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Dopamine transporter as target for drug development of cocaine dependence medications

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Abstract

Because much evidence implicates the dopamine transporter in the reinforcing effects of cocaine, development of potential medications for cocaine dependence has included the dopamine transporter as a target. The present overview covers progress in the drug development area regarding several classes of dopamine uptake inhibitors, with an emphasis on structure–activity relationships that enhance potency and selectivity at transporters for dopamine compared with those for serotonin or norepinephrine. The following categories of compounds are covered: tropane, benztropine, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR), methylphenidate, mazindol, and phencyclidine analogs. Activity at transporters as well as on behavior is highlighted.

Keywords: Cocaine; Analogue; Structure–activity relationship study; DAT (dopamine transporter); Dopamine hypothesis; Binding activity

1. Introduction

Cocaine is a naturally occurring molecule that is well known for its strong reinforcing activity and abuse potential (Johanson and Schuster, 1995). Addiction to cocaine is a major problem in our society today causing financial problems and posing a burden in securing law and order. Moreover, it has also contributed to the spreading of Human Immunodeficiency Virus (HIV) infection as needle sharing is a pervasive problem among drug abusers. At present, no effective medication is available for the treatment of cocaine addiction and there is an urgent need for the development of an effective medication. Cocaine binds to all three monoamine neurotransporter systems in the brain (Ritz et al., 1990). It has been established that binding of cocaine to the dopamine transporter, resulting in decreased clearance of neuronally released dopamine, is responsible for its strong reinforcing effects (Ritz et al., 1987; Spealman et al., 1989). The dopamine hypothesis of cocaine addiction received further support from a series of in vivo experiments (Kuhar et al., 1991; Koob and Bloom, 1988; Witkin et al., 1991) and also from molecular biological studies involving dopamine transporter knockout mice (Giros et al., 1996). Thus, binding potencies of dopamine receptor agonists and dopamine transporter specific compounds correlated very well with their relative reinforcing effects in animal drug discrimination and self-administration experiments (Spealman et al., 1989; Witkin et al., 1991). Furthermore, in a microdialysis study, it was demonstrated that cocaine increases dopamine preferentially in the nucleus accumbens in relation to its reinforcing effects (Di Chiara and Imperato, 1988). More recently, the dopamine hypothesis for cocaine's reinforcing effects was further strengthened by the demonstration that in dopamine transporter knockout mice cocaine and amphetamine increase extracellular dopamine in the nucleus accumbens but not in the caudate putamen (Carboni et al., 2001). This observation perhaps explains the results of a recent experiment showing self-administration of cocaine by dopamine transporter knockout mice (Rocha et al., 1998) as in these mice cocaine will still block the norepinephrine transporter, which is known to accumulate dopamine efficiently (Yamamoto and Novotney, 1998), in the nucleus accumbens and, thus, will increase the extracellular concentration of dopamine (Eshleman et al., 1999). Finally, in recent Positron Emission Tomography (PET) studies, it

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was very elegantly demonstrated that the subjective effect of cocaine in humans directly correlates with the extent to which it occupies the dopamine transporter (Volkow et al., 1997). These results support a drug development approach targeting the dopaminergic system as a viable avenue to develop medications for cocaine addiction (Smith et al., 1999).

As the relationship between cocaine binding to the dopamine transporter and reinforcing activity was strongly established, this transporter became a logical choice for drug development. A number of approaches have been taken to develop medications for cocaine addiction (McCance, 1997; Carroll et al., 1999; Mello and Negus, 1996; Platt et al., 2002). The two primary strategies are based on either the concept of substituting a non-addictive treatment agent, e.g. agonist, for cocaine, or the idea of antagonizing the reinforcing effect of cocaine. These ideas are primarily rooted in the successful application of these strategies in the treatment of opioid addiction. Substitution therapies with full and partial agonists have been applied with success in the treatment of heroine and nicotine addiction. An ideal profile of a pharmacotherapeutic agent in substitution therapy consists of decreasing self-administration of cocaine over a wide range of doses by depressing its overall rewarding quality. However, the drug by itself should have little or no abuse liability and it should produce some of the subjective effects of cocaine thus reducing its craving (Howell and Wilcox, 2001; Gorelick et al., 1998; Carroll et al., 1999). A potential medication for substitution therapy should have the following properties: (1) The agent should enter the brain slowly. (2) It should exhibit an appropriately long duration of action to provide a suitable dosing schedule. (3) The drug should be target specific and should exhibit minimum side effects (Glowa, 1996). Site-directed mutagenesis results suggested that cocaine and dopamine do not share identical binding sites raising the expectation for developing a cocaine antagonist (Kitayama et al., 1992). Such antagonists, if developed, will find an application in reducing cocaine self-administration, by reducing its reinforcing activity. It may also find a useful application in reducing toxicity from cocaine overdose.

2. Different structural classes of molecules targeting the dopamine transporter

A great number of structurally diverse compounds have been developed for the dopamine transporter with the aim to develop effective pharmacotherapies for cocaine addiction (Singh, 2000; Carrol et al., 2002). These compounds can be classified in the following categories: tropane, benztropine, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909)-analogues, methylphenidate, mazindol and phencyclidine analogs. The representative structure for each class of molecules is shown in Fig. 1. Due to the limited scope of this article, only important findings pertaining to structure–activity relationships for these classes of compounds are presented here. In addition, as results presented here are obtained from various laboratories, no numerical values are provided but rather relative activities are given.

2.1. Tropane derivatives

2.1.1. Transporter activity

The initial phase of drug development for the dopamine transporter was mainly focused on synthesizing compounds based on the tropane structure. (*R*)-Cocaine is a plant alkaloid and is derived from the leaves of *Erythroxylon coca*. Altogether, eight stereoisomers of cocaine were synthesized and biologically characterized. Out of these eight isomers, (*R*)-cocaine was found to be the biologically most active indicating stereospecificity in interaction with the dopamine transporter (Caroll et al., 1991a). Various structural modifications were introduced into the parent structure of cocaine which resulted in the development of various analogues some of which were found to be more potent compared to the parent cocaine. These structural modifications were made at the 2-, 3-, 6- and 7-positions along with

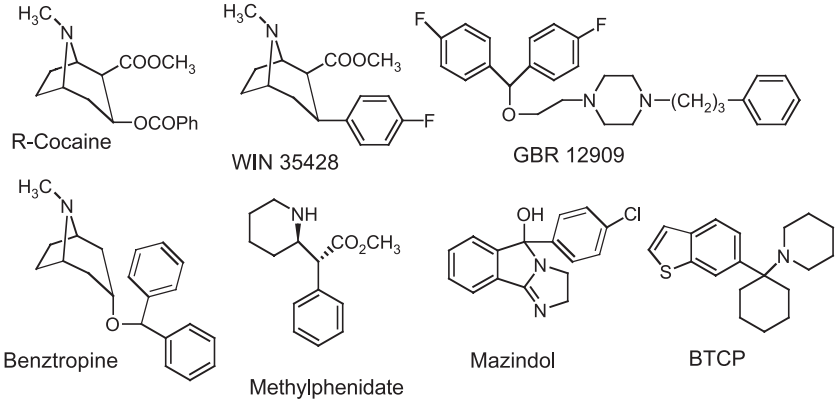

Fig. 1. Representative structures of different classes of dopamine transporter blockers.

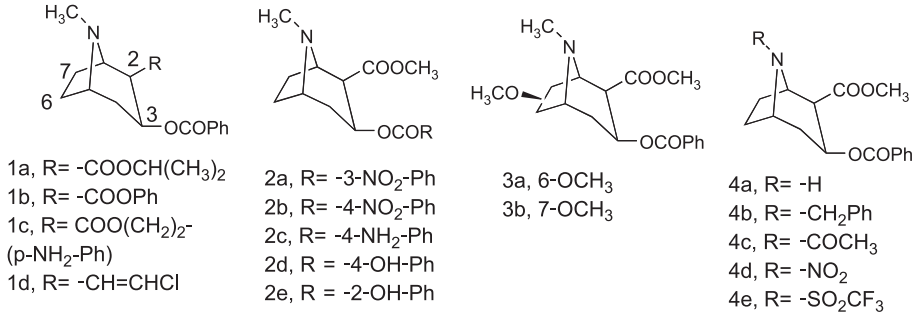

Fig. 2. Cocaine analogs with different structural modifications at different positions.

modification at the tropane nitrogen atom. Modification at the 2-position with various ester moieties produced derivatives exhibiting mostly lower activity compared to cocaine except for compound **1c** (Fig. 2) which was equipotent to cocaine (Lewin et al., 1992). On the other hand, modification at the 3β-position demonstrated interesting electronic effects as compound **2a** was twice as potent as cocaine while the phenyl-4-substituted nitro and amino derivatives **2b** and **2c** exhibited weaker potencies (Kline et al., 1991). Similarly, the hydroxy substituted derivatives **2d** and **2e** exhibited higher potencies than cocaine when **2e** was more potent than **2d** (Singh et al., 1997). Methoxy substitution at the 6- and 7-position in the phenyl ring as in **3a**, **b** did not improve potency over cocaine (Simoni et al., 1993). Finally, different N-substituted derivatives of cocaine indicated the importance of the presence of a basic N-atom in interaction with the dopamine transporter. Thus, in the series of **4a**–**d**, compounds **4c** and **4d** exhibited almost no activity (Stoelwinder et al., 1994; Abraham et al., 1992). On the other hand, trifluoromethylsulfonyl substituted **4e** was equipotent with cocaine (Kozikowski et al., 1994). Both norcocaine (**4a**) and *N*-benzyl substituted cocaine (**4b**) were less potent than cocaine (Abraham et al., 1992).

In another line of structure–activity experiments, replacement of the benzoate ester moiety by a β-phenyl group greatly increased the metabolic stability and activity for the dopamine transporter compared to cocaine (Clarke et al., 1973; Reith et al., 1986). Since that study, a large number of potent molecules has been developed including WIN 35428 (12β-carbomethoxy-3β-(4-fluorophenyl)-tropane (**5b**) or CFT) and RTI-55 (22β-carbomethoxy-3β-[4-iodophenyl]-tropane **5d**) or CIT), which are among the better known ligands that have been widely used in various neuropharmacological studies. These structure–activity relationship studies provided much useful information related to the impact of various substitutions at the 2- and 3-positions in the tropane backbone structure on their activity at the dopamine transporter, Fig. 3 (Carroll et al., 1991b, 1994; Blough et al., 1996).

Fig. 3 shows modification at the 3-position with different substituted phenyl moieties, which produced more potent compounds compared to cocaine. The dichloro substituted compound **5c** showed the highest potency for the dopamine transporter. The order of activity for the dopamine transporter in this series was **5c**>**5d**>**5b**>**5e**>**5a** (Carroll et al., 1991b, 1994). However, these compounds were not highly selective for the dopamine transporter as most of these compounds additionally showed high potency for the norepinephrine transporter especially when functional uptake inhibitory activities were taken into account (Kuhar et al., 1999). Modification of the 2β-ester moiety by bioisosteric oxadiazole and isooxazole rings produced some of the most potent derivatives. Compound **6c** is an isooxazole derivative which exhibited potency in the sub-nanomolar range (Kotian et al., 1996). Similarly, replacement of 2β-carboxy methyl ester either by an appropriate different ester or amide as in **6a** and **6b** also produced highly potent compounds for the dopamine transporter. However, selectivity of **6b** for the dopamine transporter compared to the serotonin transporter was higher than **6a** (Singh, 2000). Further modification at

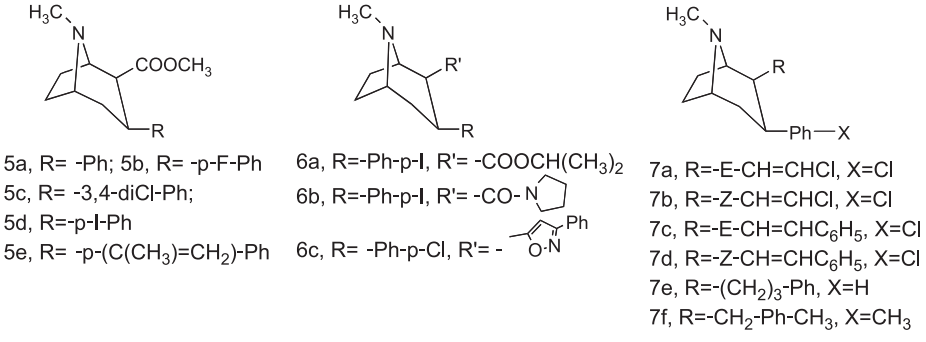

Fig. 3. Tropane analogs with different substitutions at the 2- and 3- position.

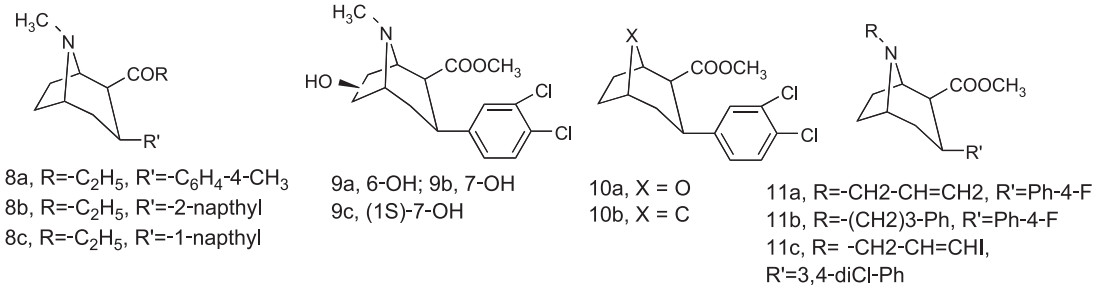

Fig. 4. Tropane derivatives with structural modifications at the 2-, 3-, 6-, 7-, and 8- position.

the 2-position by substitution with alkene moieties yielded interesting results. In the case of unsymmetrical alkenes **7a**–**c**, it was the Z-isomer which exhibited higher potency compared to the E-isomer (Kozikowski et al., 1995). The order of potency was **7d**>**7b**>**7c**>**7a**. On the other hand, replacement of 2β-carbomethoxy group by a phenyl-alkyl moiety as shown in **7e** and **7f** did not affect their potency (Xu et al., 1997, 2002). In general, substitutions at the 2-position yielded selective compounds for the dopamine transporter.

A novel entry into the tropane ring structure was developed by Davies et al. (1996) based on the Rhodium-catalyzed cyclization reaction between methyldiazobutenoate and a pyrrole derivative providing the desired tropane derivatives (Fig. 4). This approach resulted in the development of a series of potent compounds as illustrated in representative structures **8a**–**c** (Davies et al., 1996, 2001). Introduction of a tolyl group at the 3-position as in **8a**, also known as 2β-Propanoyl-3β-[4-tolyl]tropane (PTT), enhanced the activity for the dopamine transporter. Further substitutions with the isomeric α- and β-napthyl groups produced higher potency but less selectivity for the dopamine transporter. The order of activity was **8b**>**8c**>**8a**. Compound **8b** was found to be one of the most potent tropane derivatives known to date. Several 6/7-hydroxylated tropane derivatives were developed and the most potent compounds had 3β-3′,4′-dichloro-phenyl-substitution. 7-Hydroxylated derivative **9b** was more potent than 6-hydroxy derivative **9a** for the dopamine transporter (Zhao et al., 2000; Meltzer et al., 2001). Interestingly, it was found that the N-atom in the nor-tropane structure can be replaced by either an O- or C-atom, 10a and 10b, with retainment of activity (Meltzer et al., 1997, 2000). Various N-analogues of tropane derivatives were synthesized and biologically characterized. *N*-Allyl derivatives retained good potency and incorporation of a bulky substituent as shown in **11b** was also well tolerated (Millius et al., 1991).

Structurally constrained derivatives of the tropane structure were produced by fixing a tether to either the 3- or 2-carbon bridge of the tropane moiety with the N-atom (Fig. 5). Two of the most potent compounds **12** and **13** shown here have their *N*-lone pair of electrons oriented in the opposite direction (Smith et al., 1998). In another study, conversion of the tropane ring into a more flexible piperidine led to development of a series of 3,4-disubstituted derivatives. Both *cis*- and *trans*-isomers were developed and *cis*-isomers 14a-b were found to be more active (Kozikowski et al., 1998). Deviation from tropane skeleton structure resulted in the design of phenyl-bicyclo derivatives with [2.2.2] and [2.2.1] configurations. Compounds **15a** and **15b** are two such molecules in the [2.2.1] series in *trans* and *cis* configurations. Compound **15a** has a *trans*-endo structure, 4,5-dichloro-substituted, and exhibited high potency for the dopamine transporter whereas compound **15b** possesses a *cis*-exo structure with 4-chloro substitution, exerting appreciable potency for the dopamine transporter (Deutsch et al., 1999).

2.1.2. Behavioral activity

Following extensive structure–activity relationship studies as described above, several lead molecules were subjected to animal studies in various behavioral paradigms, yielding a wealth of in vivo information for this class of compounds. Among the molecules studied, compounds 3β-(4-chlorophenyl)tropane-2β-carboxylic acid phenyl ester (RTI-113) and PTT (Fig. 6) are of special interest. Both

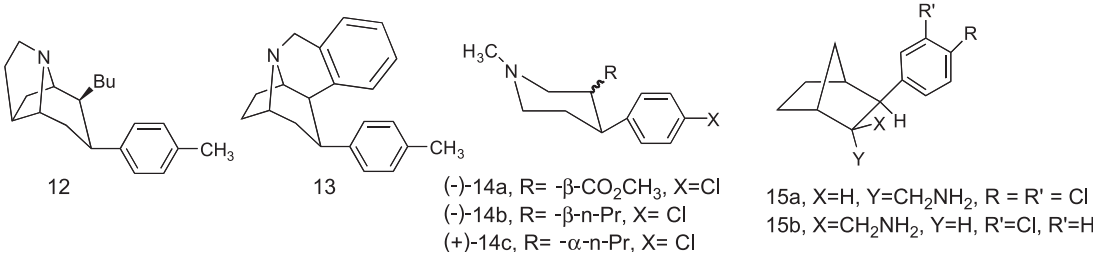

Fig. 5. Structurally constrained and flexible tropane analogs.

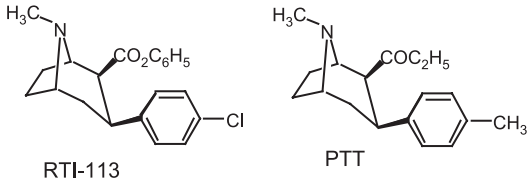

Fig. 6. Molecular structures of RTI-113 and PTT.

compounds were able to reduce self-administration of cocaine in primate experiments. Most likely, they produced some of the subjective effects of cocaine and thereby reduced the rate of cocaine self-administration. However, successful application of these drugs in substitution therapy is in doubt as they interfered with non-drug reinforcement in the same dose range that effected cocaine self-administration (Howell et al., 2000; Birmingham et al., 1998; Nader et al., 1997).

2.2. Benztropine analogs

2.2.1. Transporter activity

Benztropine (3α-diphenylmethoxytropane, cogentin) is a tropane-based dopamine uptake inhibitor that is also a potent anticholinergic agent. In the early 1970s, it was found that benztropine inhibits dopamine uptake (Horn, 1990), and now it is used clinically for the treatment of symptoms associated with Parkinson's disease. Structure–activity relationship studies were initiated by chemical modification at the *para*-position on phenyl rings which showed enhanced selectivity for the dopamine transporter. Following these initial results, detailed studies have been conducted generating more active and selective analogs at the dopamine transporter. Structure–activity relationship studies were directed to structural modifications at the 3-, 2-, 6-,7-positions and the *N*-position of the benztropane ring (Fig. 7).

The results indicated that the 3-position of the benzhydryl moiety prefers an axial stereochemistry contrary to that of cocaine and 3-aryl tropane analogs which prefer a 3β-equatorial position (Newman et al., 1995). Substitution, preferably at the 4-position on phenyl rings, can improve the potency and selectivity at the dopamine transporter, and the 4,4′-difluoro-3-(diphenylmethoxy)tropane (**16a**) (Fig. 7) is the most potent compound in this series of analogs (Newman et al., 1995; Kline et al., 1997). Substitution on the nitrogen atom of the tropane ring revealed that substantial bulk can be tolerated on this position. *N*-Butyl-phenyl substitution in **17a** produced the most potent and selective compound for the dopamine transporter. Potency decreases as the *N*-alkyl-phenyl chain length decreases, **17a**>**17b**>**17c**. Importantly, it was noted that a separation in the binding affinities at the dopamine transporter versus nonspecific muscarinic m_1 sites can be achieved by introducing alkyl and arylalkyl substitution on the nitrogen atom (Newman et al., 2001).

2-Carbomethoxy substitution in benztropine increased binding affinity and selectivity at the dopamine transporter, but unlike cocaine and 3-aryl tropane analogs, this substitution prefers S-configuration (Meltzer et al., 1994). In this series, the difluoro substituted compound **18a** was more potent than the dichloro counterparts **18b** similar to unsubstituted benztropine analogs. Replacement of the nitrogen with oxygen generated much weaker analogs, e.g. **19**, especially when compared with their 8-aza counterparts such as **10a** (Meltzer et al., 1997).

2.2.2. Behavioral activity

Benztropine analogues were generally much less potent than cocaine in producing locomotor activity despite their much higher affinity than cocaine for the dopamine transporter. In cocaine drug discrimination study, benztropine analogs were mostly very weak with an exception of fluoro-substituted molecules which produced cocaine-like behavior in animals trained to discriminate 10 mg/kg cocaine from saline (Kline et al., 1997; Katz et al., 1999). Taken together, these results indicate that the benztropine class of compounds has a different behavioral profile compared to cocaine and 3-aryl tropane analogs.

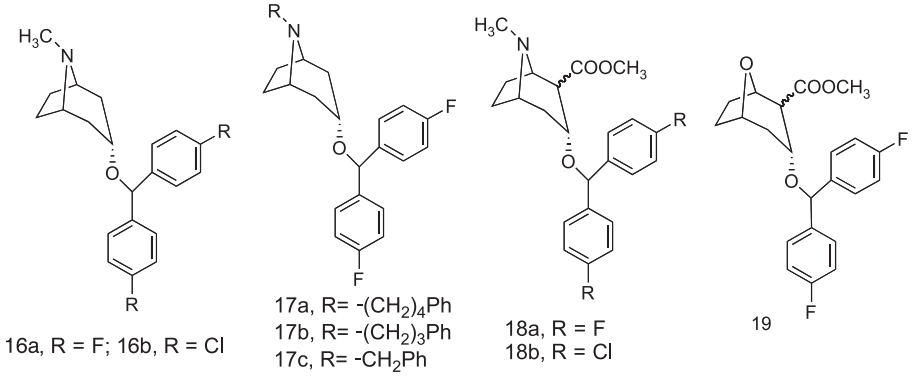

Fig. 7. Benztropine analogs with structural modifications at the 2-, 3-, 6-, 7-, 8- position.

2.3. 1,4-Dialkyl-piperazine GBR compounds and their piperidine analogs

2.3.1. Transporter activity

1,4-Dialkyl-piperazine substituted GBR compounds, originally discovered by Van der Zee et al. (1980), posses high affinity and selectivity for the dopamine transporter. Structure–activity relationship studies led to development of many potent analogs. In the original study of Van der Zee et al. (1980), several modifications were made, mainly in the distal 4-*N*-alkyl-aryl chain length along with aromatic substitutions, mostly in the diphenyl-methoxy moiety. Several potent derivatives were developed and the results demonstrated the importance of optimal *N*-propyl chain length and the effect of appropriate aromatic substitutions. Among the best known molecules that emerged from the initial studies are 1-[2-(diphenylmethoxy)-ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935), GBR 12909 and 1-[2-(diphenylmethoxy)-ethyl]4-(3-phenyl-2-propenyl)piperazine (GBR 12783) (Fig. 8) (Anderson, 1987, 1989). In general, unsubstituted aromatic moieties in the benzylhydroxy ether group produced more selective molecules for the dopamine transporter, whereas difluorosubstituted benzylhydroxy ether analogs were more potent but relatively less selective for the dopamine transporter. GBR 12935, while being more selective than GBR 12909 for binding to the dopamine compared to the serotonin transporter, exhibited comparable potency at the dopamine transporter. Further substitutions were made on the *N*-propyl side chain by introducing a hydroxyl functionality at the α- and β-positions (**20a** and **20b**) with respect to the phenyl ring which yielded hydroxylated derivatives. Some of these molecules exhibited almost two-fold higher potency compared to GBR 12909 and GBR 12935 (Lewis et al., 1996; Hsin et al., 2002). One of these hydroxyl derivatives was converted into a decanoate ester derivative, which exhibited slow-onset and long-duration of action (Glowa et al., 1996).

Alteration of the piperazine ring in GBR compounds, as shown in Fig. 8, yielded various derivatives with mixed potencies for the dopamine transporter. Introduction of a chiral methyl piperazine yielded potent compounds, e.g. **21a**, albeit less potent compared to GBR 12909 (Matecka et al., 1996). However, compound **21a** exhibited considerable selectivity compared to the reference compound GBR 12935. On the other hand, expansion of the piperazine ring into a seven membered ring also retained potency and in some cases, as in compound **22a**, exhibited higher potency and selectivity for the dopamine transporter (Matecka et al., 1996). Thus, compound **22b** was found to be six-fold more potent than GBR 12935 in inhibiting dopamine uptake and compound **22a** was 60-fold more selective in uptake activity at the dopamine compared to serotonin transporter. However, the activity in these compounds was somewhat compromised when the piperazine ring was opened up as shown in

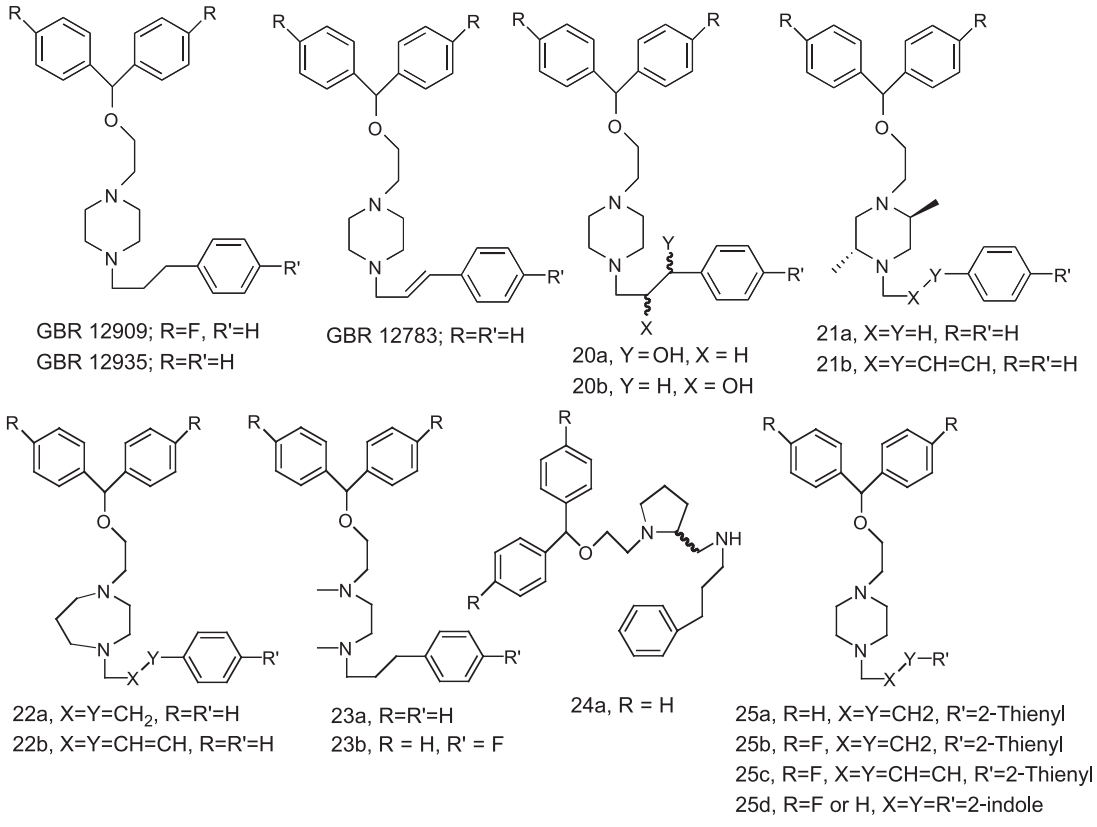

Fig. 8. Structures of lead compounds GBR 12909 and GBR 12935 and their analogs.

23a and **23b** (Choi et al., 1999; Matecka et al., 1996). On the other hand, the activity was maintained in a subset of optically active five membered pyrolidine ring derivatives like **24a** (Matecka et al., 1996). These results indicated a certain amount of tolerance to structural modifications in the central piperazine ring of GBR compounds.

Further structural modifications were made by replacing the pendant *N*-alkyl-aromatic ring by different bioisosteric aromatic heterocyclic moieties. These modifications were primarily made in order to reduce lipophilicity in these molecules. Replacement of phenyl by 2-thienyl in **25a**–**c** produced the highest potency in fluorosubstituted **25b** and **25c**, whereas as found before, the unsubstituted version **25a** produced less potency but higher selectivity (Matecka et al., 1997). In further study, replacement of the distal phenyl propyl moiety by benzo heterocycle derivatives yielded the most potent derivatives with 2-indole substitution as in **25d** (Matecka et al., 1997).

In a further structure–activity relationship study with GBR derivatives, it was demonstrated that only one of the N-atoms in the piperazine ring is required for activity and that the N-atom located distal to the diphenyl-methoxy moiety, compound **27a** in Fig. 9, is required for potency and selectivity at the dopamine transporter (Dutta et al., 1993; Madras et al., 1994). Consequently, compound **26** with the N-atom proximally located was less potent and selective for the dopamine transporter. In addition, this new piperidine analogue was shown to preferentially target the dopamine transporter since it did not show the non-selective piperazine binding activity which contributes as much as 30% to the binding of conventional GBR compounds to brain preparations under commonly used assay conditions (Madras et al., 1994). In further structure–activity relationship studies, it was shown that these piperidine analogs do not quite follow the structure–activity relationships as found in conventional GBR compounds. For example, some of the highest selectivity and potency in piperidine analogs for the dopamine transporter were exhibited in *N*-benzyl substituted compounds contrary to the *N*-propyl substitution required for optimum activity and selectivity in classical GBR compounds (Dutta et al., 1996, 1997, 1998b). Most of these novel piperidine derivatives were much more selective than GBR 12909 in interacting with the dopamine transporter. Some of the highest selective and potent compounds are shown in Fig. 9. Generally, pendant benzyl moiety substituted with electron withdrawing groups produced the most selective and potent molecules for the dopamine transporter. Binding affinity of compounds **28a** and **28b** were comparable to GBR 12909 but their selectivity for the dopamine compared to serotonin transporter was far greater than that of GBR 12909 (Dutta et al., 1997, 2001a). In this series of compounds, the rank order of potency was **28c**>**28b**>**28d**>**28a**. Bioisosteric replacement with a thiophene moiety (**29a**, **b**) maintained potency and selectivity in

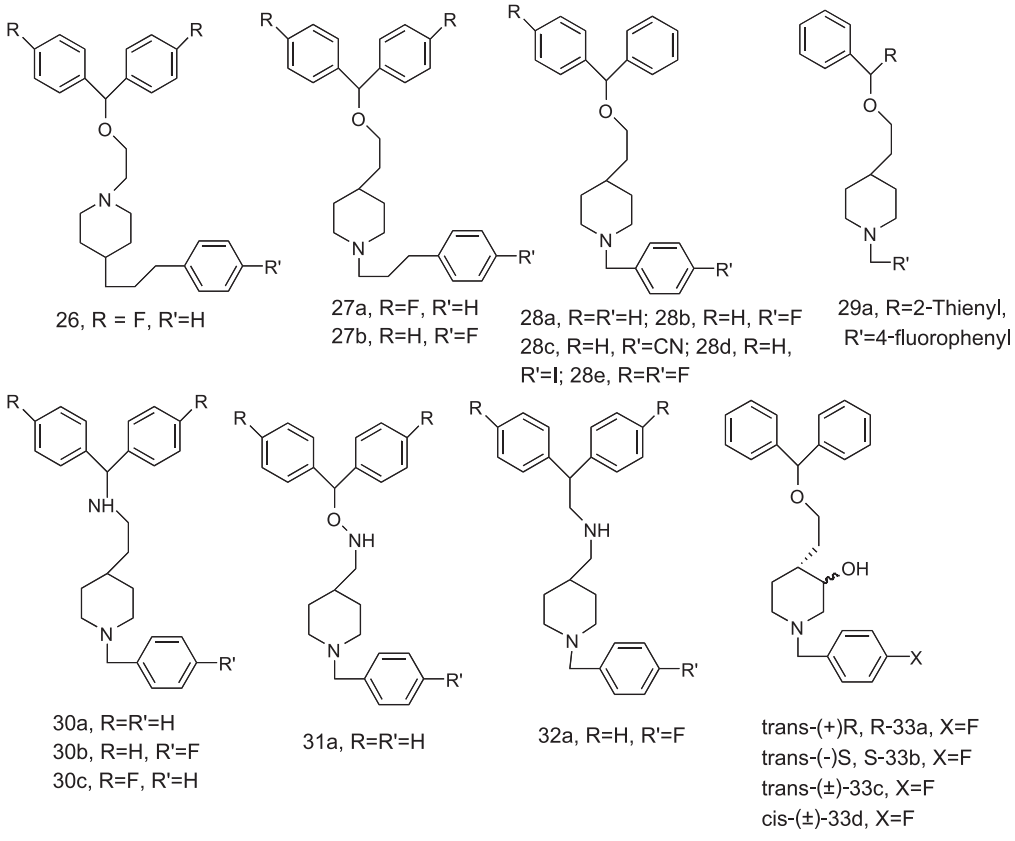

Fig. 9. Structures of piperidine analogs of GBR compounds.

these bioisosteres. Compound **29a**, which ranks among some of the most potent piperidine analogs such as **28a**, incorporates bioisosteric replacement of one of the phenyl rings by a thiophene moiety (Dutta et al., 1997). In another bioisosteric replacement of the benzhydrylether moiety with benzhydrylamine as shown in **30a**–**c**, activity was retained and in some cases, potency was increased compared to their oxy-counterpart (Dutta et al., 1998a, 2001a). Thus, in this series, **30a** was more potent than corresponding **28a** in the oxy-series. Modification of ethylene oxide side chain into an oxime derivative in **31a** did not appreciably alter the binding profile, and relocation of the N-atom in **30b** to an adjacent position as shown in **32a** did not alter the potency for the dopamine transporter but reduced the selectivity appreciably (Dutta et al., 2001a,b). In another study, introduction of a hydroxyl group in the 3-position of the piperidine ring of **28b** produced *cis* and *trans* isomers (**33a**–**d**) in which the racemic *trans* isomer **33c** exhibited higher potency than the racemic *cis*-**33d**. Moreover, optically pure (+)-*RR*-*trans*-**33a** was found to be one of the most potent and selective compounds for the dopamine transporter known to date (Ghorai et al., 2003).

In the next phase of the development of piperidine analogues, structurally constrained derivatives were designed. Thus, one of the flexible open-chain analogues (**32a**) was converted to generate structurally constrained novel *cis*- and *trans*-3,6-disubstituted piperidine derivatives which showed preferential affinity for the dopamine transporter in their *cis*-isomeric form (Dutta et al., 2001b). Further, structure–activity relationship studies confirmed that this novel cis template possesses affinity and selectivity for the dopamine transporter (Kolhatkar et al., 2003). Among these compounds, optically active fluoro and cyano substituted analogues exhibited the highest activity, **34a**>**34b**>**34c** (Fig. 10). Further conversion of this structurally constrained piperidine template led to development of bioisosteric pyran derivatives which maintained their activity for the dopamine transporter in their *cis*-isomeric form **35a-b** (Zhang et al., 2003).

2.3.2. Behavioral activity

It has been shown that GBR 12909 can attenuate the increase in extracellular dopamine induced by cocaine as measured in microdialysis experiments in rat brain, which might indicate its possible partial agonist properties against cocaine action (Rothman et al., 1991). GBR 12909 has been shown to produce the discriminative stimulus effect of cocaine. In self-administration study, GBR 12909 decreased cocaine self-administration without modulating food reinforcement (Glowa et al., 1995, 1996b). A recent study showed that GBR 12909 in low doses is not self-administered, and another study demonstrated that GBR 12909 has much less reinforcing potential when compared to cocaine (Tella et al., 1996). Taken together, these experimental lines of evidence suggest that a suitable GBR-type compound might have the potential to act as a pharmacotherapeutic agent in the treatment of cocaine addiction.

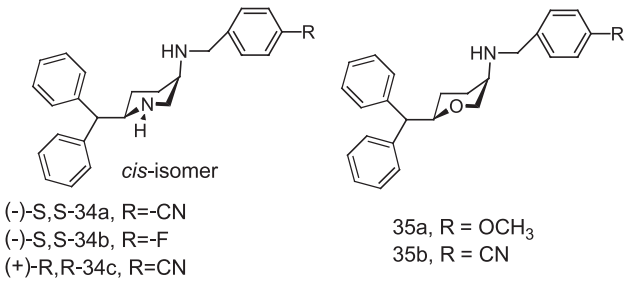

Fig. 10. Molecular structures of constrained piperidine and pyran derivatives.

2.4. Methylphenidate analogs

2.4.1. Transporter activity

Methylphenidate was first synthesized in 1944 and was recognized as a stimulant in 1954 (Meier and Tripod, 1954). DL-Threo-Methylphenidate (Ritalin) is the drug of choice for symptomatic treatment of ADD (Attention Deficit Disorder).

Structure–activity relationship studies on methylphenidate analogs have focused on modification of the piperidine ring and introduction of different aromatic substitutions. Due to the presence of two asymmetric centers, methylphenidate can exist in four isomeric forms. Within a pair of diastereomers, DL-threo **36a** (Fig. 11) was found to be more potent than DL-*erythro* **36b** at the dopamine transporter. In these threo-isomers the D form is the most active isomer possessing almost 14 times higher potency than its L-conjugate.

Apart from the separation of diastereoisomers and enantiomers, most research in this area has been focused on the modification and substitution of the phenyl ring in methylphenidate. All derivatives with substituents in the 2-position (**37a**–**e**) are much less active than those with the same substituents at the 3- or 4-position. This effect roughly correlates to the size of substituent and thus appears to be steric and not electronic in nature (Deutsch et al., 1996; Gatley et al., 1996). Thus, loss in activity (expressed as ratio of IC_{50} for position 2/4) was largest with methoxy (1200) followed by bromo (270), chloro (95), and fluoro (41). On the other hand, 3- and 4-substituted compounds **38a**–**g** and **39a**–**j** with halogen substitutions showed highest binding potency with bromine substitution being the most potent derivative. The relative order of activity for 3-substituted compounds was **38g**>**38a**>**38c**>**38b**>**38d**>**38f**>**38e**; whereas for the 4-substituted derivatives, the order of activity was **39j**>**39g**>**39a**>**39c**>**39d**>**39b**>**39f**>**39h**>**39i**. It is evident from this results that the presence of either electron withdrawing or electron donating substitutions at the 3- or 4-positions failed to produce the most active derivatives. Substitution at the 3-position produced compounds **38a**–**g** with a potency similar to or greater than substitution at the 4-position. 3, 4-Disubstituted compounds **40a**–**c** proved to

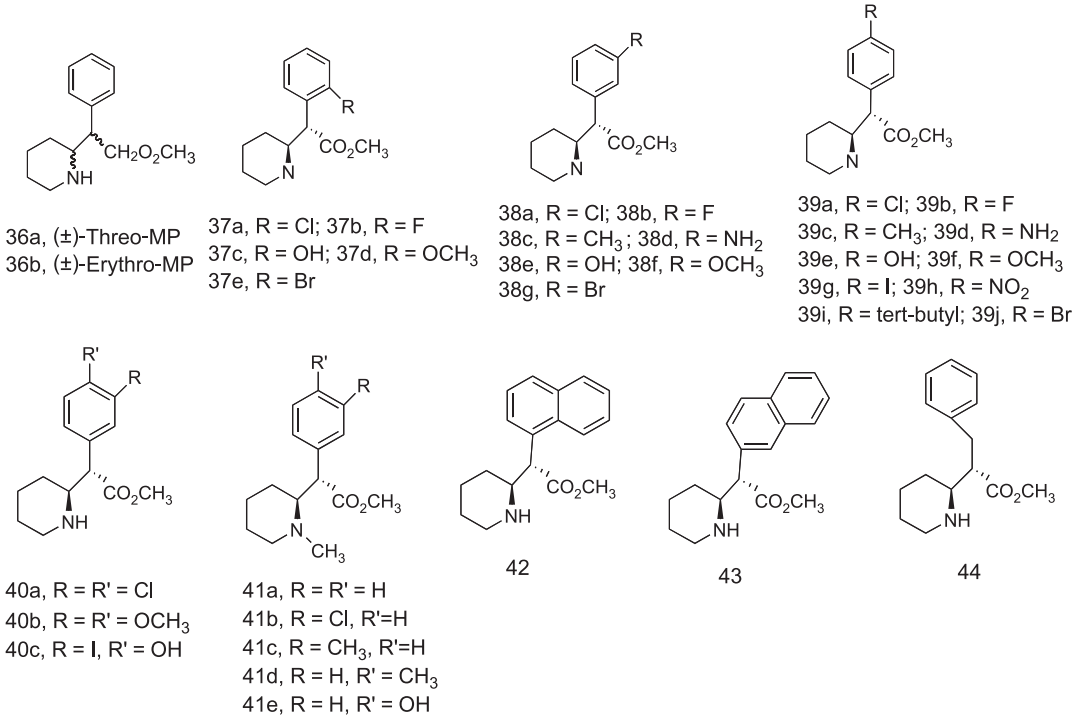

Fig. 11. Structures of methylphenidate and its analogs with modified aryl group.

have similar or less activity as 3-substituted compounds (Deutsch et al., 1996). *N*-methyl derivatives of methyl phenidate **41a**–**e** proved to be less active, reducing the potency from 4- to 30-fold as compared to unsubstituted derivatives which indicated that an equatorial *N*-methyl group may impair binding to the dopamine transporter (Froimowitz et al., 1997).

Replacement of the phenyl ring by napthyl moieties increased activity at the dopamine transporter. A seven-fold increase in binding was achieved with the 2-napthyl analog (**43**) relative to methyl phenidate (Deutsch et al., 2001). 1-Napthyl analog (**42**) was much less active as compared to the 2-napthyl analog. Changing the aryl moiety from phenyl to benzyl (**44**) significantly attenuated binding affinity (Axten et al., 1998).

Dopamine transporter affinity was also found to be sensitive to subtle changes in the piperidine ring size. Either increasing or decreasing the size of the ring (45–47) attenuated binding by 5- to 10-fold (Axten et al., 1998; Deutsch et al., 2001) compared to methylphenidate. Exchange of N by O (**49a**–**b**) proved to be interesting. Although in most cases, substitution produced less active compounds, the 3,4-dichloro analogs (**49a**, **b**) showed good activity. These derivatives maintained diastereoselectivity as observed with methylphenidate when the threo isomer (±)-**49a** was 10 times as potent as the corresponding erythro isomer (±)-**49b**. The threo isomer also showed enatioselectivity when (−)-threo 3, 4-dichloro analog of (±)-**49a** was found to be twice as potent as the (+)-threo analog (Meltzer et al., 2003) (Fig. 12).

2.4.2. Behavioral activity

Analogous to in vitro findings the D-threo isomer was more active than the L-threo isomer when tested for the ability to induce locomotor activity (Patrick et al., 1987). When administered by the intravenous route to laboratory animals, methylphenidate appears to have reinforcing effects equivalent to those of cocaine (Bergman et al., 1989). Its binding affinity for the dopamine transporter is almost two times greater when compared with cocaine. This

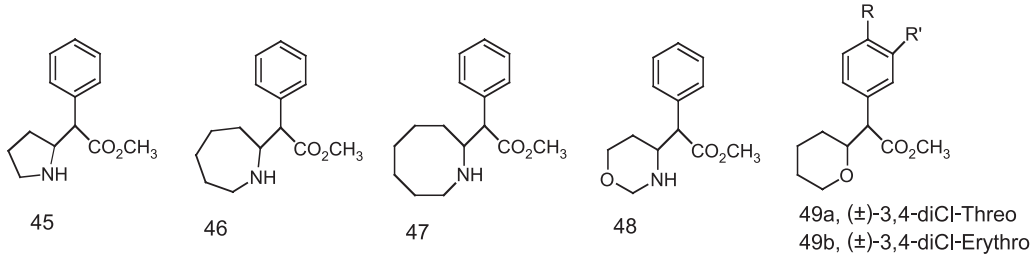

Fig. 12. Structures of modified piperidine ring analogs of methylphenidate.

correlates with its in vivo potency to block the dopamine transporter in human brain. However, its abuse liability in humans appears to be substantially lower (Volkow et al., 1999). A possible explanation for this is the longer in vivo retention of methylphenidate compared with cocaine as the half life in human brain after intravenous administration is 90 min for methylphenidate and 20 min for cocaine (Volkow et al., 1995). Recently, several methylphenidate analogs with high affinity for the dopamine transporter were tested in the drug discrimination assay. A positive correlation was obtained between potency at the dopamine transporter and the extent of generalization in the cocaine drug discrimination assay (Schweri et al., 2002).

2.5. Mazindol analogues

Mazindol, an imidazoisoindol derivative, is a tricyclic compound which was first synthesized in 1968 as a compound designed to have properties similar to those of dexamphetamine without the addiction or other side effects (Gogerty et al., 1968). Later on, it was developed as an anorectic agent and it is currently marketed for management of exogenous obesity and as an orphan drug for the treatment of Duchenne muscular dystrophy. It alters the concentration of biogenic amines in the brain by inhibiting the uptake of dopamine, norepinephrine and serotonin (Javitch et al., 1984; Angel et al., 1988).

Mazindol is known to exist in keto-enol tautomeric forms. The tricyclic form is favored in neutral media (95% ethanol) and the protonated benzophenone (keto) tautomer exists in acidic media (Houlihan et al., 1998). Since dopamine binding and uptake assays for WIN 35428 are routinely carried out at a pH close to 7.4, the 'ol' form of a mazindol analogue is expected to be the predominant tautomer in solution. Relatively low affinity of mazindol analogs that exists solely in the 'keto' form suggests the importance of 'ol' form for binding. Extensive structure–activity relationship studies have been carried out by introducing various aromatic ring substitutions and by altering the heterocyclic imidazole ring size.

Introduction of various substitutions in the 4′-position of ring D produced a variety of activities in these analogues (Fig. 13). Among the halogen substitutions, 3′,4′-dichloro and 4′-bromo substitutions produced the highest potency and the order of potency was **50d**>**50b**>**50a**>**50c** (Houlihan et al., 1996). 3′,4′-Methylenedioxy compound **50g** was moderately potent in this series and was more potent than **50f** (Houlihan et al., 2002). The lower activity in compounds **50f** and **50g** may be due to the presence of a significant amount of the keto form. Aromatic substitution in ring C produced differentially active derivatives in the representative series **51a**–**e**. The order of activity was **51c**>**51a**>**51d**>**51b**>**51e**. Expansion of the five-membered imidazole ring into a corresponding six-membered ring generally increased the binding activity by more than two- to three-fold. For example, compound **52a** was almost four-fold more active compared to mazindol, whereas compound **52e** was 50-fold more active compared to **50g**. Further expansion of the five-membered imodazole ring into a seven-membered ring did not further improve binding.

The literature is equivocal regarding the addictive potential of mazindol (Chait et al., 1987). In the clinic, mazindol was found to be non-addictive and it did not produce euphoria. Mazindol maintained self-administration behavior only in a subset of all monkeys tested.

2.6. Phencyclidine analogues

The dissociative anesthetic phencyclidine (*N*-(1-phenylcyclohexyl) piperidine, PCP) has complex interaction in the central nervous system resulting in a complex behavioral profile in human and animal studies. Behavioral effects of PCP have been attributed to its binding to both the *N*-Methyl-D-aspartate (NMDA) ion-channel complex and the dopamine transporter molecule (Vignon et al., 1988). As a result of its activity at the dopamine transporter, several structure–activity relationship studies were conducted with PCP derivatives resulting in a number of selective compounds for the dopamine transporter.

Derivatization of the phenyl ring in PCP with a nitro group as shown in **53b** resulted in moderate loss of potency at the dopamine transporter, whereas the loss of potency for interacting with the PCP binding site was more than 40-fold. In the case of *m*-hydroxy substitution, the effect was reversed. Thus, compound **53c** gained eight-fold more activity at PCP binding sites but was approximately two

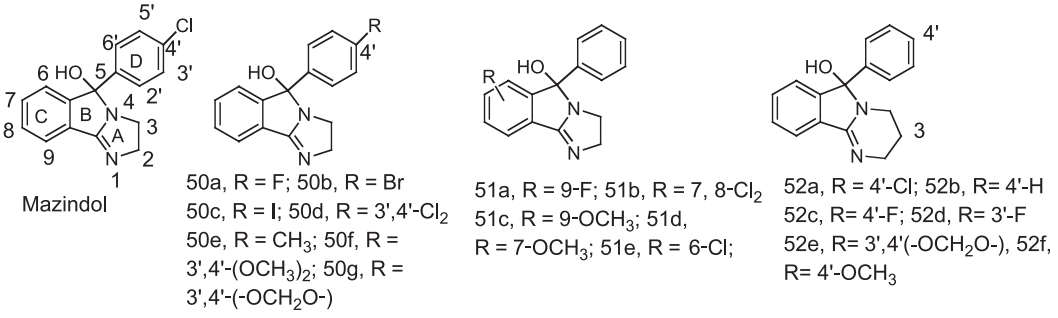

Fig. 13. Molecular structures of various mazindol derivatives.

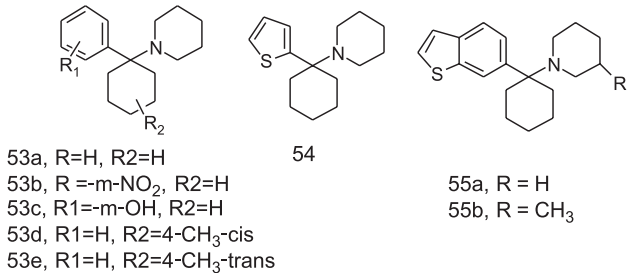

Fig. 14. Molecular structures of phencyclidine and its derivatives.

and half-fold less active at dopamine transporter sites (Chaudieu et al., 1989). Substitution in the cyclohexane ring as shown in **53d** and **53e** did not have a major impact on binding activity compared to the parent molecule (Chaudieu et al., 1989).

Interesting structure–activity relationship result was obtained when the phenyl ring in PCP was replaced by a thiophene or benzothiophene ring. Replacement of the phenyl ring by a thiophene moiety resulted in the production of *N*-[1-(2-thienyl)cyclohexyl]piperidine (TCP), compound **54**, which exhibited a 10-fold increase in potency at PCP binding sites and loss of activity at dopamine transporter sites. On the other hand, replacement by a benzothiaphene ring, compound **55**, produced *N*-[1-(2-benzo(*b*)thiophenyl)-cyclohexyl] piperidine (BTCP) which exhibited an enhancement of potency for the dopamine transporter by 62-fold (Chaudieu et al., 1989). Further structure–activity relationship studies were conducted with BTCP analogues with an effort to produce higher-affinity analogues. Modification of the carbocyclic ring led to lowering of potency compared to BTCP (He et al., 1993). Similarly, extensive modification of the amino group was tried which generally led to lower activity. However, methyl substitution at the 3-position in the piperidine ring increased activity compared to BTCP (Billaud et al., 1994) (Fig. 14).

In vivo, both PCP and BTCP produced locomotor stimulation like cocaine (Koek et al., 1989). In pigeons, PCP-like catalepsy was not observed with BTCP indicating a dissociation in behavioral activity from PCP. BTCP, like PCP, produced generalization in rats discriminating cocaine from saline (Koek et al., 1989).

3. Concluding remarks

It is clear from the above overview that intense drug development efforts have targeted the dopamine transporter in order to find leads for medications useful in the treatment of cocaine dependence. Progress has been made in the area of potential leads for a substitution therapy, with compounds identified that have a slow onset and long duration of action, such as RTI-113 (Fig. 6), PTT (Fig. 6), and GBR 12909 (Fig. 8) in decanoate form. Structural refinements will be needed to enhance behavioral selectivity towards attenuating cocaine—over non-drug-reinforcement, and to parcel out the contribution, or adverse effect, of norepinephrine uptake inhibitory activity that is inherent in all of the current leads. It has turned out to be much more difficult to find a cocaine antagonist (at the level of the dopamine transporter) that could be used as a deterrent treatment to avoid relapse to cocaine use or as a detoxifying agent in treating cocaine overdose. It is possible that the inhibitor-type molecules searched for so far, even if those do not bind to the recognition sites for dopamine, induce dopamine transporter conformations that do not allow dopamine translocation, a process that likely requires a succession of conformational changes exposing the substrate site either to the external interstitial compartment surrounding, or the internal cytosol within, nerve cells carrying the dopamine transporter.

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