# Pharmacophore Identification for Sigma-1 ($\sigma_1$) Receptor Binding: Application of the "*Deconstruction – Reconstruction – Elaboration*" Approach

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**Abstract**: At least two different types of sigma σ ($\sigma_1$ and $\sigma_2$) receptors have been identified. A structural feature common to *high-affinity* (Ki <10 nM) $\sigma_1$ ligands is: C-N(R)-X-Ph; both C and Ph are associated with regions of bulk tolerance. Numerous other ligands bind, but typically do so with lower affinity.

**Keywords:** Sigma Receptors, arylalkylamines, arylpentylamines piperazines, piperidines, pharmacophore.

## INTRODUCTION

Sigma (σ) receptors were first proposed in the mid 1970s and, due to the binding of certain benzomorphans such as *N*-allylnormetazocine (NANM; SKF 10,047; **1**) and pentazocine, were initially thought to represent a type of opioid receptor. As might be expected, this spurred early interest in this receptor population. Nevertheless, very little was reported on sigma receptors until the mid 1980s. It gradually became evident that various non-opioids bind at these receptors and on the basis of additional binding and other pharmacological studies, serious doubt was cast on their membership in the opioid receptor family. Upon finding that some benzomorphans bind at phencyclidine (PCP) binding sites, these receptors were briefly termed σ/PCP receptors. Because PCP is a drug of abuse (whose mechanism of action was not fully understood at that time), a second wave of interest in these receptors was fueled by their possible involvement in drug abuse. Due to differences in brain localization, and because of affinity differences in ligand binding at σ *versus* PCP sites (for example, haloperidol binds at certain of these sites but not others – leading to concepts such as "haloperidol-sensitive σ sites"), it became apparent that σ binding sites and PCP binding sites were not identical entities. Even with advances in the characterization of σ receptors, pharmacological studies were hampered by a lack of selective agents. For example, NANM (**1**) (with reported Ki values ranging from about 100 nM to 1,000 nM depending on the radioligand and tissue source used), perhaps considered a prototypical σ ligand, binds only with modest affinity at σ receptors and also binds at other opioid receptors, whereas haloperidol binds with higher affinity at dopamine $D_2$ receptors than at σ receptors. Identification of several, somewhat more selective, agents led to the final realization that σ sites and PCP sites are distinct; one agent in particular, ditolylguanidine (DTG; **2**), was able to differentiate these sites and is still employed today as a σ ligand (and its tritiated version is still used as a radioligand) [1]. In addition to the lack of selective agents, further confusion with receptor characterization was likely associated with the use by various laboratories of different radioligands to label σ receptors (e.g. tritiated analogs of pentazocine, NANM, haloperidol, PCP, DTG), and with the low (typically micromolar) affinity of some of the more selective early ligands. Nevertheless, it was quickly demonstrated that ligands of very diverse structure displayed affinity for σ receptors; literally hundreds of structure-types were shown to bind at σ receptors, but here too, the agents were nonselective and generally displayed only modest (i.e., high nanomolar to low micromolar) affinity. Interest in σ receptors continued with suggestions that they might represent a novel mechanism of action for antipsychotic agents, that they might be involved in drug abuse, that they might be related to metabolizing (e.g. cytochrome P450) enzymes, and that they might constitute a steroid receptor. For basic information on early investigations with σ receptors and σ receptor ligands, the reader is referred to several reviews [2-10].

At first, σ receptors were thought to represent a homogenous population of receptors; but, in 1990 it was found that at least two major populations of σ receptors exist: $\sigma_1$ and $\sigma_2$ [reviewed: 11]. These two receptor populations differed in their tissue distribution and subcellular localization. The benzomorphan (+)pentazocine displayed several hundred-fold selectivity for the former whereas DTG bound nearly equally well at both populations. The $\sigma_1$ receptor has been recently cloned from several sources including human brain; $\sigma_2$ receptors have yet to be cloned [reviewed: 12]. Recent reviews [12,13] describe the potential involvement of σ receptors in schizophrenia, movement disorders, depression, anxiety, drug abuse, pain, and inflammation (Crohn's disease, rheumatoid arthritis). Sigma-1 ($\sigma_1$) knockout mice have been generated and investigations with such animals should shed further light on the possible pharmacological relevance of this receptor population [15].

## IDENTIFICATION OF A $\sigma_1$ RECEPTOR PHARMACOPHORE

One of the long term goals of our work in this area was/is the development of high-affinity σ-selective ligands. As the foundation for such an endeavor, we undertook an investigation to identify a pharmacophore for the binding of ligands at σ receptors. As our studies were begun prior to

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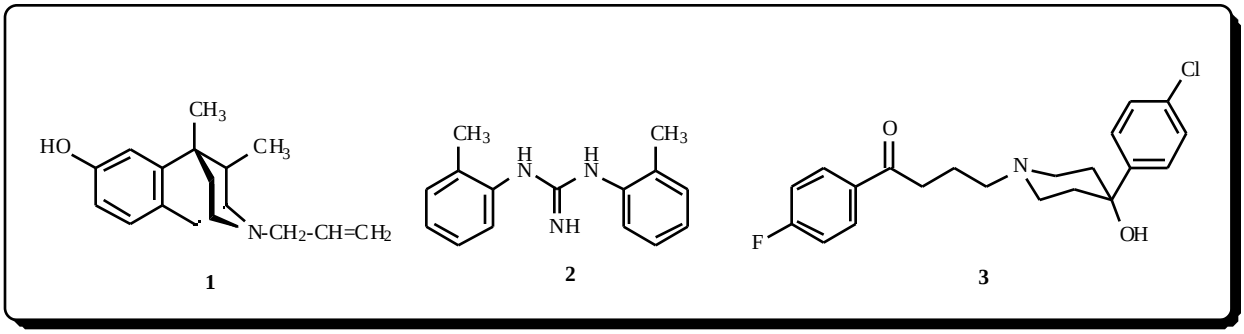


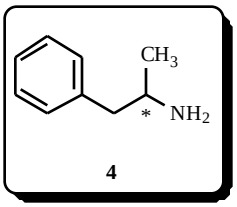

**1** **2** **3**

the discovery of $\sigma_1$ and $\sigma_2$ receptors, our conclusions required modification once $\sigma_1$ receptors were described. It might be noted that the discovery of $\sigma_1$ receptors necessitated a re-evaluation of some initial findings; although most of our structure-affinity conclusions for σ *versus* $\sigma_1$ binding did not differ substantially from a qualitative perspective, this review describes our results in the context of the newer $\sigma_1$ binding data and, unless otherwise noted, Ki values represent binding to $\sigma_1$ sites. That is, this review is based on a series of articles we published over a period of about 15 or so years [e.g. 14-29], however, in some instances, the published compounds were subsequently re-evaluated at $\sigma_1$ receptors. In certain instances then, the literature references provided below describe the compounds, their physicochemical properties, and their σ binding affinities. It was only later that $\sigma_1$ binding data were obtained on some of the earlier compounds; these $\sigma_1$ data can be found in the patent literature [28,29]. This review brings together much of the $\sigma_1$ binding data for the first time. Other groups of investigators also pursued development of novel σ-selective agents. However, because our investigations were aimed at pharmacophore development from their very outset, we take this opportunity to describe our efforts in this area. Some results obtained by others will be mentioned where pertinent.

As already alluded to, our work began in the mid 1980s. At the time, fewer than three dozen papers had been published on σ receptors. Where does one begin when the number of known σ ligands is limited? One approach frequently employed in our laboratories to investigate novel receptor populations is the "*deconstruction – reconstruction – elaboration*" approach. That is, a ligand that binds at a particular receptor population of interest is identified, pendant substituents are stripped from the ligand, and the ligand is then reconstructed by re-introducing its various *original* structural features to determine how each contributes to binding and/or selectivity. Once this process has been completed, the resultant novel structure-type is elaborated by introducing/exploring *novel* structural features to validate any structure-affinity hypotheses that might have been generated in the course of the investigation. This systematic approach has proven highly effective in several cases, and is described here for $\sigma_1$ ligands.

What was known by the mid 1980s is that benzomorphans bind at σ receptors with modest affinity, and that one of the highest affinity ligands available at the time was haloperidol. We found that the benzomorphan (±)NANM binds with modest affinity (**1**; Ki = 430 nM), that (+)NANM (Ki = 150 nM) binds with higher affinity than (−)NANM (Ki = 3,640 nM), and that haloperidol (**3**; Ki = 1 nM) binds with significantly higher affinity. We began by deconstructing the structure of NANM; later, we tackled the structure of haloperidol. All of our initial investigations employed [$^3$H]DTG as radioligand. During the course of our studies, $\sigma_1$ and $\sigma_2$ receptors were identified; hence, subsequent studies employed conditions found by others to more accurately reflect binding at each of these two sites (i.e., the more selective [$^3$H](+)pentazocine was used to label $\sigma_1$ receptors, and the nonselective [$^3$H]DTG in the presence of cold (+)pentazocine was used to label $\sigma_2$ receptors in guinea pig brain homogenates).

The structure of NANM (**1**) can be conveniently simplified (i.e., "*deconstructed*") to an arylalkylamine; it can also be simplified to a 4-(phenyl)piperidine (Fig. **1**). In the latter instance, due to the loss of conformational constraint, the phenylpiperidine will likely exist primarily as the equatorial-phenyl conformer (Fig. **1**). Racemic phenylisopropylamine (**4**) (i.e., amphetamine; Ki = 46,000 nM) binds with low affinity at σ receptors. The presence of the NANM hydroxyl group had relatively little effect on binding, as did stereochemistry about the α-methyl group [13]. During the elaboration step, other aryl substituents were explored but, generally, had little influence on affinity. Conformationally-constrained analogs were also examined [16]. The chief structural difference among the benzomorphans is their amine substituent, so attention was focused on this position. Modification of the arylalkylamine amine substituent had a significant impact on binding. As shown in Table **1** [14,16,17], increasing the length/bulk of the amine substituent resulted in a gradual enhancement of affinity such that the *S*(+)N-(5-phenyl)pentyl analog **11*S*** (σ Ki = 6.3 nM) displayed >7,000-fold greater affinity than primary amine **4**.




**4**

One of the first high-affinity σ ligands to be identified, and studied pharmacologically, was *R*(−)PPAP (**9*R***; σ Ki = 28 nM) [14]. This compound was later found to bind with similar affinity at $\sigma_1$ sites (Ki = 11 nM) [26] and consequently served as the basis for an extensive examination of the effect of aryl substituents on $\sigma_1$ affinity.

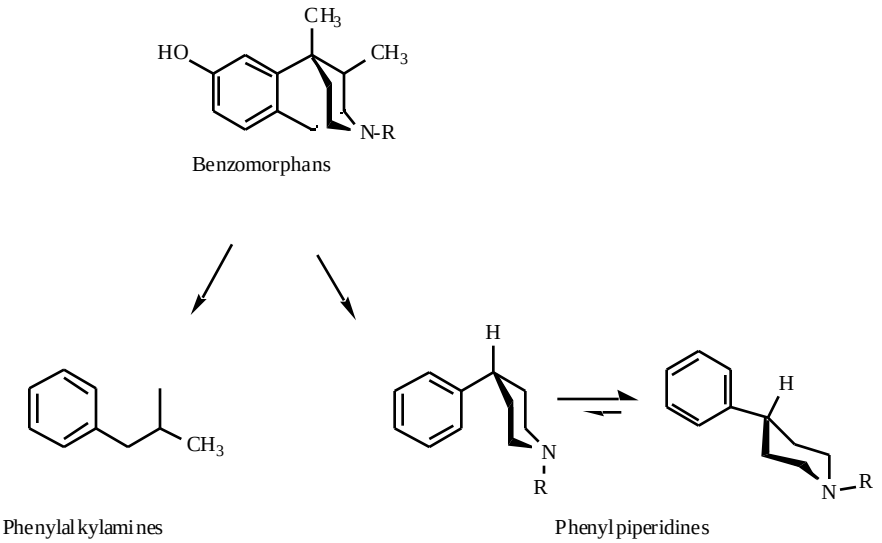
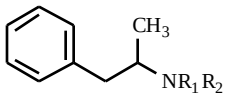



**Fig. (1).** Benzomorphans can be deconstructed to arylalkylamines (e.g. phenylalkylamines) and phenylpiperidines. Opening of the benzomorphan nucleus to a phenylpiperidine should result in the conformationally more stable equatorial phenylpiperidine as shown on the far right.

Representative results (Table **2**) showed that aryl substituents had little influence on receptor affinity [21,26].

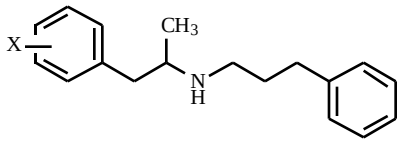
**Table 1. Binding of Simple N-Substituted Phenylisopropylamines at Sigma (σ) Receptors**




| | $R_1$ | $R_2$ | Stereochemistry | σ Ki (nM) |
|---|---|---|---|---|
| **4** | H | H | (±) | 46,000 |
| **5(*R*)** | H | Me | R(-) | 8,320 |
| **6(*R*)** | H | Et | R(-) | 660 |
| **7(*R*)** | H | $CH_2Ph$ | R(-) | 117 |
| **8(*R*)** | H | $CH_2CH_2Ph$ | R(-) | 60 |
| **8(*S*)** | | | S(+) | 30 |
| **9(*R*)** | H | $(CH_2)_3Ph$ | R(-) | 28 |
| **9(*S*)** | | | S(+) | 22 |
| **10(*S*)** | H | $(CH_2)_4Ph$ | S(+) | 6.6 |
| **11(*S*)** | H | $(CH_2)_5Ph$ | S(+) | 6.3 |
| **12(*S*)** | Me | $(CH_2)_5Ph$ | S(+) | 2.6 |

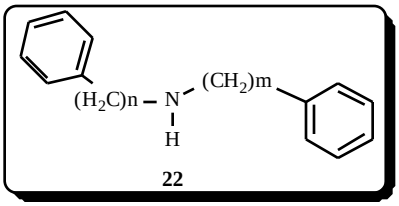
Preliminary indications (comparing **9** with **10** and **11**, Table **1**) [14,17] were that chain extension resulted in enhanced affinity. This led to a more systematic investigation and some results are shown in Table **3**. In general, the *R*(−)isomers displayed several-fold higher affinity than their *S*(+)enantiomers. This was not the case for compound **8** and slight affinity reversal was attributed to its nearly symmetrical nature indicating that it might bind in a reversed fashion at the receptors. The difference in the affinity of *S*(+)**11** for σ sites (Ki = 6.3 nM; Table **1**) versus $\sigma_1$ sites (Ki = 0.9 nM; Table **3**) show the discrepancies that can occur when comparing σ with $\sigma_1$ binding data.

**Table 2. Binding of Aryl-Substituted Phenylisopropylamines at Sigma-1 ($\sigma_1$) Receptors**



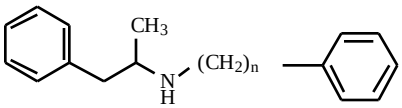

| | X | Stereochemistry | σ Ki (nM) |
|---|---|---|---|
| **9(*R*)** | H | R(-) | 11 |
| **13** | 3-$CF_3$ | (±) | 9 |
| **14** | 3-Br | (±) | 10 |
| **15** | 4-Br | (±) | 12 |
| **16(*R*)** | 4-I | R(-) | 18 |
| **17** | 4-OH | (±) | 26 |

A series of compounds **22** was prepared where the length of one or both alkyl chains was varied (Table **4**). A phenylpentyl chain was found optimal and some representative results are provided in Table **4**. It was found that, as long as one of the alkyl chains of **22** was five methylene groups in length (i.e., **22**, m = 5), the length of the second chain was not particularly important.




**22**

**Table 3. Investigation of Chain Length on Sigma-1 ($\sigma_1$) Receptor Binding**

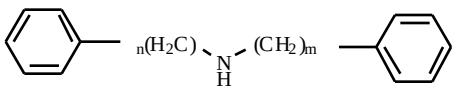


| | n | Stereochemistry | σ Ki (nM) |
|---|---|---|---|
| **8(*R*)** | 2 | R(-) | 44 |
| **8(*S*)** | 2 | S(+) | 15 |
| **9(*R*)** | 3 | R(-) | 11 |
| **9(*S*)** | 3 | S(+) | 39 |
| **10(*R*)** | 4 | R(-) | 7.4 |
| **10(*S*)** | 4 | S(+) | 19 |
| **11(*R*)** | 5 | R(-) | 0.5 |
| **11(*S*)** | 5 | S(+) | 0.9 |

Studies up to this point had been focused on compounds with two aryl groups. Are both groups necessary? Compounds **26** (Ki = 0.38 nM) and **27** (Ki = 0.25 nM) bind with high affinity [26]. Removal of one of the phenyl groups (i.e., **28**; Ki = 0.29 nM) had no effect on affinity whereas removal of the pentyl phenyl group (i.e., **29**; Ki = 48 nM) decreased affinity by about 100-fold. That the *n*-propyl substituent of **28** makes an actual contribution to binding is evidenced by the diminished affinity of **30** (Ki = 14 nM) and **31** (Ki = 418 nM) [22].

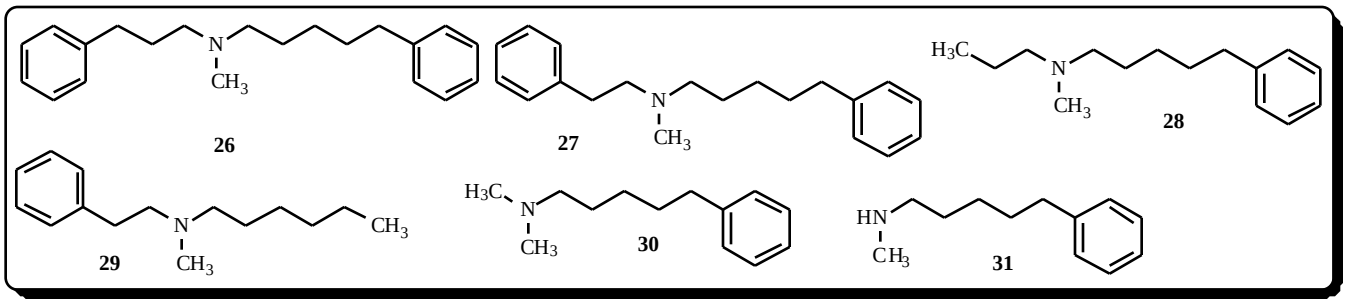
= 0.8 nM) binds with high affinity, and with an affinity comparable to its aryl counterpart (**36**, Ki = 0.2 nM) showing that the Phenyl-B ring is not essential for binding [25]. Compound **33** (Ki = 0.3 nM) binds with an affinity significantly higher than that of its aryl counterpart **30** (Ki = 14 nM). The reduced affinity of **34** (Ki = 6.8 nM) and **35** (Ki = 190 nM) again show the importance of a tertiary amine.

**Table 4. Investigation of Phenylalkylamine Chain Length on $\sigma_1$ Receptor Binding**



| | n | m | σ Ki (nM) |
|---|---|---|---|
| **18** | 2 | 2 | 11.4 |
| **19** | 2 | 3 | 11.3 |
| **20** | 2 | 4 | 2.6 |
| **21** | 2 | 5 | 0.20 |
| **23** | 1 | 5 | 0.21 |
| **24** | 3 | 5 | 0.28 |
| **25** | 4 | 5 | 0.48 |

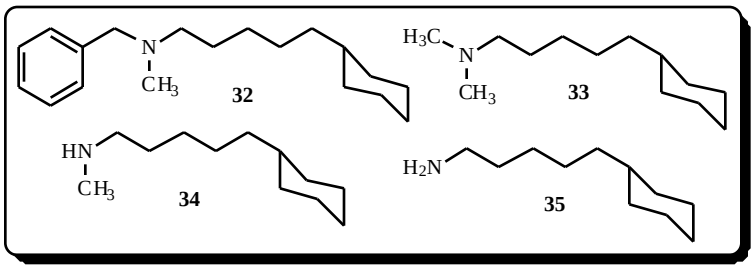
Other comparisons can be made; but, because they involve σ rather than $\sigma_1$ binding data the results cannot be compared quantitatively to the above $\sigma_1$ affinities – they do, nevertheless, allow qualitative comparisons and support the

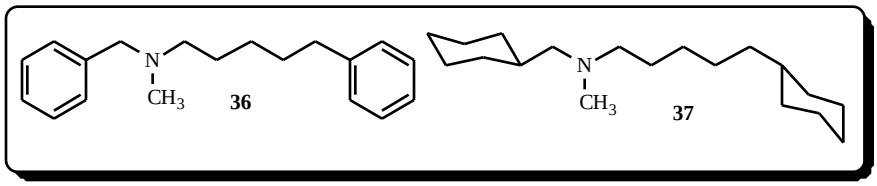



A convention was adopted whereby the benzomorphan-derived phenyl group was referred to as the Phenyl-A group, and the distal phenyl (e.g., that of the phenylpentylamine) as Phenyl-B. The above studies showed that the Phenyl-A ring is not required for high affinity binding at $\sigma_1$ receptors, and that it probably plays a less important role than Phenyl-B. Is the Phenyl-B ring required for binding? In fact, Phenyl-B can be replaced by a cyclohexyl moiety. Compound **32** (Ki

above mentioned concept that an aryl moiety is not required for high affinity binding. For example, a comparison of **36** (σ Ki = 1.0 nM) with **37** (σ Ki = 1.6 nM) shows that two aromatic moieties are not required for binding [25].

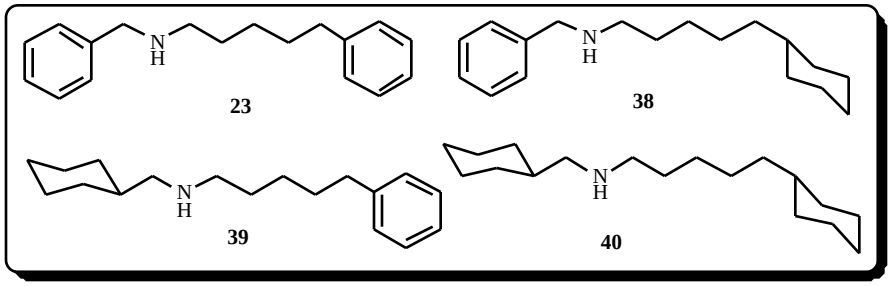
Furthermore, replacement of either phenyl group of **23** (σ Ki = 2.0 nM) with a cyclohexyl group (**38** and **39**, σ Ki = 1.2 and 3.4 nM, respectively), and replacement of both phenyl groups with cyclohexyl groups (**40**, σ Ki = 1.2 nM),






have little impact on binding affinity [25]. Nonetheless, this still requires substantiation by examination of these compounds at $\sigma_1$ sites.

The next question addressed was whether or not the amine function is actually required for binding. Several steroids had been reported to bind at $\sigma$ receptors suggesting




The results of additional structure affinity studies indicated that optimal affinity was associated with *a)* a basic amine separated from a phenyl group (or some other hydrophobic moiety [24]) by a four- to six-atom chain (with five seemingly being optimal), *b)* a second amine substituent of at least one carbon atom (with three being optimal), and *c)* a third amine substituent which could be H or a small alkyl group. Pyrrolidine **41** (Ki = 0.76 nM) provided supporting evidence that the Phenyl-A ring is not required for binding, and **42** (Ki = 1.0 nM) showed that a certain amount of bulk is tolerated in the vicinity of the amine [26]. Aminotetralin **43** (Ki = 0.6 nM) was of interest because its structure approaches that of the benzomorphans such as NANM (**1**), and yet it binds with enhanced affinity [22].

that a nitrogen atom was not necessary (although it might be noted that steroids typically display only micromolar affinity for these receptors). Complete removal of the nitrogen atom from an arylpentylamine would likely result in a water insoluble compound. Hence, a location was sought where a solubilizing amine substituent might be tolerated. Compound **44** (Ki = 35 nM) was found to bind with modest affinity; however, replacement of the more basic amine by a methylene group (**45**; Ki >36,000 nM) decreased affinity by >1,000-fold [20]. Evidently, the arylpentylamine nitrogen atom is necessary for binding at $\sigma_1$ receptors, and affinity is diminished if the amine is not in the proper location.

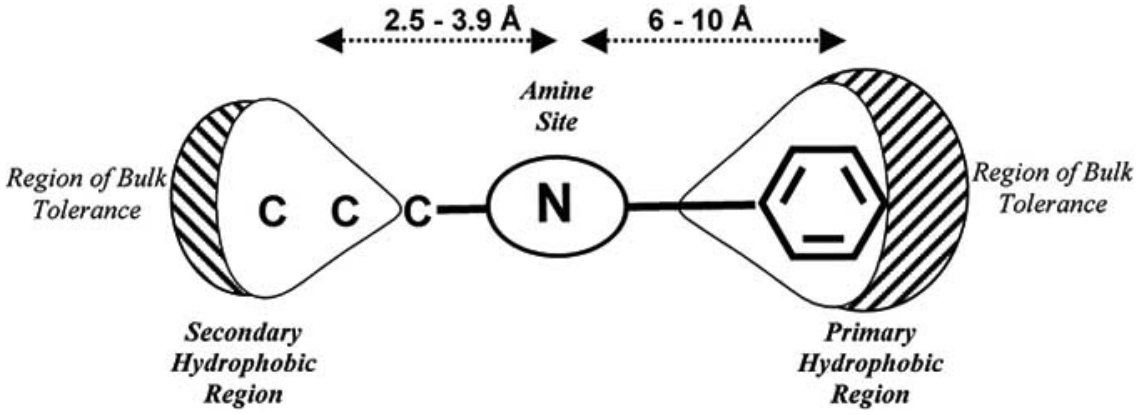
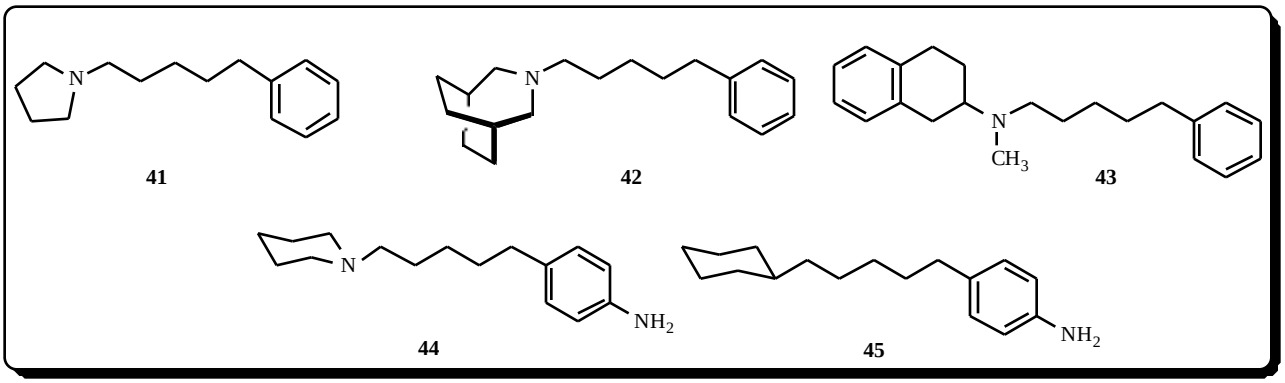
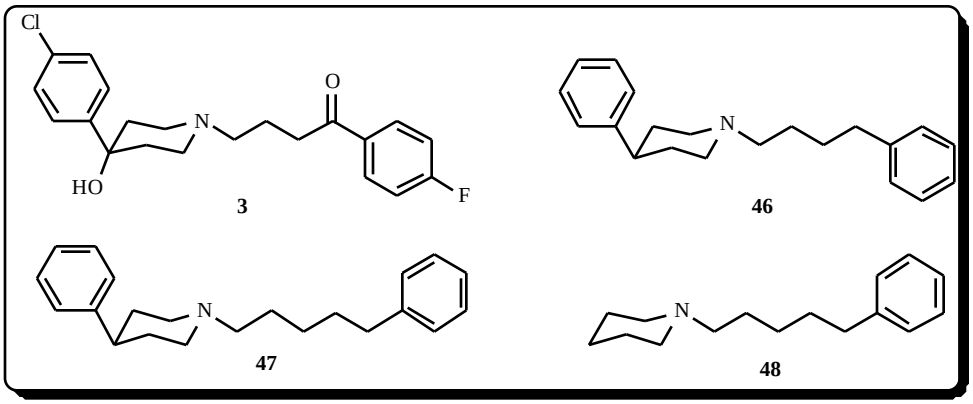



**Fig. (2).** General structural features found optimal for $\sigma_1$ receptor binding. The binding site consists of an amine site flanked by two hydrophobic regions. The primary hydrophobic region is situated such that a 5-(phenyl)pentyl or 5-(cyclohexyl)pentyl moiety is optimally accommodated. The pentyl group may bear a polar substituent (i.e., it need not be a simple alkyl chain) but in these cases affinity is somewhat reduced depending upon the nature and position of this substituent. The secondary hydrophobic site is probably somewhat smaller than the primary site and optimally accommodates a three-atom chain. Both hydrophobic sites are associated with regions of bulk tolerance such that additional bulk can be added but typically fails to enhance affinity. The amine can be secondary or tertiary; however, if a third substituent is present, relatively small substituents are optimal. The amine moiety can also be embedded in a cyclic structure such as a pyrrolidine, piperidine, or piperazine ring. When the amine is part of a piperazine ring, the possibility exists that either nitrogen atom can bind at the amine site and, consequently, the structural requirements for the binding of piperazine derivatives are not necessarily identical to the requirements for their corresponding piperidine counterparts.



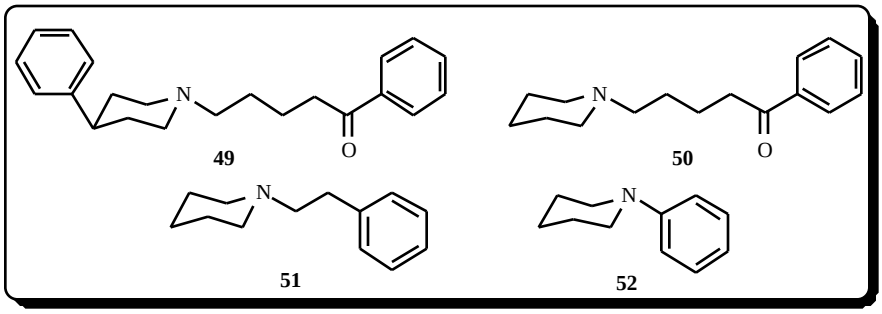

The overall structure-affinity findings emanating from investigations of benzomorphan-derived arylalkylamines are summarized in (Fig. **2**). Next investigated was the phenylpiperidine portion of the benzomorphans (Fig. **1**). Although the phenylalkylamines were the initial structure-types examined, the phenylpiperidine project ran almost concurrently, with our first study being published in 1991 [16]. Simple inspection of the haloperidol (**3**) structure shows that it, too, possesses a phenylpiperidine moiety. Furthermore, haloperidol also possesses a second aromatic moiety situated at the end of a four-atom chain. Hence, rather than investigating simple phenylpiperidines, we immediately applied the "*deconstruction – reconstruction – elaboration*" approach to haloperidol. Pendent substituents of haloperidol (**3**; Ki = 1.0 nM) were removed to afford the structurally simpler **46** (Ki = 1.0 nM); compound **46** displayed an affinity identical to haloperidol. Because **46** is a phenylbutylamine, extension to a phenylpentylamine should result in enhanced affinity. Indeed, compound **47** (Ki = 0.15 nM) displayed improved affinity [16,21,23]. As shown above, two aryl groups are not required for the binding of arylpentylamines; hence, it should be possible to remove the piperidine phenyl group without detriment to affinity. Here too, the results were as expected; that is, compound **48** (Ki = 0.38 nM) essentially retained the affinity of **47**.

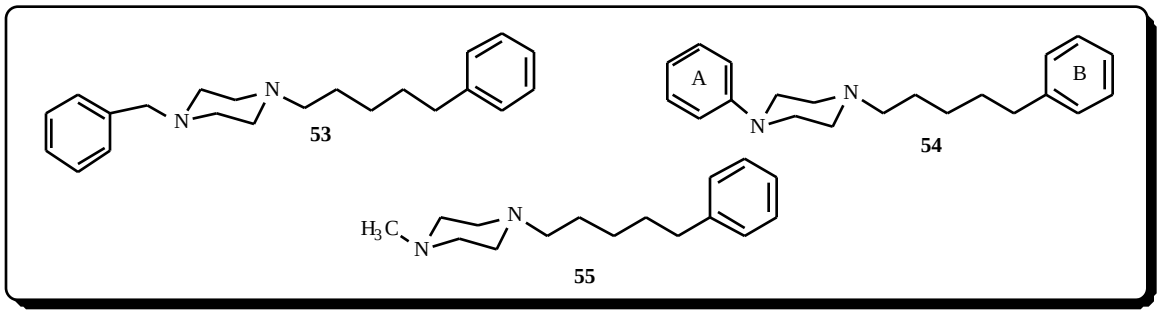
Re-incorporation of the haloperidol carbonyl group to provide valerophenone **49** showed that the C=O group makes no contribution to $\sigma_1$ binding (**49**; Ki = 0.12 nM) relative to **47** (Ki = 0.15 nM) [20]. And here too, the piperidine phenyl group could be removed with no change in affinity (**50**; Ki = 0.18 nM) [20].




At this point, we once again addressed the importance of the five-membered chain. The chain length of **48** (Ki = 0.38 nM) was shortened, and compounds such as **51** (Ki = 49 nM) and **52** (Ki = 24,000 nM) displayed >100-fold reduced affinity [23].

Subsequent studies showed that the piperidine phenyl group of **47**-type compounds could be replaced by a benzyl group. In addition, the piperidine ring could be replaced by a piperazine ring, generally with retention of affinity [16]. For example, **53** (Ki = 0.2 nM) binds with an affinity






comparable to **47** [22]. Also, it was demonstrated, depending upon alkyl chain length, that removal of the Phenyl-A ring (i.e., replacement of the phenyl group by a methyl group) of **54** was less detrimental to binding than removal of the Phenyl-B ring. These findings are consistent with the aforementioned observation that two aryl groups are not required for binding. For example, compound **55** (Ki = 1.4 nM) retains, albeit slightly reduced, high affinity for the receptors.

affinity comparable to **55**, and that the affinity of **57** should be enhanced because the latter can interact with the major hydrophobic site while at the same time removing the amine from association with the secondary hydrophobic site. The affinity of **56** (Ki = 1.3 nM) was found to be essentially identical to that of **55**, whereas the affinity of **57** (Ki = 0.07 nM) was 20-fold enhanced [20,27].

There have been a number of suggestions that sigma receptors might represent a novel antiphyschotic mechanism

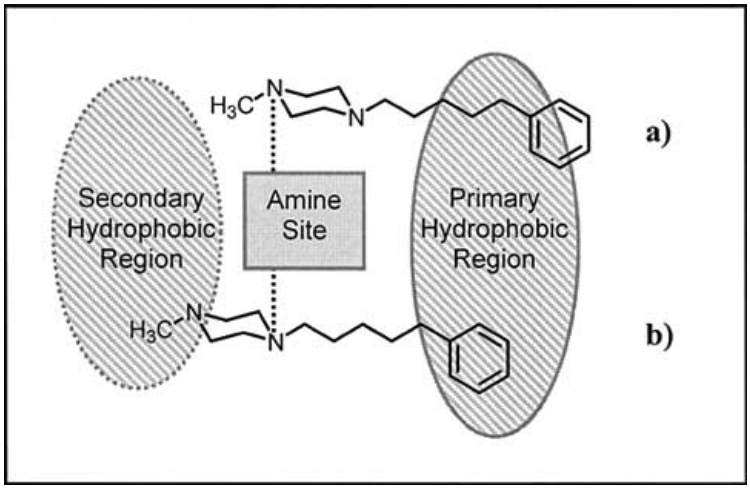
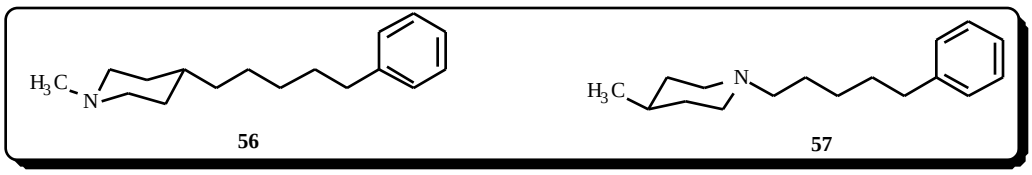



**Fig. (3).** Two possible modes of interaction of **55** with $\sigma_1$ receptors. Either of the piperazine amine functions might interact with the amine binding site (see text for extended discussion).

Alkyl chain length seemingly plays a somewhat more important role in the binding of the piperazine derivatives than in the binding of their piperidine counterparts. This might be explained relative to the proposed model shown in (Fig. **2**) [20]. Either of the two amines can conceivably interact at the amine binding site (shown for **55** in Fig. **3**)). If compound **55** binds in such a manner so as to avoid projecting one of the amine nitrogen atoms into a hydrophobic region, the aryl moiety might not optimally utilize the major hydrophobic binding area (Fig. **3a**); in contrast, if **55** optimizes its interaction with the major hydrophobic site, one of the basic amine groups is thrust into the secondary hydrophobic site (Fig. **3b**). If this is the case, we reasoned that compound **56** should bind with an

because antipsychotic agents such as haloperidol, certain phenothiazines, and certain thioxanthenes bind with high to modest affinity [e.g. 9,30]. Thioxanthenes such as **58** (Fig. **4**) possess an embedded arylalkylamine moiety. Deconstruction of **58** led to a pair of geometric isomers (**59** and **60**; Ki = 30 nM) which displayed equivalent affinity for $\sigma_1$ receptors [27]. This finding is rather unusual and suggests that both olefinic phenyl groups are accommodated by the receptor equally well. Reduction of the double bond of **59**/**60** led to **61** (Ki = 20 nM). Also, using the same rationale employed in the design of **57**, one of the piperazine nitrogen atoms was replaced by a methylene group (i.e. **62**; Ki = 0.7 nM) to result in a high-affinity compound [27].



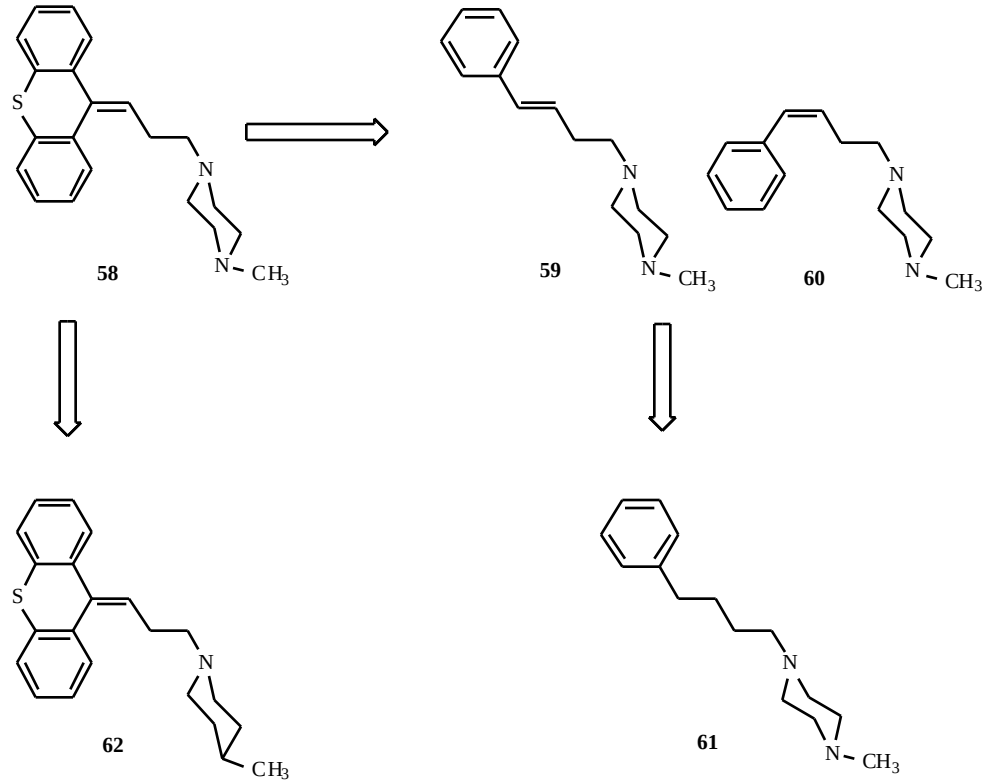


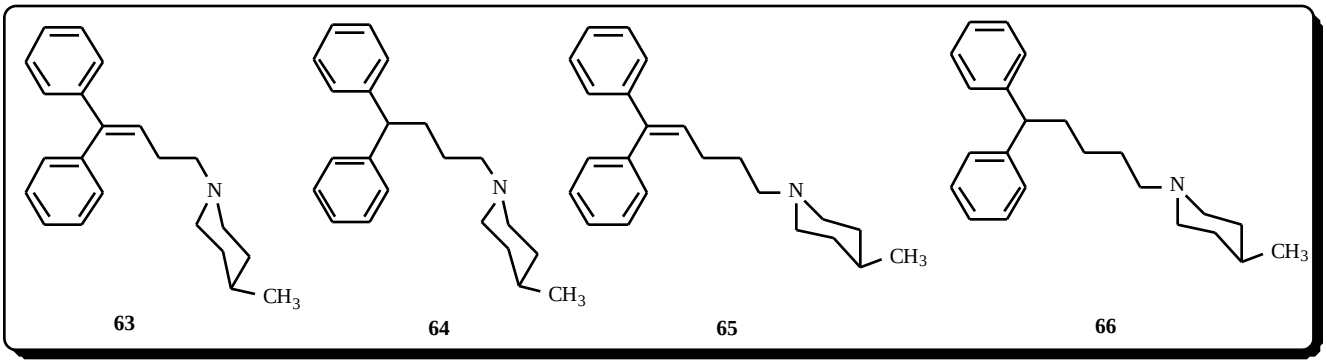

**Fig. (4).** Deconstruction of the thioxanthenes.

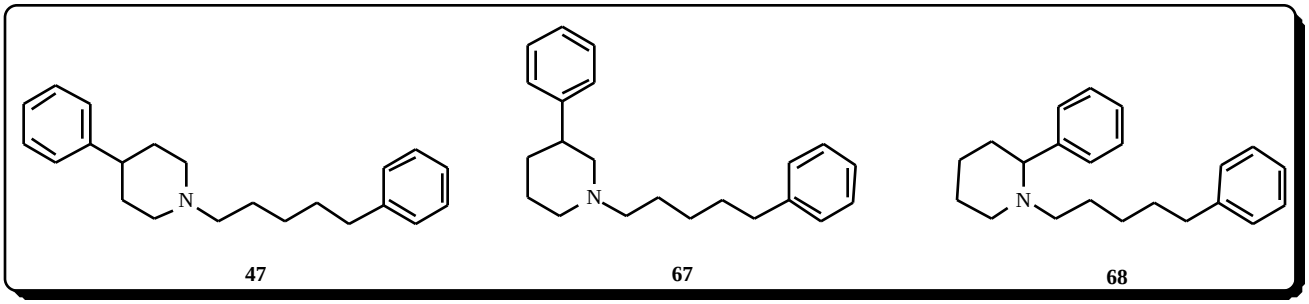
Deletion of the sulfur atom of **62** (i.e., **63**; Ki = 1.9 nM) had little consequence on affinity, as did reduction of the double bond (i.e., **64**; Ki = 1.0 nM). Being phenylbutylamines, it was surmised that chain extension of **63** and **64** to their phenylpentylamine counterparts should result in enhanced affinity. Compound **65** (Ki = 0.13 nM) displayed >10 times the affinity of **63** [27]. Likewise, compound **66** (Ki = 0.09 nM) binds with 10-fold enhanced affinity relative to **64**. Because the affinity of **66** is similar to that of **57** (Ki = 0.07 nM), it would seem that $\sigma_1$ receptors have a region of bulk tolerance associated with the Phenyl-B ring binding region (see structure **54** and (Fig. **2**).

At this time it is not known with certainty whether a sigma mechanism plays any role in the action of classical antipsychotic agents. However, it seems that some antipsychotic agents might bind at $\sigma_1$ receptors because they approximate the model shown in (Fig. **2**); moreover, chain extension (as with haloperidol and the thioxanthene-derived agents) further enhances their affinity.

These latter studies support the concept of bulk tolerance associated with the Phenyl-B region. Evidence for a region of bulk tolerance associated with the Phenyl-A region was presented above. In order to further probe the Phenyl-A region, several compounds were evaluated, including **67** and **68** [22]. Both of these compounds (**67**, Ki = 0.14 nM; **68**, Ki = 0.17 nM) displayed an affinity comparable to that of **47** (Ki = 0.15 nM). In fact, all three compounds displayed affinities similar to that of the *des*-phenyl compound **48** (Ki = 0.38 nM).

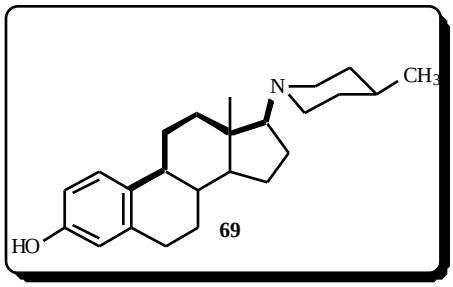
Steroids have long been known to bind at $\sigma$ sites [9] leading to early speculation that $\sigma$ receptors might represent steroid receptors, or the steroid binding site on an enzyme. Although steroids typically bind only with micromolar affinity, it is possible that their $C_{17}$ substituent interacts with the $\sigma_1$ receptor amine site (although, alternatively, they might bind in an altogether different manner). To test this hypothesis, and because these steroids possess an embedded arylpentyl moiety, compound **69** was prepared for






**47** **67** **68**

evaluation. Although compound **69** (Ki = 60 nM) binds at $\sigma_1$ receptors with lower affinity than many of the arylpentylamines described above, it binds with higher affinity than any other known steroid. Its low affinity, considering it is an arylpentylamine, might be a consequence of the added bulk, or the severe conformational constraint imposed by the cyclopentanoperhydrophenanthrene ring.

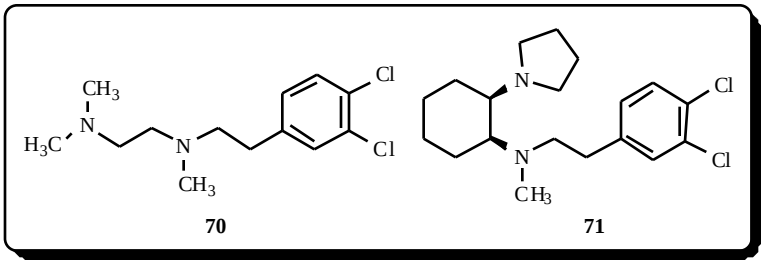



**69**

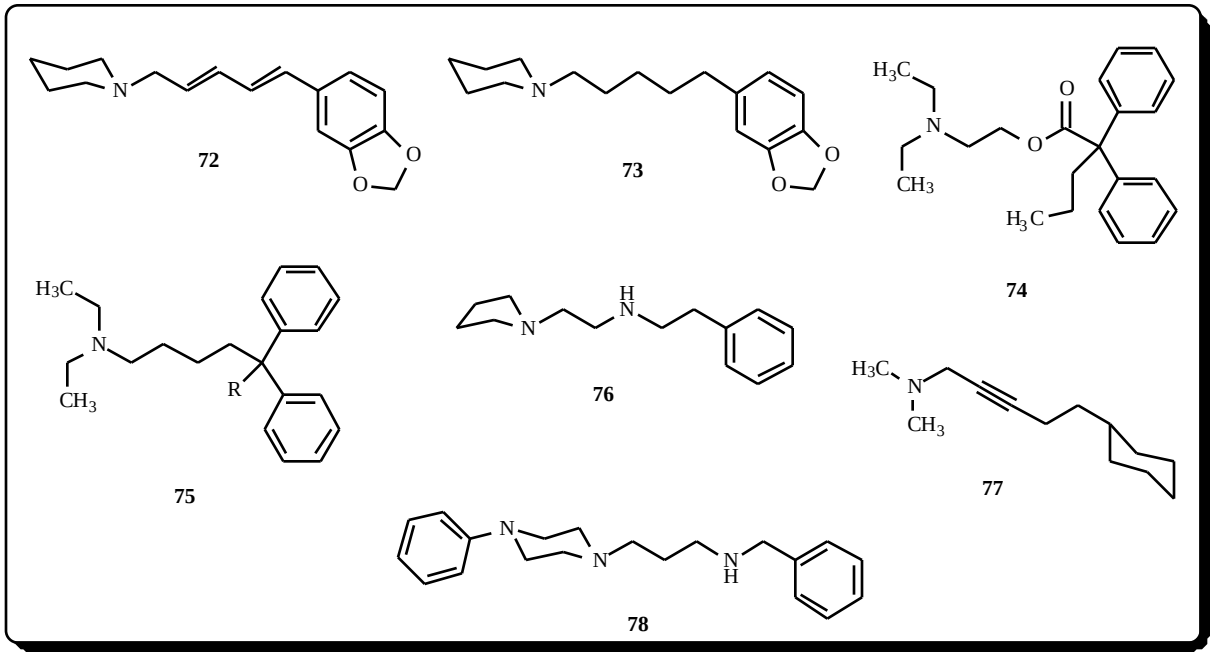
Another issue to be considered is whether or not $\sigma_1$ agonists and antagonists bind in a similar fashion at the receptors. Due primarily to the lack of functional data on most sigma ligands, this question remains unanswered at present. However, BD-737 (**70**; Ki = 2 nM) has been reported to be a $\sigma$ agonist, and BD-1047 (**71**; Ki = 86 nM) an antagonist [reviewed: 12]. First, it is worth noting that both agents possess aryl groups separated from a basic tertiary amine by a five-atom chain (e.g. compare with **41**). Though it is not known how these two agents bind relative to one another, their structural similarity argues that they might be binding in a similar manner. In fact, the only structural difference between these compounds rests with substitution adjacent to the amine function. Given the structural similarity of **70** and **71**, this region of the molecules should prove rich for further exploration of functional activity.

Several review articles have described the promiscuity of sigma ligands. It is probably not surprising, given the model shown as (Fig. **2**), particularly with its two regions of bulk tolerance, that a large variety of agents might be accommodated. This is especially true when it is considered that many of the agents bind in the low micromolar to mid nanomolar range. Nevertheless, those agents displaying high nanomolar affinity typically possess an aryl moiety separated from a terminal amine by a four- to six-atom chain, with five atoms being the most common. We and others have shown that the chain need not be a methylene chain, and that the chain can contain heteroatoms, unstauration, branching, and/or be part of a ring system. Some examples are provided here; others will be mentioned later. For example, diamine **70** binds with high affinity, and diene **72 (**Ki = 0.86 nM) binds with an affinity comparable to that of **48** and **50**, and only 3-fold lower than that of its saturated counterpart **73** (Ki = 0.32 nM) [22]. Even ester **74** (Ki = 17 nM) binds with high affinity, but with an affinity lower than that of **75a** (**75**, R = H; Ki = 1.4 nM) [27], suggesting that the presence of polar substituents in the chain (or the *n*-propyl substituent), while tolerated, might have a small affinity detracting influence. Indeed, **75b** (**75**, R = OH; Ki = 14 nM) with a polar hydroxyl group binds with lower affinity than **75a** [27]. Compound **76** (Ki = 83 nM) is similar in structure to **71** (Ki = 86 nM) and binds with similar affinity [22]; however, it might be the polar NH group that accounts for its 100-fold reduced affinity relative to **41** (Ki = 0.76 nM). Pentyne **77** (Ki = 9.1 nM) binds with 30-fold lower affinity than its pentyl analog **33** (Ki = 0.3 nM) suggesting perhaps that conformation might be important as well. Compound **78** (Ki = 2 nM) represents another variation of the same theme whereby a basic amine is separated from an aryl group by a 5-membered non-aliphatic chain. Thus, what seems to be a common denominator among *high-affinity* $\sigma_1$ ligands is: **C-N(R)-X-Ph** where **C** is a short alkyl (cyclic or noncyclic) chain with three atoms being optimal, **R** is a small alkyl group (typically methyl), **X** is a chain of preferably about five atoms, and **Ph** is either a phenyl ring or some other hydrophobic group. Both **C** and **Ph** are associated with regions of bulk tolerance.

A two-dimensional model for overall $\sigma$ binding, similar to that shown as (Fig. **2**), was first developed in our laboratories in the late 1980s. The model was modified using $\sigma_1$ binding data in the 1990s to accommodate the



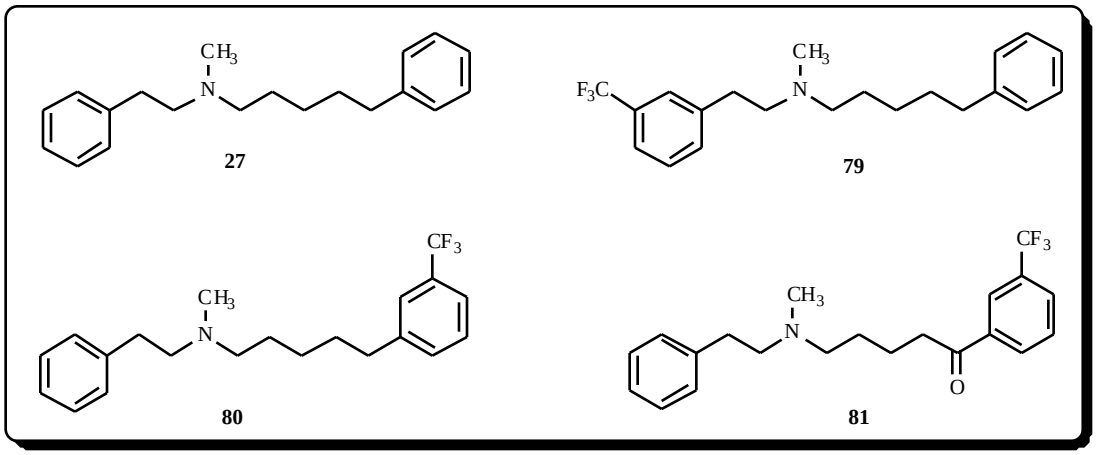

**70** **71**

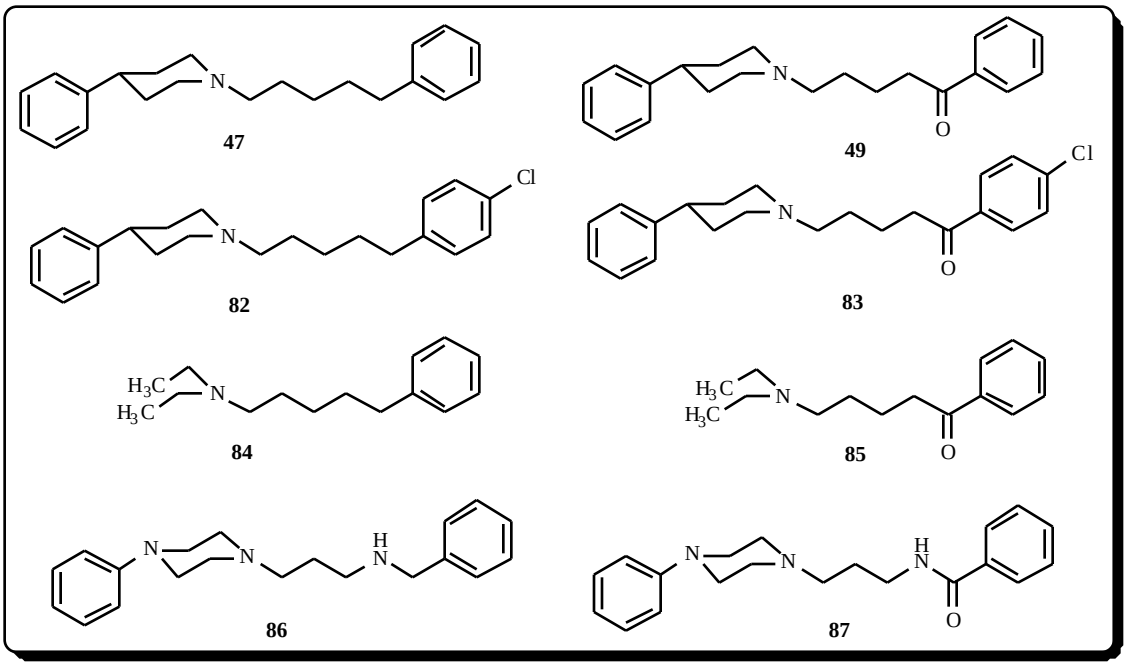



existence of $\sigma_1$ and $\sigma_2$ receptors. Actually, the two models were quite similar; this might reflect the observation that most of the compounds examined in the initial studies were subsequently shown to bind with higher affinity at $\sigma_1$ than $\sigma_2$ receptors, even though most showed only 10- to 200-fold selectivity for the former. During the course of our work, Gilligan *et al.* [31] independently described a model for the binding and oral activity of $\sigma_1$ ligands. Their model, though derived from different types of structures, is not unlike that shown in (Fig. **2**) and consists of an amine site and two hydrophobic regions separated by distances very similar to that proposed by us. Their model also contains a hydrogen bonding site; this site might be important for oral activity, or it might simply be a consequence of the structures that they investigated – nearly all of which possessed a polar oxygen substituent in the alkyl chain. These models lack three-dimensionality because most of the compounds examined are quite conformationally flexible. QSAR studies also have been conducted and, for example, a CoMFA (Comparative Molecular Field Analysis) model has been described [21]; but such models rely on the arbitrary alignment of conformationally-flexible ligands – some of which possess two amine groups – that produce results that are alignment dependent. What is required now are novel high-affinity ligands with greater conformational constraint so that reliable three-dimensional models might be developed.

There are other problems in defining a sigma pharmacophore. That is, there are certain inconsistencies that have yet to be fully accounted for. For example, introduction of a 3-trifluoromethyl group decreases the affinity of **27** (Ki = 0.25 nM) by 7-fold when incorporated into the Phenyl-A ring (**79**; Ki = 1.7 nM) [16,23], but decreases affinity only by 2-fold when incorporated into the Phenyl-B ring (**80**; Ki = 0.6 nM). The presence of a carbonyl group has no influence on the affinity of the latter compound (**81**; Ki = 0.7 nM); similar structural modifications don't always have the same effect in other compounds.

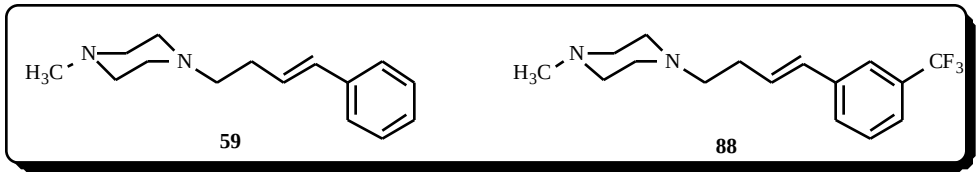
Introduction of the carbonyl function has no effect on the affinity of **49** (Ki = 0.12 nM) relative to **47** (Ki = 0.15 nM), or **83** (Ki = 0.10 nM) relative to **82** (Ki = 0.16 nM), and the affinity of carbonyl compound **85** (Ki = 24 nM) is only 4-fold lower than that of its alkyl counterpart **84** (Ki = 5.9



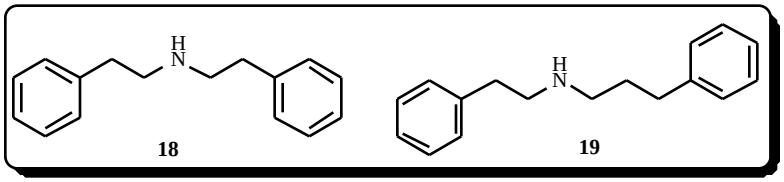



nM). However, that of **87** (Ki = 94 nM) is 50-fold lower than that of **86** (Ki = 2.0 nM) [20].

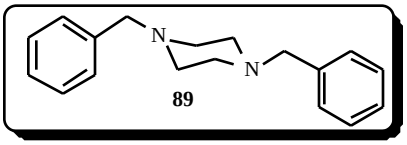
Introduction of the Phenyl-B 3-trifluoromethyl group had minimal effect on the affinity of **27**. However, introduction of a 3-trifluoromethyl group enhanced the affinity of **59** (Ki = 29 nM) by >20-fold (**88**; Ki = 1.4 nM).




We have arbitrarily assigned Phenyl-A/Phenyl-B designations to certain *bis*(arylalkyl) amine derivatives for "bookkeeping" purposes; but in many instances there is no confidence as to which ring represents the Phenyl-A ring and which represents the Phenyl-B ring. This is epitomized by compound **18** (Ki = 11.4 nM) where such a distinction is meaningless. If one of the alkyl chains is lengthened by a single methylene group (i.e., **19**; Ki = 11.3 nM) the assignment of Phenyl-A and Phenyl-B is no more obvious.



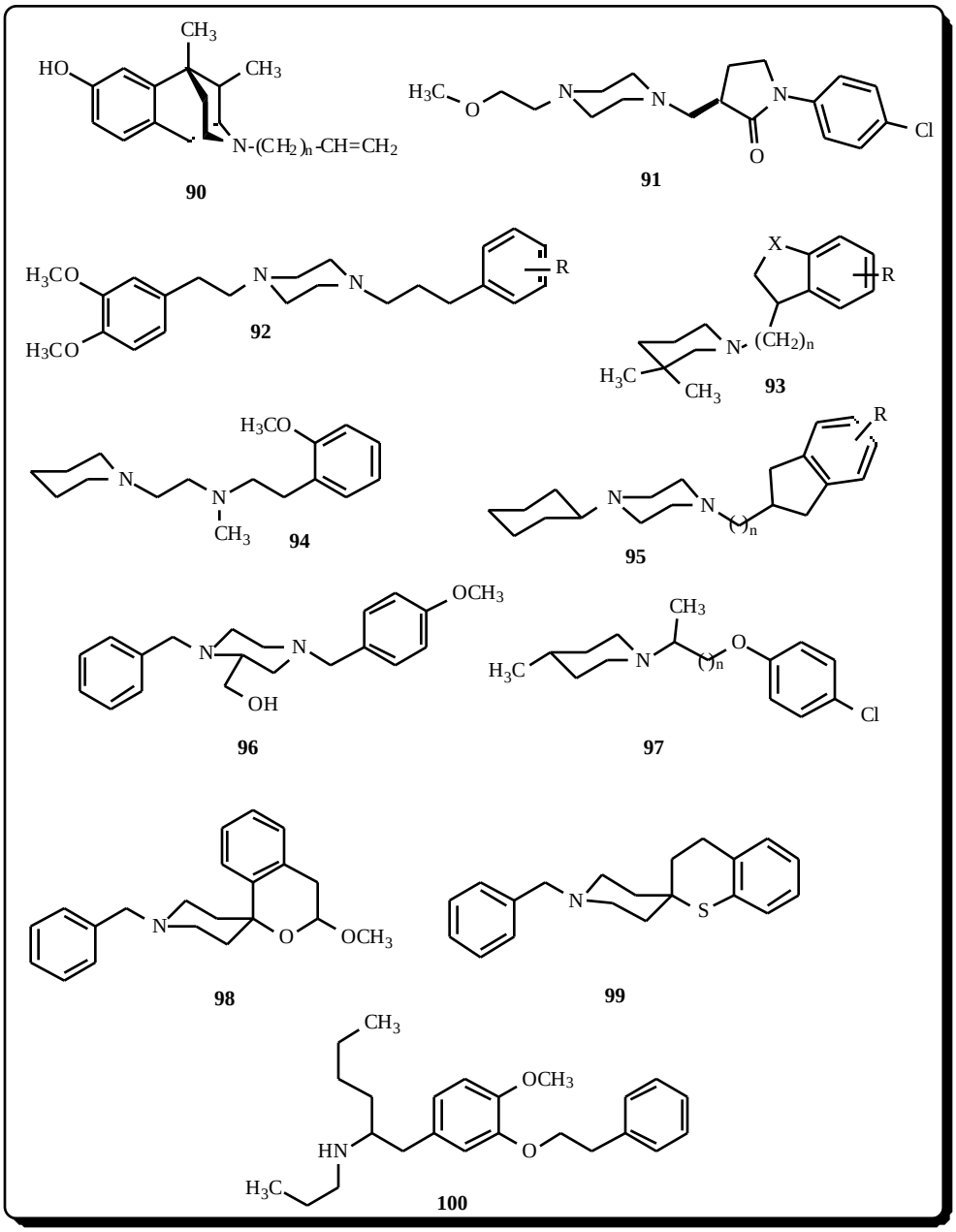

Given the two hydrophobic binding regions, and their associated regions of bulk tolerance, it can be appreciated that certain ligands might bind in either one (or both) of two fashions. Introduction of aryl substituents only complicates the possibilities. That is, a particular Phenyl-A substituted compound might bind in one manner (e.g. using the secondary hydrophobic site), but when the substituent is moved to a different ring position the ligand might bind in the reverse manner (with the aryl ring now utilizing the primary hydrophobic site) [20]. Another complicating issue is the presence in the ligands of more than one basic amine moiety (e.g., as with piperazines) where either one of the amine groups could interact with the amine binding site [20]. Compound **89** would epitomize such a case in the arylpiperazine series. Depending upon which amine interacts with the amine site, alterations on one side of the molecule or changes in chain length might not give consistent results for these types of compounds. These issues have confounded formulation of a more detailed pharmacophore model. In addition, due to the quasi-symmetrical nature of the currently proposed pharmacophore (Fig. **2**), conclusions drawn from QSAR studies (including those published by us) that require alignment of specific structural features are suspect when alignment is made in the absence of information as to exactly how the molecules should be aligned.

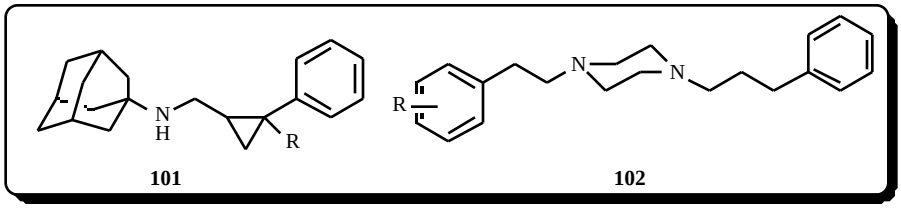


Instances of "reverse" modes of binding might be explained on this basis; for example, **8*R*** (Ki = 44 nM) binds with lower affinity than **8*S*** (Ki = 15 nM), but its homologous **9*R*** (Ki = 11 nM) binds with higher affinity than **9*S*** (Ki = 39 nM). Although the enantioselectivity of these two ligands is small, it might present clues to how the compounds are binding, and suggests that further investigation of chiral compounds be targeted.

## NEWER AGENTS

Given the above caveats, other investigators have described a number of new sigma receptor ligands which, gratifyingly, seem to conform to the concepts we have previously espoused. For example, May *et al.* [32] have shown that as the length of the nitrogen substituent is increased, $\sigma_1$ affinity of benzomorphans **90** (1*S*,5*S*,9*S* series) increases (i.e., **90**: Ki = 300 nM, 15 nM, 2.1 nM, and 1.1 nM when n = 1-4, respectively). With MS-377 (**91**; Ki = 73 nM) [33], one wonders which of the two basic amines is the more important and whether replacing one of them with a methylene group will result in enhanced affinity. Compounds **92** (SA4503; $IC_{50}$ = about 10 to 100 nM) [34], and compounds **93** where X is $-CH_2-$ or $-CH_2CH_2-$ and n is varied from 3 to 5 [35] possess arylalkylamine moieties. Compound **94** (Ki = 32 nM) [36] binds with an affinity similar to that of **76**. Piperazines **95** where n = 1-6 bind in the low nanomolar range [37], and **96** (Ki ca 12 nM) [38] might be viewed as an additional example of a arylalkylamines bearing a heteroatom (i.e., N atom) in the chain. Compounds **97**, which also bind in the low nanomolar range when n = 1 or 2 [39], bears an oxygen atom in the chain and possesses a piperidinyl 4-methyl group as found in **57**. Spiro compound **98** (which binds with a Ki = 1.29 nM and displays >2,700-fold selectivity over $\sigma_2$ receptors) [40], and **99** [41] represents a sulfur-






**101** **102**

containing compound. Compound **100** ($IC_{50}$ = 2.1 nM) is a structurally complex phenylalkylamine [42].

The binding of adamantyl amine analogs **101** in the low micromolar range [43] is not inconsistent with a region of bulk tolerance, as is the finding that substituents R (e.g. methoxy, chloro, nitro) have no influence on the affinity of **102** (Ki = 1-4 nM) [44].

## SUMMARY

Application of the "*deconstruction – reconstruction – elaboration*" approach has aided the recognition of those structural features that contribute to $\sigma_1$ binding, and the "elaboration" aspect has resulted in the identification of a number of high-affinity (i.e., Ki <1 nM) ligands. Given the simplicity and generality of the current model (Fig. **2**), additional studies are certainly required to better define a pharmacophore. As shown, numerous structure types bind at $\sigma_1$ receptors, and many do so with low nanomolar affinity. Due to the promiscuity of $\sigma_1$ binding, it is suggested that future studies focus on high-affinity ligands. As already mentioned, inclusion of stereochemical "markers" might be useful, and conformationally constrained compounds would also be of value for development of reliable three-dimensional pharmacophore models. Finally, because the intent of this review was to identify pharmacophoric features for $\sigma_1$ binding, and although selective agents are not required to formulate pharmacophore models, the issue of $\sigma_2$ binding was ignored. Interestingly, however, many structural features that contribute to $\sigma_1$ binding also contribute to $\sigma_2$ binding [reviewed: 45]. Nevertheless, there is still a need to identify agents with greater subtype selectivity for $\sigma_1$ versus $\sigma_2$ receptors in order to better investigate sigma pharmacology.

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