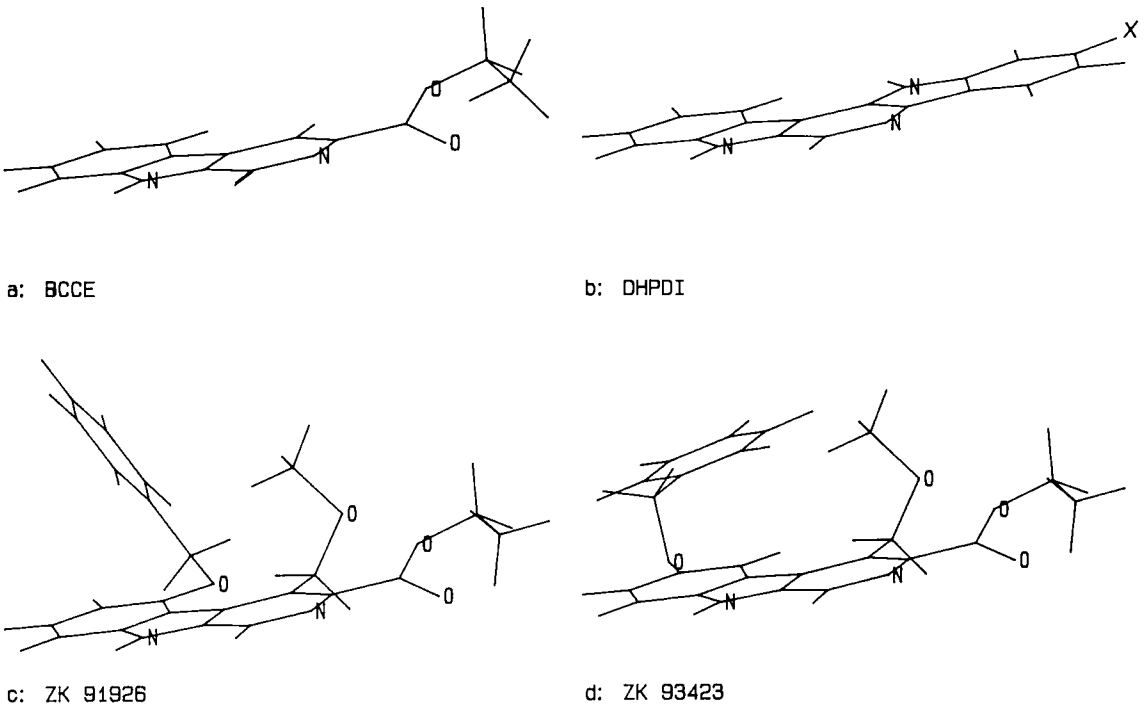


Fig. 12. β-Carboline derivatives: β-CCE (a); DHPDI (b); ZK 91926 (c); and ZK 93423 (d).

(f) Possible existence of an additional hydrophobic region (AHR) which accommodates the phenyl rings (probably in a coplanar conformation to the PAR system) of PK and of Ro 23-1590.

3. *Discussion of the model*

To prove its validity, our model has to account for the affinities and the activity profiles of the whole, heterogeneous set of BZ-receptor ligands; in addition, it has to possess some heuristic value. For these reasons we shall successively apply it to the different ligand classes, considering the structure-activity relationships (SAR) of the classical and the newer benzodiazepines, the β-carbolines, the triazolopyridazines, the cyclopyrrolones, the pyrazoloquinolines, the phenylquinolines and the imidazopyridines.

SAR in the benzodiazepine series

The classical structure-activity relationships published by Haefely et al. [2] show that four critical elements are essential for a good affinity:

(i) Presence of the C(2) carbonyl or carbonyl isostere (diazepam, IC_{50} = 8.1 nM; medazepam, which results from the change of the 2-C = 0 to a 2-CH_2, IC_{50} = 3850 nM [39]).

(ii) Presence of the C(5)-N(4) imino function (4,5-dihydrodiazepam – Ro 05-2881; IC_{50} > 1000 nM [2]). All compounds with a single C-N bond in position 4,5 are inactive in vitro. The activity found in vivo is probably due to partial metabolic dehydrogenation.

(iii) Presence of a phenyl (or aryl) ring in position 5. Replacement by a methyl group as found in 5-methyl-nordiazepam (Ro 05-3663 [2]), or by a benzyl group (Ro 05-3580 [2]) yields IC_{50} values higher than 1000 nM.

(iv) Necessity of the A ring (benzo or isostere). Not much has been published about deletion of this element which seems implicitly considered as crucial.

In relation to our model, we may observe that the 5-phenyl ring in diazepam lies out of the benzo plane and occupies the OPR zone (Fig. 11a). This orientation may explain the agonist properties of DZ, especially since the benzodiazepines *do not* occupy the FRA zone, which is the second important place able to confer agonist properties.

So far our conclusions on benzodiazepine SAR are consistent with those of Fryer et al. [8] and Borea et al. [9].

The fact that the stereochemical requirements for the classical benzodiazepines are similar to those of the imidazobenzodiazepine series [2, 40] suggest that in the antagonist Ro 15-1788, the imidazolic C=N has to be placed in the δ_1 and the amide function in the δ_2 regions (Fig. 11a,b). For the antagonist Ro 15-1788 the non-occupation of neither the FRA nor the OPR spaces probably accounts for the disappearance of the agonist potential normally present in benzodiazepines.

Conversely the introduction of correctly oriented additional residues in the FRA space can lead to a shift in the agonist direction (Ro 15-1788 → Ro 19-0528 [16]; Fig. 11b,d).

In the 3-substituted benzodiazepines a high degree of stereoselectivity is encountered. A possible explanation is that in the 'wrong' isomer the substituent in position 3 hinders the access to the A ring, whose interaction is crucial for activity (Fig. 13). This explanation holds for the *S,R*-couple of 3-methyl-flunitrazepam (IC_{50} = 7 nM for the (*S*)-CH_3 Ro 11-6896 and IC_{50} > 1000 nM for the (*R*)-CH_3 Ro 11-6893 [2, 40]), as well as for the bridged imidazobenzodiazepines Ro 14-5974 (3-(*S*); IC_{50} = 6.4 nM [2, 40]) and Ro 14-7527 (3-(*R*); IC_{50} > 1000 nM [2, 40]) as illustrated in Fig. 13 (a,b,c,d).

SAR in the β-carboline series

In assuming that in β-CCM the dominant group for δ_1 interaction is the C=N imine dipole and that it corresponds to the carbonyl group of CGS, we preferred a mode of superposition entirely different from that proposed by Codding et al. [8] and Fryer et al. [9]. Recent observations from the Skolnick group strengthen our point of view. Thus Trudell et al. [41] found that rigid and planar dihydropyrido-diindoles ('DHPDI'; X=H, IC_{50} = 15.1 nM; X=Cl, IC_{50} = 10 nM) are potent [^{3}H] diazepam displacers although they no longer possess the 'indispensable' carbonyl function in position 3. Consequently, a π aromatic system can replace the carbonyl group and vice versa (Fig. 12a,b). The structural and electronic similarities between the DHPDIs and the CGS pyrazoloquinolines [41] are also paralleled by similar substituent effects, the shift from H to Cl entailing a loss of inverse agonist activity. Finally the formerly free rotating aromatic (FRA) ring is completely frozen in a rigid and planar structure, suggesting that coplanarity is a requisite for good receptor fit. Here too it is encouraging to observe that, even when a free rotation of the phenyl ring is possible (CGS, CLO), the crystallographically observed conformations are practically planar (dihedral angle of the phenyl group and the heterocyclic PAR system: 10.1° for CGS [7]; 16.6° for CLO [9]).

The second paper from the Skolnick group studies the replacement of the ZK benzyloxy moiety in position 6 by a benzylamino group [42]. The two interesting observations which support our

present arguments are that deletion of the carbonylic function in position 3 gives rise to still highly potent compounds (IC_{50} = 106 nM) and, moreover, that the suppression of the imino dipole in ring C of the β-carboline nucleus (carboline → carbazole) leads to a total loss of affinity (IC_{50} > 5000 nM). This latter point again illustrates the importance of the electron density in ring C of the β-carbolines.

The substitution of β-CCE in positions 4 and 5 or 4 and 6 are illustrated in Fig. 12(c,d). This figure shows that the 4,6-disubstituted ZK 93423 produces a better fit with the OPR zone than does the 4,5-analogue ZK 91926; this may explain why the former compound is a weak agonist whereas the latter is only a partial agonist.

SAR in the triazolopyridazines series

As mentioned by Borea et al. [9] CL 218872 does not seem to be of great use in determining the receptor geometry. The much more potent (in terms of affinity) triazolo phthalazines [33] which contain freely rotating phenyl rings in position 3 and various aliphatic amine groups in position 6 gave us the necessary hint for a good fit with CGS and CIN. Applied to CL 218872 our mode of orientation shows that this compound does not occupy the FRA zone and, due to the rotation of the meta-tri-fluoro-phenyl substituent, can only cover partially the OPR (Fig. 14), suggesting

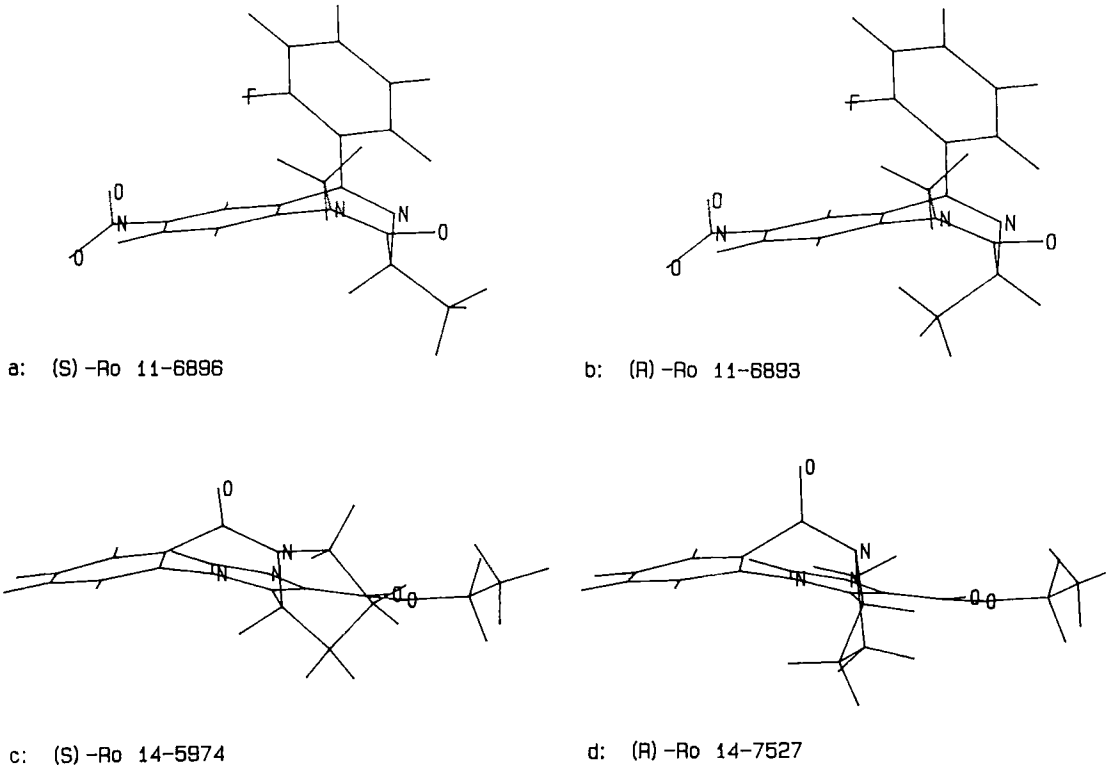


Fig. 13. Perspective views of the optical antipodes (*S*)-Ro 11-6896 (a); (*R*)-Ro 11-6893 (b); (*S*)-Ro 14-5974 (c); and (*R*)-Ro 14-7527 (d).

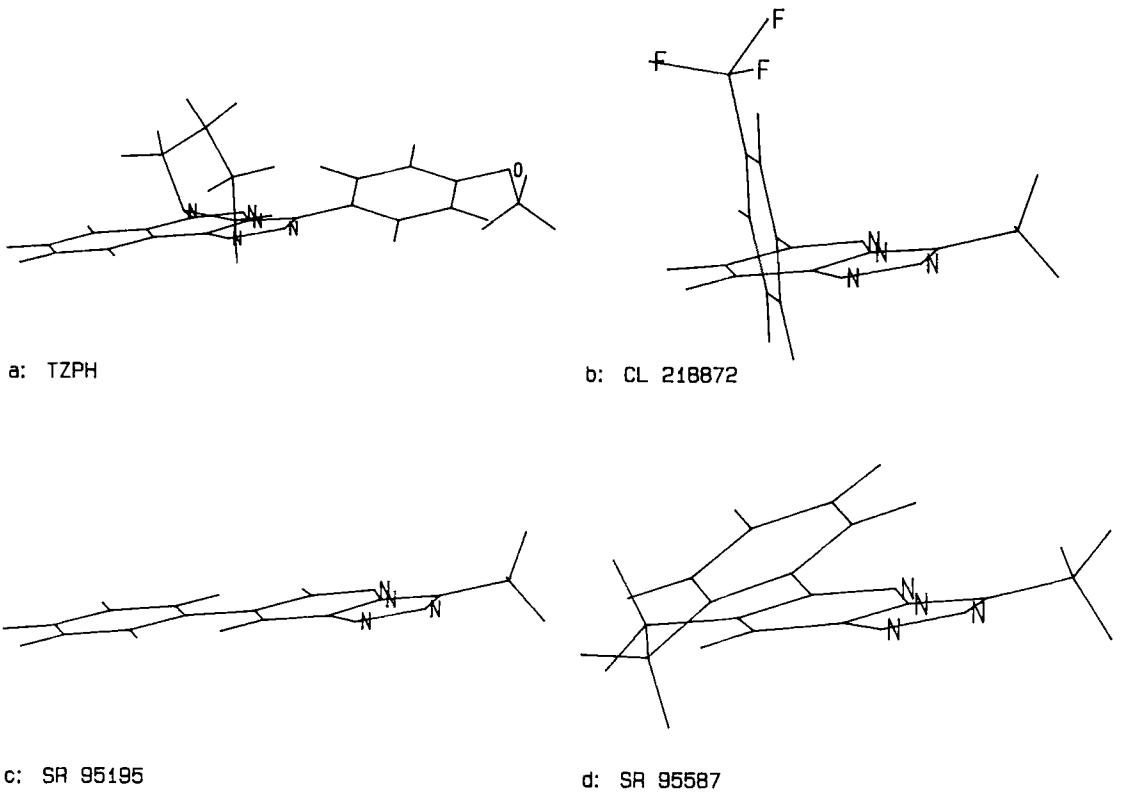


Fig. 14. Triazolopyridazine derivatives: TZPH (a) and CL-218872 (b), perspective view; SR 95195 (c) and SR 95587 (d), front view.

a limited agonistic potential. This is in agreement with the proposition of partial agonist properties made for CL 218872 by Haefely and Polc [43].

As mentioned earlier, we observed that in displacing the 6-aryl group of the triazolopyridazines to position 7 we generate inverse agonist properties [15, 24]. A possible explanation is that 7-aryl analogues (e.g. SR 95195; Fig. 14c) no longer occupy the OPR zone. As they already lack interaction with the FRA zone no agonist directing elements remain present.

In compound SR 95587 (Fig. 14d), a bridged analogue of CL 218872 that we prepared recently, the phenyl ring is forced to be coplanar. This was confirmed by crystallographic studies which indicated a dihedral angle of approximately 19° between the phenyl and the pyridazine rings (F. Durant, Namur, personal communication). Although bearing a phenyl ring *in the same* position as CL 218872, compound SR 95587 behaves as a BZ-receptor antagonist. Here again the reason for the loss of agonistic properties becomes evident: in forcing the phenyl ring to be coplanar with the whole system we render the occupation of the OPR impossible.

SAR in the cyclopyrrolone series

The available data from Julou et al. [44] totally support our model insofar as high affinities for zopiclone (RP 27267) analogues are dependent on:

(a) the nature of the OPR substituent; replacement of the *N*-methyl-piperazino group by only an amino group (RP 24131) yields a practically inactive compound.

(b) the steric and electronic effects in the FRA region; introduction of substituents or additional rings can induce drastic changes. Especially ortho-substitution, which prohibits coplanarity, is detrimental (RP 25664); but electronic factors also play an important role as is seen for chloro substituents in the 4,5 or 6 position of the pyridine ring, which cause affinity changes varying from 13 to > 1000 nM (RP 25700 vs. RP 25664 [44]).

(c) the presence of a benzo-like A ring, for which various isosteric replacements seem to be allowed, as benzo, α-pyrido, β-pyrido, pyrazino and dihydrothiazino rings always give active compounds.

The combination of a dihydro-thiazine ring in the PAR zone and a 7-chloro-1,8-naphtyridine ring in the FRA yielded a particularly potent compound, suriclone (RP 31264), which is presently in an advanced stage of development as an anxiolytic.

SAR in the pyrazoloquinoline series

The first representative of this class of compounds was CGS 8216 whose pharmacological profile is that of a partial inverse agonist [17, 18]. Introduction of substituents in the para position of the phenyl ring induce dramatic changes in the pharmacological profile. The *p*-chloro derivative (CGS 9896) behaves as a partial agonist [19] while the corresponding *p*-methoxy derivative possesses full agonist properties at low doses and antagonist properties at high doses [20]. These striking differences in intrinsic activity highlight once more the sensitivity of this region of space to electronic effects. In the absence of detailed structure-activity relationship studies in the CGS series it is however encouraging to find that potent substituent effects are found on the FRA cycles of the thienyl isosteres of CGS recently reported by Takada et al. [45]. When a methyl group occupies the same place as the chlorine in the para position of CGS 9896 the resultant compound, S-135, is a potent and orally active inverse agonist. The displacement of the methyl group to the adjacent carbon atom transforms S-135 to an antagonist which still possesses high affinity.

SAR in the phenylquinoline series

The partial agonists [46] PK 8165 and PK 9084 (Fig. 2E) show moderate in vitro affinity (IC_{50} = 100 and 380 nM respectively [2]) in comparison to the more recent analogues such as 'PK' (IC_{50} = 8.0 nM [35]) or Ro 23-1590 (IC_{50} = 4 nM [36]). The explanation lies in the presence, for the two

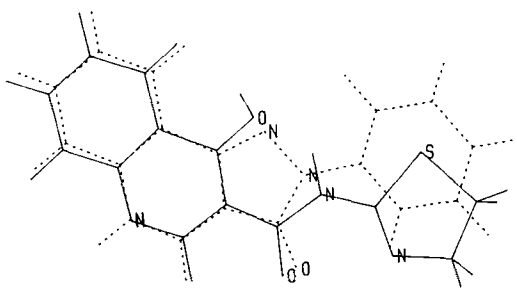


Fig. 15. Superposition of compound RU 40904 with CGS.

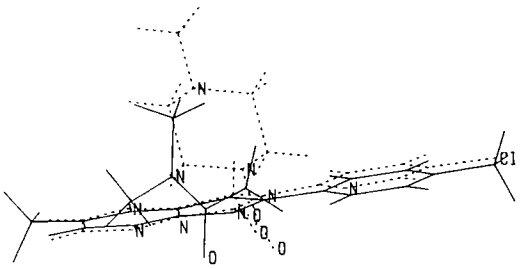


Fig. 16. Superposition of zolpidem with zopiclone.

latter compounds, of exocyclic oxygen or nitrogen atoms overlapping their lone pairs with the δ_2 zone thus giving a stronger interaction with the receptor than PK 8165 and PK 9084. The occupation of the OPR space is however possible for all four compounds, causing agonist profiles.

Although chemically related to the quinolines, compound RU 40904 has to be considered as an open bioisostere of CGS with which it superimposes perfectly (Fig. 15).

SAR in the imidazo-pyridine series

Zolpidem, an imidazo [1,2-a] pyridine derivative shows high affinity for the benzodiazepine receptor [47, 48] and resembles very close analogues (Fig. 2F) which have already been investigated in 1969 as anticonvulsants [49]. Its agonistic, central depressant and anticonvulsant profile is perfectly compatible with our pharmacophore model (simultaneous FRA and OPR occupation, Fig. 16).

CONCLUSION

The model presented in this paper has been elaborated stepwise, applying the active analog approach to the largest possible set of centrally active Bz-receptor ligands. This approach may be subject to some criticism as it implies static receptor-ligand interactions, based on the occupation-non occupation theory and therefore does not necessarily account for dynamic aspects such as ligand-induced conformational changes. However, for comparative studies within a series of relatively rigid structures, its use is still justified. The model assumes the existence of a unique recognition site for agonists, antagonists and inverse agonists. The pharmacophore geometry we observed is generally flat and comprises an aromatic (mostly heterocyclic) system (PAR), two electron-rich regions (δ_1 and δ_2) attached to the PAR rings and, eventually, an additional aromatic ring or delocalized π system (FRA). These features can be completed by bulky polar moieties or aromatic rings attached at the proximity of the δ_2 region and pointing out of the mean plane (OPR). The OPR seems to be associated with the agonist character of the corresponding ligand. Compared to published models of BZ-receptor ligand pharmacophores, our model has the following advantages:

(i) The model is exhaustive in that it includes all the central BZ-receptor ligands described in the literature: benzodiazepines, pyrazoloquinolines, benzocinnolones, cyclopyrrolones, phenylquinolines, imidazopyridines and β-carbolines.

(ii) The model is consistent with the observed stereochemical structure-affinity relationships. It explains the sensitivity to steric hindrance in the vicinity of the δ_1 interaction zone. Furthermore it

accounts for the differential affinities of the *S* and *R* stereosomers of 3-substitued benzodiazepines which seem to be due, for the less active *R* isomers, to a hindered access to the A ring. Finally it implies that the conformation of the FRA ring has to be nearly coplanar with the PAR system, as illustrated by the high affinity of DHPI[41] and the inactivity of RP 25664 [44].

(iii) One of the salient features of our model is its capacity to explain how, in a given ligand, the agonist character is induced through the occupation of either the OPR or the FRA region, or both. Thus our interpretation of the agonist vs, the non-agonist character of BZ-receptor ligands differs from that of other authors [7–9]. Fryer's interpretation [8] is based on the distance between the A-ring centroid and the π_1 dipole. Codding and Muir [7] correlate the agonistic profile to the existence of the δ_2-dipole. Borea et al. [9] attribute the inverse agonist activity to a different localization mode of the ligand within the receptor active site. The main argument advanced by these authors is that BZ-receptors are still able to interact with an inverse agonist, such ad β-CCM, after irreversible photolabeling with an agonist (flunitrazepam). This argument is however seriously weakened by the fact that the binding of other agonists, such as suriclone or CL 218872, is only moderately affected by previous flunitrazepam photolabeling [9,44].

(iv) Although the model does not allow the discrimination between antagonists and inverse agonists, it highlights the crucial role played in this discrimination by the nature of the chemical entity occupying the FRA region. Substituent effects in this region induce drastic changes in the affinity and in the intrinsic activity of the ligand.

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ABBREVIATIONS

Pyrazoloquinolines: CGS (2-phenyl-2,5-dihydro pyrazolo [4,3-c] quinoline-3 (3H)-one).
Cinnolinones: CIN (2-(4-methoxyphenyl)-benzo [h] 3-cinnolinone.
Triazolophthalazines: TZPH (3-(4-methoxyphenyl)-6 pyrrolidinotriazolo [4,3-a] phthalazine.
Cyclopyrrolones: RP 27267, CLO ([6-(5-chloro-2-pyridyl)-6,7-dihydro-7 oxo- 5H-pyrrolo [3,4-b]pyrazin-5-yl] 4-methyl-1-piperazine carboxylate).
Phenylquinolines: PK (phenyl-2 (morpholinocarbonyl methyl oxy)-4 quinoline.
β-Carbolines: BCC (3-carboethoxy-β-carboline).
Benzodiazepines: Diazepam: DZ (7-chloro-1,3-dihydro-1-methyl-5 phenyl-2H-1,4-benzodiazepin-2-one).