Chapter 2

MEDICINAL CHEMISTRY: NEW CHEMICAL CLASSES AND SUBTYPE-SELECTIVE LIGANDS

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1. INTRODUCTION AND HISTORY

Previous reviews of the area of σ ligand structure activity relationships have covered most of the early ligands, but many of the pharmacological conclusions based on early ligands were confusing due to the ligands interacting with several other biological systems. This chapter attempts to briefly discuss the history behind the development of early σ ligands, but maintains a greater focus on the more recent σ-selective ligands, which have been developed over the past decade. For a more detailed discussion of the earlier ligands the reader is directed to these excellent reviews (1-3).

σ Receptors were initially described by Martin as a subtype of opioid receptors based on the actions of the benzomorphans, specifically racemic SKF-10,047 (**1**) (4). This was a confusing birth for the σ receptor system, as the actions attributed to the effects of SKF-10,047 at σ receptors were probably due to the interaction of the (+)-isomer of the benzomorphan with σ receptors, whereas the (-)-isomer was the agent responsible for the opioid effects (5). The situation was further confused when σ sites were believed to be part of the phencyclidine binding site (ionophore site) or polyamine site of the NMDA receptor complex.

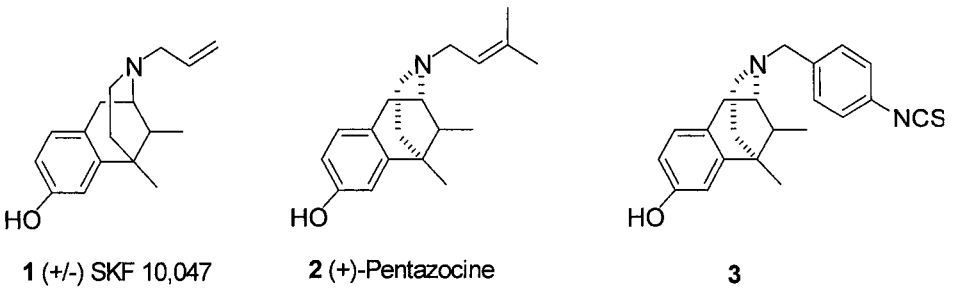


Figure 2-1. Benzomorphan-based σ ligands

1.1 Benzomorphans

As discussed above, the σ activity of the benzomorphan SKF-10,047 was probably due to the actions of the (+)-enantiomer, and this led to the discovery of (+)-pentazocine (**2**) as a selective σ ligand. Through the use of this ligand, and others including 3-PPP (3-(3-hydroxyphenyl)-N-(1-propyl)piperidine) and DTG (di-o-tolylguanidine), the σ receptor system was finally characterized as unique (6-10). Additional ligands of the benzomorphan class have also been found to possess affinity for the σ system, and are covered in the review by Walker et al. (2).

Since the initial cloning of the σ_1 receptor (11), studies have concentrated on the development of ligands to further characterize and purify these receptors. A recent investigation into the development of selective σ_1 receptor probes has led to a (+)-benzomorphan-based irreversible ligand. Ronsisvalle et al. (12) showed that the introduction of an isothiocyanate into the (+)-N-benzyl benzomorphan derivative gave a ligand (**3**) (Figure 2-1) which appears to show promise as such an agent (see review by Ronsisvalle on irreversible ligands in Chapter 3).

1.2 σ_1 and σ_2 receptors

It was eventually found that σ receptors consisted of a heterogeneous population of sites, now termed σ_1 and σ_2 (13-15). The discovery of the heterogeneity of σ receptors prompted concentrated efforts into the search for compounds with selectivity for each σ receptor subtype. (+)-Benzomorphans display selectivity for σ_1 receptors, and indeed tritiated (+)-benzomorphans are used in σ_1 receptor binding assays (16).

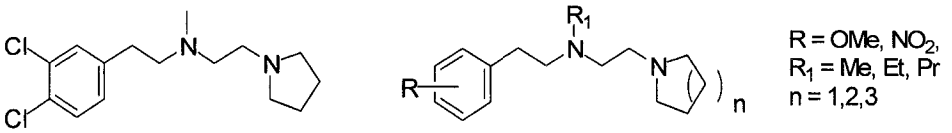
σ_2 Receptor-selective ligands have proven less common, with the currently accepted radioligand being the subtype nonselective [^{3}H]DTG in the presence of a (+)-benzomorphan to block binding to σ_1 sites. The pharmacological effects of activating both subtypes are described elsewhere in this volume. Briefly, σ_1 receptors have been associated with numerous conditions including cognitive effects, neuroprotection, and may be involved in the actions of cocaine (see Chapters 12, 15). σ_2 Receptors have been less well studied, but activation of σ_2 receptors appears to affect movement and posture and has been associated with inhibition of cell proliferation and induction of apoptotic cell death (see Chapter 11).

Many of the ligands discovered prior to about 1992 were only evaluated using binding assays against [^{3}H](+)-pentazocine, which is primarily an assay for σ_1 receptor binding affinity (17). Thus, little can be stated about the activity of these compounds for σ_2 receptors. This review will concentrate on the compounds where affinity at both receptors has been established.

2. ENDOGENOUS LIGANDS

The endogenous ligand for σ receptors remains elusive. Several laboratories have identified brain extracts, which show affinity for σ receptors (18,19). Furthermore, physiological studies have suggested depolarization- and calcium-dependent release of σ-active substances from brain slices (20,21). To date, however, none of the substances have been identified.

The search for an endogenous ligand for σ receptors did, however, lead to the discovery that certain neurosteroids possess affinity for σ_1 receptors, notably progesterone (22). From a chemical point of view, this is an interesting finding as the majority of ligands with affinity for σ receptors contain a basic nitrogen. Indeed, most models of ligand recognition include the requirement of a basic nitrogen, yet progesterone is a lipophilic steroid lacking any basic or acidic groups. This finding, along with information gleaned from cloning, lead to the hypothesis that σ_1 receptors are distantly related to enzymes of steroid biosynthesis (23). The merits of this hypothesis are discussed elsewhere in this volume.



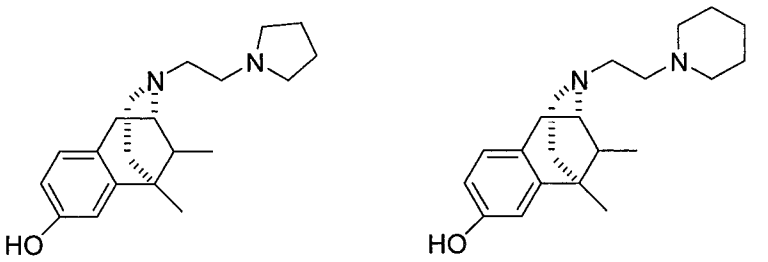
4 **5**

Figure 2-2. Phenylethylene diamine-based σ ligands

3. σ SELECTIVE AGENTS

Initial studies with early σ ligands were limited due to the effects on other systems influencing the pharmacology of the ligand. Obviously, what was needed were compounds that did not interact with other biological systems. One of the most widely studied class of compounds are the phenylethylene diamines: the protypical member of this class is BD1008 (**4**) (Figure 2-2) (24). BD1008 contains 3,4-dichloro substitution on the aromatic ring, a substitution pattern which leads to high affinity at both σ_1 and σ_2 receptors. Numerous other substituents have been introduced, but it appears that lipophilic substituents are preferred for high affinity agents (25). A range of substitutions that have been investigated on phenylethylene diamines (**5**) are shown in Figure 2-2.

In order to exploit the activity of the (+)-benzomorphans and phenylethylene diamines, hybrid structures were prepared where the basic amine and aromatic ring of the benzomorphan skeleton was taken as the "phenethyl" group of the phenylethylene diamines (26). Compounds such as **6** and **7** (Figure 2-3) did indeed display excellent affinity at σ_1 receptors (K_i < 10 nM), lower affinity at σ_2 receptors, and little activity at opioid receptors.



6 **7**

Figure 2-3. Hybrids of the benzomorphans and the phenylethylene diamines

A class of compounds that share structural similarities to the phenylethylene diamines are the phenylpentylamines (such as **8** and **9**, Figure 2-4), which show high σ receptor affinity against [^{3}H](+)-pentazocine, with a K_i of about 1 nM (27) (further discussed by Ablordeppey and Glennon elsewhere in this volume). This class can be viewed as phenylethylene diamine analogs that lack one of the basic nitrogens, and suggests that the second basic nitrogen is not essential for σ_1 affinity. As binding was only performed in assays to measure σ_1 affinity, little can be concluded about their affinity for σ_2 receptors. However, the recent report that AC915 (**10**) (Figure 2-4), an ester derivative of the phenylpentylamines, is a σ_1 ligand with excellent selectivity over σ_2 receptors (2000-fold), suggests that this is a class where additional σ_1 selective agents may be developed (28). Indeed, this compound may find use as a masking agent in σ_2 binding assays replacing the (+)-benzomorphans. Recently, a related class of phenoxyalkyl amines (**11**) have also been reported to possess excellent affinity for both σ_1 and σ_2 receptors, and the introduction of stereochemistry onto the alkyl chain was interestingly shown to influence affinity and selectivity (**12**) (29).

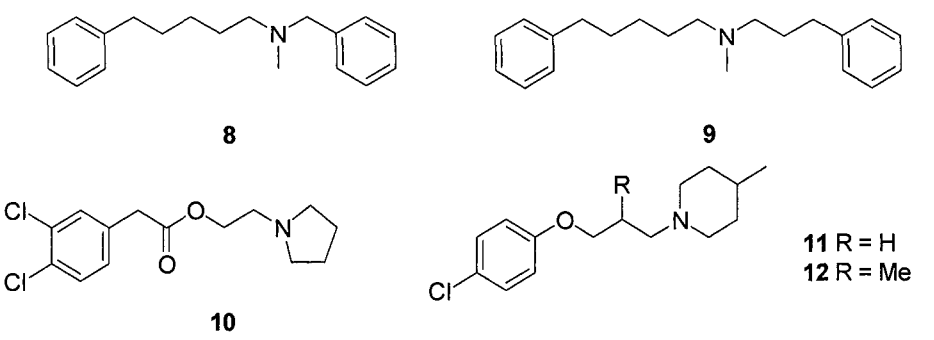


Figure 2-4. Phenyl pentyl amines, AC915, and phenoxyalkylamines

4. σ SUBTYPE SELECTIVE AGENTS

4.1 σ_1 ligands

4.1.1 Haloperidol derivatives

Compounds related to haloperidol are shown in Figure 2-5. Haloperidol (**13**) has been shown to possess high affinity for σ-receptors, with a slight preference for σ_1 over σ_2 (30). When the ketone was reduced to give reduced haloperidol (**14**), the dopamine D_2 affinity of haloperidol was greatly decreased, to give a compound relatively selective for σ receptors over other systems. These studies led to the development of the related E-5842 (**15**) as a σ_1 agent, with excellent selectivity over a range of other biological systems. E-5842 has been shown to possess promise as an antipsychotic agent (31).

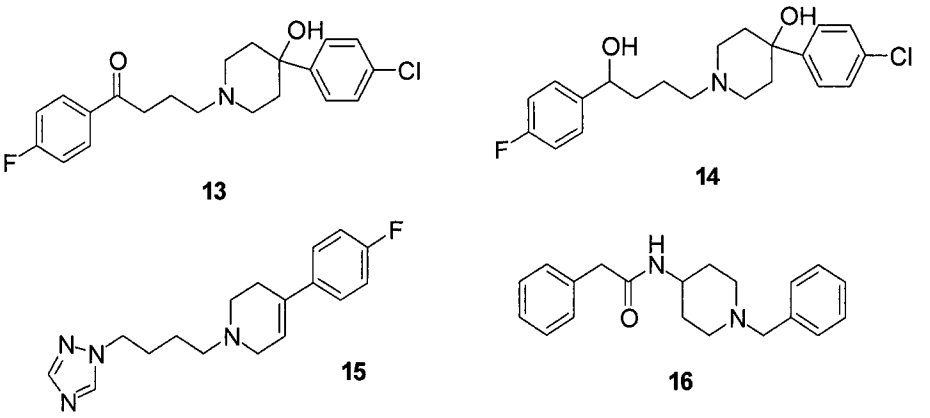


Figure 2-5. σ Ligands based on haloperidol

4.1.2 Phenylacetamides

N-(1-Benzylpiperidin-4-yl)phenylacetamides (such as **16**, Figure 2-5) share a similar skeleton to E-5842 discussed above. These compounds have been shown to possess excellent selectivity for σ_1 receptors, with affinities in the low nanomolar range, and selectivities over σ_2 up to 200-fold (32). Further studies into the structure-activity relationships of this series of compounds showed that replacing the aromatic ring with heterocyclic rings led to compounds with reduced affinity, but that the introduction of a halogen on both aromatic rings led to an increase in selectivity for σ_1 receptors over σ_2 (33).

4.1.3 NE-100

NE-100 (N,N-di-isopropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl] ethyl-amine (**17**) (Figure 2-6) is a simple amine with only two carbons between the amine and the aromatic ring. This compound shows high affinity for σ_1 receptors, and moderate selectivity over σ_2 receptors (34). Studies of this interesting class of compound have shown that both propyl groups are not necessary for affinity at σ receptors, and that the mono-propyl analog **18** possesses significant affinity (34). Further studies showed that the introduction of alkyl groups alpha to such a secondary amine (to give **19**) actually led to increases in affinity and selectivity for σ_1 receptors (Figure 2-6) (35).

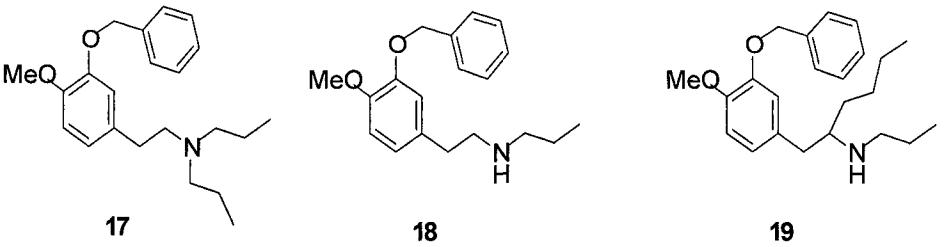


Figure 2-6. NE-100 and secondary amine analogs

4.2 σ_2 ligands

4.2.1 Benzylidine phenylmorphans

Perhaps the most widely studied σ_2 selective agents are the benzylidene phenylmorphans (Figure 2-7), typified by CB-64D (**20**) and CB-184 (**21**) (36). Both compounds show high affinity and excellent selectivity for σ_2 receptors over σ_1 receptors, with CB-184 showing the greater selectivity. Both contain the aryl morphinan skeleton present in a class of opioids, but with an additional benzylidene group. It has been suggested that this dichlorinated ring may occupy similar space on the receptor as the equivalent ring in BD1008 (37). These compounds have shown excellent activity in functional assays (38-40) and have indeed proved to be valuable tools in delineating σ_2 ligand pharmacology and the possible role of σ_2 receptors in regulation of cell growth and survival (reviewed by Bowen in Chapter 11). Even so, this class of compounds suffers from the major problem of their interaction with opioid receptors, as they display potent mu opioid agonism *in vivo*. Hence, further study of this class is required in order to develop analogs lacking the opioid component, but which maintain σ_2 receptor selectivity.

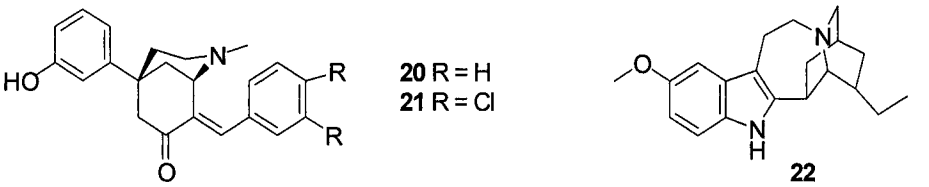


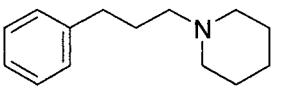
Figure 2-7. σ Ligands based on phenylmorphans and ibogaine

4.2.2 Ibogaine

Another compound that demonstrates relative selectivity for σ_2 receptors over σ_1 receptors is ibogaine (**22**) (Figure 2-7), although its affinity for σ_2 receptors is modest (41). Ibogaine gained notoriety due to its reported actions as an anti-addiction agent and has been useful as a tool to study the cytotoxicity mediated by σ_2 receptors *in vitro* (42). However, it interacts with a variety of biological systems in addition to σ_2 receptors and therefore cannot be used to study the actions of σ_2 receptors in *in vivo* assays.

4.2.3 Arylpropylamines

A recent report discussed the fact that ibogaine and CB-184 contain arylpropyl amines and display σ_2 selectivity, whereas compounds with affinity for σ_1 sites (such as NE-100) tend to possess a phenylethylamine moiety (37). Based on this observation, a simple range of phenethyl and phenylpropyl amines were studied. It was shown that phenylpropyl-piperidine (**23**) (Figure 2-8) demonstrated a preference for σ_2 sites (four-fold) and that the preference could be increased with other substituents to give **24** as a high affinity ligand for σ_2 receptors with moderate selectivity (Figure 2-8) (37). It is anticipated that this finding may lead the way to agents optimized for σ_2 receptors.



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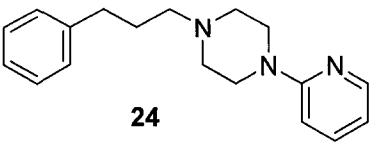

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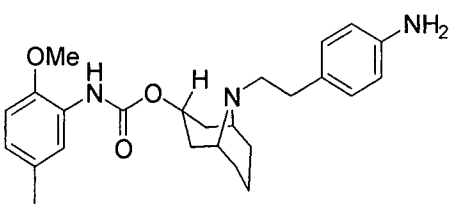
Figure 2-8. Simple phenylalkylamines

4.2.4 Tropane analogs

A recent report by Mach et al. (43) described a novel tropane-based ligand (**25**) (Figure 2-9) which is reported to possess an affinity at σ_2 receptors of 5 nM, and a selectivity over σ_1 receptors of greater than 500-fold. The para-amine substitution was shown to aid in the selectivity, as the unsubstituted phenyl analog demonstrated much reduced selectivity for σ_2 receptors.

The related tropane-containing ligand (±)-SM-21 (**26**) (Figure 2-9) has been shown to posses significant affinity for σ_2 receptors (44) and is currently used as a σ_2 preferring antagonist in behavioral assays (45).



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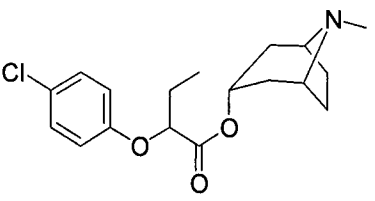

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Figure 2-9. Tropane-based σ ligands

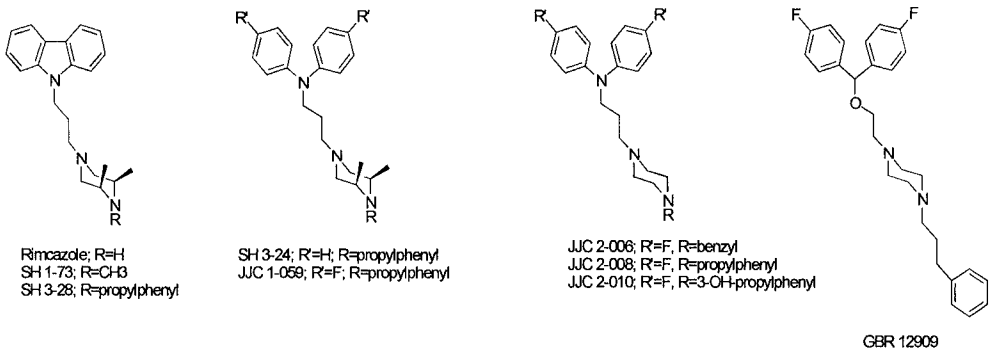

Figure 2-10. Rimcazole and other piperazine analogs

5. DUAL PROBES FOR σ_1 RECEPTORS AND THE DOPAMINE TRANSPORTERS

5.1 Rimcazole analogues

Over the past decade, several lines of evidence have linked σ receptors and cocaine. For example, cocaine was reported to bind with low to moderate affinity to σ receptors and these concentrations were shown to be achievable *in vivo* (46). In addition, several σ ligands such as rimcazole and BMY 14802 (Figure 2-10) have been shown to attenuate locomotor and rewarding effects of cocaine (47,48). Recently, the σ_1 receptor antagonists NE-100 and BD1047 showed significant attenuation of cocaine-induced place preference (48). Other studies showed that σ receptor antagonists block the development of sensitization to cocaine in rats (49). Furthermore, attenuation of cocaine's convulsive and lethal effects by the selective σ antagonists BD1047, LR172 and N-alkyl substituted and conformationally restricted analogues of BD1008 has also been reported (50-53).

Curiously, there also seems to be a structural linkage to the cocaine binding site on the dopamine transporter (DAT) and the σ antagonist binding site, despite no apparent homology between the DAT and σ_1 receptor protein structures. Namely, an iodoazido-analogue of cocaine was reported to photolabel a 26 kDa polypeptide in rat brain that displayed the pharmacology of a σ receptor (54,55). Furthermore, the potent DAT inhibitor GBR 12909 was reported to potently displace [^{3}H]3-PPP from σ receptors in rat brain (IC_{50} = 48 nM) (56). More recently, an isothiocyanato

analogue of the σ antagonist rimcazole has been shown to bind irreversibly to the DAT, in rat caudate-putamen (57).

These early linkages prompted an experiment evaluating nine structurally diverse σ ligands for displacement of [^{3}H]WIN 35,428 binding at DAT and inhibition of dopamine uptake, in rat caudate-putamen (58). Although most of these compounds did not bind with high affinity to DAT, rimcazole displaced [^{3}H]WIN 35,428 from DAT with an affinity of 103 nM. Rimcazole had previously been reported to attenuate the locomotor stimulant effects of cocaine at doses that were not themselves behaviorally active (47). These discoveries lead to the design and synthesis of a series of rimcazole analogues as potential dopamine uptake inhibitors and structure-activity relationships at DAT, serotonin transporter (SERT), norepinephrine transporter (NET), and σ_1 receptors were determined. It was discovered that in general, substitutions on the carbazole ring system of rimcazole served to decrease binding affinities at both σ_1 receptors and the DAT (57,59). Data for other rimcazole analogues is shown in Table 2-1. N-methylation of the terminal piperazine nitrogen (SH 1-73) resulted in a small increase in binding affinity at σ_1 receptors (K_i = 552 nM) but in a slightly less active DAT compound (K_i = 436 nM) (59). Alternatively, placing a propylphenyl group, on the terminal piperazine nitrogen (SH 3-28), as seen with GBR 12909, served to improve and restore σ_1 receptor and DAT binding affinities, respectively. Likewise, when the carbazole ring system was replaced with a diphenylamine, coupled with the N-propylphenyl substituent, a moderately potent rimcazole analogue SH 3-24 resulted (K_i = 97 nM at σ_1 and 61 nM at DAT) (59). Adding fluoro-groups to the para-positions of the diphenylamine moiety (JJC 1-059) served to significantly improve both σ_1 receptor and DAT binding (K_i =11.1 nM and 22.8 nM, respectively) (60,61). Removal of the 2,6-dimethyl groups on the piperazine ring (JJC 2-008) served to reduce lipophilicity and also reduced σ_1 receptor binding affinity (K_i = 66.2 nM) while retaining high affinity for DAT (K_i = 18 nM). Interestingly, the N-benzyl analogue (JJC 2-006) showed the highest affinity for σ_1 receptors in the demethylated series (K_i = 13.1 nM) (61).

Rice's laboratory synthesized analogues of GBR 12909 and showed that GBR 12935 and several analogues displaced [^{3}H](+)-pentazocine from σ_1 receptors with high affinity (K_i range = 8.6 - 231 nM) (62). Many of these compounds show structural similarity to the rimcazole analogues and bind with high affinity to both σ_1 receptors and DAT (62,63). The most potent σ_1 ligand in these series was the trans 2,5-dimethylpiperazinyl analogue of GBR 12909 (62). Comparing the SAR derived from the rimcazole analogues to these compounds, the presence of a dimethylated piperazine,

regardless of position and stereochemistry, appears to improve binding affinity at σ_1 receptors as compared to the unsubstituted piperazines.

Behavioral evaluation of rimcazole, SH 1-73, SH 3-24, and SH 3-28 has shown that all of these ligands produced dose-related decreases in locomotor activity and decreased cocaine-induced locomotor activity. Furthermore, rimcazole and its analogues did not generalize to the cocaine discriminative stimulus in rats trained to discriminate 10 mg/kg of cocaine from saline (64). Interestingly, SH 3-28 decreased cocaine-appropriate responding as well. Another preliminary study with JJC 1-059, in comparison to cocaine, GBR 12909 and rimcazole demonstrated that, like its parent compound, JJC 1-059 did not produce locomotor stimulation in mice (61,65). Furthermore, rimcazole and its analogues attenuated cocaine-induced convulsions in mice (66). In total, these results are curious, as all of these compounds bind to the dopamine transporter, some with higher affinity than cocaine. Hence, it has been hypothesized that despite their actions at DAT, perhaps σ_1 receptor antagonism is involved in the blockade of cocaine's actions demonstrated by rimcazole and its analogues. The recent proposal that DAT-mediated cocaine-like actions, including reinforcement, might be modulated by σ_1 receptors (67,68) further supports the development of dual DAT/σ_1 probes to investigate whether or not these combined actions might provide a novel approach to cocaine-abuse medication discovery.

Table 2-1. Binding Results at σ_1 Receptors and Dopamine Transporters (DAT)

Compound	[^{3}H](+)-Pentazocine (σ_1)	[^{3}H]WIN 35,428 (DAT)	σ_1/DAT
Cocaine	8830 ± 860[b]	187 ± 19[a]	47
GBR 12909	318 ± 18[a]	11.9 ± 1.9[a]	27
Rimcazole	908 ± 99[a]	224 ± 16[a]	4.1
SH 3-24	97.2 ± 14.0[a]	61.0 ± 6.1[a]	1.6
SH 1-73	552 ± 110[a]	436 ± 44[a]	1.3
SH 3-28	104 ± 0.4[a]	263 ± 34[a]	0.4
JJC 1-059	11.1 ± 0.8[b]	22.8 ± 2.0[b]	0.5
JJC 2-008	66.2 ± 3.6[b]	18.1 ± 2.7[b]	3.7
JJC 2-006	13.1 ± 1.2[b]	27.6 ± 3.9[b]	0.5
JJC 2-010	372 ± 21[b]	8.5 ± 0.8[b]	44

Ki in nM. Data from ref. (59)[a] and ref. (61)[b].

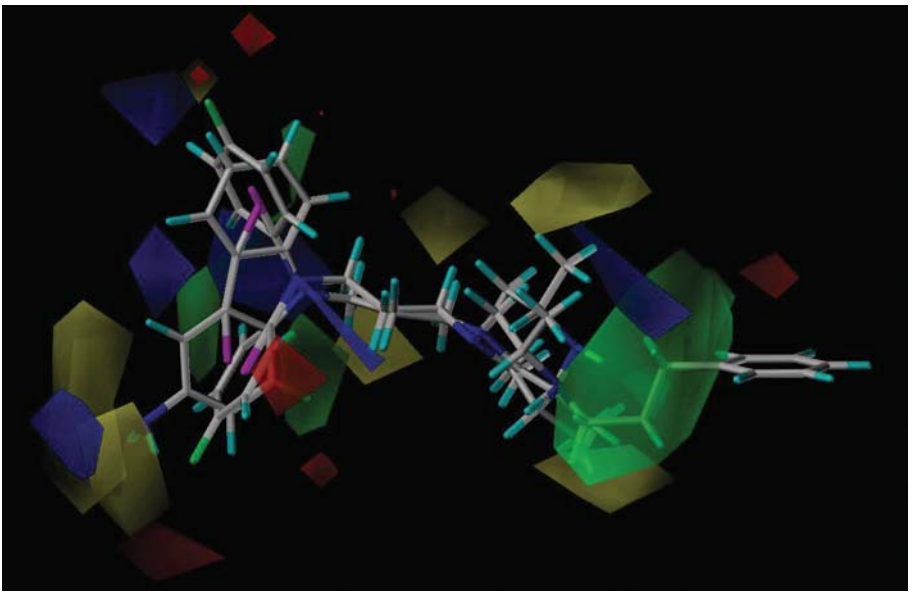

Figure 2-11. The CoMFA Contour Graphs for the Activity on the σ_1 Receptor (61). The sterically favored and unfavored (contribution at 80% and 20%, respectively) are shown as green and yellow fields and positive charges favorable and unfavorable (contribution at 80% and 20%, respectively) are shown as blue and red fields respectively.

5.2 Molecular models

Several CoMFA models were derived for σ_1 receptor binding of the rimcazole analogues and have been recently reported (61). Figure 2-11 shows the steric and electrostatic contour maps derived using σ_1 binding affinities. A sterically favored green region was observed near the terminal piperazine nitrogen substituent, supporting a strong steric interaction in this region of the molecule. Also, the scattered yellow regions around the molecule define the limits for size and shape of the substituents. Positive charge favoring regions shown as blue contours were observed in the vicinity of the para-position of the diaryl ring system. Hence small electron-withdrawing substituents, e.g. F, are predicted to improve σ_1 binding affinities. Putative binding site characteristics for the σ_1 receptor have been proposed (33,69,70) and are reviewed elsewhere in this volume by Ablordeppey and Glennon (Chapter 4). The CoMFA results describing optimal binding features of the rimcazole analogues were interpreted to be comparable to those previously described (61). As such, the substituent on the terminal piperazine nitrogen of the rimcazole analogues could be binding

in the described primary hydrophobic site and the diaryl amine could be accessing the secondary binding site, which seems to tolerate bulk in this region. The region between the terminal piperazine nitrogen and the terminal phenyl ring is less tolerant to electron releasing or hydrophilic substituents as the 3-OH group of JJC 2-010 overlaps in the blue contour, which is unfavorable for activity. Likewise, comparison with the previously proposed σ model (70) would suggest that hydrophilic interactions in this region would reduce affinity towards the σ_1 receptor.

6. SUMMARY

Over the past decade, advances have been made in discovering novel σ receptor probes and developing structure-activity relationships for σ_1 and σ_2 receptor selectivity. These compounds have provided useful tools to further investigate the physiological role that central and peripheral σ receptors play. Furthermore, many of these compounds have been investigated for their *in vivo* actions, and particularly promising is their ability to attenuate cocaine-induced behaviors such as locomotor stimulation and conditioned place preference, as well as cocaine-induced toxicities. These *in vivo* studies are described in other chapters in this book and the interested reader is referred to these. Compounds that have dual actions at both σ_1 receptors and the dopamine transporter may prove to be a novel strategy for the development of a cocaine-abuse medication and is being investigated toward this goal. Compounds selective at σ_2 receptors may be useful as antineoplastic agents or for control of cell survival in neurodegenerative disease. The design and synthesis of novel and selective σ_1 and σ_2 receptor selective agonists and antagonists will undoubtedly provide the required molecular tools to elucidate both structure and function of these receptors.

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