Dopamine D_5 Receptors: A Challenge to Medicinal Chemists

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Abstract: Due to the lack of highly selective dopamine D_1 or D_5 receptor ligands, only few data about activation or blocking of these receptor subtypes are available. The present review collects the available information about molecules with notable affinity for D_5 receptor subtype with the purpose to help the researchers to design novel D_5 selective ligands, whose discovery may enrich the knowledge about the physiological function of such a receptor, provide information about its topography, as well as lead to novel potential therapeutic tools.

Key Words: Dopamine receptor ligands, D_1 subtype, D_5 subtype, structure-activity relationship (SAR), pharmacophore.

INTRODUCTION

Dopamine (DA) is a neurotransmitter interacting with specific receptors widely distributed both in peripheral and central nervous system [1-3]. Since their discovery, DA receptors have been considered attractive therapeutic targets because dysfunction of the dopaminergic signal transduction is strongly involved in neuropsychiatric disorders, such as schizophrenia, drug dependence, and Parkinson's disease [4].

On the basis of pharmacological studies, performed with several different classes of DA receptor ligands, DA receptors have firstly been divided into two types, named D_1 and D_2 [5-8]. Later, molecular cloning studies identified five distinct genes encoding the DA receptors (D_1, D_2, D_3, D_4, and D_5), all belonging to the superfamily of G protein coupled receptors (GPCRs). D_1 [9] and D_5 [10, 11] receptors in humans, corresponding to D_{1A} and D_{1B} respectively in rodents, belong to the D_1-like family, whose activation is linked to the stimulation of cAMP synthesis. Instead, D_2 [12], D_3 [13], and D_4 [14] receptors are included in the D_2-like family, which is linked to the inhibition of cAMP synthesis or coupling to other effector systems. Analysis of the amino acid sequences of the cloned DA receptor subtypes reveals that the members, belonging to the same family, share most of their structural features [15].

D_1-like receptors are widely distributed in different areas of the brain and are involved in cognition, sleep, motor control, endocrine function, behavioural reinforcement and sensitization, under both normal and pathological conditions. In particular, in the last few years, new information concerning distribution and role of D_5 receptors has been reported. Ciliax *et al*. found that D_{1B} immunoreactivity was widespread in rat brain, including perikarya and proximal dendrites in cortex, basal forebrain, hippocampus, basal ganglia diencephalons, brainstem, and cerebellum [16, 17]. Preliminary studies developed on D_5 receptor "knockout" mice suggested that D_5 receptors exert subtle, but not negligible, regulation of discrete topographies of behavior [18]. Zeng *et al*. reviewed studies on D_5 receptor null mice showing that the disruption of the D_5 receptors results in hypertension. Such an effect is probably caused by the activation of the sympathetic nervous system consequent to the stimulation of oxytocin, V_1 vasopressin, and non-NMDA receptors in the central nervous system, indicating that D_5 receptors play a role in blood pressure regulation [19]. Therefore, D_5-selective ligands have been suggested to be useful tools for the treatment of hypertension [20]. Moreover, it has been reported that activation of DA D_5 receptors in the ventral tegmental area (VTA) neurons may contribute to the addictive properties [21] and locomotor stimulant effects of cocaine [22], suggesting that DA D_5 antagonists might be employed in the treatment of cocaine addiction. Recently, DA D_5 receptor subtype has been shown to mediate DA action on male and female sexual behavior, perhaps *via* a reward pathway [23].

Over the last twenty years, several structurally different compounds have been developed as ligands more or less selective toward one DA receptor subtype. Some of them proved to be clinically useful tools for the treatment of neurological diseases. While ligands selective for D_2, D_3 and D_4 receptors are well known and a rich panel of data concerning the topographic requirements of these DA receptor subtypes is available from the literature [4], the discrimination between D_1 and D_5 receptors is still an almost unexplored field. The aim of the present review is to collect the available information about molecules with remarkable affinity for DA D_5 receptor subtype with the purpose to help the researchers to design novel D_5 selective ligands, whose discovery may enrich the knowledge about the function of such a receptor, provide information about its topography, as well as lead to novel potential therapeutic tools.

DA D_5 RECEPTOR LIGANDS

Several examples of agents selective for the D_1-like receptors are known [24], but only few data about the selective activation or blocking of one of the two subtypes belonging to this receptor family are available. The 80% sequence homology within the highly conserved seven transmembrane spanning domains of DA D_1 and D_5 receptors [25, 26] is probably responsible for the lack of highly selective D_1 or D_5 receptor ligands.

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1389-5575/08 $55.00+.00

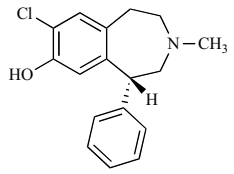
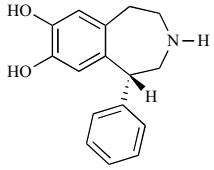
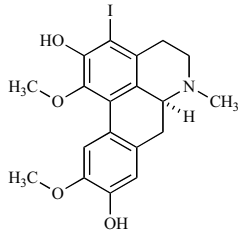
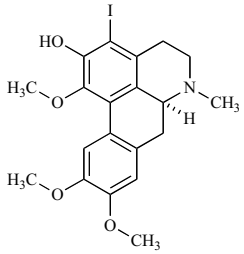
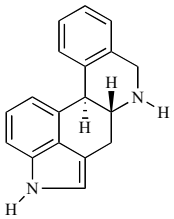
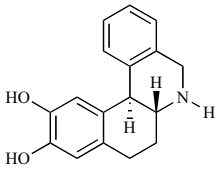
Table 1. Affinities (K_i, nM) for [^{3}H]SCH23390 Binding to D_1 and D_5 Receptors in COS-7 Cells. Data from Ref. [10]

	D_1	D_5	D_1/D_5
Agonists			
(+)-SKF82526	28.0	15.0	1.86
SKF38393	150	100	1.50
β-Ergocriptine	-	113	-
Dopamine	2,340	228	10.29
Apomorphine	680	363	1.87
Bromocriptine	672	454	1.48
SKF76783	645	530	1.22
(-)-SKF82526	1,818	800	2.27
(+)-ADTN	4,600	909	5.06
Pergolide	1,363	918	1.48
N0437	2,172	986	2.20
NPA	1,816	1,136	1.59
Serotonin	9,690	3,000	3.23
Noradrenaline	50,000	12,000	4.16
LY171555	>20,000	>20,000	-
Antagonists			
SCH23390	0.35	0.30	1.17
SKF83566	0.30	0.40	0.75
α-Flupenthixol	4.0	8.0	0.50
Fluphenazine	21.0	14.0	1.50
(+)-Butaclamol	3.0	27.0	0.11
SCH23388	41.0	35.0	1.17
Haloperidol	27.0	48.0	0.56
Chlorpromazine	73.0	133	0.55
Clozapine	141	250	0.56
Thioridazine	100	300	0.33
Ketanserin	190	2,500	0.07
Spiperone	220	4,500	0.05
Etichlopride	18,000	19,000	0.95
(+)-Sulpiride	20,454	28,636	0.71
(-)-Sulpiride	36,000	77,270	0.46

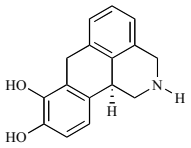
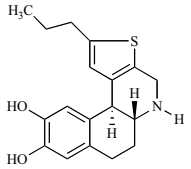
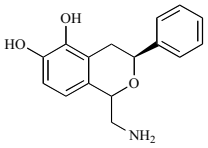
After the cloning of the D_5 receptor, several DA agonists and antagonists were tested for their affinity for DA D_1 and D_5 receptors expressed in COS-7 cells (Table **1**) [10].

D_5 receptor bound the tested drugs with a pharmacological profile similar to that of the cloned D_1 receptor, but displayed a 10-fold higher affinity for the endogenous agonist DA, providing the first evidence of discrimination between the two different DA D_1-like receptor subtypes. Such an evidence was in agreement with the results of the study by Tiberi *et al*. who reported that membranes of cells expressing

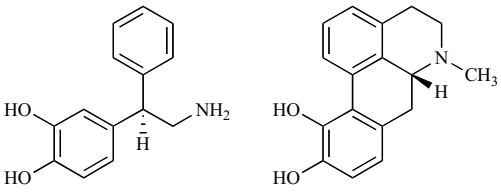
Table 2. Affinities (K_i, nM) for Compounds 1-9

Compd.	D_1-like			D_2-like		
	D_1	D_5	D_1/D_5	D_2	D_3	D_4
 **1: SCH23390**[a]	~0.2	0.3	0.67	~1,100	~800	~3,000
 **2: SKF38393**[b]	1.0	~0.5	2.0	~150	~5,000	~1,000
 **3: Iodo-Boldine**[c]	2.0		-	68.0		
 **4: Iodo-Predicentrine**[c]	6.0		-	831		
 **5: CY208243**	417[d]	40.0[d]	10.4	92.0[e]		
 **6: Dihydrexidine**	2.2[f]	14.0[g]	0.16	180[h]	15.0[h]	14.0[g]

(Table 2. Contd….)

Compd.	D_1-like			D_2-like		
	D_1	D_5	D_1/D_5	D_2	D_3	D_4
 **7: Dinapsoline**	5.5[f]	10.0[g]	0.55	140[j]	10.0[g]	60.0[g]
 **8: A86929[k]**	51.0	15.0	3.4	750	1,000	350
 **9: A68930[i]**	3.0		-	776		

[a] data from Ref. [37]; [b] data from Ref. [38]; [c] data from Ref. [39]; [d] data from Ref. [40]; [e] data from Ref. [45]; [f] data from Ref. [41]; [g] data from Ref. [42]; [h] data from Ref. [43]; [j] data from Ref. [33]; [k] data from Ref. [44]; [i] data from Ref. [36].



10: Apomorphine

Fig. (1). Pharmacophore of D_1-like full agonists and structure of apomorphine.

D_{1B} receptors displayed higher affinities for agonists, such as DA, rather than those expressing D_{1A} receptors, whereas antagonist affinities were lower at the D_{1B} rather than at the D_{1A} receptors [27].

The core molecules that have shown DA D_1-like selective properties include: phenylbenzazepines [28], such as SCH23390 (**1**) and SKF38393 (**2**) and their constrained analogs [29]; aporphine alkaloids [30], such as iodo-boldine (**3**) and iodo-predicentrine (**4**); benzergoline [31], such as CY208243 (**5**); dihydrexidine (**6**) [32] and dinapsoline (**7**) [33]; isoquinoline and thieno[*c*]quinoline analogs [34, 35], such as A86929 (**8**); isochromans [36], such as A68930 (**9**) (Table **2**).

From structure-activity relationship studies the "*trans*-β-phenyldopamine", reported in Fig. (**1**), has been proposed as the pharmacophore for D_1-like full agonists. Such a pharmacophore was later confirmed by the synthesis of dihydrexidine (**6**) and dinapsoline (**7**). In addition, apomorphine (**10**), which embeds DA in a *trans*-α-rotameric conformation, behaves as a partial agonist at the D_1-like receptors in striatal tissue [45].

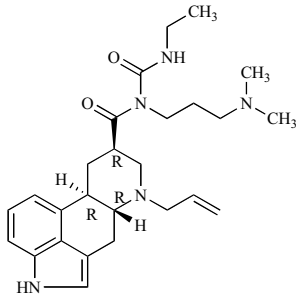
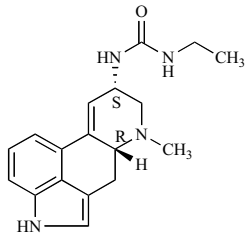
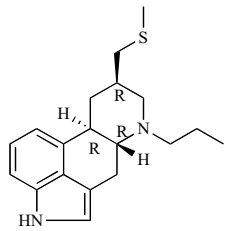
Although the structural requirements for D_1-like antagonism are less depicted, on the basis of molecular modeling studies, the size, shape and spatial orientation of the pharmacophore hydrophobic moiety seem to be important [46].

None of the ligands reported in Table **2** markedly distinguishes D_5 from D_1 receptors, except for CY208243, which is 10-fold selective for D_5 toward D_1.

CY208243 belongs to the family of ergoline derivatives, among which cabergoline (**11**), lisuride (**12**) and pergolide (**13**) are also included. Although the ergoline derivatives reported in Table **3** show higher affinity values for D_2-like receptor subtype family, within the D_1-like family, their D_5 receptor affinity has been reported to be from about 10- to 20-fold higher than that for D_1 [47].

From the binding data reported in the same study by Millan *et al*. [47] an interesting DA receptor binding profile was shown by apomorphine (**10**) and the aminotetraline TL99 (**14**) (Table **4**). Although these compounds displayed the highest binding affinity toward DA D_4 and D_3 subtypes, respectively, they both proved to be selective for D_5 with respect to D_1 (25-fold for **10** and 34-fold for **14**).

Table 3. Affinities, Expressed as pK_i (K_i, nM), of Cabergoline, Lisuride and Pergolide at Recombinant, Human DA Receptors. Data from Ref. [47]

Compd.	hD_1	hD_5	hD_{2S}	hD_{2L}	hD_3	hD_4
 **11: Cabergoline**	6.67 (214)	7.65 (22.4)	9.21 (0.62)	9.02 (0.95)	9.10 (0.79)	7.25 (56.2)
 **12: Lisuride**	7.19 (64.6)	8.45 (3.55)	9.47 (0.34)	9.18 (0.66)	9.55 (0.28)	8.34 (4.57)
 **13: Pergolide**	6.47 (339)	7.48 (33.1)	7.50 (31.6)	7.59 (25.7)	8.26 (5.50)	7.23 (58.9)

It has recently been reported that the introduction of an aryl group (phenyl, *p*-OH-phenyl, *p*-F-phenyl, or *p*-CH_3-phenyl) in the position 2 of apomorphine (**10**) decreased binding to the DA D_1 and D_5 receptors when compared to that of apomorphine. In particular, D_5 receptor affinity decreased (from 31- to 494-fold) more than D_1 affinity (from 1.7- to 12-fold) (Table **5**) [48].

Phenylbenzazepines have contributed to searches for DA D_1 receptor-based therapeutic agents. The agonist SKF38393 (**2**) and SCH23390 (**1**) that still remains the primary selective DA D_1-like receptor antagonist, represented a breakthrough in the characterization of D_1 receptor function. The most important structural feature of both of these benzazepines and their derivatives was the pendant phenyl ring, which

Table 4. Affinities, Expressed as pK_i (K_i, nM), of TL99 and Apomorphine at Recombinant, Human DA Receptors. Data from Ref. [47]

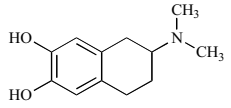
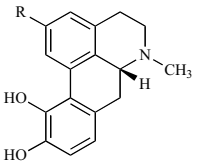
Compd.	hD_1	hD_5	hD_{2S}	hD_{2L}	hD_3	hD_4
 **14: TL99**	5.57 (2692)	7.11 (77.6)	7.22 (60.3)	7.17 (67.6)	8.60 (2.51)	7.24 (57.5)
10: Apomorphine	6.43 (372)	7.83 (14.8)	7.46 (34.7)	7.08 (83.2)	7.59 (25.7)	8.36 (4.37)

Table 5. Affinities (K_i, nM) of 2-Arylapomorphines on Cloned D_1 and D_5 Receptors. Data from Ref. [48]

	R	D_1	D_5
	H (**10**)	101[a]	10.0[a]
	C_6H_5	755	2359
	p-OH-C_6H_5	167	313
	p-F-C_6H_5	1217	4936
	p-CH_3-C_6H_5	1129	4255

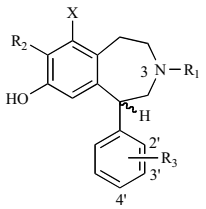
[a] data from Ref. [67].

appeared to impart D_1-like selectivity to this family of molecules. Later, structure-activity relationship studies demonstrated that such a phenyl ring was pseudoequatorially oriented and almost perpendicular to the substituted benzazepine phenyl ring [49].

Neumeyer *et al*. [28] reported that relatively minor changes in the 3- and 3'-alkyl, and 6-halo substituents of the partial agonist SKF83959 affected DA D_1 activity (Table **6**). Among the novel compounds, racemic MCL204 was the most potent DA D_1 receptor ligand (K_i = 0.11 nM) and displayed high selectivity for D_1 *vs*. D_2 receptors (762-fold) and *vs*. D_5 receptors (109-fold).

Racemic MCL209 was the most potent novel compound at D_5 receptors (K_i = 0.88 nM), yet it also retained high D_1 receptor affinity (K_i = 0.60 nM). Moreover, it showed the greatest D_1/D_2 receptor-selectivity (8333-fold). The greatest separation of DA D_1/D_5 receptor potencies (140-fold) was found with MCL201. The authors concluded that, although the number of the assayed compounds was too few to support secure structure-activity relationships, the compounds with high D_1 receptor potency had a halogen at the position 6; the methyl substitution on the accessory phenyl ring seemed to be optimal at position 3' and important for D_1 receptor interaction. The 3-*N*-substituent seemed to play a

Table 6. Receptor Potencies of Phenylbenzazepines. Data from Ref. [28]



Compd.		X	R_1	R_2	R_3	Receptor Potencies (K_i, nM)			
						D_1	D_5	D_2	D_3
MCL204	*RS*(±)	Br	$CH_2CH=CH_2$	OH	3'-CH_3	0.11	12.0	83.8	283
MCL203	*RS*(±)	Br	CH_3	OH	3'-CH_3	0.19	2.47	440	>10,000
MCL207	*RS*(±)	Cl	CH_3	OH	2'-CH_3	0.46	2.32	226	177
MCL202	*R*(+)	Cl	CH_3	OH	3'-CH_3	0.49	1.53	515	374
MCL210	*RS*(±)	Cl	$CH_2CH=CH_2$	OH	3'-CH_3	0.52	9.94	119	334
MCL209	*RS*(±)	Cl	H	OH	3'-CH_3	0.60	0.88	≥5,000	>10,000
MCL214	*RS*(±)	Br	CH_3	OH	2'-CH_3	1.10	3.42	409	467
SKF83959	*RS*(±)	Cl	CH_3	OH	3'-CH_3	1.18	7.56	920	399
MCL216	*RS*(±)	Br	H	OH	2'-CH_3	1.81	1.95	19.5	20.4
MCL206	*RS*(±)	Cl	CH_3	OH	4'-CH_3	1.93	3.96	362	>10,000
MCL205	*RS*(±)	Br	H	OH	3'-CH_3	4.41	13.7	1,072	>10,000
MCL212	*RS*(±)	Br	CH_3	OH	4'-CH_3	19.3	4.36	1,031	≥10,000
MCL201	*S*(-)	Cl	CH_3	OH	3'-CH_3	21.3	≥3,000	2,136	659

role as well: MCL209, which lacks an *N*-substituent, showed the highest potency at D_5 receptor, whereas the corresponding *N*-allyl (MCL204) and *N*-methyl (MCL203) derivatives were the most potent D_1 receptor ligands in the series. Moreover, in the authors' opinion the optical resolution of the most interesting racemic compounds and the pharmacological characterization of the obtained enantiomers might be of interest. In fact, it had previously been reported that receptor affinity in the 1-phenyl-1*H*-3-benzazepine series was associated specifically with *R* enantiomers [50]. From the data reported in Table **6** it can already be argued that the stereochemistry may play an important role in the discrimination between D_1 and D_5 receptors. In fact, considering the racemic form SKF83959 and its corresponding enantiomers (*R*)-(+)-MCL202 and (*S*)-(-)-MCL201, it can be observed that D_1 receptor affinity is only slightly affected by the stereochemistry, whereas for D_5 receptors the higher affinity resides in the *R* enantiomeric form MCL202.

Information about the orientation of the pendant phenyl ring has been provided by the preparation of conformationally restricted analogs of the D_1-like antagonist SCH23390 (**1**), in which the phenyl ring was unequivocally locked in the molecular skeleton [49]. The data reported in this study have clearly shown that the most interesting compound was the SCH39166 (**15**) and that the receptor affinity was associated with the conformationally rigid *trans* series. Moreover, among the four diastereoisomers, the 6a*S*,13b*R* isomer **15** (structure reported in Table **7**) had significantly higher D_1-like affinity and selectivity when compared with D_2-like receptors.

In a recent work by Wu *et al*. SCH23390 (**1**) and SCH39166 (**15**) have been used as lead compounds for the development of new phenol bioisosteric D_1-like ligands with improved pharmacokinetic profile (compounds **16**-**20**, Table **7**), to demonstrate the crucial importance of the directionality of the hydrogen bond donor group [29]. In such a study compound **15** had significantly greater D_1 and D_5 affinities when compared with D_2 and D_4 receptors.

Thus, the replacement of the phenol group of SCH39166 (**15**) with various heterocycles bearing hydrogen bond donor groups afforded the very potent D_1/D_5 benzimidazolones **16** and **17**, and benzothiazolones **18** and **19**. These compounds showed excellent D_1-like selectivity over D_2-like receptors. In sharp contrast, similar phenolic replacements in SCH23390 (**1**) dramatically decreased the binding affinity, presumably due to a conformational change of the pendant phenyl group. However, among the indazole derivatives of **1**, compound **20** proved to be a potent and selective D_1/D_5 ligand when compared with D_2 and D_4 receptor subtypes.

Several conformationally constrained compounds, such as dihydrexidine (**6**), dinapsoline (**7**), dinoxyline (**21**), and doxanthrine (**22**), embedding the pharmacophore of D_1-like full agonists "β-phenyldopamine" shown in Fig. (**1**), have been reported (Table **8**).

Dihydrexidine (**6**) was developed by Brewster *et al*. and showed a 10-fold selectivity for D_1 *vs*. D_2 receptors [32]. More importantly, it was the first full D_1 agonist. Moreover, it was observed that N-methyl, N-*n*-propyl and N-allyl substituents were detrimental for the affinity for D_1 sites and negatively affected the ability to stimulate adenylate cyclase. Tethering the two phenyl rings of dihydrexidine (**6**) through a methylene bridge, and removing the C(7)-C(8) ethano bridge led to dinapsoline (**7**) that conserved the relative orientation of all the essential elements of the hypothesized molecular pharmacophore necessary for high D_1 affinity and full intrinsic efficacy [33]. In fact dinapsoline (**7**) proved to be a high-affinity ligand at rat striatal D_1 receptors and was endowed with full intrinsic activity. To determine whether similar SAR existed between dinapsoline and dihydrexidine series, six analogs of dinapsoline (**7**) were synthesized (compounds **23**-**28** reported in Table **9**) and studied by Qandil *et al*. [52]. In such studies, affinity data of dihydrexidine (**6**), dinapsoline (**7**) and its six analogs for DA receptors were reported. As expected, N-allyl or N-*n*-propyl analogs showed a decreased affinity for both D_1 and D_5. The addition of a methyl group at position 6 (compound **25**) increased D_1/D_2 selectivity. Such a modification, as well as the addition of a methyl group to position 4 (compound **26**), did not strongly affect the D_1 and D_5 affinities.

Comparing the D_5 affinity values of dihydrexidine (**6**) and dinapsoline (**7**) reported in Table **9** it can be observed that the latter has 3-fold higher affinity for such a receptor subtype.

Using a bioisosteric approach and on the basis that an ether linkage could be substituted for the methylene tether of dinapsoline (7), Grubbs *et al*. [42] prepared dinoxyline (21) (structure reported in Table **8**). Compound 21 was the first ligand with high affinity for all DA receptors, and behaved as a potent full agonist at all of them. Thus, it may represent the first drug that can be considered a true high affinity DA replacement (Table **10**). Once again, the N-allyl and N-*n*-propyl derivatives of 21 showed a decrease in D_1 and D_5 affinity values. The incorporation of other heteroatoms, such as a sulfur or nitrogen, into the tether did not afford active compounds [53].

The search for other oxygen bioisosters led to the preparation of doxanthrine (**22**) (structure reported in Table **8**), an analog of **6** in which the ethyl tether between the catechol and tetrahydroisoquinoline substructures was replaced by an oxymethylene ether bridge [51]. Such a compound possessed high affinity (K_i = 20-30 nM) for D_1-like receptors in native porcine striatal tissue and full intrinsic activity at cloned human DA D_1 receptors, but had much lower affinity for DA D_2-like receptors (K_i = 3000 nM).

Interestingly, compound **22**, submitted to a screening program on cloned DA receptor subtypes (Table **8**), showed affinity for the DA D_1 (K_i = 98 nM) and D_5 (K_i = 7 nM) receptors.

To obtain moderately constrained compounds with the tryptamine and α-phenylethylamine structure incorporated into a 10-membered azecine ring, Witt *et al*. reported the synthesis and the DA receptor affinities of some 6,7,8,9,14,15-hexahydro-5*H*-benz[*d*]indolo[2,3-*g*]azecine derivatives [54].

Table 7. Affinities (K_i, nM) of Compounds 16-20, SCH23390 (1), and SCH39166 (15). Data from Ref. [29]

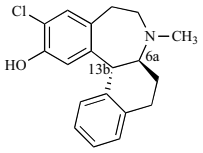
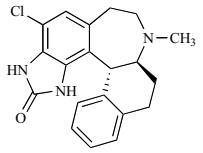
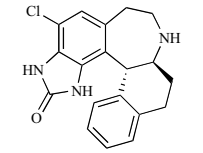
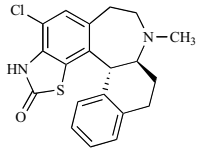
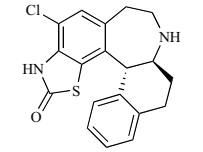
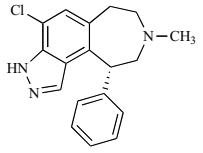
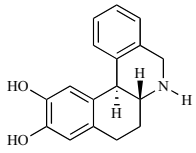
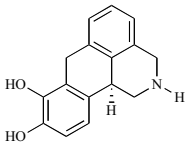
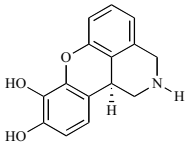
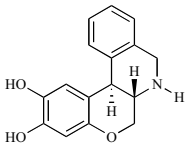
Compd.	D_1	D_5	D_1/D_5	D_2	D_4
1: SCH23390	1.4	2.8	0.5	1,000	-
 **15: SCH39166**	1.2	2.0	0.60	980	5,520
 **16**	7	4.2	1.67	1,023	10,000
 **17**	16.5	2.4	6.86	3,270	10,000
 **18**	2.1	2.8	0.75	257	10,000
 **19**	6.5	1.7	3.82	661	10,000
 **20**	14	30	0.47	3,550	10,000

Table 8. Affinities (K_i, nM) for Compounds 6, 7, 21, and 22

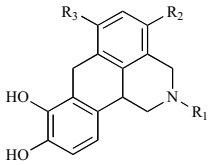
Compd.	D_1	D_5	D_5/D_1	D_2	D_3	D_4
 **6: Dihydrexidine**	2.2[a]	14.0[b]	0.16	180[c]	15.0[c]	14.0[b]

(Table 8. Contd….)

Compd.	D_1	D_5	D_5/D_1	D_2	D_3	D_4
 **7: Dinapsoline**	5.5[a]	10.0[b]	0.55	140[d]	10.0[b]	60.0[b]
 **21: Dinoxyline[b]**	3.9	3.8	1.02	86.0	1.0	1.1
 **22: Doxanthrine[e]**	98.0	7.0	14.0	1910	390	90.0

[a] data from Ref. [41]; [b] data from Ref. [42]; [c] data from Ref. [43]; [d] data from Ref. [33]; [e] data from Ref. [51].

Table 9. Affinities of Dinapsoline Analogs 23-28 for DA Receptors. Data from Ref. [52]



Drug	R_1	R_2	R_3	Clonal Lines ($K_{0.5}$ nM)				
				C-6 D_{1A}	HEK D_5	C-6 D_{2L}	C-6 D_3	CHO D_4
6: Dihydrexidine	-	-	-	2.2[a]	16.0	180	18.0	13.0
7: Dinapsoline	H	H	H	6.1	5.0	53.0	10.0	60.0
23	allyl	H	H	23.0	290	81.0	14.0	48.0
24	*n*-propyl	H	H	170	1,500	39.0	2.8	17.0
25	H	H	Me	9.8	8.1	190	28.0	220
26	H	Me	H	32.0	20.0	36.0	18.0	83.0
27	allyl	Me	H	56.0	690	36.0	4.3	63.0
28	*n*-propyl	Me	H	200	2,400	23.0	2.5	28.0

[a] datum from Ref. [41].

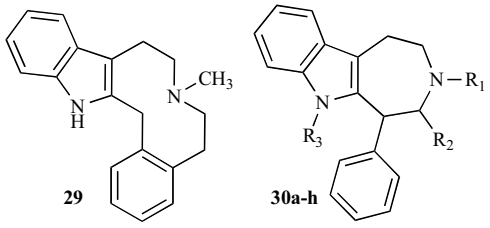
In binding assays with rat striatal receptors, the 7-methyl-derivative (LE-300, **29** reported in Fig. **2**) showed higher affinity for the D_1 binding site (K_i = 0.08 nmol for displacement of [^{3}H]SCH23390), than standards, such as butaclamol and SCH23390 (**1**). This compound was characterized as a DA antagonist by conditioned avoidance response test with mice and, since then, has represented the lead compound of a new class of DA antagonists. The indoloazepines **30** reported in Fig. (**2**), analogs of LE300 (**29**), SCH23390 (**1**) and SKF38393 (**2**), were synthesized and evaluated for their affinity for DA D_1, D_{2L}, D_4 and D_5 receptor subtypes [55]. All the compounds showed affinity in the micromolar range to both DA receptor subtype families with higher selectivity for the D_1 receptor family. Interestingly, when compared to SKF38393 (**2**) compound **30a** was a partial agonist, while compounds **30c** and **30e** behaved as inverse agonist, and neutral ligand (antagonist), respectively.

In order to estimate the importance of the indole moiety in this highly active benz-indolo-azecine, a series of benzazecines and benzazonines, reported in Fig. (**3**), was synthe-

Table 10. Binding Affinities and Potency at Cloned DA Receptors. Data from Ref. [42]

Compd.	Binding Affinities $K_{0.5}$ (nM) for Cloned DA Receptors					Potency at DA Receptors – EC_{50} (nM)				
	C-6 D_1	HEK D_5	C-6 D_{2L}	C-6 D_3	CHO D_4	D_1-like (striatum)	C-6 D_1	HEK D_5	D_{2L} C-6	CHO D_4
DA	-	-	-	-	-	5,000	530	110	20.0	1,800
6: dihydrexidine	2.2	14.0	180	15.0	14.0	70.0	34.0	12.0	110	1,400
7: dinapsoline	5.5	10.0	140	10.0	60.0	30.0	44.0	9.7	550	-
21: dinoxyline	3.9	3.8	86.0	1.0	1.1	87.0	8.6	12.0	26.0	52.0
N-allyl-dinoxyline	110	1,500	290	9.0	6.3	-	610	-	>10,000	170
N-*n*-propyl-dinoxyline	190	570	98.0	0.93	2.6	-	760	2,600	99.0	25.0

sized and pharmacologically investigated [56]. Unlike LE-300 (**29**), the new compounds did not show any significant affinity toward the D_1, D_2, D_4, and D_5 receptors. The authors concluded that an indole moiety, or at least another second aromatic system at the central azecine ring, is part of the pharmacophore and thus essential for high biological activity.



29 **30a-h**

	R_1	R_2	R_3
a	H	H	H
b	$C_6H_5CH_2CH_2$	H	H
c		H	H
d	CH_2=CH-CH_2	H	H
e	CH_3	H	H
f	CH_3	H	CH_3
g	H	CH_3	H
h	CH_3	CH_3	H

Fig. (2). Structures of compounds LE300 (29) and 30a-h.

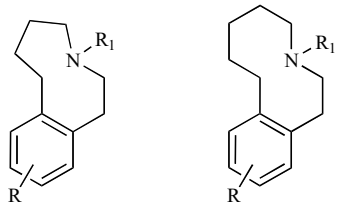


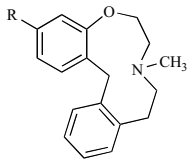
Fig. (3). General structures of benzazecines and benzazonines.

SAR studies on the N-substituent of LE300 have shown that by increasing the size of such a substituent the affinity to all the tested DA receptor subtypes decreased [57].

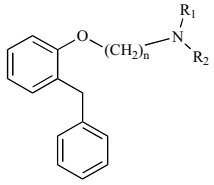
A real *"step toward dopamine D_5 receptor selectivity"* was reported by Wittig *et al*. [58]. Originating from the lead LE300 (**29**), the authors synthesized compounds with an enlarged central ring system and an isosteric replacement of the methylene group in position 5 with an oxygen atom, leading to a lower degree of rigidization and a different electronic situation in the central ring system as well as induction of a different electrostatic field in the respective benzene moiety. Compounds **31** and **32** were screened for their binding affinities to human cloned DA receptor subtypes expressed in HEK 293 or CHO cells by *in vitro* radioligand binding studies (Table **11**). The oxaazacycloundecenes **31** and **32** showed nanomolar affinities at both D_1 and D_5 receptor subtypes. Noteworthy, compound **32**, the highest affinity drug for D_5, showed a moderate D_5/D_1 selectivity ratio. On the other hand, the 3-methoxylated derivative **31**, even being endowed with lower affinity values, showed higher D_5/D_1 affinity ratio.

Both heterocycles displayed weaker affinities at the D_2-like receptor family. Concerning D_5 over D_{2L} binding selectivity, the hydroxylated **32** showed a significant ratio of almost 1:500, while the methoxylated derivative **31** still reached a ratio of 1:88. Compound **32** investigated for its functional behavior at the hD_1 receptor, proved to be a blocker of D_1 activity. Compound **31** revealed high binding affinities in the nanomolar range to D_1-like receptors and represented the first step to a structurally new class of dopaminergic ligands with binding selectivity to the hD_5 receptor subtype, thus giving chances for evaluating the properties of D_5 receptors in the brain.

Table 11. Affinities (K_i, nM) of Dibenz[*g,i*]-1-oxa-4-azycycloundecenes (31 and 32) and Open-Chain Analogues (33-38) at DA Receptor Subtypes. Data from Ref. [58]



		K_i (nM)					
Compd.	**R**	**D_1**	**D_5**	**D_5/D_1**	**D_{2L}**	**D_3**	**$D_{4.4}$**
31	OCH_3	35.5	1.8	0.05	158	1,601	546
32	OH	3.2	0.57	0.18	274	1,384	375



Compd.	**n**	**R_1**	**R_2**	**D_1**	**D_5**	**D_5/D_1**	**D_{2L}**	**D_3**	**$D_{4.4}$**
33	2	CH_3	CH_3	343	414	1.21	2,740	>10,000	2,765
34	2	pyrrolidinyl		379	951	2.51	>10,000	1,818	1,428
35	2	piperidinyl		33.7	43.0	1.28	1,015	7,804	4,476
36	2	morpholinyl		201	366	1.82	>10,000	>10,000	6,484
37	2	phenylpiperazinyl		1,480	620	0.42	4,188	2,860	>10,000
38	3	pyrrolidinyl		113	113	1	1,006	2,923	1,261

To investigate the effect of rigidization of these substances on the binding affinities, the open-chain analogs (2-benzylphenoxy)alkylamines **34**-**38** were also synthesized (Table **11**) [58]. When compared to the heterocycles, all the non-rigidized analogs showed lower affinities to all the binding sites and consistently higher D_1 over D_2 binding affinities. The D_1 and D_5 receptor affinity of the 2-benzylphenoxyethylamines reached a maximum with **35**, carrying a piperidine ring as the alkylamino moiety.

Recently, a variety of structural modifications of the benz [*d*]indolo[2,3-*g*]azecine LE300 (**29**), reported in Fig. (**4**), was performed by Hoefgen *et al*. and structure-activity relationships were deduced [59].

The inhibitory activities at human cloned D_1, D_{2L}, and D_5 receptors were measured by using a simple fluorescence microplate reader based calcium assay (Table **12**). Subsequently, the affinities of active compounds were estimated by radioligand binding experiments (Table **13**).

As already reported above for other series [57], larger substituents at the aliphatic nitrogen atom in LE300 negatively affected the inhibitory activities and affinities: methyl seemed to be the optimum in terms of activity. Deleting one of the aromatic rings (compounds **41**, **42**, and **45**), as well as replacing the non-indole aromatic ring with a phenyl moiety (compounds **44** and **49**), almost completely abolished the inhibitory activities. Contraction of the 10-membered central ring (compound **40**) significantly decreased them. The replacement of indole with thiophene (compound **46**) or N-methylpyrrole (compound **47**) reduced the inhibitory activity, whereas replacing the indole with benzene (compounds **43a,c,d**; Table **12**) increased it. Finally, the hydroxylated dibenz[*d,g*]azecine derivative **43d** (LE404) was found to be more active than the lead LE300 in the functional calcium assay as well as in radioligand displacement experiments, where it showed low nanomolar affinities for D_1 and D_5 receptor subtypes.

A comprehensive binding and functional receptor profile of the dibenzazecine derivatives **43a-e**, shown in Fig. (**4**), at all human DA receptors has been reported by Hamacher *et al*. (Table **14**) [60].

Compounds **43d** (LE404) and **43a** (LE410) behaved as competitive antagonists with pK_b derived from functional analyses in accordance with pK_i values derived from inhibition curves.

Different positions of the hydroxy/methoxy groups, and the introduction of an amino group or chlorine atoms on the substituted benzene ring of hexahydro-dibenz[*d,g*]azecines **43** were evaluated for their affinities and selectivity profiles toward DA receptors (Table **15**) [61].

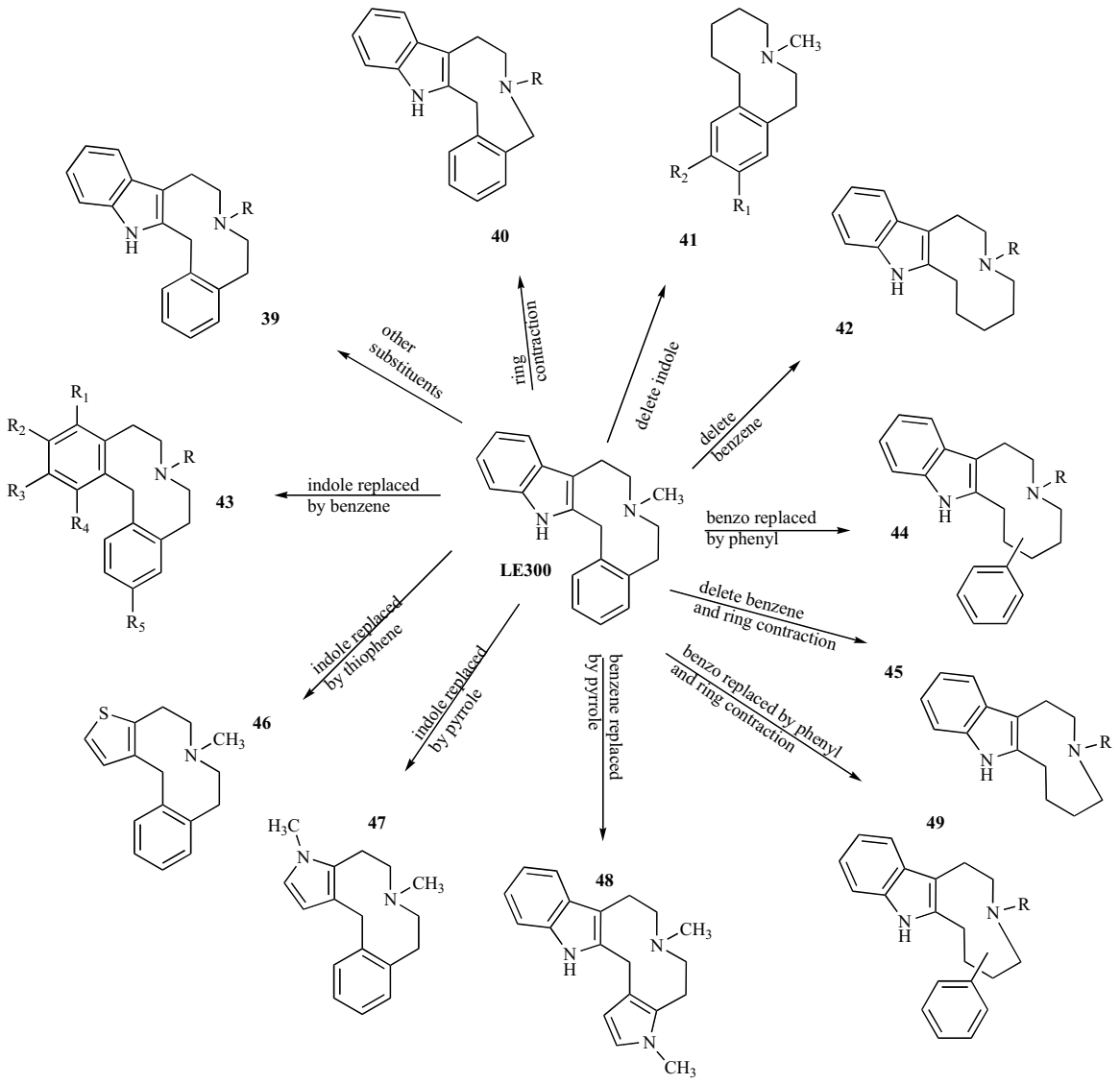


Fig. (4). Structural modifications of the lead compound LE300 (**29**).

Table 12. Inhibitory Activities at Human Cloned DA Receptors – Ca^{2+} Assay Data. Data from Ref. [59]

Compd[a]	R	R_2	R_3	K_i (nM)		
				hD_1	hD_5	hD_{2L}
29: LE300	-	-	-	60.4	12.7	19.0
39a	C_2H_5	-	-	170	22.3	82.6
43a[b]	CH_3	H	H	40.4	3.09	8.47
43c[b]	CH_3	OH	OH	20.7	2.32	65.7
43d[b]	CH_3	OH	H	6.93	1.69	33.5
46	-	-	-	207	33.7	92.0
47	-	-	-	~1,000	337	264
49a[c]	CH_3	-	-	742	112	943

[a] Structures reported in Fig. (**4**). [b] R_1=R_4=R_5=H. [c] 6-phenyl derivative.

Table 13. Affinities (K_i, nM) for Human Cloned DA Receptors – Radioligand Binding Data. Data from Ref. [59]

Compd.[a]	R	R_2	R_3	K_i (nM)			
				hD_1	hD_5	hD_{2L}	hD_4
29: LE300	-	-	-	1.9	7.5	44.7	109
39a	C_2H_5	-	-	16.4	14.7	253	378.5
39b		-	-	767	893	>5,000	>5,000
43a[b]	CH_3	H	H	4.5	11.2	56.5	134
43b[b]	CH_3	OCH_3	OCH_3	509	2,610	>5,000	2,514
43c[b]	CH_3	OH	OH	341	1,078	>5,000	165
43d[b]	CH_3	OH	H	0.39	1.5	17.5	11.3
46	-	-	-	10.7	79.1	198	299
47	-	-	-	61	361	712	1,647

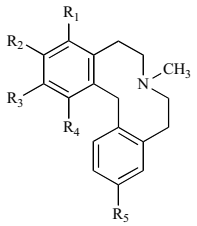
[a] Structures reported in Fig. (4). [b] R_1=R_4=R_5=H.

Table 14. Characterization of Compounds 43a-e, 46 and LE300 (29) by Heterologous Competition Binding and Their Inhibitory Potencies on Agonist-Induced Effects on $[cAMP]_i$, $[Ca^{2+}]_i$, and $[S^{35}]$-GTPγS Binding. Data from Ref. [60]

Compd.[a]	pK_i (K_i, nM)					p$K_{i\ app}$ (K_i, nM)					
						$[cAMP]_i$		$[Ca^{2+}]_i$		$[S^{35}]$-GTPγS binding	
	hD_1	hD_5	hD_{2L}	hD_3	$hD_{4.4}$	hD_1	hD_{2L}	hD_1	hD_{2L}	hD_1	hD_{2L}
29: LE300	7.98 (10.5)	7.99 (10.2)	7.19 (64.6)	6.48 (331)	6.46 (347)	7.55 (28.2)	8.73 (1.86)	7.22 (60.3)	7.93 (11.7)	7.75 (17.8)	8.14 (7.24)
43a[b]	7.76 (17.4)	7.78 (16.6)	7.54 (28.8)	6.86 (138)	6.32 (479)	7.35 (44.7)	8.63 (2.34)	7.39 (40.7)	8.13 (7.41)	8.02 (9.55)	8.13 (7.41)
43b[b]	5.58 (2630)	5.44 (3631)	5.90 (1259)	5.28 (5248)	4.79 (>10,000)	5.35 (4467)	6.88 (132)	<5 (>10,000)	<5 (>10,000)	6.25 (562)	6.39 (407)
43c[b]	7.94 (11.5)	7.84 (14.5)	6.43 (372)	6.14 (724)	6.26 (550)	7.02 (95.5)	7.23 (58.9)	7.57 (26.9)	7.14 (72.4)	7.48 (33.1)	7.20 (63.1)
43d[b]	8.47 (3.39)	8.53 (2.95)	7.10 (79.4)	6.73 (186)	7.23 (58.9)	7.95 (11.2)	8.01 (9.77)	8.20 (6.31)	7.71 (19.5)	8.10 (7.94)	8.13 (7.41)
43e[b,c]	4.77 (>10,000)	4.79 (>10,000)	5.06 (8710)	4.83 (>10,000)	<4 (>10,000)	5.0 (10,000)	<5 (>10,000)	<5 (>10,000)	<5 (>10,000)	<5 (>10,000)	<5 (>10,000)
46	6.89 (129)	6.92 (120)	6.64 (229)	6.07 (851)	5.83 (1479)	6.44 (363)	7.69 (20.4)	6.73 (186)	7.08 (83.2)	7.17 (67.6)	7.51 (30.9)

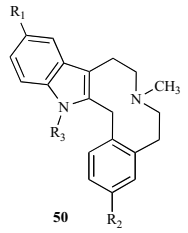
[a] Stuctures reported in Fig. (4). [b] R_1=R_4=R_5=H. [c] R_2=R_3=OCH_3.

Table 15. Affinities (K_i, nM) of Dibenz[*d,g*]azecines 43d-r at DA-Receptor Subtypes Measured by Radioligand-Binding Studies (Affinities) and an Intracellular Calcium Assay (Inhibitory Activities). Data from Ref. [61]



Compd	R_1	R_2	R_3	R_4	R_5	K_i (nM) (Radioligand Binding Studies/Calcium Assays)			
						D_1	D_5	D_{2L}	D_3
29: LE300	-	-	-	-	-	1.9 60.4	7.5 12.7	44.7 19.0	n.d. n.d.
43d	H	OH	H	H	H	0.39 1.35	1.5 1.69	17.5 33.5	47.5 n.d.
43f	H	OCH_3	H	H	H	28.5 24.1	n.d. 7.2	13.0 0.55	75.7 n.d.
43g	Cl	OH	H	H	H	0.83 0.46	0.057 0.053	4.0 6.1	24.6 n.d.
43h	Cl	OH	Cl	H	H	3.2 3.8	n.d. 1.2	88.0 37.5	n.d. n.d.
43i	H	OH	NH_2	H	H	9.3 20.9	226.5 39.5	37.3 26.1	n.d. n.d.
43j	H	H	OH	H	H	8.9 76.5	42.3 37.5	36.9 2.6	296 n.d.
43k	H	H	OCH_3	H	H	82.0 567	n.d. 1,050	62.0 21.1	150.5 n.d.
43l	H	Cl	OCH_3	Cl	H	25.3 34.0	n.d. 59.0	210 2.5	415 n.d.
43m	H	H	OH	Cl	H	0.46 6.8	0.98 1.6	0.99 0.13	1.88 n.d.
43n	H	Cl	OH	H	H	3.1 10.8	4.9 3.1	2.0 4.8	27.7 n.d.
43o	H	H	H	OH	H	8.7 64.1	n.d. 10.4	84.0 7.9	215 n.d.
43p	H	H	H	OCH_3	H	7.6 37.6	n.d. 54.6	164 39.9	1,833 n.d.
43q	H	OH	H	H	OH	2.0 3.3	1.68 0.54	58.7 19.0	342 n.d.
43r	H	OCH_3	H	H	OCH_3	9.4 26.9	12.6 11.5	6.5 0.62	101.5 n.d.

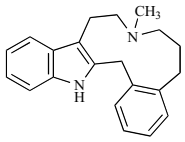
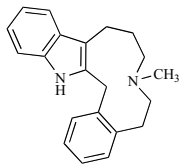
Table 16. Affinities (K_i, nM) for DA Receptor D_1-D_5 Subtypes Determined by Radioligand-Binding Experiments. Data from Ref. [62]



50

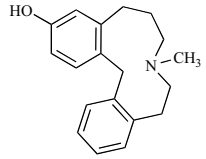
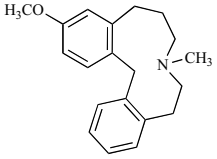
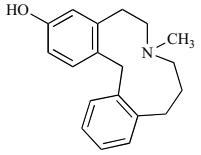
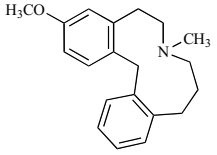
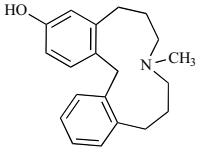
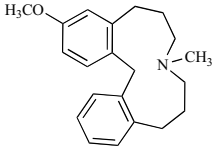
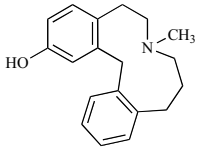
Compd	R_1	R_2	R_3	K_i (nM)				
				HEK D_1	HEK D_5	CHO D_{2L}	CHO D_3	CHO $D_{4.4}$
29: LE300	H	H	H	1.9	7.5	44.5	40.3	109
43a	-	-	-	4.5	11.2	56.5	52.5	134
43d	-	-	-	0.39	1.5	17.5	47.0	11.3
43f	-	-	-	28.5	38.3	13.0	75.7	43.4
50a	OCH_3	H	H	0.82	3.6	11.9	475	266
50b	OH	H	H	0.56	0.39	38.4	944	398
50c	H	OCH_3	H	19.0	31.5	22.8	1,135	92.6
50d	H	OH	H	3.7	5.4	74.7	2,070	1,359
50e	OCH_3	H	CH_3	2.0	0.23	1.7	3.78	21.55

Table 17. Affinities (K_i, nM) for DA D_1/D_5 Receptor Subtypes, Determined by Radioligand Binding Experiments and Inhibitor Activities (K_i, nM) at DA D_1, D_2, and D_5 Receptor Subtypes Generated by a Functional Calcium Assay. Data from Ref. [63]

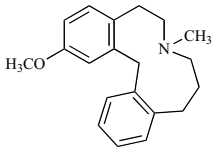
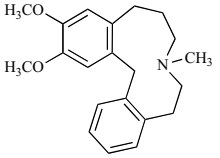
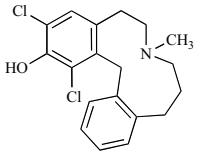
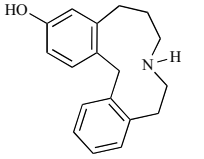
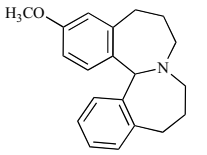
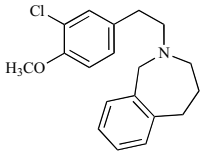
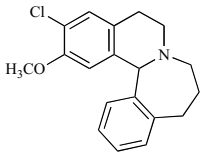
Compd.	K_i (nM)							
	Binding Affinities					Inhibitory Activities		
	HEK D_1	HEK D_5	CHO D_{2L}	CHO D_3	CHO $D_{4.4}$	HEK D_1	HEK D_5	HEK D_{2L}
29: LE300	1.9	7.5	44.5	25.9	108	60.4	12.7	19.0
 **51**	2.2	0.61	14.5	277.5	98.4	2.8	2.8	1.5
 **52**	163.5	92	143	521	184	304	71.6	38

All the reported dibenz-[*d,g*]azecines **43g-r**, as well as the dibenz[*d,g*]azecines **43d** and **43f**, showed antagonist properties in the calcium assay. Concerning the influence of the position of the hydroxy and methoxy groups among the new dibenz[*d,g*]azecines, the highest D_1-like affinities were shown by the 3-hydroxy/methoxy compounds (**43d** and **43f**). 2-Amino-3-hydroxy-7-methyl-5,6,7,8,9,14-hexahydrodibenz-[*d,g*]azecine (**43i**) proved to be a nanomolar ligand. Concerning both selectivity and affinity, the most interesting

Table 18. Affinities (K_i Values) for DA Receptor Subtypes Measured by Radioligand Binding Studies. Data from Ref. [64]

Compd	K_i (nM)				
	D_1	D_5	D_{2L}	D_3	$D_{4.4}$
29: LE300	1.9	7.5	44.7	40.35	74.9
43d	0.39	1.5	17.5	47.5	11.3
43f	28.5	54.0	13.0	75.7	43.4
 **53**	13.9	17.0	518	6,122	2,258
 **54**	29.4	55.0	25.0	3,136	1,103
 **55**	3.2	9.8	74.0	100	60.15
 **56**	18.5	4.6	87.0	507	271
 **57**	83.0	95.0	382	3,964	422
 **58**	23.5	53.0	172	1,349	2,869
 **59**	70.0	69.0	63.0	776	3,751

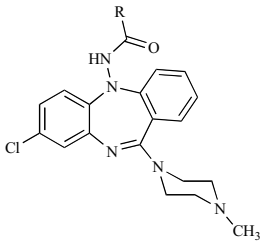
(Table 18. Contd….)

Compd	K_i (nM)				
	D_1	D_5	D_{2L}	D_3	$D_{4.4}$
 **60**	137	75.0	1,396	23,903	2,763
 **61**	579	510	1,028	14,830	5,048
 **62**	124	89.0	11.0	-	15.0
 **63**	291	314	857	6,896	4,256
 **64**	>10,000	>10,000	>10,000	>10,000	>10,000
 **65**	>10,000	>10,000	>10,000	>10,000	>10,000
 **66**	>10,000	>10,000	>10,000	>10,000	>10,000
Clozapine	266	255	184	269	24.0

compounds were the chlorinated derivatives **43g,h** and **43l-n**. Compound **43m** was a subnanomolar ligand at all the DA receptors tested, but lacked in selectivity. Compound **43g** (LEPM436) showed subnanomolar affinities toward D_1 receptor (>30-fold selectivity D_1>D_2) with an even higher affinity toward D_5 (K_i = 57 pM, 15-fold selectivity D_5>D_1) and, therefore, representing one of the most potent D_5 antagonist.

To investigate whether the effects of methoxylation and hydroxylation of azecine derivatives with regard to the affinities and selectivities for all the DA receptor subtypes were beneficial, methoxy and hydroxy groups were attached to the aromatic rings of the lead LE300 [62]. Structure-activity relationship studies concerning the influence of the hydroxy- methoxy- substitution of the benz-indolo-azecines **50** on the affinity for the D_1 receptor were similar to those found for the dibenzazecines **43**. Among the indolic compounds **50a-d**, the phenolic derivatives **50b**,**d** displayed higher binding affinities to D_1 and D_5 receptor subtypes when compared to their methoxy analogs **50a,c**. Compound **50e**, bearing an N-methylated indole moiety, showed increased affinities toward D_2-D_5 receptors compared to the desmethyl derivative **50a**, whereas the affinity for D_1 receptor decreased. Thus, **50e** proved to be 9-fold more selective for D_5 with respect to D_1 receptors. Moreover, the high affin-

Table 19. Affinities (K_i, nM) for DA Receptor Subtypes Measured by Radioligand Binding Studies. Data from Ref. [65]



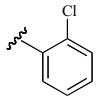
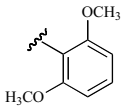
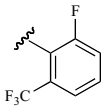
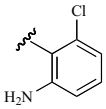
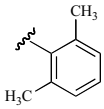
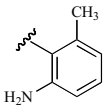
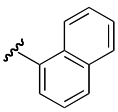
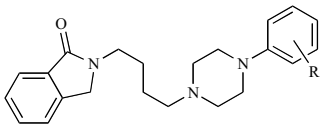
Compd	R	K_i (nM)			
		D_1	D_5	D_2	D_4
67a		4.0	73.0	181	870
67b		1.6	38.0	340	1,810
67c		2.5	7.0	383	2,000
67d		2.6	53.0	496	808
67e		3.0	31.0	287	1,870
67f		3.2	58.0	1,015	4,846
67g		3.8	105	458	977

Table 20. Affinities (K_i, nM) for DA Receptor Subtypes Measured by Radioligand Binding Studies. Data from Ref. [66]



Compd.	R	K_i (nM)			
		D_2	D_3	D_4	D_5
68a	H	189	35.8	216	80.0
68b	4-Cl	760	660	142	33.6
68c	3-CF_3	387	14.0	368	80.8
68d	2-OCH_3	319	30.0	134	75.4

ity for the D_1 and D_2 receptors, the very high affinity for D_5 and the considerable affinities for D_3 and D_4, shown by compound **50e**, represented a quite unusual affinity profile for the azecine-like DA receptor antagonists.

Enlarging the 10-membered ring of LE300 yielded two higher homolog antagonists (**51** and **52**) [63]. Compared to the lead compound LE300, the compound with the ring enlargement next to the indole moiety (**52**) showed a decrease in affinities for all the receptor subtypes. Maintaining the tryptamine template and elongating phenylethyl to phenylpropyl moiety (**51**) showed to be much more favorable. Noteworthy, the affinity of the phenylpropyl homolog (**51**) for D_5 increased from K_i = 7.5 nM (LE300) to K_i = 0.61 nM, displaying a 3.5-fold selectivity with regard to D_1. However, similar selectivity was not observed in functional assays. These findings suggested that the optimal distance between the indole moiety and the nitrogen is measured by a two-carbon chain.

A similar SAR study was performed homologizing the methoxylated and hydroxylated hexahydrodibenz[*d,g*]azecines **43** (Table **18**). The resulting 11- and 12-membered heterocycles were investigated with respect to their affinities and selectivity profiles for the D_1-D_5 receptors [64].

The most interesting compounds behaved as antagonists or inverse agonists, preferentially at the D_1-like family. Enlarging the dibenzazecines to the corresponding dibenzazacycloundecenes and dibenzazacyclododecenes generally maintained the high antagonist affinity for D_1/D_5. As previously observed for compounds **51** and **52**, the position of the nitrogen in relation to the substituted benzene ring was crucial, the compounds with the nitrogen closer to the substituted benzene ring (**55** and **56**) showing higher affinity than the other regioisomers **53** and **54**. The hydroxylated dibenzazacycloundecenes **53**, **55** and **59** exhibited higher affinities for the D_1 receptor when compared to their methoxylated analogs **54**, **56** and **60**, respectively. Interestingly, the replacement of the *N*-methyl with NH (compound **63**) was detrimental for the affinity for all receptor subtypes. Expanding the size of the central N-heterocycle from 11 to 12 (compounds **57** and **58**) proved to be less favorable. Surprisingly, the dichlorinated compound **62** was reported to be the first "dibenzazecine-type" DA receptor antagonist without selectivity toward the D_1 receptor family. Compound **65** and the more constrained tetracyclic compounds **64** and **66** did not display any significant affinity.

In the last two years, clozapine derivatives and lactam derivatives, not related to the structures above discussed, showed appreciable affinity to DA D_5 receptors. Clozapine derivatives have been reported as D_1 DA receptor subtype selective antagonists. Among all the synthesized compounds, the affinity values toward D_1, D_2, D_4 and D_5 DA receptor subtypes (Table **19**) were reported only for seven of them [65].

The results showed that all the compounds had good D_1-like selectivity with D_1 over D_5 preference except for compound **67c**, which did not display significant differences in the affinity values for D_1 and D_5 receptor subtypes.

Finally, lactam derivatives, bearing various phenylpiperazinylbutyl side chains attached to the amide nitrogen, have been described and evaluated for their DA receptor affinity [66]. Binding data revealed general affinity of the target compounds toward the DA receptor D_2-like family. Interestingly, isoindolinone derivatives **68a-d** showed DA D_5 receptor affinity although no data about D_1 receptor affinity are reported.

SUMMARY

Since DA D_5 receptor cloning, research has provided some advances in our understanding of receptor-ligand interactions and functions. There is still considerable territory to be explored to better understand the structure requirements to activate or inhibit selectively such a receptor subtype mainly over the D_1 subtype. The discovery of highly selective DA D_5 ligands, which will greatly contribute to determine the functional role for this receptor subtype and its involvement in numerous diseases, still proves to be an extremely difficult goal to be achieved. This is an open challenge for medicinal chemists working in this field.

REFERENCES

[1] Huang, X.; Lawler, C.P.; Lewis, M.M.; Nichols, D.E.; Mailman, R.B. *Int. Rev. Neurobiol.*, **2001**, *48*, 65.

[2] Amenta, F.; Ricci, A.; Tayebati, S.K.; Zaccheo, D. *Italian J. Anat. Embryol.*, **2002**, *107*, 145.

[3] Khan, Z.U.; Gutiérrez, A.; Martín, R.; Pañafiel, A.; Rivera, A.; De La Calle, A. *Neuroscience*, **2000**, *100*, 689.
[4] Zhang, A.; Neumeyer, J.L.; Baldessarini, R.J. *Chem. Rev.,* **2007**, *107*, 274.
[5] Kebabian, J.W.; Petzold, G.L.; Greengard, P. *Proc. Natl. Acad. Sci. U.S.A.,* **1972**, *69*, 2145.
[6] Garau, L.; Govoni, S.; Stefanini, E.; Trabucchi, M.; Spano, P.F. *Life Sci.*, **1978**, *23*, 1745.
[7] Kebabian, J.W.; Calne, D.B. *Nature*, **1979**, *277*, 93.
[8] Neve, K.A.; Seamans, J.K.; Trantham-Davidson H. *J. Recept. Signal Transduct.*, **2004**, *24*, 165.
[9] Dearry, A.; Gingrich, J.A.; Falardeau, P.; Fremeau, R.T., *Jr.*; Bates, M.D.; Caron, M.G. *Nature*, **1990**, *347*, 72.
[10] Sunahara, R.K.; Guan, H.-C.; O'Dowd, B.F.; Seeman, P.; Laurier, L.G.; Ng, G.; George, S.R.; Torchia, J.; van Tol, H.H.M.; Niznik, H.B. *Nature*, **1991**, *350*, 614.
[11] Tiberi, M.; Jarvie, K.R.; Silvia, C.; Falardeau, P.; Gingrich, J.A.; Godinot, N.; Bertrand, L.; Yang-Feng, T.L.; Fremeau, R.T. Jr; Caron, M.G. *Proc. Natl. Acad. Sci. U.S.A.*, **1991**, *88*, 7491.
[12] Bunzow, J.R.; van Tol, H.H.M.; Grandy, D.K.; Albert, P.; Salon, J.; Christie, M.; Machida, C.A.; Neve, K.A.; Civelli, O. *Nature*, **1988**, *336*, 783.
[13] Sokoloff, P.; Giros, B.; Martres, M.P.; Bouthenet, M.L.; Schwartz, J.C. *Nature*, **1990**, *347*, 146.
[14] Van Tol, H.H.M.; Bunzow, J.R.; Guan, H.C.; Sunahara, R.K.; Seeman, P.; Niznik, H.B.; Civelli, O. *Nature*, **1991**, *350*, 610.
[15] Missale, C.; Nash, S.R.; Robinson, S.W.; Jaber, M.; Caron, M.G. *Physiol. Rev.*, **1998**, *78*, 189.
[16] Ciliax, B.J.; Nash, N.; Heilman, C.; Sunahara, R.; Hartney, A.; Tiberi, M.; Rye, D.B.; Caron, M.G.; Niznik, H.B.; Levey, A.I. *Synapse*, **2000**, *37*, 125.
[17] Montague, D.M.; Striplin, C.D.; Overcash, J.S.; Drago, J.; Lawler, C.P.; Mailman, R.B. *Synapse*, **2001**, *39*, 319.
[18] O'Sullivan, G.J.; Clifford, J.J.; Tomiyama, K.; Koshikawa, N.; Drago, J.; Sibley, D.R.; Croke, D.T.; Waddington J.L. *J. Recept. Signal Transduct.*, **2004**, *24*, 107.
[19] Zeng, C.; Yang, Z.; Asico, L.D.; Jose, P.A. *Cardiovasc. Hematol. Agents Med. Chem.*, **2007**, *5*, 241.
[20] Yang, Z.; Sibley, D.R.; Jose, P.A. *J. Recept. Signal Transduct.*, **2004**, *24*, 149.
[21] Schilström, B.; Yaka, R.; Argilli, E.; Suvarna, N.; Schumann, J.; Chen, B.T.; Carman, M.; Singh, V.; Mailliard, W.S.; Ron, D.; Bonci, A. *J. Neurosci*., **2006**, *26*, 8549.
[22] Elliot, E.E.; Sibley, D.R.; Katz, J.L. *Psychopharmacology,* **2003**, *169*, 161.
[23] Kudwa, A.E.; Dominguez-Salazar, E.; Cabrera, D.M.; Sibley, D.R.; Rissman, E.F. *Psychopharmacology*, **2005**, *180*, 206.
[24] Kebabian, J.W.; Tarazi, F.I.; Kula, N.S.; Baldessarini, R. J. *Drug Disc. Today,* **1997**, *2*, 333.
[25] Gingrich, J.A.; Caron, M.G. *Rev. Neurosci.*, **1993**, *16*, 299.
[26] O'Dowd, B.F. *J. Neurochem.*, **1993**, *60*, 804.
[27] Tiberi, M.; Caron, M.G. *J. Biol. Chem.*, **1994**, *269*, 27925.
[28] Neumeyer, J.L.; Kula, N.S.; Bergman, J.; Baldessarini, R.J. *Eur. J. Pharmacol.*, **2003**, *474*, 137.
[29] Wu, W.-L.; Burnett, D.A.; Spring, R.; Greenlee, W.J.; Smith, M.; Favreau, L.; Fawzi, A.; Zhang, H.; Lachowicz, J.E. *J. Med. Chem.*, **2005**, *48,* 680.
[30] Zhang, A.; Zhang, Y.; Branfman, A.R.; Baldessarini, R.J.; Neumeyer, J.L. *J. Med. Chem.*, **2007**, *50*, 171.
[31] Temlett, J.A.; Chong, P.N.; Oertel, W.H.; Jenner, P.; Marsden, C.D. *Eur. J. Pharmacol.*, **1988**, *156*, 197.
[32] Brewster, W.K.; Nichols, D.E.; Riggs, R.M.; Mottola, D.M.; Lovenberg, T.W.; Lewis, M.H.; Mailman, R.B. *J. Med. Chem.*, **1990**, *33*, 1756.
[33] Ghosh, D.; Snyder, S.E.; Watts, V.J.; Mailman, R.B.; Nichols, D.E. *J. Med. Chem.*, **1996**, *39*, 549.
[34] Michaelides, M.R.; Hong, Y.; Didomenico, S., Jr.; Bayburt, E.K.; Asin, K.E.; Britton, D.R.; Lin, C.W.; Shiosaki, K. *J. Med. Chem.*, **1997**, *40*, 1585.
[35] Asin, K.E.; Domino, E.F.; Nikkel, A.; Shiosaki, K. *J. Pharmacol. Exp. Ther.*, **1997**, *281*, 454.
[36] DeNinno, M.P.; Schoenleber, R.; Asin, K.E.; MacKenzie, R.; Kebabian, J.W. *J. Med. Chem.*, **1990**, *33*, 2948.
[37] Bourne, J.A. *CNS Drug Rev.*, **2001**, *7*, 399.
[38] Seeman, P.; Van Tol, H.H.M. *Trends Pharmacol. Sci.*, **1994**, *15*, 264.
[39] Asencio, M.; Hurtado-Guzmán, C.; López, J.J.; Cassels, B.K.; Protais, P.; Chagraoui, A. *Bioorg. Med. Chem.*, **2005**, *13***,** 3699.
[40] Demchyshyn, L.L.; McConkey, F.; Niznik, H.B. *J. Biol. Chem.*, **2000**, *275*, 23446.
[41] Lewis, M.M.; Watts, V.J.; Lawler, C.P.; Nichols, D.E.; Mailman, R.B. *J. Pharmacol. Exp. Ther.*, **1998**, *286*, 345.
[42] Grubbs, R.A., Lewis, M.M.; Owens-Vance, C.; Gay, E.A.; Jassen, A.K.; Mailman, R.B.; Nichols, D.E. *Bioorg. Med. Chem.*, **2004**, *12*, 1403.
[43] Watts, V.J.; Lawler, C.P.; Gilmore, J.H.; Southerland, S.B.; Nichols, D.E.; Mailman, R.B. *Eur. J. Pharmacol.*, **1993**, *242*, 165.
[44] Shiosaki, K.; Jenner, P.; Asin, K.E.; Britton, D.R.; Lin. C.W.; Michaelides, M.; Smith, L.; Bianchi, B.; Didomenico, S.; Hodges, L.; Hong, Y.F.; Mahan, L.; Mikusa, J.; Miller, T.; Nikkel, A.; Stashko, M.; Witte, D.; Williams, M. *J. Pharmacol. Exp. Ther*., **1996**, *276*, 150.
[45] Andersen, P.H.; Jansen, J.A. *Eur. J. Pharmacol.*, **1990**, *188,* 335.
[46] Charifson, P.S.; Bowen, J.P.; Wyrick, S.D.; Hoffman, A.J.; Cory, M.; McPhail, A.T.; Mailman, R.B. *J. Med. Chem.*, **1989**, *32*, 2050.
[47] Millan, M.J.; Maiofiss, L.; Cussac, D.; Audinot, V.; Boutin, J.-A.; Newman-Tancredi, A. *J. Pharmacol. Exp. Ther*., **2002**, *303*, 791.
[48] Søndergaard, K.; Kristensen, J.L.; Palner, M.; Gillings, N.; Knudsen, G.M.; Roth, B.L.; Begtrup, M. *Org. Biomol. Chem.*, **2005**, *3*, 4077.
[49] Berger, J.G.; Chang, W.K.; Clader, J.W.; Hou, D.; Chipkin, R.E.; McPhail, A.T. *J. Med. Chem.*, **1989**, *32*, 1913.
[50] O'Boyle, K.M.; Waddington, J.L. *J. Neurochem.*, **1987**, *48*, 1039.
[51] Cueva, J.P.; Giorgioni, G.; Grubbs, R.A.; Chemel, B.R.; Watts, V.J.; Nichols D.E. *J. Med. Chem.*, **2006**, *49*, 6848.
[52] Qandil, A.M.; Lewis, M.M.; Jassen, A.; Leonard, S.K.; Mailman, R.B.; Nichols, D.E. *Bioorg. Med. Chem.*, **2003**, *11*, 1451.
[53] Sit, S.-Y.; Xie, K.; Jacutin-Porte, S.; Boy, K.M.; Seanz, J.; Taber, M.T.; Gulwadi, A.G.; Korpinen, C.D.; Burris, K.D.; Molski, T.F.; Ryan, E.; Xu, C.; Verdoorn, T.; Johnson, G.; Nichols, D.E.; Mailman, R.B. *Bioorg. Med. Chem.* **2004**, *12*, 715.
[54] Witt, T.; Hock, F.J., Lehmann, J. *J. Med. Chem.*, **2000**, *43*, 2079.
[55] Decker, M.; Lehmann, J. *Arch. Pharm. Pharm. Med. Chem.*, **2003**, *336***,** 466.
[56] El-Subbagh, H.; Wittig, T.; Decker, M.; Elz, S.; Nieger, M.; Lehmann, J. *Arch. Pharm. Pharm. Med. Chem.*, **2002**, *9,* 443.
[57] Kassack, M.U.; Höfgen, B.; Decker, M.; Eckstein, N.; Lehmann, J. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **2002**, *366*, 543.
[58] Wittig, T.W.; Decker, M.; Lehmann J. *J. Med. Chem.*, **2004**, *47*, 4155.
[59] Hoefgen, B.; Decker, M.; Mohr, P.; Schramm, A.M.; Rostom, S.A.F.; El-Subbagh, H.; Schweikert, P.M.; Rudolf, D.R; Kassack, M.U.; Lehmann, J. *J. Med. Chem*., **2006**, *49*, 760.
[60] Hamacher, A.; Weigt, M.; Wiese, M.; Hoefgen, B.; Lehmann, J.; Kassack, M.U. *BMC Pharmacology*, **2006**, *6*:11. This open access article is available at: http://creativecommons.org/licenses/by/2.0
[61] Mohr, P.; Decker, M.; Enzensperger, C.; Lehmann, J. *J. Med. Chem.*, **2006***, 49*, 2110.
[62] Enzensperger, C.; Kilian, S.; Ackermann, M.; Koch, A.; Kelch, K.; Lehmann, J. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 1399.
[63] Enzensperger, C.; Lehmann, J. *J. Med. Chem.*, **2006**, *49*, 6408.
[64] Enzensperger, C.; Müller, F.K.U.; Kilian, S.; Schmalwasser, B.; Wiecha, P.; Traber, H.; Lehmann, J. *J. Med. Chem.*, **2007**, *50*, 4528.
[65] Sasikumar, T.K.; Burnett, D.A.; Zhang, H.; Smith-Torhan, A.; Fawzi, A.; Lachowicz, J.E. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 4543.
[66] Awadallah, F.M.; Müller, F.; Lehmann, J.; Abadi, A.H. *Bioorg. Med. Chem.*, **2007**, *15*, 5811.
[67] Hsieh, G.C.; Hollingsworth, P.R.; Martino, B.; Chang, C.; Terranova, M.A.; O'Neill, A.B.; Lynch, J.J.; Moreland, R.B.; Donnelly-Roberts, D.L.; Kolasa, T.; Mikusa, J.P.; McVey, J.M.; Marsh, K.C.; Sullivan, J.P.; Brioni, J.D. *J. Pharm. Exp. Ther.*, **2004**, *308*, 330.

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