Revista Brasileira de Ciências Farmacêuticas
Brazilian Journal of Pharmaceutical Sciences
vol. 41, n. 1, jan./mar., 2005

RBCF

Binding characteristics of σ_2 receptor ligands

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Sigma (σ) receptors, once considered a type of opioid receptor, are now recognized as representing a unique receptive entity and at least two different types of σ receptors have been identified: σ_1 and σ_2 receptors. Evidence suggests that these receptors might be targeted and exploited for the development of agents potentially useful for the treatment of several central disorders. This review primarily describes some of our efforts to understand those structural features that contribute to σ_2 receptor binding, and some recent work by other investigators is also included. Despite an inability to formulate a unified pharmacophore model for σ_2 binding due to the diversity of structure-types that bind at the receptor, and to the conformational flexibility of these ligands, significant progress has been made toward the development of some very high-affinity agents.

Uniterms
- σ_2 receptor
- σ_2 ligand structural features
- σ_2 pharmacophore model

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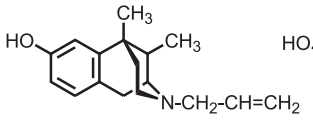
INTRODUCTION

The possible existence of putative sigma (σ) opioid receptors was proposed by William Martin in the mid 1970s to account for the binding of benzomorphans such as *N*-allylnormetazocine (NANM; SKF 10,047; **1**) and pentazocine (**2**). It soon became evident that various non-opioids (e.g. haloperidol; **3**) bind at these receptors and that some benzomorphans bind at phencyclidine (PCP) binding sites; the sites were subsequently termed σ/PCP receptors. Due to differences in brain localization, and because of affinity differences in ligand binding at σ *versus* PCP sites, it became apparent that σ binding sites and PCP binding sites were distinct receptor types. Identification of agents such as ditolylguanidine (DTG; **4**) led to the final realization that σ sites and PCP sites are distinct (Scherz *et al.*, 1990). Eventually, at least two major populations of σ receptors were identified: σ_1 and σ_2 (reviewed: Bowen, 2000). These two receptor populations differed in their tissue distribution and subcellular localization. The benzomorphan (+)pentazocine displays several hundred-fold selectivity for the former whereas DTG binds nearly equally well at both populations. The σ_1 receptor has been recently cloned from several sources including human brain; σ_2 receptors have yet to be cloned (reviewed: Guitart *et al.*, 2004). A very recent review (Guitart *et al.*, 2004) describes the potential involvement of σ receptors in schizophrenia, movement disorders, depression, anxiety, drug abuse, and pain. For general reviews of early investigations with σ receptors and σ receptor ligands, the reader is referred to: Abou-Gharbia *et al.*, 1993; Chavkin, 1990; Domino and Kamenka, 1988; Itzhak, 1994; Itzhak and Stein, 1990; Junien and Leonard, 1989; Musacchio *et al.*, 1989; Quirion *et al.*, 1987; Snyder and Largent, 1989; Walker *et al.*, 1990.



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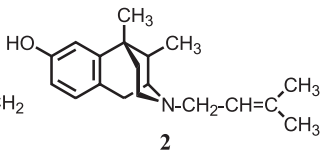

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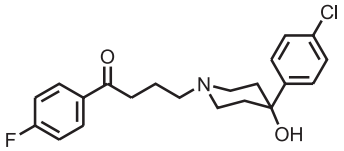

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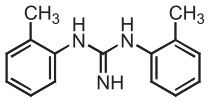

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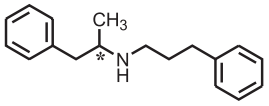
Binding character of σ_2 receptor ligands

One goal of our work has been the development of high-affinity σ-selective ligands, and as the foundation for such an effort we have attempted to identify a pharmacophore for the binding of ligands at σ receptors. Because our studies were begun in the mid 1980s prior to the discovery of σ_1 and σ_2 receptors, our conclusions required modification once two distinct σ receptor types were described. That is, the discovery of σ_1 and σ_2 receptors necessitated a re-evaluation of some initial findings. Although our primary focus was on σ_1 receptors, nearly all of the compounds we prepared were also evaluated at σ_2 receptors. Hence, a by-product of our work was the formulation of structure-affinity relationships for the binding of these ligands at σ_2 sites. We have not previously reviewed these latter findings and take this opportunity to do so. That is, this review is based on ligands we reported in a series of articles published over the past 15 years (e.g. Ablordeppey *et al.*, 1991; Ablordeppey *et al.*, 1992a; Ablordeppey *et al.*, 1992b; Ablordeppey *et al.*, 1993; Ablordeppey *et al.*, 1998; Ablordeppey *et al.*, 2000; Ablordeppey *et al.*, 2002; El-Ashmawy *et al.*, 1992; Glennon *et al.*, 1991a; Glennon *et al.*, 1991b; Glennon *et al.*, 1991c; Glennon *et al.*, 1994; Glennon, 2000; Glennon, and Fischer 2000; Glennon *et al.*, 2004); these papers can be consulted for the synthesis and physicochemical properties of most of the compounds described here. Sigma-2 receptor binding data were obtained using guinea pig brain homogenates and the nonselective [^{3}H]DTG in the presence of cold (+)pentazocine to block σ_1.

One of the first sigma ligands we reported was *R*(-)PPAP (**5*R***); the structure might be viewed as an *N*-phenylpropyl derivative of a ring-opened benzomorphan. *R*(-)PPAP was found to bind at σ_1 sites (Ki = 11 nM) with slightly higher affinity than it displayed for σ_2 sites (Ki = 61 nM; Table I). However, because selectivity is not a requirement for pharmacophore development or structure-affinity studies, we used *R*(-)PPAP as the basis for a more detailed structure-affinity investigation.



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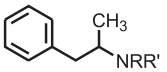
PPAP showed little stereoselectivity of binding with *S*(+)PPAP (Ki = 38 nM) binding with only about twice the affinity of it enantiomer. Chain length was extended from propyl to *n*-butyl (i.e. **6**) and *n*-pentyl (i.e., **7**) with relatively little change in affinity (Table I). Nevertheless, it was curious that with the longer-chain compounds (i.e., **7**) the *R*(-) isomers displayed slightly higher affinity than their *S*(+)enantiomers.

TABLE I - Binding of simple N-substituted phenylisopropylamines at σ_2 receptors

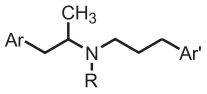


	R'	Stereochemistry	σ_2 Ki (nM)
5(*R*)	$(CH_2)_3Ph$	R(-)	61
5(*S*)	$(CH_2)_3Ph$	S(+)	38
6	$(CH_2)_4Ph$	(±)	53
6(*R*)	$(CH_2)_4Ph$	R(-)	48
6(*S*)	$(CH_2)_4Ph$	S(+)	36
7(*R*)	$(CH_2)_5Ph$	R(-)	10
7(*S*)	$(CH_2)_5Ph$	S(+)	19

Next examined was the effect of aryl substituents on binding. Table II shows that neither electron withdrawing nor electron donating substituents had much impact on affinity. First one, then the other, phenyl group of PPAP was replaced by either a 1-naphthyl or 2-naphthyl group (i.e. **13-16**; Ki = *ca* 200 nM) indicating that such changes were not beneficial to affinity. *N*-Monomethylation of *S*(+)PPAP (i.e., **17**; Ki = 5 nM) resulted in about 7-fold enhanced affinity, however the N-benzyl analog **18** (Ki = 470 nM) displayed >10-fold reduced affinity.

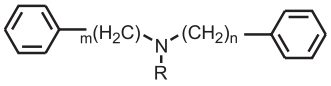
Due to the apparent reversal in stereoselectivity seen upon extension of chain length, and because the butyl and pentyl analogs **6** and **7** retained the affinity of PPAP, the α-methyl group was removed and chain-length on both sides of the amine was investigated (Table III). Comparing the phenylethyl derivatives **23-25**, (Ki = 90 nM, 120 nM, and 15 nM, respectively) it seems that a pentyl chain (i.e., **25**) is optimal among the three; furthermore, comparing **25** with **29**, it also seems that the phenylethyl moiety can be replaced with a phenylpropyl chain. As was seen with PPAP, *N*-monomethylation induced a slight enhancement of affinity. The overall result is that phenylethyl and

TABLE II - Binding of aryl-substituted phenylisopropylamines at σ_2 receptors



	Ar	Ar'	R	Stereochemistry	σ_2 Ki (nM)
5	Phenyl	Phenyl	H	(±)	48
8	3-CF_3 Ph	Phenyl	H	(±)	20
9	3-Br Ph	Phenyl	H	(±)	26
10	4-Br Phenyl	Phenyl	H	(±)	39
11	4-OH Phenyl	Phenyl	H	(±)	15
12	4-OEt Phenyl	Phenyl	H	(±)	34
13	Phenyl	1-Naphthyl	H	*R*(-)	280
14	Phenyl	2-Naphthyl	H	*R*(-)	260
15	1-Naphthyl	Phenyl	H	(±)	152
16	2-Naphthyl	Phenyl	H	(±)	220
17	Phenyl	Phenyl	Me	*S*(+)	5
18	Phenyl	Phenyl	Bn	*S*(+)	470

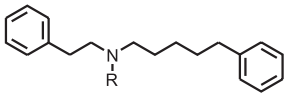
TABLE III - Investigation of phenylalkylamine chain length on σ_2 receptor binding



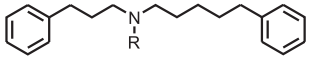
	m	n	R	σ_2 Ki (nM)
19	1	4	H	162
20	1	5	H	34
21	1	5	Me	13
22	1	7	H	39
23	2	3	H	90
24	2	4	H	120
25	2	5	H	15
26	2	5	Me	5.0
27	2	7	H	33
28	3	3	H	64
29	3	5	H	9.8
30	3	5	Me	6.3
31	4	5	H	58

phenylpropyl compounds **25** (Ki = 15 nM) and **29** (Ki = 9.8 nM) bind with fairly similar affinity, and their *N*-monomethyl tertiary amine counterparts **26** (Ki = 5.0 nM) and **30** (Ki = 6.3 nM) bind with slightly higher affinity.

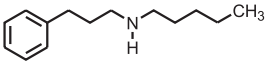
Removal of the phenylpentyl phenyl group of **29** (i.e. replacement of the phenyl group by H) afforded **32** (Ki = 240 nM) which binds with nearly 25-fold reduced affinity;



25 R = H
26 R = Me



29 R = H
30 R = Me



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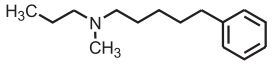

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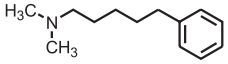

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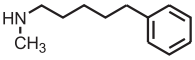
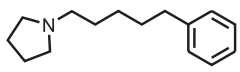


35

in contrast, removal of the phenylpropyl phenyl group of **30** (i.e., **33**; Ki = 40 nM) decreased affinity only by about 4-fold. Further shortening of the propyl chain of **33** to a methyl group (**34**; Ki = 965 nM) or replacement by H (**35**; Ki = 7,900 nM) resulted in dramatic decreases in affinity. On the other hand, cyclization of the alkyl substituents of **33** (Ki = 40 nM) to a five- (**36**; Ki = 70 nM) or six-membered (**37**; Ki = 50 nM) ring had much less impact on affinity. Replacement of the phenylpentyl phenyl ring with a cyclohexyl moiety had varying effects on affinity comparing **34** with **38** (Ki = 195 nM), and **35** with **39** (Ki = 350 nM).

Other modification of *des*-methyl PPAP that were examined included conformational constraint, aryl substitution, and certain side chain modifications. For example, compound **40** (Ki = 170 nM) is an analog of an extended PAPP compound that possesses an aminotetralin



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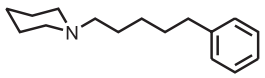

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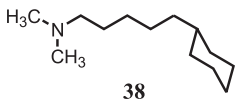

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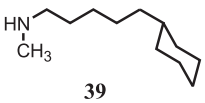

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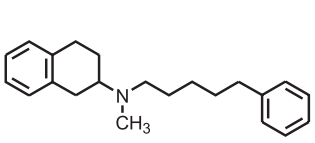
moiety similar to that found in the benzomorphans; yet **40** binds with >10-fold reduced affinity relative to its ring-open counterpart **25** (Ki = 15 nM).



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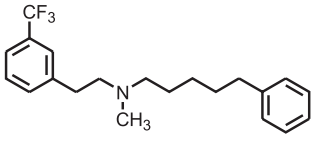

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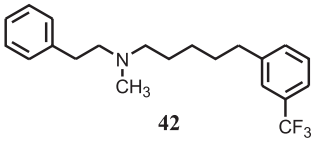

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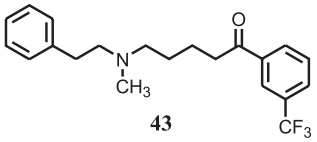

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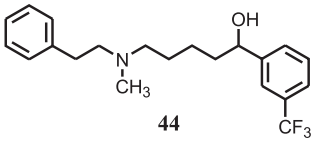
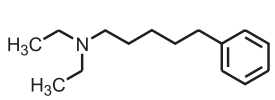


44

The trifuoromethylphenyl compound **41** (Ki = 14 nM) binds with an affinity similar to that of **25**, but its positional isomer **42** (Ki = 3.6 nM) binds with several-fold enhanced affinity. Compounds **43** (Ki = 1.3 nM) and racemate **44** (Ki = 3.0 nM) suggest that polar substituents are tolerated in the side chain. Additional compounds of this type require examination.

Cyclic compound **36** (Ki = 70 nM) was ring-opened to **45** (Ki = 90 nM) but, interestingly, introduction of a polar carbonyl oxygen atom decreased affinity (**46**; Ki = 750 nM). A second phenyl ring was tolerated (**47**; Ki = 50 nM), but here too, introduction of a polar hydroxyl substituent resulted in decreased affinity (**48**; Ki = 800 nM).



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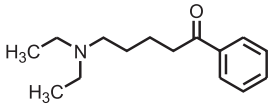

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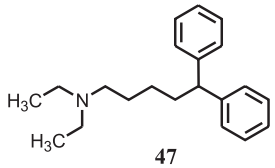

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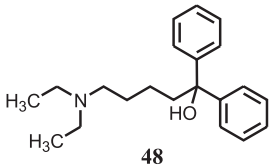
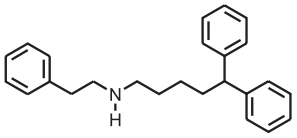


48

Gem-diphenyl substitution was also tolerated by **25** (i.e., **49**; Ki = 32 nM); however here, rather than enhancing affinity *N*-monomethylation decreased affinity by 3-fold (i.e., **50**; Ki = 100 nM).



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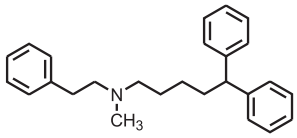
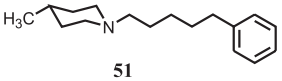


50

Focusing on the piperidine ring of **37**, 4-position substitution was investigated. Introduction of both a 4-methyl group (**51**; Ki = 7.1 nM) or a 4-benzyl group (**52**; Ki = 2.8 nM) resulted in about a 10-fold increase in affinity.



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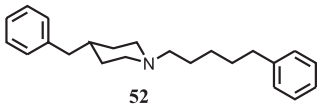
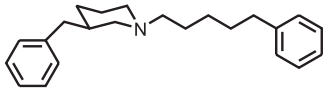


52

The benzyl group of **52** was moved to the 3-position (**53**; Ki = 4.2 nM) and 2-position (**54**; Ki = 10 nM), where it was shown to be tolerated at each of the three positions; nevertheless, the 3- and 4-substituted derivatives displayed the highest affinity. Both the 3- (**55**; Ki = 5.3 nM) and 2-phenyl (**56**; Ki = 4.7 nM) derivatives also retained high affinity.



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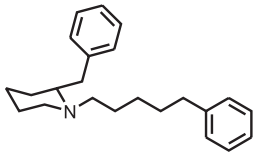

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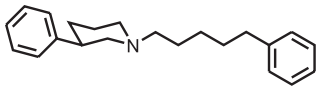

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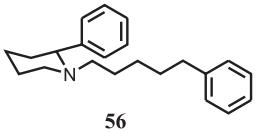
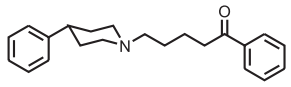
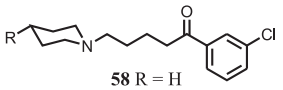


56

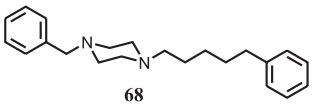
At this point, the role of carbonyl derivatives, aryl substitution, and the importance of the piperidino phenyl group was reinvestigated. Compound **57** (Ki = 3.1 nM) binds with high affinity; introduction of a 3-chloro substituent (**59**; Ki = 1.6 nM) has little effect on affinity as does moving the 3-chloro substituent to the 4-position (**61**; Ki = 1.0 nM). But in both of the latter cases, removal of the piperidino phenyl group decreases affinity by about 20-fold (i.e. **58** and **60**; Ki = 33 and 25 nM, respectively). In contrast, removal of the carbonyl oxygen atom of **61** (**62**; Ki = 2.1 nM) indicates that it likely does not participate in binding.



57



58 R = H
59 R = Ph



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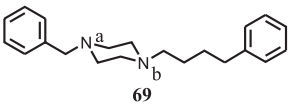

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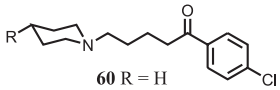
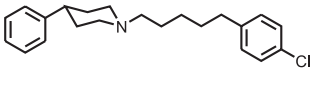


60 R = H
61 R = Ph



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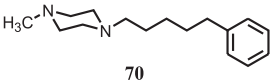

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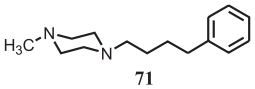
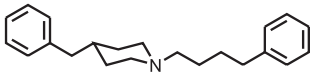


71

What emerged from these studies is that the σ_2 receptor likely consists of an amine binding site flanked by two hydrophobic sites; in fact, there is striking similarity to what we have previously proposed for σ_1 binding requirements (and, indeed, most compounds bind both at σ_1 and σ_2 receptors).

Subsequently, the phenylpentyl chain of **52** was shortened to a phenylbutyl (**63**: Ki = 3.1 nM) and phenylpropyl (**64**; Ki = 3.3 nM) chain; affinity was retained independent of the length of the chain. In the phenylbutyl series, the benzyl group could be replaced with a benzoyl group (**65**; Ki = 11 nM), and the benzoyl group could be reduced to its corresponding racemic alcohol (**66**; Ki = 13 nM). However, the *gem* diphenyl analog **67** (Ki = 235 nM) displayed reduced affinity.



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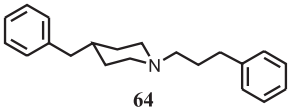
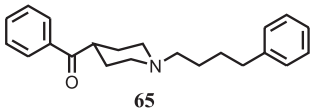
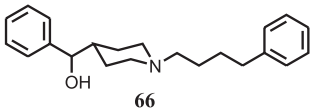


64



65



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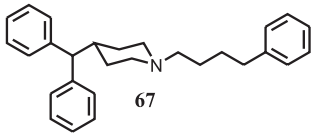
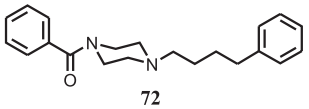


67

Interesting is that 4-benzylpiperidine derivatives **52**, **63**, and **64**, which vary only with respect to the length of their N-alkyl chain, bind with similar high affinity (Ki = 2.8 to 3.3 nM). This is in contrast to the affinities of N-substituted phenylethylamines **23**-**25** where the N-pentyl analog **25** (Ki = 15 nM) binds with 8-fold higher affinity than its N-butyl counterpart **24** (Ki = 120 nM). This same type of inconsistency was observed with piperazine **68** (Ki = 11 nM) where shortening of the alkyl chain by a single methylene group (i.e., **69**; Ki = 8.2 nM) had negligible effect on affinity. However, in the absence of the benzylic phenyl group, pentyl analog **70** (Ki = 79 nM) displayed 10-fold higher affinity than its butyl counterpart **71** (Ki = 900 nM).

These inconsistencies argue for different modes of binding. This is especially germaine to piperazine derivatives where either one of the two basic nitrogen atoms might interact with the amine binding site. Because a benzylic carbonyl group seems to be tolerated by the receptor (e.g. comparing **63** with **65**), it was reasoned that the affinity of **69** should remain unchanged following reduction of the basicity of the N_a nitrogen atom if it is the N_b nitrogen atom that interacts with the amine binding site; however, the reduced affinity of **72** (Ki = 965 nM) suggests that the N_a, not the N_b, nitrogen atom of **69** might be the more important. But, comparing **70** (Ki = 965 nM) with its *des*-amino analogs **51** (Ki = 7.1 nM) and **73** (Ki = 97 nM), it would seem that interaction of N_b with the amine binding site leads to higher affinity. Evaluation of a number of related compounds produced results that are equally difficult to interpret. Similar inconsistencies were observed in an earlier investigation of compounds at σ_1 receptors. To account for this we suggested that multiple modes of binding are possible. That is, we proposed two different modes of binding for piperidine derivatives, and four possible modes of binding for piperazine derivatives (Ablordeppey *et al.*, 2000), depending upon the presence of one or two basic nitrogen atoms, the length of the alkyl chain, and substituents that might be present in aryl portions of the chain. A similar argument can be made here for the binding of these ligands at σ_2 receptors (Figure 1).



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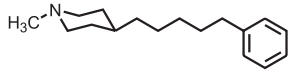


73

Figure 1 contains an amine binding site and presupposes the necessity of an amino group in the ligand. To determine whether such an amine is actually required for binding, we prepared the *para*-amino analog of **37** (i.e., **74**) so that when the more basic amine was removed the compound would retain aqueous solubility. Compound **74** (Ki = 830 nM) possessed 16-fold lower affinity than **37**; however when the basic nitrogen atom of **74** was replaced by a methylene group, the resultant compound

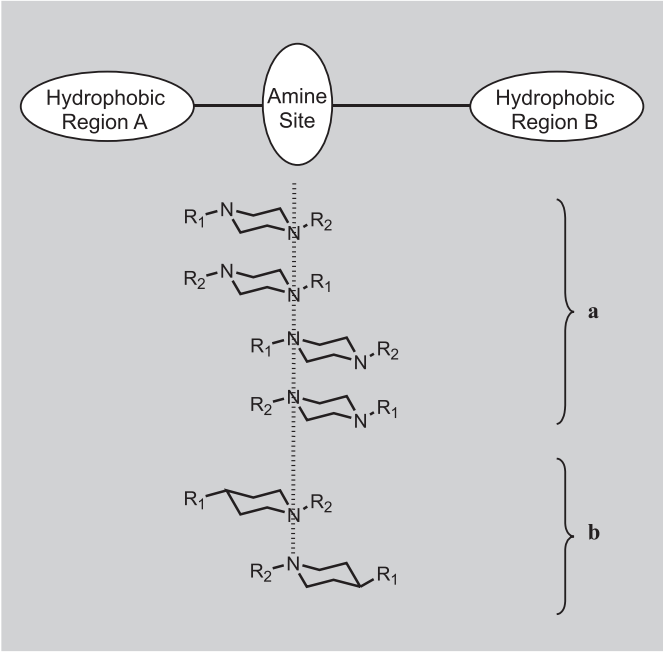
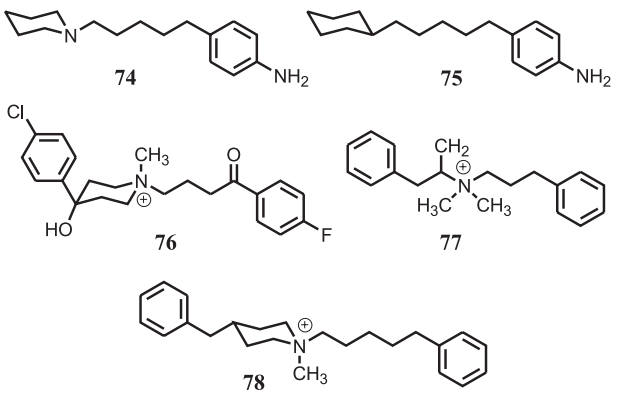


FIGURE 1 - Sigma-2 receptors appear to consist of an amine binding site flanked by two hydrophobic sites. Hydrophobic Region A is situated such that a distance of four carbon atoms is optimal, whereas Hydrophobic Region B seems better able to accommodate a phenyl group at a distance of several atoms away with a chain length of five atoms appearing to be optimal. Data suggest that piperazine derivatives (**a**) might be accommodated in any one of four orientations depending upon chain length, amine basicity, and the nature of the R substituents; whereas piperidines (**b**) might be accommodated in either of two different orientations.

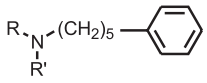
(**75**; Ki >50,000 nM) lacked affinity for the receptors. This provides support for the concept that the amine moiety must be present for optimal binding. [It might be noted that quaternary amines are also accommodated; for example, haloperidol (**3**) binds at σ_2 receptors with high affinity (Ki = 11 nM) and its N-methyl quaternary amine analog **76** (Ki = 23 nM) binds with similarly high affinity. The quaternary amine analog of *R*(-)PPAP (i.e., **77**; Ki = 87 nM) displays modest affinity, and the quaternary amine analog of **52** (i.e., **78**; Ki = 4.0 nM) binds with an affinity similar to that of **52**. Too few quaternary amines were investigated, however, to allow any general conclusions to be drawn at this time.]

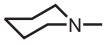
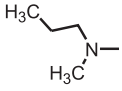
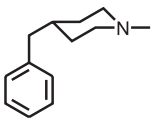
Turning to Figure 1, we further examined the existence of two possible hydrophobic binding regions. It would appear that occupation of one of these regions, in addition to interaction at the amine site, is insufficient to impart high affinity. For example, compound **79** (Ki >10,000 nM) which, in theory, should be accommodated by



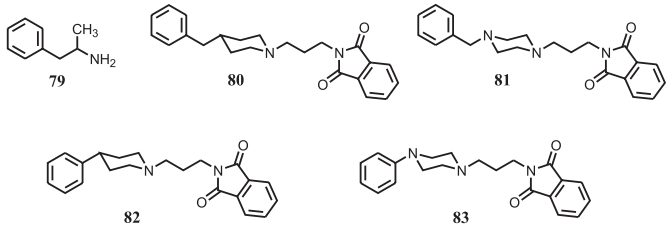
Hydrophobic Region A and the amine site, lacks significant affinity. Likewise, phenylpentylamine compound **35** (Ki = 7,900 nM; Table IV) binds only with low affinity. However, comparison of a series of 5-(phenyl)pentylamines showed that as the lipophilicity (bulk?) of the amine substituent increased, affinity increased. If it is assumed that the common phenyl moiety interacts with Hydrophobic Region B, it would appear that at least four carbon atoms are required (in the diection of Hydrophobic Region A) to achieve optimal affinity (*to wit*: compare **37** with **51**, Table IV). The necessity of an aromatic ring to interact with Hydrophobic Region A does not seem to be a requirement. Nevertheless, such a ring is accommodated by the recep-

TABLE IV - Influence of amine substituents on σ_2 receptor affinity of 5-(phenyl)pentylamines.

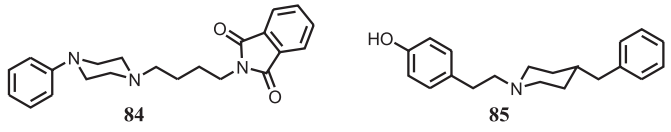


	-NRR'	σ_2 Affinity (Ki, nM)
35	CH_3-NH-	7,900
34	$(CH_3)_2$N-	965
45	$(CH_3CH_2)_2$N-	90
36		70
37		50
33		40
51		7.1
52		2.8

tor (in a region of bulk tolerance?). It is proposed that there exists two binding regions (tentatively termed hydrophobic regions) flanking an amine site. Hydrophobic Region A is situated such that it optimally accommodates four carbon atoms. Hydrophobic Region B is situated such that it seems capable of accommodating somewhat longer chains. But the semi-symmetrical nature of the binding site makes it very difficult to determine exactly how compounds bind. For example, if the 5-(phenyl)pentyl chain is shortened by a single methylene group, the phenyl ring might now bind at Hydrophobic Region A rather than Hydrophobic Region B. This might be further influenced by any substituents that might be present on the phenyl ring. Of course, this is further complicated when the ligand possesses a piperidine or piperazine ring (see Figure 1). Evidence for the possibility of "reverse" modes of binding might be that *S*(+)PPAP (**5*S***) binds with twice the affinity of its enantiomer **5*R***, whereas for the corresponding pentyl homolog **7*R*** binds with twice the affinity of **7*S***, and for the butyl analog (i.e., **6**) the two optical isomers bind with nearly identical affinity. Other such evidence might come from the observations that conversion of piperidine **52** (Ki = 2.8 nM) to its corresponding piperazine **68** (Ki = 11 nM), or piperidine **63** (Ki = 3.1 nM) to piperazine **69** (Ki = 8.2 nM) results in very small changes in affinity, whereas conversion of piperidine **80** (Ki = 13 nM) to piperazine **81** (Ki = 290 nM), or piperidine **82** (Ki = 12 nM) to piperazine **83** (Ki = 149 nM), lead to substantial decreases in affinity.

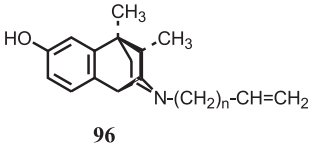


The proposed model, simplistic though it might be, provides a framework for further evaluation. Although it accounts for the binding of certain compounds, it is not particularly robust in explaining the binding of other agents. For example, it does not provide a satisfactory explanation for why chain extension of **83** to **84** (Ki = 20 nM) results in enhanced affinity, nor why compound **85** (Ki = 0.7 nM) binds with such high affinity.



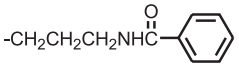
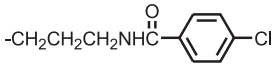
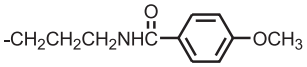
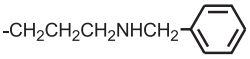
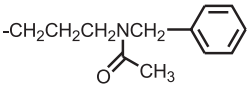
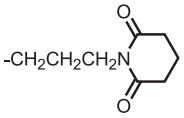
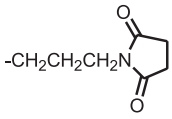
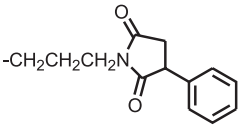
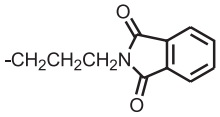
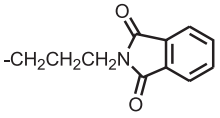
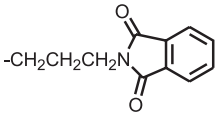
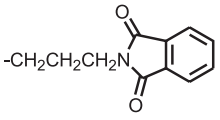
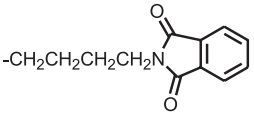
There are relatively few agents that display significant selectivity for σ_2 *versus* σ_1 receptors. Most of the compounds described herein bind with at least equivalent, and in most cases, with higher affinity at σ_1 receptors than at σ_2 receptors. Our interest in the above phthalimido analogs was heightened by the observation that introduction of a carbonyl group adjacent to an amine present in the alkyl chain showed a tendency to modulate selectivity. For example, amide **88** (Ki = 490 nM) displayed modest affinity and no selectivity for σ_2 receptors (Table V); the corresponding amine **91** (Ki = 100 nM) showed 5-fold enhanced affinity for σ_2 receptors, but was 50-fold selective for σ_1 receptors. It was surmised that the basicity of the amine might be responsible for these effects. Accordingly, we investigated amide **92** and imides **93**-**95**; these compounds displayed varying affinities for σ_2 receptors but showed enhanced σ_2 selectivity. Compound **83** (σ_2 Ki = 149 nM, 87.9-fold σ_2 selectivity) displayed modest affinity but good selectivity. Conversion of the 1-phenylpiperazine **83** to 4-phenylpiperidine **82** enhanced affinity at both receptor populations but resulted in loss of selectivity. Replacement of the piperazine phenyl group of **83** with a benzyl group (i.e., **81**) reduced both affinity and selectivity. Lengthening of the alkyl chain of **83** by a single methylene group (i.e. **84**) enhanced σ_2 affinity by about 7-fold, but reduced its selectivity by a corresponding amount. Among the compounds examined, compound **83** was a compromise between high affinity and selectivity.

Most of our studies were conducted in the mid to late 1990s. Since then, a number of exciting results have been reported by others; some of these will be described. Although benzomorphans do not typically bind with high affinity at σ_2 receptors, May and co-workers (2000) have found that the σ_2 receptor affinity of a series of (1*R*,5*R*,9*R*)-benzomorphans **96** increases as n is increased from 1-4 (Ki = 3,200 nM, 620 nM, 180 nM, and 80 nM, respectively). These results are consistent with those features we have found to be important for binding (Figure 1).

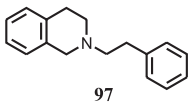


Maeda *et al.* (2002) prepared a series of *bis*(aralkyl)amines, such as those shown in Table III, and found they could be cyclized to tetrahydroisoquinolines; for example **97** (Ki = 57.3 nM) and **98** (Ki = 14.2 nM) bind with high affinity. However, **97** binds with 10-fold greater affinity at σ_1 receptors than at σ_2 receptors whereas **98**

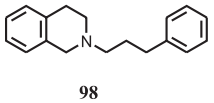
TABLE V - Comparison of σ_2 *versus* σ_1 receptor affinity for selected piperazine-related compounds

	R_1	X	R_2	σ_2 (Ki, nM)	σ_1 (Ki, nM)	σ_2 Selectivity
86	Ph	N	$-CH_2CH_2CH_3$	540	82	0.2
87	Ph	N	$-CH_2CH_2CH_2NH_2$	13,500	3,300	0.2
88	Ph	N		490	94	0.2
89	Ph	N		89	36	0.4
90	Ph	N		646	780	1.2
91	Ph	N		100	2	0.02
92	Ph	N		186	132	0.7
93	Ph	N		965	7,760	8.0
94	Ph	N		2,220	6,460	2.9
95	Ph	N		237	189	0.8
83	Ph	N		149	13,100	87.9
82	Ph	CH		12	25	2.1
81	$PhCH_2$	N		290	57	0.2
80	$PhCH_2$	CH		13	90	6.9
84	Ph	N		20	195	9.8

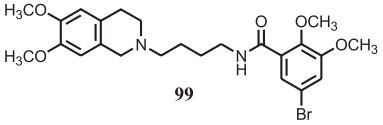
binds equally well at both population; on the basis of these and related studies they suggested that phenylpropyl derivatives, rather than phenylethyl derivatives, might provide leads to agents with greater σ_2 selectivity. More recently, Mach *et al.* (2004) have reported on a very interesting series of tetrahydroisoquinolines. Compound **99** (Ki = 8.2 nM), for example, binds with high affinity, and displays 1,573-fold selectivity *versus* σ_1 binding; interestingly, the related but shorter analog **100** (Ki = 89.4 nM) binds with lower affinity and is several-fold selective for σ_1 receptors.



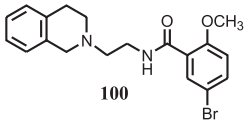
97



98

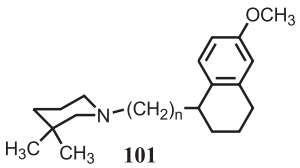


99

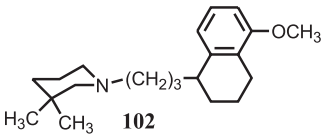


100

Piperidine and piperazine derivatives continue to receive considerable attention (e.g. Berardi *et al.*, 1998; Berardi *et al.*, 2004; Fujimura *et al.*, 1997; Kawamura *et al.*, 2003; Mach *et al.*, 2004; Maeda *et al.*, 2002; Maier and Wunsch, 2002; Marrazzo *et al.*, 2001; Matsumoto *et al.*, 2004). Of particular interest is a series of substituted tetralins bearing a *gem*-dimethyl piperidine. Compound **101** (n = 4; IC_{50} = 0.016 nM) is a very high affinity σ_2 ligand with >100,000-fold selectivity over σ_1 receptors; remarkably, when n=5 (IC_{50} = 0.03 nM) the compound binds only with 21-fold selectivity. Also remarkable is that removal of the methoxy group from the latter compound (i.e., *des*-methoxy **101**, n = 5; IC_{50} = 0.008 nM) once again results in >100,000-fold σ_2-selectivity. The shorter relative **102** (IC_{50} = 119 nM) binds with much lower affinity and with 1,340-fold σ_1 selectivity (Berardi *et al.*, 1998).

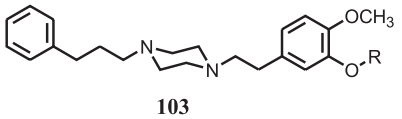


101



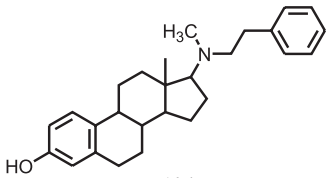
102

Kawamura *et al.* (2003) have also found that small structural differences have a major impact on affinity and selectivity. Compound **103** binds at σ_2 receptors with low affinity and 106-fold selectivity for σ_1 sites when R is a methyl group (σ_2 Ki = 1,800 nM), but binds with higher affinity at, and nearly 3-fold selectivity for, σ_2 sites when R is an ethyl group (σ_2 Ki = 13 nM).

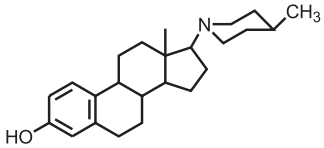


103

Some of the more structurally unusual compounds to be examined are steroids **104** and **105**, and tropane **106**. Curious as to just how far we might push the 5-(phenyl)pentylamine concept with respect to conformation and bulk, we prepared steroid analogs **104** and **105** (*unpublished finding*; Glennon, Ismaiel, Fischer). Both compounds possess an embedded 5-(phenyl)pentyl chain in their cyclic structure, with a different amine function appended. Compound **104** (Ki = 125 nM) binds with unexpectedly high affinity and with 6-fold selectivity over σ_1 receptors. Compound **105** (Ki = 24 nM) binds with even higher affinity, but with 3-fold selectivity. Surprisingly, the affinity of **105** is not much unlike that of the structurally simpler 4-methyl-N-(5-phenyl)pentylpiperazine (**51**; Ki = 7.1 nM). Evidently, the receptors can tolerate considerable bulk.

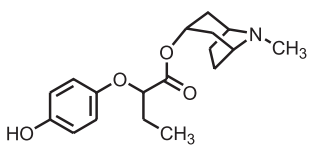


104



105

SM-21 (**106**) has seen application as a σ_2 antagonist. SM-21 was initially reported to bind at σ_2 receptors (rat liver; Ki = 67.5 nM) with >14-fold selectivity over σ_1 receptors (Mach *et al.*, 1999). However, a more recent investigation demonstrated that SM-21 binds with somewhat lower affinity (guinea pig; σ_2 Ki = 434 nM; σ_1 Ki >1000 nM); in that same study, neither optical isomer retained the affinity of the racemate (σ_2 Ki = 703 nM and 2,169 nM for the *R*(+) and *S*(-)isomers, respectively) (Prezzavento *et al.*, 2002). The lower affinity of SM-21 in the latter study was attributed to differences in the tissue source that was used.



106

This review has attempted to highlight progress made in the identification of the binding character of σ_2 ligands since the σ_1/σ_2 receptor concept was first proposed 15 years ago. It should now be evident that a pharmacophore

model to account for the binding of ligands at σ_2 receptors remains elusive. Nevertheless, outstanding progress has been made, despite lack of a pharmacophore model, towards the development of various high-affinity σ_2 ligands. While σ_2-selective ligands were not the intended focus of the review, it should be noted that several such agents also have been identified.

Important binding features common to many σ_2 ligands are shown in Figure 1. The nearly symmetrical nature of these binding features presents problems as to exactly how specific compounds might bind at the receptors, and this is only further complicated when the ligands possess two basic amine groups (e.g. as with piperazine derivatives). To some extent, binding character is not unlike that proposed for σ_1 ligands (Gilligan *et al.*, 1992; Glennon *et al.*, 1994; Ablordeppey *et al.*, 2000); indeed, most compounds bind at both σ receptor subtypes with, frequently, <10-fold selectivity. On the other hand, compounds such as **101**-**103** suggest that real differences exist – subtle though they may be. What is now required to better define pharmacophore models for σ_1 and σ_2 receptor binding are high-affinity, conformationally-constrained ligands. It would certainly not come as a surprise if multiple pharmacophores are eventually identified for one or both σ receptor types.

RESUMO

Características estruturais de ligantes do receptor σ_2

Receptores sigma (σ), considerados como um tipo de receptor opióide, são hoje considerados como uma entidade receptora singular. Pelo menos dois subtipos desses receptores foram identificados: σ_1 e σ_2. Há evidências de que esses receptores devam ser explorados como alvo para o desenvolvimento de agentes potencialmente úteis para o tratamento de várias disfunções centrais. Esta revisão descreve, principalmente, alguns dos nossos esforços para compreender as características estruturais que contribuem para a ligação no receptor σ_2, e incluem-se alguns trabalhos recentes desenvolvidos por outros pesquisadores. Apesar da incapacidade de formular um modelo de farmacóforo único para ligação no receptor σ_2, em razão da diversidade de estruturas que a ele se ligam e da flexibilidade conformacional desses ligantes, houve progresso significativo no desenvolvimento de agentes de alta afinidade.

Unitermos: Receptor opióide σ_2. Características estruturais do ligante σ_2. Modelo farmacóforo σ_2.

ACKNOWLEDGMENTS

Dr. Abd Ismaiel and Dr. Ho Law are acknowledged for their contributions to this project.

REFERENCES

ABLORDEPPEY, S. Y.; EL-ASHMAWY, M. B.; GLENNON, R. A. Analysis of the structure-activity relationships of sigma receptor ligands. *Med. Chem. Res.*, v.1, p.425-438, 1991.

ABLORDEPPEY, S. Y.; EL-ASHMAWY, M. B.; GLENNON, R. A. Analysis of the structure-activity relationships of sigma receptor ligands. *Med. Chem. Res.*, v.2, p.425-438, 1992a.

ABLORDEPPEY, S. Y.; FISCHER, J. B.; GLENNON, R. A. Is a nitrogen atom an important pharmacophoric element in sigma ligand binding? *Bioorg. Med. Chem.,* v.8, p.2105-2111, 2000.

ABLORDEPPEY, S. Y.; ASHMAWY, M.; FISCHER, J. B.; GLENNON, R. A. A CoMFA investigation of sigma receptor binding affinity: Reexamination of a spurious sigma ligand. *Eur. J. Med. Chem.,* v.33, p.625-633, 1998.

ABLORDEPPEY, S. Y.; FISCHER, J. B.; LAW, H.; GLENNON, R. A. Probing the proposed phenyl-A region of the sigma-1 receptor. *Bioorg. Med. Chem*., v.10, p.2759-2765, 2002.

ABLORDEPPEY, S. Y.; ISSA, H.; FISCHER, J. B.; BURKE HOWIE, K. J.; GLENNON, R. A. Synthesis and structure-affinity relationship studies of sigma ligands related to haloperidol. *Med. Chem. Res.*, v.3, p.131-138, 1993.

ABLORDEPPEY, S. Y.; FISCHER, J. B.; BURKE HOWIE, K. J.; GLENNON, R. A. Design, synthesis and binding of sigma receptor ligands derived from butaclamol. *Med. Chem. Res*., v.2, p.368-375, 1992b.

ABOU-GHARBIA, M.; ABLORDEPPEY, S. Y.; GLENNON, R. A. Sigma receptors and their ligands: The Sigma Enigma. *Ann. Rep. Med. Chem*., v.28, p.1-10, 1993.

BERARDI, F.; SANTORO, S.; PERRONE, R.; TORTORELLA, V.; GOVONI, S.; LUCCHI, L. N-[ω-(Tetralin-1-yl)alkyl] derivatives of 3,3-dimethylpiperidine are highly potent and selective σ_1 or σ_2 ligands. *J. Med. Chem.*, v.41, p.3940-3947, 1998.

BERARDI, F.; FERORELLI, S.; ABATE, C.; COLABUFO, N. A.; CONTINO, M.; PERRONE, R.; TORTORELLA, V. 4-(Tetralin-1-yl)- and 4-(naphthalene-1-yl)alkyl derivatives of 1-cyclohexylpiperazine as s receptor ligands with agonist σ_2 activity. *J. Med. Chem*., v.47, p.2308-2317, 2004.

BOWEN, W. D. Sigma receptors: Recent advances and new clinical potentials. *Helv. Pharm. Acta*, v.74, p.211-218, 2000.

CHAVKIN, C. The sigma enigma. *Trends Pharmacol. Sci*., v.11, p.213-215, 1990.

DOMINO, E. F.; KAMENKA, J.-M. (Eds.) Sigma and Phencyclidine-like Compounds as Molecular Probes in Biology. Ann Arbor: NPP Books. 1988.

EL-ASHMAWY, M.; ABLORDEPPEY, S. Y.; ISSA, H.; GAD, L.; FISCHER, J. B.; BURKE HOWIE, K. J.; GLENNON, R. A. Further investigation of 5-phenylpentylamine derivatives as novel σ receptor ligands. *Med. Chem. Res*., v.2, p.119-126, 1992.

FUJIMURA, K.; MATSUMOTO, J.; NIWA, M.; KOBAYASHI, T.; KAWASHIMA, Y.; IN, Y.; ISHIDA, T. Synthesis, structure and quantitative structure-activity relationships of σ receptor ligands, 1-[2-(3,4-dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazines. *Bioorg. Med. Chem*., v.5, p.1675-1683, 1997.

GILLIGAN, P. J.; CAIN, G. A.; CHRISTOS, T. E.; COOK, L.; DRUMMOND, S.; JOHNSON, A. L.; KERGAY, A. A.; MCELROY, J. F.; ROHRBACH, K. W.; SCHMIDT, W. K.; TAM, S. W. Novel piperidine σ receptor ligands as potential antipsychotic drugs. *J. Med. Chem.*, v.35, p.4344-4361, 1992.

GLENNON, R. A. Sigma receptor ligands and the use thereof. U.S. Patent, v.6, p.057,371, May 2, 2000.

GLENNON, R. A.; FISCHER, J. B. Sigma receptor ligands and the use thereof. U.S. Patent, v.6, p.087,346. July 11, 2000.

GLENNON, R. A., SMITH, J. D., ISMAIEL, A. M., EL-ASHMAWY, M., BATTAGLIA, G., FISCHER, J. B. Identification and exploitation of the sigma opiate pharmacophore. *J. Med. Chem*., v.34, p.1094-1098, 1991a.

GLENNON, R. A.; YOUSIF, M. Y.; ISMAIEL, A. M.; EL-ASHMAWY, M. B.; HERNDON, J. L.; FISCHER, J. B.; SERVER, A. C.; BURKE-HOWIE, K. J. Novel 1-phenylpiperazines and 4-phenylpiperidines as high-affinity sigma ligands. *J. Med. Chem*., v.34, p.3360-3365, 1991b.

GLENNON, R. A., ISMAIEL, A., SMITH, J. D., YOUSIF, M. Y., EL-ASHMAWY, M., HERNDON, J. L., FISCHER, J. B., BURKE HOWIE, K. J., SERVER, A. A. Binding of substituted and conformationally restricted derivatives of N-(3-phenyl-*n*-propyl)- 1-phenyl-2-aminopropane at σ receptors. *J. Med. Chem*., v.34, p.1855-1859, 1991c.

GLENNON, R. A.; ABLORDEPPEY, S. Y.; ISMAIEL, A. M.; EL-ASHMAWY, M. B.; FISCHER, J. B.; AND BURKE-HOWIE, K. Structural features important sigma-1 (σ-$_1$) receptor binding. *J. Med. Chem*., v.37, p.1214-1219, 1994.

GLENNON, R. A.; ISMAIEL, A. M.; ABLORDEPPEY, S.; EL-ASHMAWY, M.; FISHER, J. B. Thioxanthene-derived analogs as σ_1 receptor ligands. *Bioorg. Med. Chem. Lett*., v.14, p-2217-2220, 2004.

GUITART, X.; CODONY, X.; MONROY, X. Sigma receptors: Biology and therapeutic potential. *Psychopharmacology*, v.174, p.301-319, 2004.

ITZHAK, Y. (ed.) *Sigma receptors*. Academic Press, San Diego. 1994.

ITZHAK, Y.; STEIN, I. Sigma binding sites in the brain: An emerging concept for multiple sites and their relevance for psychiatric disorders. *Life Sci*., v.47, p.1073-1081, 1990.

JUNIEN, J. L.; LEONARD, B. E. Drugs acting on σ receptors: A review of their nature, function, and possible therapeutic importance. *Clin. Neuropharmacol*., v.12, p.353-374, 1989.

KAWAMURA, K.; ELSINGA, P. H.; KOBAYASHI, T.; ISHII, S.; WANG, W,-F.; MATSUNA, K.; VAALBURG, W.; ISHIWATA, K. Synthesis and evaluation of ^{11}C- and ^{18}F-labeled 1-[2-(4-alkoxy-3-methoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazines as sigma receptor ligands for positron emission tomography studies. *Nucl. Med. Biol.*, v.30, p.273-284, 2003.

MACH, R. H.; WU, L.; WEST, T.; WHIRRETT, B. R.; CHILDERS, S. R. The analgesic tropane analogue (±)SM-21 has a high affinity for sigma$_2$ receptors. *Life Sci*., v.64, PL131-137, 1999.

MACH, R. H.; HUANG, Y.; FREEMAN, R. A.; WU, L.; VANGVERAVONG, S.; LUEDTKE, R. R. Conformationally-flexible benzamide analogues as dopamine D$_3$ and σ_2 receptor ligands. *Bioorg. Med. Chem. Lett.*, v.14, p.195-202, 2004.

MAIER, C. A.; WUNSCH, B. Novel spiropiperidines as highly potent and subtype selective σ-selective ligands. *J. Med. Chem*., v.45, p.438-448, 2002.

MARRAZZO, A.; PREZZAVENTO, O.; PASQUINUCCI, L.; VITTORIO, F.; RONISVALLE, G. Synthesis and pharmacological evaluation of potent and enantioselective σ_1 and σ_2 ligands. *Farmaco*, v.56, p.181-189, 2001.

MATSUMOTO, R.; POTELLERET, F. H.; MACK, A.; POUW, B.; ZHANG, Y.; BOWEN, W. D. Structure-activity comparison of YZ-069, a novel σ ligand, and four analogs in receptor binding and behavioral studies. *Pharmacol. Biochem. Behav.,* v.77, p.775-781, 2004.

MAY, E. M.; JACOBSON, A. E.; MATTSON, M. V.; TRAYNOR, J. R.; WOODS, J. H.; HARRIS, L. S.; BOWMAN, E. R.; ACETO, M. D. Synthesis and in vitro and in vivo activity of (-)-(1R,5R,9R)- and (+)-(1S,5S,9S)-N-alkenyl, N-alkynyl, and N-cyanoalkyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan homologues. *J. Med. Chem.*, v.43, p.5030-5036, 2000.

MAEDA, D. Y.; WILLIAMS, W.; KIM, W. E.; THATCHER, L. N.; BOWEN, W. D.; COOP, A. N-Arylalkylpiperidines as high-affinity sigma-1 and sigma-2 receptor ligands: Phenylpropylamines as potential leads for selective sigma-2 agents. *Bioorg. Med. Chem. Lett*., v.12, p.497-500, 2002.

MUSACCHIO, J. M.; KLEIN, M.; CANOLL, P. D. Dextromethorphan and sigma ligands: Common site but diverse effects. *Life Sci*., v.45, p.1721-1732, 1989.

PREZZAVENTO, O.; GUALTIERI, F.; MARRAZZO, A.; ROMANELLI, M. N.; RONSISVALLE, G.; TEODORI, E. Sigma receptor binding profile of a series of analgesic tropane derivatives. *Arch. Pharm*., v.335, P.39-43. 2002.

QUIRION, R.; CHICHEPORTICHE, R.; CONTRERAS, P. C.; JOHNSON, K. M.; LODGE, D.; TAM, S. W.; WOODS, J. H.; ZUKIN, S. R. Classification and nomenclature of phencyclidine and sigma sites. *Trends Neurol. Sci*.,v.11, p.444-446, 1987.

SCHERZ, M. W.; FIALEIX, M.; FISCHER, J. B.; REDDY, N. L.; SERVER, A. C.; SONDERS, M. S.; TESTER, B. C.; WEBER, E.; WONG, S. T.; KEANA, J. F. W. Synthesis and structure-activity relationships of N,N-di-o-tolylguanidine analogues: High-affinity ligands for the haloperidol-sensitive σ receptor. *J. Med. Chem*., v.33, p.2421-2429, 1990.

SNYDER, S. H.; LARGENT, B. L. Receptor mechanisms in antipsychotic drug action: Focus on sigma receptors. *J. Neuropsychiat*., v.1, p.7-15, 1989.

WALKER, J. M.; BOWEN, W. D.; WALKER, F. O.; MATSUMOTO, R. R.; DECOSTA, B.; RICE, K. C. Sigma receptors: Biology and function. *Pharmacol. Rev*., v.42, p.355-404, 1990.

Recebido para publicação em 27 de janeiro de 2005.
Aceito para publicação em 02 de fevereiro de 2005.