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Original article

Synthesis, dopamine D_2 receptor binding studies and docking analysis of 5-[3-(4-arylpiperazin-1-yl)propyl]-1*H*-benzimidazole, 5-[2-(4-arylpiperazin-1-yl)ethoxy]-1*H*-benzimidazole and their analogs

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Abstract

5-[3-(4-Arylpiperazin-1-yl)propyl]-1*H*-benzimidazoles and 5-[2-(4-arylpiperazin-1-yl)ethoxy]-1*H*-benzimidazoles were synthesized and their affinity for the D_1, D_2 and 5-HT_{1A} receptors examined. They expressed a rather high affinity for the D_2 dopamine receptor. The main features of ligand–D_2 receptor interactions revealed by docking analyses were: salt bridge between piperazine ring protonated N_1 and Asp 86, hydrogen bonds of ligand bezimidazole part with Ser 141, Ser 122 and His 189, edge-to-face interactions of arylpiperazine aromatic ring with Phe 178, Tyr 216 and Trp 182 and hydrogen bond between ethereal oxygen in ethylenoxy ligands and hydrogen of Phe 185 or Trp 115. The most active 5-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]ethoxy}-1,3-dihydro-2*H*-benzimidazole-2-thione **(27)** has a maximal number of attractive interactions. A satisfactory correlation between docking of the compounds into the D_2 receptor and competition binding results was observed.

Keywords: Arylpiperazines; D_2 receptor; 5-HT_{1A} receptor; Binding pocket; Docking analysis

1. Introduction

Since the introduction of chlorpromazine, a known dopamine (DA) antagonist, DA-ergic drugs have been widely used in medicinal practice. During the last years, different DA agonists and antagonists have been applied in human medicine for the treatment of numerous disorders, e.g. Parkinson's disease, schizophrenia, some neurohumoral disturbances, etc. Since a great number of these compounds expressed undesirable side effects, the need for new drugs with side effects reduced to a minimum is increasing. Scientific literature in this field of interest is numerous and can be found summarized in recent review articles [1,2].

Within the scope of the program aimed at the discovery of new DA-ergic ligands, we have synthesized a series of benzimidazoles that can be considered as non-catechol bioisosteres of catecholamines [3]. The most active compounds were obtained by connecting benzimidazole ring through a flexible spacer with *N*-arylpiperazines, structure of one in such a way obtained ligand is presented in Chart 1. It was observed that the affinity of the obtained ligands for the binding to the D_2 DAR depends on both the structure of benzimidazole and arylpiperazine part of the molecule and the spacer itself [4,5]. In order to obtain a better structure/DA-ergic activity relationship and to evaluate the influence of the spacer in this type of ligands on their binding affinity, we have synthesized,

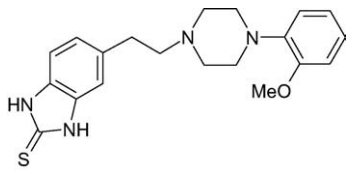

Chart 1.

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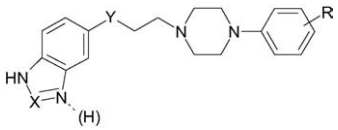
evaluated and performed docking analyses of a series of new compounds, derivatives of 5-[3-(4-arylpiperazin-1-yl)propyl]-1*H*-benzimidazole and 5-[2-(4-arylpiperazin-1-yl)ethoxy]-1*H*-benzimidazole. On the other hand oxygen/methylene isosteric replacement that introduce an aryl–O bond has significant effect on both electronic and conformational properties studied compounds [6,7] and therefore can effect ligand–receptor interaction on several levels. Our previous finding shows that interactions between benzoimidazole type ligand and D_2 DAR are sensitive to above mentioned molecular properties [5,8] what initiated investigations presented in this paper.

Binding pocket of the D_2 DAR was defined according to Teeter and DuRand [10], while docking analysis was done using Insight II software packet from Accelerys.

2. Chemistry

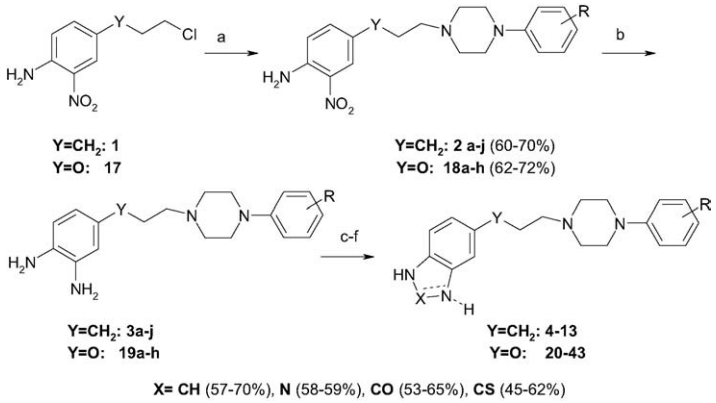
The derivatives **1**–**13** and **20**–**43** (Table 1) were synthesized as outlined in Scheme 1. Compounds with propylene spacer were prepared according to previously described strategy [4] starting from 4-(3-chloropropyl)-2-nitroaniline **(1)**. Ethylenoxy compounds were obtained starting from 4-(2-

Table 1
Chemical structure and binding affinity of the examined arylpiperazine ligands for the D_1 and D_2 dopamine and 5-HT_{1A} serotonin receptors



Number	X	Y	R	K_i (nM) ± S.E.M.		
				D_1	D_2	5-HT_{1A}
4	CH	CH_2	H	>1000	129 ± 19	71.1 ± 8.2
5	CH	CH_2	3-CF_3	>1000	>1000	>1000
6	CH	CH_2	4-Cl	>1000	>1000	>1000
7	CH	CH_2	4-OMe	>1000	>1000	>1000
8	CH	CH_2	2-OMe	>1000	97 ± 21	47 ± 4.0
9	C=S	CH_2	H	>1000	3.0 ± 0.2	177 ± 29
10	C=S	CH_2	3-CF_3	>1000	5.8 ± 0.4	>1000
11	C=S	CH_2	4-Cl	>1000	20 ± 2.8	>1000
12	C=S	CH_2	4-OMe	>1000	33 ± 4.2	>1000
13	C=S	CH_2	2-OMe	>1000	0.4 ± 0.05	8.6 ± 1.1
20	CH	O	H	>1000	274 ± 18	>1000
21	CH	O	3-CF_3	>1000	>1000	>1000
22	CH	O	2-MeO	>1000	12.6 ± 0.9	284 ± 35
23	CH	O	3-MeO	>1000	>1000	330 ± 42
24	CH	O	4-MeO	>1000	>1000	>1000
25	C=S	O	H	>1000	0.67 ± 0.06	>1000
26	C=S	O	3-CF_3	>1000	5.94 ± 0.3	>1000
27	C=S	O	2-MeO	>1000	0.19 ± 0.06	308 ± 29
28	C=S	O	3-MeO	>1000	13.2 ± 1.2	>1000
29	C=S	O	4-MeO	>1000	105 ± 8.1	>1000
30	C=S	O	2-Cl	446 ± 32	2.75 ± 0.4	>1000
31	C=S	O	3-Cl	>1000	7.06 ± 0.9	>1000
32	C=S	O	4-Cl	>1000	>1000	>1000
33	C=O	O	H	>1000	7.53 ± 1.0	>1000
34	C=O	O	3-CF_3	>1000	26.1 ± 4.2	>1000
35	C=O	O	2-MeO	>1000	1.30 ± 0.1	>1000
36	C=O	O	3-MeO	>1000	44.8 ± 6.2	>1000
37	C=O	O	4-MeO	>1000	>1000	>1000
38	C=O	O	2-Cl	405 ± 30	>1000	>1000
39	C=O	O	3-Cl	>1000	45.5 ± 3.0	>1000
40	C=O	O	4-Cl	>1000	>1000	>1000
41	N	O	H	>1000	>1000	>1000
42	N	O	3-CF_3	>1000	>1000	>1000
43	N	O	2-MeO	>1000	1.51 ± 0.4	>1000

Structure of 5-[3-(4-arylpiperazine-1-yl)propyl]-1*H*-benzimidazoles and 5-[2-(4-arylpiperazine-1-yl)ethoxy]-1*H*-benzimidazoles and their analogs tested for the binding to the D_1 and D_2 dopamine and 5-HT_{1A} serotonin receptors is shown. K_i values are the means of three independent experiments done in triplicate performed at eight to 10 competing ligand concentrations.



Scheme 1. Synthesis of 5-[3-(4-arylpiperazine-1-yl)propyl]-1*H*-benzimidazole, 5-[2-(4-arylpiperazine-1-yl)ethoxy]-1*H*-benzimidazole and their analogs. (a) Arylpiperazine, K_2CO_3, DMF; (b) Ra–Ni, N_2H_4, EtOH, DCE; (c) HCOOH, reflux; (d) CS_2, KOH, EtOH; (e) 1,1′-carbonyldiimidazole and (f) $NaNO_2$, AcOH. Obtained yields are clamed in parentheses.

chloroethoxy)-2-nitroaniline **(17)** as a parent compound (Scheme 2). Briefly, 1-(2-chloroethoxy)-4-nitrobenzene **(14)** was prepared by alkylating 4-nitrophenol with 1,2-dichloroethane. A simultaneous reduction and acetylation with zinc dust in acetanhydride/acetic acid mixture afforded acetanilide **(15)**. Nitration of **15** in boiling 20% nitric acid gave *o*-nitroacetanilide **(16)** that was further hydrolyzed to *o*-aniline **17** with boiling HCl. Compound **17** readily alkylates *N*-phenyl-piperazine in DMF in the presence of K_2CO_3 and KI at elevated temperature affording 2-nitro-4-[2-(4-arylpiperazin-1-yl)ethoxy]-anilines (**18a**–**h**). Further reduction with Ra–Ni/hydrazine afforded *o*-phenylenediamines **19a**–**h**.

Target benzimidazoles (**4**–**8** and **20**–**24**), benzimidazole-2-thiones (**9**–**13** and **25**–**32**), benzimidazole-2-ones (**33**–**40**) and benzotriazoles (**41**–**43**) were prepared as described earlier [3].

3. Pharmacology

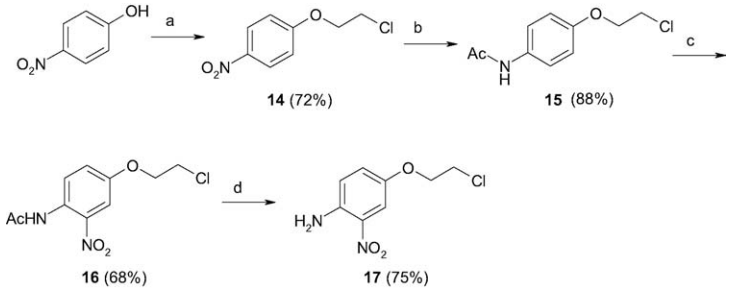
All compounds were evaluated for the binding affinity to the D_1 and D_2 dopamine and serotonin 5-HT_{1A} receptors by in vitro competition displacement of the specific radioligands ([^{3}H]SCH 23390, [3H]spiperone and 8-OH-[^{3}H]DPAT, respectively) from synaptosomal membranes prepared from fresh bovine caudate nuclei (D_1 and D_2 receptor) and hippocampi (5-HT_{1A} receptor). K_i values of individual compounds were calculated from the displacement curves obtained in three independent experiments done in triplicate and performed at eight to 10 competing ligand concentrations. The pharmacological results are listed in Table 1.

4. Results

None of the compounds synthesized and evaluated for the DA-ergic/serotonergic activity throughout the present study expressed the affinity for the binding to the D_1 DAR. Ligands **5**–**7**, **24**, **37**, **38** and **40**–**42** were completely inactive competitors in both D_2 and 5-HT_{1A} binding assays. Besides, compounds **10**–**12**, **20**, **25**, **26**, **28**–**31**, **33**–**36**, **39** and **43** acted only as [^{3}H]spiperone displacers. Also, only one of the ligands **(23)** acted as an active displacer of [^{3}H]8-OH-DPAT. The remaining novel compounds (**4**, **8**, **9**, **13**, **22** and **27**) behaved as efficient displacers of [^{3}H]spiperone and [^{3}H]8-OH-DPAT, expressing the binding affinity for the corresponding receptor in a nanomolar range of concentrations.

Among all tested ligands, compound **27** with a K_i value of 0.19 nmol was the most potent displacer of [^{3}H]spiperone, while in [^{3}H]-OH-DPAT binding assay compound **13** with a K_i of 8.6 nmol was the most active (Table 1).

Binding pocket of the D_2 DAR was defined according to Teeter and DuRand [10]. The main features of the D_2 DAR model shown in Fig. 1 using compound **27** as a ligand were: (a) salt bridge between protonated N1 of the piperazine ring



Scheme 2. Synthesis of 4-(2-chloroethoxy)-2-nitrophenylamine **(17)**. (a) DCE, K_2CO_3, MEK; (b) Zn, Ac_2O/AcOH; (c) 20% HNO_3, Δ; (d) HCl, H_2O. Obtained yields are clamed in parentheses.

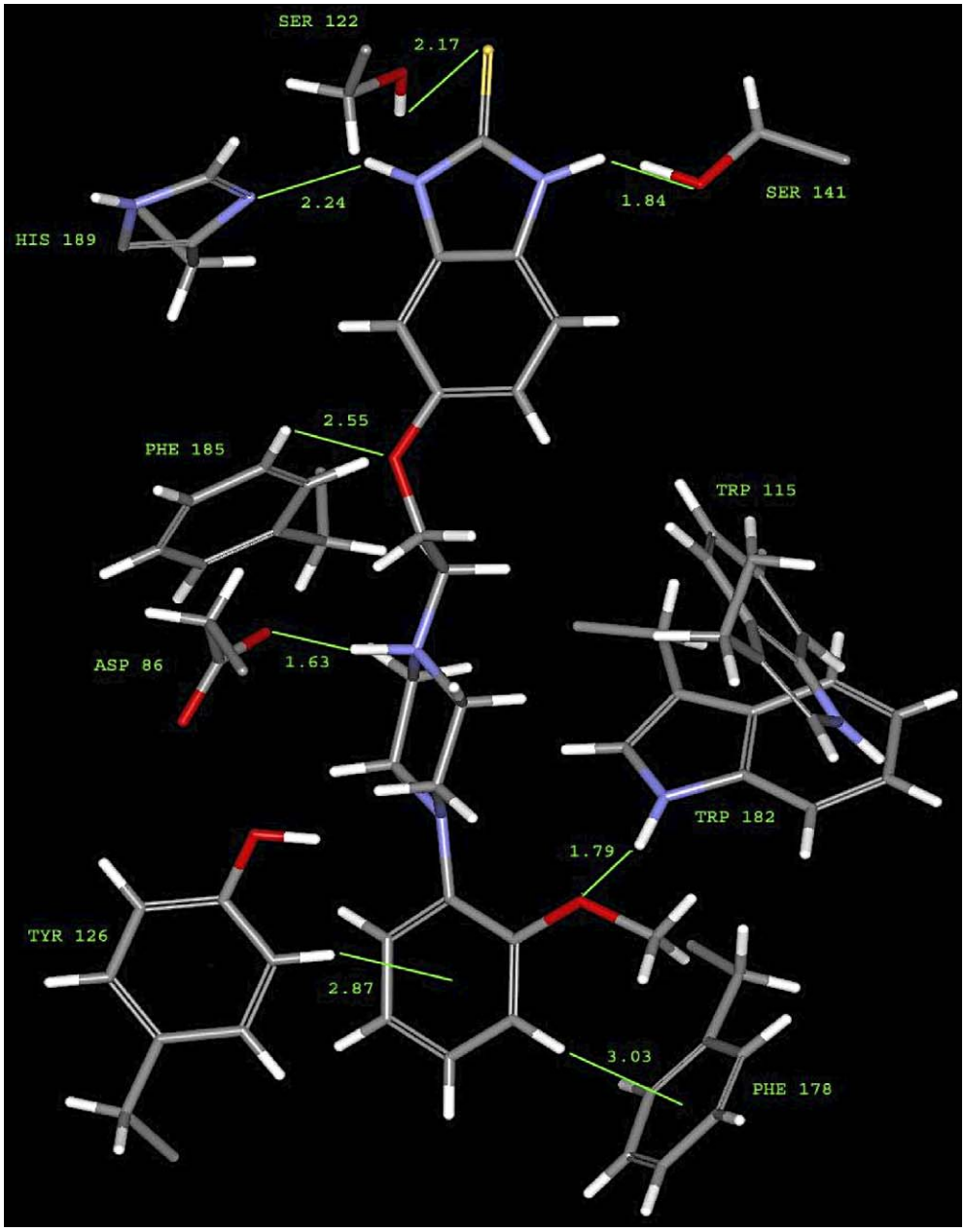


Fig. 1. Schematic representation of ligand **27** interaction with the D_2 dopamine receptor. Schematic model of the proposed interaction of the studied compound **27** with the D_2 DAR. 3D model describes a possible interaction of compound **27** and theoretical dopamine D_2 receptor model.

and negatively charged Asp 86 (calculated distance 1.63 Å); (b) hydrogen bonds between benzimidazole part of the ligand and Ser 141, Ser 122 and His 189; (c) edge-to-face interactions of the aromatic ring or arylpiperazine part of the ligand with Phe 178, Tyr 216 and Trp 182 of the receptor. Similar results were obtained with other substituents in piperazine ring; (d) in addition, 2-methoxypiperazine derivatives **8**, **13**, **22**, **27**, **35** and **43** could build one more hydrogen bond with Trp 182 and (e) interaction of ethereal oxygen of ethylenoxy ligands (**20**–**43**) with hydrogen of Phe 185 or Trp 115. To calculate free energy (ΔG) of this particular interaction, the structures obtained in docking analysis were used. Coordinates of a ligand and Phe 185 of the receptor were frozen, while all other elements of the receptor were removed. Single point calculation was employed to define total energy of thereof defined system. In the following step, the ligand was translated along *X*-axis by 10 Å and the calculation was repeated. The difference in the obtained energies was taken as a measure of the system stabilization. For this calculation, B3LYP method was used with basis set 6–31 g* in a Gaussian 98W software [11,12]. The obtained free energy value ranged from 2.5 to 3.8 kcal mol^{-1}.

5. Discussion

Arylpiperazines have been known to have the activity profiles similar to those of atypical antipsychotics [13–15]. In

order to design new drugs with desired pharmacological profiles, we have investigated the effects of various structural modifications of these molecules on their DA-ergic and serotonergic activity [3–5]. Based on these results, we have found of interest to prepare derivatives with ethylenoxy and propylene bridge between arylpiperazine and heteroaryl part of the molecule and to examine their DA-ergic and serotonergic activity. In contrast to a high D_2 DAR binding affinity of a number of tested compounds, only a few were active in the 5-HT_{1A} competition binding assay, while none of them acted as a displacer of [^{3}H]SCH 23390. Therefore, our efforts were directed toward a better understanding of the D_2 DAR–ligand interactions at molecular level using docking of newly designed ligands in the binding pocket of this receptor.

Docking was performed by considering all receptor amino acids that could interact after initial positioning of the ligands against Asp 86, Ser 141 and Ser 122 residues. The binding pocket designed in this way provided the data that matched the experimental results obtained in competition binding assays.

Generally, uncaring points of a ligand docked in the binding pocket of the D_2 DAR are localized around benzimidazole and arylpiperazine part of the ligands separated by polymethylene spacer. Benzimidazole structural motif interacts with the receptor through hydrogen bonds that involve amino acids Ser 122 and Ser 141 in TM II and TM III, that are a part of catechol binding site of the D_2 DAR [10,16]. In addition, docking analysis revealed that His 189 in TM VI could be also involved in hydrogen bond formation. Arylpiperazine part of the ligands interacts with the receptor through hydrophobic (edge-to-face) type of interactions [17,18]. A cluster of aromatic amino acid residues that are highly conserved in GPCR family of class A are responsible for this kind of interactions. Bondensgaard et al. [19] postulated that this amino acid cluster builds an ancillary pocket in GPCRs that is involved in docking of so-called "privileged structures". This aspect of ligand–receptor interaction that is more elaborated in our previous paper [9] can be summarized as follows: aryplpiperazine structural motif can be considered as a privileged structure fitting within the ancillary pocket of the D_2 DAR that is preserved in most of the GPRCs. The aryplpiperazine moiety is positioned in the ancillary pocket spanned by the three conserved aromatic residues, i.e. Phe 178, Trp 182 and Tyr 216, providing favorable aromatic–aromatic interactions. The results of docking studies revealed that close interaction of protonated N1 of the piperazine ring with Asp 86, and edge-to-face interactions of the aromatic ring or arylpiperazine part of the ligand with Phe 178, Trp 182 and Tyr 216 of the receptor, represent the main stabilizing forces. In addition, 2-methoxy derivative (**8**, **13**, **22**, **27**, **35**, **43**) could build one additional hydrogen bond with Trp 182. Bulky substituents in position 4 of the aromatic part of phenylpiperazine ring are not tolerated because of the unfavorable steric interactions with Phe 178. Substituents in position 2 and 3 of phenylpiperazine are sterically well tolerated. Electron attractive groups decreased the binding affinity, while electron donors like—OMe increased the affinity for the binding at the D_2 DAR in comparison with the unsubstituted phenylpiperazine. These effects can be explained by stronger edge-to-face interactions of negative ESP in the center of aromatic residues of the ligands and positive ESP of the protons of the receptor aromatic residues. Methoxy group, increase the affinities of ligands if attached to position 2, since one additional hydrogen bond could be formed with Trp 182.

Methylene groups connecting benzimidazole part of the ligands to arylpiperazine part can be defined as spacers that determine the distance between two clusters of ligand uncaring points. Therefore, the number of methylene groups in the spacer critically influenced the binding affinity of the ligands. Compounds with a single methylene group had the lowest affinity that originates from partial occupation of receptor binding site. Ligands with ethylene and propylene (or ethylenoxy) spacer can completely occupy the binding site of the receptor. The main difference between these two groups of ligands comes from the involvement of His 189 in binding of propylene or ethylenoxy ligands. This increases the number of hydrogen bonds and consequently, the binding affinity to the D_2 DAR (Fig. 2).

Ethylenoxy ligands expressed a higher binding affinity compared to propylene counterparts because of one additional attractive interaction with the receptor molecule. This stabilizing interaction may originate in the formation of an atypical hydrogen bond between aromatic hydrogens in Phe 185 or Trp 115 and ethereal oxygen of the ligands. This type of hydrogen bonds was postulated and studied in some other biological systems [20]. It was shown that these weak interactions, typically 3–4 Å long, represent an important stabilizing factor in protein folding [21]. In docking analyses performed in the present study, distances between the aromatic hydrogen and ethereal oxygen ranging from 2.55 to 3.25 Å were found, what makes the formation of this bond quite likely (Fig. 3). Calculated free energy (ΔG) of this particular interaction was 2.14 kcal mol^{-1}. This value is slightly exceeding the value of 0.3–1.9 kcal mol^{-1} claimed by Gu et al. [22] but this can be explained by limitations of the available model of the D_2 DAR binding site. In addition to this, some recent published data [6,7] shows significant difference in torsional potential between aryl–CH_2 and aryl–O. In studied anisol like systems, the global minimum corresponds to the eclipsed structure in which the methoxy group is coplanar with the aromatic ring. A significant decrease of the calculated rotational barrier at the orthogonal structure by 1.5–3.0 kcal mol^{-1} was observed. This is an additional constrain that itself can influence overall stability of receptor–ligand complex.

Differences in binding affinity among the ligands that differ only in benzimidazole part of the molecule can be ascribed to the number of possible hydrogen bonds formed with Ser 122 (TM IV), Ser 141 (TM V) and His 189 (TM VI). Benzimidazole derivatives **4**–**8** and **20**–**24** are capable of building only one hydrogen bond with Ser 141 (Fig. 2a), while benzotriazoles **41**–**43** are interacting by forming one or two hydrogen bonds with Ser 141 and His 189 (Fig. 2b).

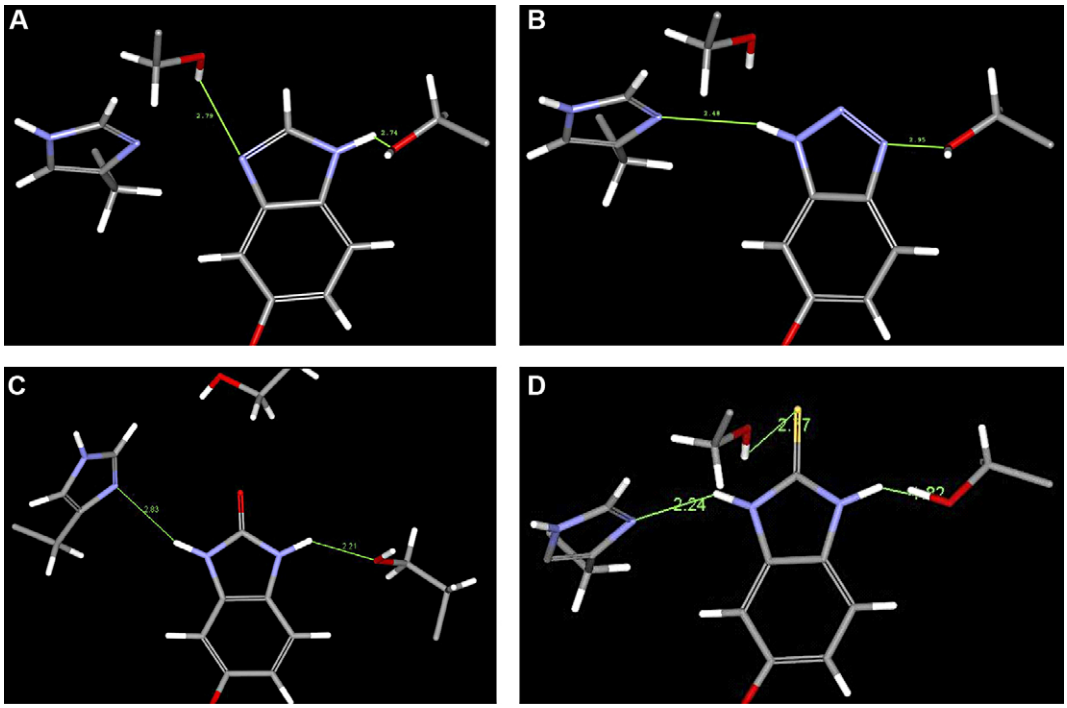


Fig. 2. Interaction of benzimidazole part of the ligands with the D_2 dopamine receptor. Schematic representation of the interaction of **(a)** benzimidazole, **(b)** benzotriazole, **(c)** benzimidazole-2-one and **(d)** benzimidazole-2-thione derivatives with Ser 141, Ser 122 and His 189 of the D_2 DAR.

Benzimidazole-2-ones **33**–**40** are building two hydrogen bonds, one with Ser 141 and one with His 189 (Fig. 2c), while benzimidazole-2-thiones **9**–**13** and **25**–**32** are capable of building one additional hydrogen bond with Ser 122 via long C=S bond (Fig. 2d). Accordingly, benzimidazole-2-thione derivatives **9**–**13** and **25**–**32** should have the highest binding affinity. They are followed by benzimidazole-2-ones **33**–**40**, benzotriazoles **41**–**43** and benzimidazoles **4**–**8** and **20**–**24**, as confirmed by experimental results obtained in competition binding assays listed in Table 1.

The effects of substituents introduced in arylpiperazines on DA-ergic activity could be summarized as follows: (a) sub-

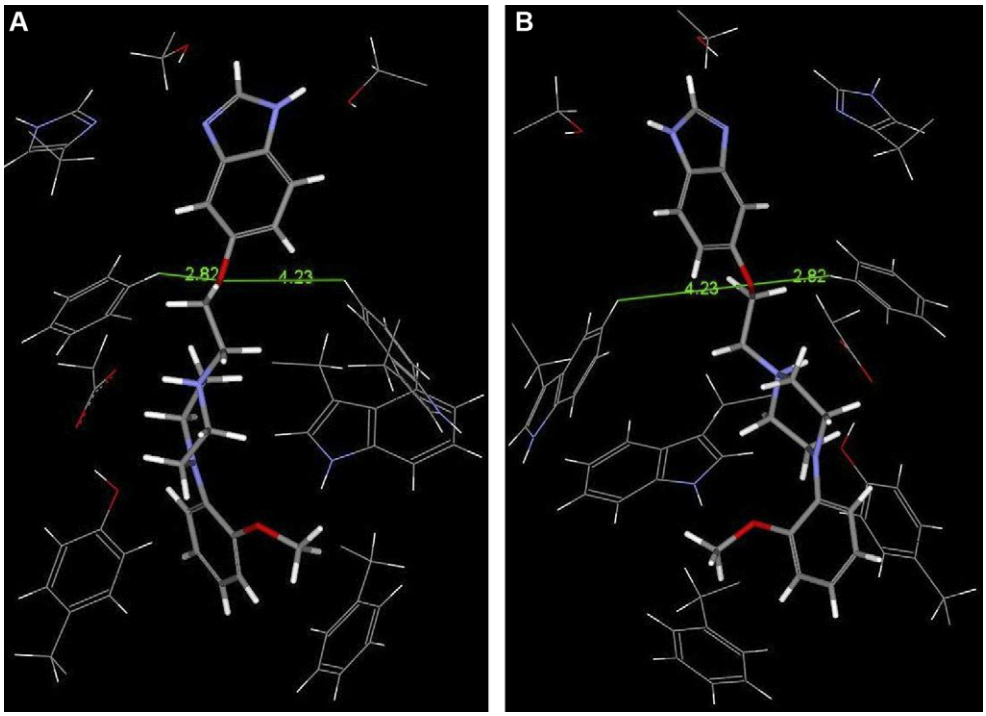


Fig. 3. Interaction of ethereal oxygen of 5-[2-(4-phenylpiperazine-1-yl)ethoxy]-1*H*-benzimidazole type of ligands with the D_2 DAR. Interaction of ethereal oxygen of 5-[2-(4-phenylpiperazine-1-yl)ethoxy]-1*H*-benzimidazole (**20**) with. Phe 185 and Trp 115. Figures **a** and **b** are showing docked ligand from two different angles.

stituents in position 2 and 3 of piperazine phenyl ring are sterically well tolerated; (b) substituents with electron withdrawal effect in this position, such as 3-trifluoromethyl (**5**, **10**, **21**, **26**, **34** and **42**) and 3-chloro (**31** and **39**) are affecting the affinity by decreasing electron density in the benzene ring of these ligands that results in the reduction of the energy of edge-to-face interactions; (c) electron donor groups, e.g. methoxy (**8**, **13**, **22**, **27**, **28**, **35**, **36** and **43**), expressed a beneficial effect on ligand binding by facilitating edge-to-face interactions; (d) in the case of ligands with methoxy group in position 2 (**8**, **13**, **22**, **27**, **35** and **43**), one additional hydrogen bond can be built with Trp 182, and as a consequence, these ligands were the most active among all analogs; (e) ligands with bulky substituents in position 4 of arylpiperazine ring (**6**, **7**, **11**, **12**, **24**, **29**, **32**, **37** and **40**) are involved in unfavorable steric interaction of substituents with Phe 178 in the receptor binding pocket what reduced the affinity of these ligands.

6. Conclusions

The results of docking studies on newly synthesized compounds—D_2 DAR complexes revealed that these ligands interact through protonated N1 of the piperazine ring with Asp 86 (salt bridge), hydrogen bonds between bezimidazole part of a ligand and Ser 141, Ser 122 and His 189, edge-to-face interactions of the aromatic ring or arylpiperazine part of a ligand with Phe 178, Tyr 216 and Trp 182 of the receptor. In addition, 2-methoxy derivatives could build one additional hydrogen bond with Trp 182. Similarly, ethereal oxygen of ethylenoxy derivatives interacts with hydrogen of Phe 185 or Trp 115. Compound of the highest affinity 5-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2*H*-benzimidazole-2-thione **(27)** is the one with the maximal number of the above listed attractive interactions. The results presented here provide a basis for further rational design of DA-ergic compounds.

7. Experimental protocols

7.1. General

A Boetius PHMK apparatus (VEB Analytic, Dresden, Germany) was used to determine melting points (m.p.), presented here as uncorrected. ^{1}H NMR spectra recorded on a Gemini 2000 spectrometer (Varian, Palo Alto, CA, USA) with $CDCl_3$ as a solvent unless otherwise stated are reported in ppm downfield from the internal standard tetramethylsilane.

The IR spectra were run on a Perkin–Elmer 457 Grating Infrared Spectrophotometer (Perkin–Elmer, Beaconsfield, UK). High resolution mass spectra were acquired on a Bruker Biflex MALDI TOF (Bruker, Bremen, Germany). Elemental analyses were performed by a Perkin–Elmer 240B microanalyzer. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values.

For analytical thin-layer chromatography E. Merck (Darmstadt, Germany) F-256 plastic-backed thin-layer silica gel plates were used. Chromatographic purifications were performed on Merck-60 silica gel columns, 230–400 mesh ASTM, under medium pressure (MPLC). Solutions were routinely dried over anhydrous Na_2SO_4 *prior* to evaporation.

7.2. Molecular modeling

Ligand models were constructed using a Hyperchem v. 7.0 software (Hypercube Inc., Gainesville, USA) and inbuilt PM3 routine for molecular geometry optimization. It was postulated that the ligands are bound to the receptors in protonated form [23,24], therefore, a formal charge of +1 was added to piperazine nitrogen (1N). The results obtained were further optimized in a Gaussian 98W, Rev. A.9 (Gaussian Inc., Pittsburgh, USA) using the DFT B3LYP method and a 6–31 g basis set [11,12].

Modeling of the ligand–D_2 DAR complexes was done using the Docking module within an INSIGHT II software (Accelerys Inc., Cambridge, UK) on a SGI Octane 2 workstation (Silicon Graphics Inc., Mountain View, USA). Docking of the ligands described here was performed initially, using SA docking algorithm and up to 100 structures were generated applying a Monte Carlo method. Each structure was further minimized for 4000 cycles or until 0.01 kcal mol^{-1} Å^{-1} was reached. Minimization was performed by fixing all the protein backbone atoms and keeping ligand and amino acid residues in the binding site flexible. In this way, the relaxation of Van der Waals interactions was permitted. Subsequently, all structures were filtered using the general rule that the best structure is the one with the shortest salt bridge between the ligand and Asp 86, and with a maximum number of hydrogen bonds with the D_2 DAR. The obtained results were visualized using a DS View software (Accelerys Inc., Cambridge, UK).

7.3. Chemistry

7.3.1. Syntheses

*7.3.1.1. Preparation of 1-(2-chloroethoxy)-4-nitrobenzene **(14)**.* To 0.5 mol 4-nitrophenol solution in 350 ml of ethyl–methyl–ketone, 60 g (0.5 mol) of anhydrous potassium carbonate and 150 ml 1,2-dichloroethane were added with stirring. The mixture was refluxed (36 h) with a constant stirring. After the reaction was completed, the reaction mixture was cooled to ambient temperature and poured into 1 l water. The product was extracted three times with 300 ml methylene chloride. After evaporation, crude product was crystallized from 90% ethanol. Yield: 72%; m.p. 48 °C; IR (cm^{-1}): 1258, 1339, 1507, 1596; ^{1}H NMR: δ 3.87 (t, 2H, J = 5.8 Hz), 4.33 (t, 2H, J = 5.6 Hz), 7.00 (d, 2H, J = 9.2 Hz, ArH), 8.22 (d, 2H, J = 9.4 Hz, ArH).

*7.3.1.2. Preparation of N-[4-(2-(chloroethoxy)phenyl]acetamide **(15)**.* 1-(2-Chloroethoxy)-4-nitrobenzene **(14)** (0.25 mol) was dissolved in the mixture of 300 ml acetic acid and 250 ml

acetanhydride. The mixture was stirred and upon adding 195 g zinc dust in three portions, heated in an oil bath (80 °C) with constant stirring. After the reaction was completed (about 6 h), the mixture was filtered through sinter glass and the filtrate evaporated under reduced pressure. Crude product was crystallized from ethanol. Yield: 88%; m.p. 110 °C; IR (cm^{-1}): 1256, 1530, 1663, 3313; ^1H NMR: δ 2.16 (s, 3H, CH_3), 3.80 (t, 2H, J = 6 Hz), 4.21 (t, 2H, J = 6 Hz), 6.89 (d, 2H, J = 9 Hz, ArH), 7.18 (s, 1H, NH), 7.41 (d, 2H, J = 9 Hz, ArH).

*7.3.1.3. Preparation of N-[4-(2-chloroethoxy)-2-nitrophenyl]acetamide **(16)**.* Finely powdered *N*-[4-(2-(chloroethoxy)phenyl]acetamide **(15)** (0.20 mol) was added in portions into 300 ml of boiling 20% nitric acid with stirring. After 1 h, the reaction mixture was cooled and poured onto 500 g crushed ice. The precipitate was filtered and washed with cold water. Crude product was crystallized from 90% ethanol. Yield: 68%; m.p. 95 °C; IR (cm^{-1}): 1242, 1277, 1508, 1697, 3366; ^1H NMR: δ 2.28 (s, 3H, CH_3), 3.84 (t, 2H, J = 5.6 Hz), 4.28 (t, 2H, J = 5.6 Hz), 7.27 (d, 1H, J = 3 Hz, J = 6.1 Hz, ArH), 7.70 (d, 1H, J = 3 Hz, ArH), 8.68 (d, 1H, J = 9.4 Hz, ArH), 10.09 (s, 1H, NH).

*7.3.1.4. Preparation of 4-(2-chloroethoxy)-2-nitroaniline **(17)**.* *N*-[4-(2-Chloroethoxy)-2-nitrophenyl]acetamide **(16)** (0.15 mol) was resuspended in 120 ml 4 N HCl and refluxed for 4 h. The solution was cooled to ambient temperature and transferred in a refrigerator overnight. The crystals were separated by filtration and recrystallized from 90% EtOH. Yield: 75%; m.p. 77 °C; IR (cm^{-1}): 1114, 1453, 1510, 1669, 2356, 3057; ^1H NMR: δ 3.81 (t, 2H, J = 5.8 Hz), 4.20 (t, 2H, J = 5.6 Hz), 6.02 (s, 2H, NH_2), 6.80 (d, 1H, J = 8.8 Hz, ArH), 7.70 (d, 1H, J = 2.8 Hz, J = 6.2 Hz, ArH), 7.53 (d, 1H, J = 3 Hz, ArH).

7.3.2. General alkylation procedure

Solution of **1** or **17** (20 mmol) was mixed with 22 mmol arylpiperazines, 25 ml dimethylformamide (DMF) and the mixture of 3.18 g K_2CO_3 and 1.0 g KI. The resulting mixture was stirred (24 h, 80 °C). After cooling, the precipitate was discarded and the filtrate evaporated in vacuo. The residue was chromatographed on silica gel and recrystallized from hot isopropanol.

*7.3.2.1. 2-Nitro-4-[3-(4-phenylpiperazin-1-yl)propyl]aniline **(2a)**.* Yield: 60%; m.p. 98 °C; IR (cm^{-1}): 1343, 1520, 3475; ^1H NMR: δ 1.83 (m, 2H), 2.45 (t, 2H, J = 7 Hz), 2.60 (m, 6H), 3.24 (m, 4H), 6.05 (s, 2H, NH_2), 6.75 (d, 1H, J = 8 Hz, ArH), 6.84 (t, 2H, J = 8 Hz, ArH), 6.92 (d, 1H, J = 8 Hz, ArH), 7.24 (m, 3H, ArH), 7.98 (s, 1H, ArH).

*7.3.2.2. 2-Nitro-4-(3-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}propyl)aniline **(2b)**.* Yield: 62%; m.p. 99 °C; IR (KBr) (cm^{-1}): 1408, 1449, 1566, 1642, 2950, 3083; ^1H NMR: δ 1.82 (quint, 2H, J = 8 Hz), 2.41 (t, 2H, J = 8 Hz), 2.60 (m, 6H), 3.25 (t, 4H, J = 5 Hz), 5.98 (s, 2H, NH_2), 6.76 (d, 1H, J = 8.6 Hz, ArH), 7.05–7.35 (m, 5H, ArH), 7.96 (d, 1H, J = 2 Hz, ArH).

*7.3.2.3. 2-Nitro-4-{3-[4-(4-chlorophenyl)piperazin-1-yl]-propyl}aniline **(2c)**.* Yield: 70%; m.p. 118 °C; IR (cm^{-1}): 1451, 1499, 1640, 2825, 3045; ^1H NMR: δ 1.81 (quint, 2H, J = 8 Hz), 2.39 (t, 2H, J = 7.8 Hz), 2.59 (m, 6H), 3.17 (t, 4H, J = 5 Hz), 5.98 (s, 2H, NH_2), 6.75 (d, 1H, J = 8.4 Hz, ArH), 6.84 (dd, 1H, J = 4.6 Hz, J = 2.2 Hz, ArH), 7.21 (m, 4H, ArH), 7.95 (d, 1H, J = 2.2 Hz, ArH).

*7.3.2.4. 2-Nitro-4-{3-[4-(4-methoxyphenyl)piperazin-1-yl]-propyl}aniline **(2d)**.* Yield: 68%; m.p. 109 °C; IR (cm^{-1}): 1242, 1513, 1635, 2822, 3320; ^1H NMR: δ 1.81 (quint, 2H, J = 8 Hz), 2.39 (t, 2H, J = 7.8 Hz), 2.59 (m, 6H), 3.17 (t, 4H, J = 5 Hz), 5.98 (s, 2H, NH_2), 6.75 (d, 1H, J = 8.4 Hz, ArH), 6.84 (dd, 1H, J = 4.6 Hz, J = 2.2 Hz, ArH), 7.21 (m, 4H, ArH), 7.95 (d, 1H, J = 2.2 Hz, ArH).

*7.3.2.5. 2-Nitro-4-{3-[4-(2-methoxyphenyl)piperazin-1-yl]-propyl}aniline **(2e)**.* Yield: 65%; m.p. 110 °C; IR (cm^{-1}): 1248, 1512, 1641, 2818, 3060; ^1H NMR: δ 1.80 (quint, 2H, J = 8 Hz), 2.41 (t, 2H, J = 7.8 Hz), 2.62 (m, 6H), 3.11 (m, 4H), 3.82 (s, 3H, OCH_3), 5.99 (s, 2H, NH_2), 6.73 (d, 1H, J = 8.4 Hz, ArH), 6.82–7.00 (m, 4H, ArH), 7.11 (dd, 1H, J = 2 Hz, J = 4.8 Hz, ArH), 7.97 (d, 1H, J = 2.2 Hz, ArH).

*7.3.2.6. 2-Nitro-4-[2-(4-phenylpiperazin-1-yl)ethoxy]aniline **(18a)**.* Yield: 70%; m.p. 127 °C; IR (cm^{-1}): 1217, 1246, 1417, 1509, 1596, 2944, 3372; ^1H NMR: δ 2.74 (m, 4H), 2.83 (t, 2H, J = 6 Hz), 3.21 (m, 4H), 4.12 (t, 2H, J = 5.8 Hz), 5.93 (s, 2H, NH_2), 6.81–6.97 (m, 4H, ArH), 7.23–7.34 (m, 3H, ArH), 8.11 (s, 1H, ArH).

*7.3.2.7. 2-Nitro-4-(2-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}ethoxy)aniline **(18b)**.* Yield: 72%; m.p. 85 °C; IR (cm^{-1}): 1215, 1315, 1416, 1513, 2828, 3171; ^1H NMR: δ 2.75 (t, 4H, J = 5 Hz), 2.87 (t, 2H, J = 5.4 Hz), 3.38 (t, 4H, J = 5 Hz), 4.12 (t, 2H, J = 4.8 Hz), 5.92 (s, 2H, NH_2), 6.77 (d, 1H, J = 9.2 Hz), 7.05–7.39 (m, 5H, ArH), 7.59 (d, 1H, J = 2.8 Hz).

*7.3.2.8. 2-Nitro-4-{2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethoxy}aniline **(18c)**.* Yield: 62%; m.p. 120 °C; IR (cm^{-1}): 1254, 1517, 1596, 2945; ^1H NMR (d_6DMSO): δ 2.62 (m, 4H), 2.73 (t, 2H, J = 5.6 Hz), 2.96 (m, 4H), 3.77 (s, 3H, OCH_3), 4.06 (t, 2H, J = 5.4 Hz), 6.87–6.95 (m, 4H, ArH, NH_2), 7.00 (d, 1H, J = 9.4 Hz, ArH), 7.19 (dd, 1H, J = 2.8 Hz, J = 6.2 Hz, ArH), 7.27 (s, 2H, ArH), 7.42 (d, 1H, J = 3 Hz, ArH).

*7.3.2.9. 2-Nitro-4-{2-[4-(3-methoxyphenyl)piperazin-1-yl]-ethoxy}aniline **(18d)**.* Yield: 66%; m.p. 119 °C; IR (cm^{-1}): 1138, 1256, 1419, 1515, 1596, 2944; ^1H NMR (d_6DMSO): δ 3.23 (m, 4H), 3.32 (m, 2H), 3.63 (m, 4H), 3.73 (s, 3H, OCH_3), 4.36 (t, 2H, J = 4 Hz), 4.51 (s, 2H, NH_2), 6.43–6.61 (m, 4H, ArH), 7.05 (d, 1H, J = 9.2 Hz, ArH), 7.26 (dd, 1H, J = 3 Hz, J = 9.2 Hz, ArH), 7.52 (d, 1H, J = 3 Hz, ArH).

*7.3.2.10. 2-Nitro-4-{2-[4-(4-methoxyphenyl)piperazin-1-yl]-ethoxy}aniline **(18e)***. Yield: 65%; m.p. 132 °C; IR (cm^{-1}): 1210, 1417, 1512, 2825; ^1H NMR: δ 2.76 (t, 4H, J = 2.8 Hz), 2.86 (t, 2H, J = 5.6 Hz), 3.13 (t, 4H, J = 4.6 Hz), 3.77 (s, 3H, OCH_3), 4.11 (t, 2H, J = 5.6 Hz), 6.02 (s, 2H, NH_2), 6.75–6.94 (m, 5H, ArH), 7.00 (dd, 1H, J = 3 Hz, J = 6.2 Hz, ArH), 7.58 (d, 1H, J = 3 Hz, ArH).

*7.3.2.11. 2-Nitro-4-{2-[4-(2-chlorophenyl)piperazin-1-yl]-ethoxy}aniline **(18f)***. Yield: 72%; m.p. 139 °C; IR (cm^{-1}): 1417, 1456, 1594, 2825, 3312; ^1H NMR: δ 2.78 (t, 4H, J = 4.6 Hz), 2.88 (t, 2H, J = 5.8 Hz), 3.12 (t, 4H, J = 4.6 Hz), 4.12 (t, 2H, J = 5.8 Hz), 6.22 (s, 2H, NH_2), 6.83 (d, 1H, J = 9.2 Hz, ArH), 6.93–6.14 (m, 3H, ArH), 7.12–7.26 (m, 1H, ArH), 7.36 (dd, 1H, J = 1.6 Hz, J = 6.4 Hz, ArH), 7.57 (d, 1H, J = 2.8 Hz, ArH).

*7.3.2.12. 2-Nitro-4-{2-[4-(3-chlorophenyl)piperazin-1-yl]-ethoxy}aniline **(18g)***. Yield: 68%; m.p. 110 °C; IR (cm^{-1}): 1216, 1255, 1513, 1595, 2830; ^1H NMR: δ 2.72 (m, 4H), 2.83 (t, 2H, J = 5.4 Hz), 3.23 (t, 4H, J = 5 Hz), 4.11 (t, 2H, J = 5.4 Hz), 5.91 (s, 2H, NH_2), 6.74–6.82 (m, 3H, ArH), 6.87–6.89 (m, 1H, ArH), 7.08–7.21 (m, 2H, ArH), 7.59 (d, 1H, J = 2.8 Hz, ArH).

*7.3.2.13. 2-Nitro-4-{2-[4-(4-chlorophenyl)piperazin-1-yl]-ethoxy}aniline **(18h)***. Yield: 65%; m.p. 106 °C; IR (cm^{-1}): 1207, 1416, 1509, 1642, 2819; ^1H NMR: δ 2.74 (m, 4H), 2.86 (t, 2H, J = 5.6 Hz), 3.19 (t, 4H, J = 5 Hz), 4.11 (t, 2H, J = 5.8 Hz), 5.91 (s, 2H, NH_2), 6.73–6.88 (m, 3H, ArH), 7.07–7.23 (m, 3H, ArH), 7.58 (d, 1H, J = 2.8 Hz, ArH).

7.3.3. General procedure for reduction

Ra–Ni (0.4–0.5 g) was added in small portions to a stirring solution of 6.5 mmol of either nitro compound (**2a–j**, **18a–h**) in 12 ml EtOH, 12 ml 1,2-dichloro-ethane and 2 ml (20 mmol) hydrazine hydrate at 30 °C. After the addition of Ra–Ni was completed, the mixture was heated in a water bath (50 °C, 60 min) and filtered through celite. The filtrate was evaporated in vacuo and crude product used for further syntheses.

7.3.4. General procedure for the synthesis of 1H-benzimidazoles

Two milimoles of diamine **3** or **19** and 5.6 mmol 96% formic acid were heated (100 °C, 2 h). After cooling to ambient temperature, 5 ml of 10% $NaHCO_3$ were added, the product extracted with CH_2Cl_2 and concentrated in vacuo. The resulting 1*H*-benzimidazoles were purified by chromatography or recrystallized from hot EtOH.

*7.3.4.1. 5-[3-(4-Phenylpiperazin-1-yl)propyl]-1H-benzimidazole **(4)***. Yield: 70%; m.p. 199–200 °C; IR (cm^{-1}): 1408, 1677, 2948, 3503; ^1H NMR of (d_6DMSO): δ 2.02 (m, 2H), 2.75 (t, 2H, J = 7.6 Hz), 3.04 (t, 2H, J = 7.6 Hz), 3.23 (m, 4H), 3.29 (m, 4H), 6.84 (t, 1H, J = 7.2 Hz, ArH), 6.98 (d, 2H, J = 8 Hz, ArH), 7.09 (d, 1H, J = 8 Hz, ArH), 7.25 (t, 2H, J = 7.8 Hz, ArH), 7.45 (s, 1H, ArH), 7.53 (d, 1H, J = 8 Hz, ArH), 8.23 (s, 1H, CH). MS: *m/e* 320.198. Anal.: $C_{20}H_{24}N_4$ (C, H, N).

*7.3.4.2. 5-(3-{4-[3-(Trifluoromethyl)phenyl]-piperazin-1-yl}propyl)-1H-benzimidazole **(5)***. Yield: 60%; m.p. oil; IR (cm^{-1}): 1451, 1495, 1612, 2947, 3029; ^1H NMR: δ 1.92 (quint, 2H, J = 8 Hz), 2.47 (t, 2H, J = 8.2 Hz), 2.62 (t, 4H J = 4.8 Hz), 2.79 (t, 2H, J = 7.6 Hz), 3.25 (t, 4H, J = 7 Hz), 7.03–7.16 (m, 4H, ArH), 7.34 (d, 1H, J = 7.6 Hz, ArH), 7.47 (s, 1H, ArH), 7.58 (d, 1H, J = 8.2 Hz, ArH), 8.05 (s, 1H, CH). MS: *m/e* 388.181. Anal.: $C_{21}H_{23}F_3N_4$ (C, H, N).

*7.3.4.3. 5-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-1H-benzimidazole **(6)***. Yield: 66%; m.p. 177 °C; IR (cm^{-1}): 1451, 1495, 1627, 2939, 3122; ^1H NMR: δ 1.80 (quint, 2H, J = 7.6 Hz), 2.33 (t, 2H, J = 6.8 Hz), 2.51 (m, 4H), 2.71 (t, 2H, J = 7.6 Hz), 3.18 (t, 4H, J = 4.8 Hz), 6.93 (d, 2H, J = 7.2 Hz, ArH), 7.05 (d, 1H, J = 8.2 Hz, ArH), 7.22 (d, 2H, J = 9 Hz, ArH), 7.45 (m, 2H, ArH), 8.14 (s, 1H, CH), 12.31 (s, 1H, NH). MS: *m/e* 354.160. Anal.: $C_{20}H_{23}ClN_4$ (C, H, N).

*7.3.4.4. 5-{3-[4-(4-Methoxyphenyl)piperazin-1-yl]propyl}-1H-benzimidazole **(7)***. Yield: 70%; m.p. 189 °C; IR (cm^{-1}): 1246, 1512, 1630, 2951, 3060, 3411; ^1H NMR: δ 1.88 (quint, 2H, J = 7.6 Hz), 2.43 (t, 2H, J = 6.8 Hz), 2.60 (m, 4H), 280 (t, 2H, J = 7.6 Hz), 3.15 (t, 4H, J = 4.8 Hz), 3.79 (s, 3H, OCH_3), 6.80–6.93 (m, 4H, ArH), 7.13 (dd, 1H, J = 1.4 Hz, J = 7 Hz, ArH), 7.45 (m, 1H, ArH), 7.57 (d, 1H, J = 8 Hz, ArH), 8.05 (s, 1H, CH), 12.31 (s, 1H, NH). MS: *m/e* 350.209. Anal.: $C_{21}H_{26}N_4O$ (C, H, N).

*7.3.4.5. 5-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl}-1H-benzimidazole **(8)***. Yield: 66%; m.p. oil; IR (cm^{-1}): 1242, 1513, 1638, 2942, 3083; ^1H NMR: δ 1.92 (quint, 2H, J = 8 Hz), 2.49 (t, 2H, J = 8.2 Hz), 2.68 (m, 4H), 2.79 (t, 2H, J = 7.6 Hz), 3.12 (m, 4H), 3.86 (s, 3H, OCH_3), 6.85–6.97 (m, 4H, ArH), 3.13 (d, 1H, J = 8.2 Hz, ArH), 7.44 (s, 1H, ArH), 7.57 (d, 1H, J = 8.6 Hz, ArH), 8.01 (s, 1H, CH). MS: *m/e* 350.212. Anal.: $C_{21}H_{26}N_4O$ (C, H, N).

*7.3.4.6. 5-[2-(4-Phenylpiperazin-1-yl)ethoxy]-1H-benzimidazole **(20)***. Yield: 62%; m.p. 74 °C; IR (cm^{-1}): 1165, 1238, 1452, 1598, 2823, 3149; ^1H NMR: δ 2.77 (t, 4H, J = 4.8 Hz), 2.90 (t, 2H, J = 5.6 Hz), 3.23 (t, 4H, J = 5.2 Hz), 4.18 (t, 2H, J = 5.6 Hz), 6.83–6.97 (m, 3H, ArH), 7.10 (d, 1H, J = 2 Hz, ArH), 7.23–7.29 (m, 3H, ArH), 7.54 (d, 1H, J = 9 Hz, AH), 7.98 (s, 1H, CH), 12.23 (s, 1H, NH). MS: *m/e* 322.183. Anal.: $C_{19}H_{22}N_4O$ (C, H, N).

*7.3.4.7. 5-(2-{4-[3-(Trifluoromethyl)phenyl]piperazin-1-yl}-ethoxy)-1H-benzimidazole **(21)***. Yield: 64%; m.p. oil; IR (cm^{-1}): 1121, 1314, 1453, 1615, 2952; ^1H NMR: δ 2.71 (t, 4H, J = 4.8 Hz), 2.85 (t, 2H, J = 5 Hz), 3.22 (t, 4H, J = 5 Hz), 4.11 (t, 2H, J = 5.4 Hz), 6.89–7.13 (m, 4H, ArH), 7.30 (t, 2H,

J = 7.8 Hz, ArH), 7.54 (d, 1H, *J* = 8.8 Hz, ArH), 8.08 (s, 1H, CH), 9.69 (s, 1H, NH). MS: *m/e* 390.166. Anal.: $C_{20}H_{21}F_3N_4O$ (C, H, N).

*7.3.4.8. 5-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy}-1H-benzimidazole **(22)**.* Yield: 57%, m.p. oil; IR (cm^{-1}): 1242, 1528, 1598, 2943; ^1H NMR: δ 2.83 (m, 6H), 3.12 (m, 4H, *J* = 5.2 Hz), 3.81 (s, 3H, OCH_3), 4.10 (t, 2H, *J* = 5.4 Hz), 6.81 (s, 1H, ArH), 6.85–6.89 (m, 3H, ArH), 6.95–7.04 (m, 2H, ArH), 7.46 (d, 1H, *J* = 8.8 Hz, AH), 8.05 (s, 1H, CH). MS: *m/e* 352.184. Anal.: $C_{20}H_{24}N_4O_2$ (C, H, N).

*7.3.4.9. 5-{2-[4-(3-Methoxyphenyl)piperazin-1-yl]ethoxy}-1H-benzimidazole **(23)**.* Yield: 60%; m.p. oil; IR (cm^{-1}): 1166, 1208, 1499, 1604, 2652; ^1H NMR: δ 2.76 (t, 4H, *J* = 5 Hz), 2.91 (t, 2H, *J* = 5.6 Hz), 3.24 (t, 4H, *J* = 4.8 Hz), 3.78 (s, 3H, OCH_3), 4.19 (t, 2H, *J* = 5.8 Hz), 6.40–6.47 (m, 2H, ArH), 6.55 (dd, 1H, *J* = 1.8 Hz, *J* = 6.6 Hz, ArH), 6.96 (dd, 1H, *J* = 2.4 Hz, *J* = 6.4 Hz, ArH), 7.13 (t, 1H, *J* = 2 Hz, ArH), 7.20 (d, 1H, *J* = 8.2 Hz, ArH), 7.55 (d, 1H, *J* = 8.8 Hz, AH), 7.98 (s, 1H, CH). MS: *m/e* 352.182. Anal.: $C_{20}H_{24}N_4O_2$ (C, H, N).

*7.3.4.10. 5-{2-[4-(4-Methoxyphenyl)piperazin-1-yl]ethoxy}-1H-benzimidazole **(24)**.* Yield: 57%; m.p. 115 °C; IR (cm^{-1}): 1151, 1251, 1458, 1512, 1632, 2827; ^1H NMR (d_6DMSO): δ 2.66 (t, 4H, *J* = 4.6 Hz), 2.79 (t, 2H, *J* = 5.6 Hz), 3.03 (t, 4H, *J* = 4 Hz), 3.68 (s, 3H, OCH_3), 4.14 (t, 2H, *J* = 5.6 Hz), 6.78–6.92 (m, 5H, ArH), 7.11 (s, 1H, ArH), 7.47 (d, 1H, *J* = 8.8 Hz, ArH), 8.09 (s, 1H, CH), 12.20 (s, 1H, NH). MS: *m/e* 352.191. Anal.: $C_{20}H_{24}N_4O_2$ (C, H, N).

7.3.5. General procedure for the synthesis of benzimidazole-2-thiones

Carbon disulfide (0.28 ml, 5.8 mmol) and KOH (0.28 g in 0.6 ml water) were added to 2.0 mmol of diamine **3** or **19** previously dissolved in 5 ml EtOH. After refluxing for 3 h, activated charcoal was added and the suspension filtered through celite. The solvent was removed in vacuo and the residue resuspended in 10% $NaHCO_3$, extracted with CH_2Cl_2 and concentrated in vacuo. The resulting benzimidazole-2-thiones were purified by chromatography or recrystallized from hot EtOH.

*7.3.5.1. 5-[3-(4-(Phenylpiperazin-1-yl)propyl]-1,3-dihydro-2H-benzimidazole-2-thione **(9)**.* Yield: 62%; m.p. >250 °C; IR (cm^{-1}): 1183, 1465, 1493, 1620, 2927; ^1H NMR (d_6DMSO): δ 1.75 (m, 2H), 2.31 (t, 2H, *J* = 6.8 Hz), 2.48 (m, 4H), 2.64 (t, 2H, *J* = 6.8 Hz), 3.12 (m, 4H), 6.76 (t, 1H, *J* = 7.4 Hz, ArH), 6.89–7.07 (m, 5H, ArH), 7.20 (t, 2H, *J* = 8 Hz, ArH). MS: *m/e* 352.172. Anal.: $C_{20}H_{24}N_4S$ (C, H, N).

*7.3.5.2. 5-(3-{4-[3-(Trifluoromethyl)phenyl]piperazin-1-yl}-propyl)-1,3-dihydro-2H-benzimidazole-2-thione **(10)**.* Yield: 58%; m.p. >250 °C; IR (cm^{-1}): 1162, 1460, 1614, 2836, 3081; ^1H NMR (d_6DMSO): δ 1.76 (quint, 2H, *J* = 7 Hz), 2.32 (t, 2H, *J* = 3.6 Hz), 2.52 (m, 4H), 2.65 (t, 2H, *J* = 7.4 Hz), 3.22 (m, 4H), 6.97–7.08 (m, 4H, ArH), 7.19 (s, 1H, ArH), 7.23 (d, 1H, *J* = 8.2 Hz, ArH), 7.40 (d, 1H, *J* = 8.2 Hz, ArH), 12.45 (s, 2H, NH). MS: *m/e* 420.161. Anal.: $C_{21}H_{23}F_3N_4S$ (C, H, N).

*7.3.5.3. 5-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-1,3-dihydro-2H-benzimidazole-2-thione **(11)**.* Yield: 55%; m.p. >250 °C; IR (cm^{-1}): 1241, 1497, 1596, 2829, 3034; ^1H NMR (d_6DMSO): δ 1.75 (quint, 2H, *J* = 7 Hz), 2.32 (t, 2H, *J* = 7.4 Hz), 2.50 (m, 4H), 2.64 (t, 2H, *J* = 7.2 Hz), 3.12 (m, 4H), 6.91–7.08 (m, 5H, ArH), 7.23 (s, 1H, ArH), 7.24 (s, 1H, ArH), 12.40 (s, 2H, NH). MS: *m/e* 386.133. Anal.: $C_{20}H_{23}ClN_4S$ (C, H, N).

*7.3.5.4. 5-{3-[4-(4-Methoxyphenyl)piperazin-1-yl]propyl}-1,3-dihydro-2H-benzimidazole-2-thione **(12)**.* Yield: 61%; m.p. >250 °C; IR (cm^{-1}): 1243, 1513, 1623, 2827, 3089, 3418; ^1H NMR (d_6DMSO): δ 1.74 (quint, 2H, *J* = 7 Hz), 2.30 (t, 2H, *J* = 6.8 Hz), 2.50 (m, 4H), 2.64 (t, 2H, *J* = 7.4 Hz), 3.00 (m, 4H), 3.68 (s, 3H, OCH_3), 6.78–6.91 (m, 4H, ArH), 6.91–7.07 (m, 3H, ArH), 12.45 (s, 2H, NH). MS: *m/e* 382.184. Anal.: $C_{21}H_{26}N_4OS$ (C, H, N).

*7.3.5.5. 5-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-thione **(13)**.* Yield: 55%; m.p. 127 °C; IR (cm^{-1}): 1241, 1511, 1625, 2831, 3092, 3418; ^1H NMR (d_6DMSO): δ 1.80 (m, 2H), 2.49 (t, 2H, *J* = 7.4 Hz), 2.65 (m, 6H), 3.01 (m, 4H), 3.77 (s, 3H, OCH_3), 6.87–7.09 (m, 7H, ArH), 12.48 (s, 2H, NH). MS: *m/e* 382.181. Anal.: $C_{21}H_{26}N_4OS$ (C, H, N).

*7.3.5.6. 5-[2-(4-Phenylpiperazin-1-yl)ethoxy]-1,3-dihydro-2H-benzimidazole-2-thione **(25)**.* Yield: 45%; m.p. 262 °C; IR (cm^{-1}): 1170, 1226, 1463, 1498, 1598, 2819, 3165; ^1H NMR (d_6DMSO): δ 2.63 (t, 4H, *J* = 4.6 Hz), 2.75 (t, 2H, *J* = 5.8 Hz), 3.13 (t, 4H, *J* = 5 Hz), 4.10 (t, 2H, *J* = 5.4 Hz), 6.73–6.80 (m, 3H, ArH), 6.91–7.05 (m, 3H, ArH), 7.17–7.25 (m, 2H, ArH), 12.40 (d, 2H, *J* = 9.4 Hz, NH). MS: *m/e* 354.153. Anal.: $C_{19}H_{22}N_4OS$ (C, H, N).

*7.3.5.7. 5-(2-{4-[3-(Trifluoromethyl)phenyl]piperazin-1-yl}-ethoxy)-1,3-dihydro-2H-benzimidazole-2-thione **(26)**.* Yield: 55%; m.p. 76 °C; IR (cm^{-1}): 1122, 1316, 1456, 1629, 2948; ^1H NMR (d_6DMSO): δ 2.69 (t, 4H, *J* = 4.8 Hz), 2.80 (t, 2H, *J* = 5.2 Hz), 3.25 (t, 4H, *J* = 4.8 Hz), 4.12 (t, 2H, *J* = 4.8 Hz), 6.73 (s, 1H, ArH), 6.76 (d, 1H, *J* = 8 Hz, ArH), 7.06 (t, 2H, *J* = 5.8 Hz, ArH), 7.17–7.24 (m, 2H, ArH), 7.42 (t, 1H, *J* = 7.8 Hz, ArH), 12.39 (s, 1H, NH), 12.45 (s, 1H, NH). MS: *m/e* 422.140. Anal.: $C_{20}H_{21}F_3N_4OS$ (C, H, N).

*7.3.5.8. 5-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-thione **(27)**.* Yield: 48%; m.p. 99 °C; IR (cm^{-1}): 1170, 1242, 1462, 1499, 1635, 2829; ^1H NMR (d_6DMSO): δ 2.68 (m, 4H), 2.86–2.90 (m, 2H), 2.97 (m, 4H), 3.78 (s, 3H, OCH_3), 4.10 (t, 2H, *J* = 5.6 Hz), 6.73 (s, 1H, ArH), 6.78 (d, 1H, *J* = 2.2 Hz), 6.88–6.93 (m, 4H, ArH),

7.03 (d, 1H, *J* = 8.4 Hz, ArH), 12.39 (s, 1H, NH), 12.44 (s, 1H, NH). MS: *m/e* 384.162. Anal.: $C_{20}H_{24}N_4O_2S$ (C, H, N).

*7.3.5.9. 5-{2-[4-(3-Methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-thione **(28)**.* Yield: 52%; m.p. 201 °C; IR (cm^{-1}): 1168, 1208, 1461, 1607, 2826; ^1H NMR (d_6DMSO): δ 2.62 (t, 4H, *J* = 4.8 Hz), 2.74 (t, 2H, *J* = 5.4 Hz), 3.13 (t, 4H, *J* = 4.4 Hz), 3.71 (s, 3H, OCH_3), 4.09 (t, 2H, *J* = 5.6 Hz), 6.36 (dd, 1H, *J* = 2 Hz, *J* = 6 Hz, ArH), 6.44 (t, 1H, *J* = 2 Hz, ArH), 6.52 (dd, 1H, *J* = 1.8 Hz, *J* = 6.4 Hz, ArH), 6.73 (s, 1H, ArH), 6.77 (d, 1H, *J* = 2.2 Hz, ArH), 7.02–7.14 (m, 2H, ArH), 12.40 (s, 2H, NH). MS: *m/e* 384.160. Anal.: $C_{20}H_{24}N_4O_2S$ (C, H, N).

*7.3.5.10. 5-{2-[4-(4-Methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-thione **(29)**.* Yield: 49%; m.p. 255 °C; IR (cm^{-1}): 1168, 1253, 1462, 1512, 1629, 2825; ^1H NMR (d_6DMSO): δ 2.67 (t, 4H, *J* = 4.6 Hz), 2.76 (t, 2H, *J* = 5.4 Hz), 3.02 (t, 4H, *J* = 4.2 Hz), 3.68 (s, 3H, OCH_3), 4.10 (t, 2H, *J* = 5.6 Hz), 6.72–6.91 (m, 6H, ArH), 7.03 (d, 1H, *J* = 8.4 Hz, ArH), 12.39 (s, 1H, NH), 12.44 (s, 1H, NH). MS: *m/e* 384.164. Anal.: $C_{20}H_{24}N_4O_2S$ (C, H, N).

*7.3.5.11. 5-{2-[4-(2-Chlorophenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-thione **(30)**.* Yield: 62%, m.p. 232 °C; IR (cm^{-1}): 1170, 1464, 1632, 3131; ^1H NMR (d_6DMSO): δ 2.73 (m, 4H), 2.85 (m, 2H), 3.00 (m, 4H), 4.12 (t, 2H, *J* = 5.4 Hz), 6.73 (s, 1H, ArH), 7.00–7.08 (m, 2H, ArH), 7.11–7.18 (m, 2H, ArH), 7.26–7.34 (m, 1H, ArH), 7.39–7.46(m, 1H, ArH), 12.42 (d, 2H, *J* = 9.6 Hz, NH). MS: *m/e* 388.112. Anal.: $C_{19}H_{21}ClN_4OS$ (C, H, N).

*7.3.5.12. 5-{2-[4-(3-Chlorophenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-thione **(31)**.* Yield: 58%; m.p. 170 °C; IR (cm^{-1}):1171, 1463, 1498, 1596, 2829; ^1H NMR (d_6DMSO): δ 2.62 (t, 4H, *J* = 4.8 Hz), 2.74 (t, 2H, *J* = 5.4 Hz), 3.18 (t, 4H, *J* = 4.6 Hz), 4.10 (t, 2H, *J* = 5.8 Hz), 6.72–6.79 (m, 3H, ArH), 6.88–6.94 (m, 2H, ArH), 7.01 (d, 1H, *J* = 8.8 Hz, ArH), 7.20 (t, 1H, *J* = 8 Hz, ArH), 12.38 (broad s, 2H, NH). MS: *m/e* 388.114. Anal.: $C_{19}H_{21}ClN_4OS$ (C, H, N).

*7.3.5.13. 5-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-thione **(32)**.* Yield: 50%; m.p. 194 °C; IR (cm^{-1}): 1168, 1463, 1498, 1596, 1632, 2825; ^1H NMR (d_6DMSO): δ 2.62 (t, 4H, *J* = 5.2 Hz), 2.74 (t, 2H, *J* = 5.2 Hz), 3.13 (t, 4H, *J* = 4.4 Hz), 4.09 (t, 2H, *J* = 5.8 Hz), 6.72–6.76 (d, 2H, *J* = 8.2 Hz, ArH), 6.91–7.05 (m, 3H, ArH), 7.22 (d, 2H, *J* = 8.8 Hz, ArH), 12.36 (broad s, 2H, NH). MS: *m/e* 388.110. Anal.: $C_{19}H_{21}ClN_4OS$ (C, H, N).

7.3.6. General procedure for the synthesis of benzimidazole-2-ones

1,1′-Carbonyldiimidazole (1.4 g, 8.6 mmol) was added step-wise to the stirred solution of 12 ml dried acetonitrile and diamine **19** (0.72 g, 2 mmol) at ambient temperature. When the addition was completed, the mixture was stirred for additional 30 min and transferred in a refrigerator overnight. The solvent was removed in vacuo and the residue recrystallized in $CHCl_3$/EtOH mixture.

*7.3.6.1. 5-[2-(4-Phenylpiperazin-1-yl)ethoxy]-1,3-dihydro-2H-benzimidazole-2-one **(33)**.* Yield: 58%; m.p. 243 °C; IR (cm^{-1}): 1170, 1499, 1701, 1754, 2824; ^1H NMR (d_6DMSO): δ 2.63 (t, 4H, *J* = 5 Hz), 2.73 (t, 2H, *J* = 5.8 Hz), 3.13 (t, 4H, *J* = 5 Hz), 4.05 (t, 2H, *J* = 5.6 Hz), 6.51–6.55 (m, 2H, ArH), 6.75–6.82 (m, 2H, ArH), 6.90–6.95 (m, 2H, ArH), 7.17–7.25 (m, 2H, ArH), 10.38 (s, 1H, NH), 10.52 (s, 1H, NH). MS: *m/e* 338.176. Anal.: $C_{19}H_{22}N_4O_2$ (C, H, N).

*7.3.6.2. 5-(2-{4-[3-(Trifluoromethyl)phenyl]piperazin-1-yl}-ethoxy)-1,3-dihydro-2H-benzimidazole-2-one **(34)**.* Yield: 53%; m.p. 226 °C; IR (cm^{-1}): 1166, 1503, 1696, 2972; ^1H NMR (d_6DMSO): δ 2.63 (t, 4H, *J* = 5 Hz), 2.73 (t, 2H, *J* = 5.6 Hz), 3.23 (t, 4H, *J* = 5.2 Hz), 4.06 (t, 2H, *J* = 6 Hz), 6.51 (d, 1H, *J* = 2.4 Hz, ArH), 6.55 (s, 1H, ArH), 6.80 (d, 1H, *J* = 9.2 Hz, ArH), 7.06 (d, 1H, *J* = 7.2 Hz, ArH), 7.16 (s, 1H, ArH), 7.21 (d, 1H, *J* = 8.2 Hz, ArH), 7.41 (t, 1H, *J* = 8.2 Hz, ArH), 10.38 (s, 1H, NH), 10.52 (s, 1H, NH). MS: *m/e* 406.163. Anal.: $C_{20}H_{21}F_3N_4O_2$ (C, H, N).

*7.3.6.3. 5-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazol-2-one **(35)**.* Yield: 56%; m.p. 182 °C; IR (cm^{-1}): 1168, 1242, 1502, 1643, 1703, 2927; ^1H NMR: δ 2.85 (m, 6H), 3.15 (m, 4H), 3.86 (s, 3H, OCH_3), 4.11 (t, 2H, *J* = 5.4 Hz), 6.58 (d, 1H, *J* = 2.4 Hz, ArH), 6.62 (s, 1H, ArH), 6.84–7.05 (m, 5H, ArH), 9.22 (s, 1H, NH), 9.48 (s, 1H, NH). MS: *m/e* 368.184. Anal.: $C_{20}H_{24}N_4O_3$ (C, H, N).

*7.3.6.4. 5-{2-[4-(3-Methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazol-2-one **(36)**.* Yield: 54%; m.p. 238 °C; IR (cm^{-1}): 1171, 1580, 1608, 1703, 2835; ^1H NMR (d_6DMSO): δ 2.61 (t, 4H, *J* = 5 Hz), 2.72 (t, 2H, *J* = 5.6 Hz), 3.13 (t, 4H, *J* = 4.6 Hz), 3.71 (s, 3H, OCH_3), 4.05 (t, 2H, *J* = 5.8 Hz), 6.36 (dd, 1H, *J* = 2.4 Hz, *J* = 5.8 Hz, ArH), 6.44 (s, 1H, ArH), 6.49–6.55 (m, 3H, ArH), 6.80 (d, 1H, *J* = 9.2 Hz, ArH), 7.10 (t, 1H, *J* = 8.2 Hz, ArH), 10.38 (s, 1H, NH), 10.52 (s, 1H, NH). MS: *m/e* 368.182. Anal.: $C_{20}H_{24}N_4O_3$ (C, H, N).

*7.3.6.5. 5-{2-[4-(4-Methoxyphenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-one **(37)**.* Yield: 55%; m.p. 185 °C; IR (cm^{-1}): 1172, 1253, 1511, 1755, 2830; ^1H NMR (d_6DMSO): δ 2.63 (t, 4H, *J* = 4.8 Hz), 2.72 (t, 2H, *J* = 5.6 Hz), 3.01 (t, 4H, *J* = 5.2 Hz), 3.68 (s, 3H, OCH_3), 4.05 (t, 2H, *J* = 5.8 Hz), 6.51 (d, 1H, *J* = 2.4 Hz, ArH), 6.55 (s, 1H, ArH), 6.56–6.91 (m, 5H, ArH), 10.38 (s, 1H, NH), 10.51 (s, 1H, NH). MS: *m/e* 368.186. Anal.: $C_{20}H_{24}N_4O_3$ (C, H, N).

*7.3.6.6. 5-{2-[4-(2-Chlorophenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazol-2-one **(38)**.* Yield: 65%; m.p. 217 °C; IR (cm^{-1}): 1170, 1479, 1713, 2943; ^1H NMR

(d_6DMSO): δ 2.67 (m, 4H), 2.75 (t, 2H, J = 5.6 Hz), 2.99 (m, 4H), 4.05 (t, 2H, J = 5.6 Hz), 6.51–6.56 (m, 2H, ArH), 6.80 (d, 1H, J = 9 Hz, ArH), 6.91–7.04 (m, 1H, ArH), 7.18 (dd, 1H, J = 1.4 Hz, J = 6.6 Hz, ArH), 7.26–7.33 (m, 1H, ArH), 7.40 (dd, 1H, J = 1.4 Hz, J = 6.2 Hz, ArH), 10.39 (s, 1H, NH), 10.53 (s, 1H, NH). MS: *m/e* 372.137. Anal.: $C_{19}H_{21}ClN_4O_2$ (C, H, N).

*7.3.6.7. 5-{2-[4-(3-Chlorophenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-one **(39)**.* Yield: 61%, m.p. 183 °C; IR (cm^{-1}): 1169, 1594, 1698, 1751, 2836; ^{1}H NMR (d_6DMSO): δ 2.62 (t, 4H, J = 4.8 Hz), 2.73 (t, 2H, J = 5.6 Hz), 3.18 (t, 4H, J = 4.6 Hz), 4.05 (t, 2H, J = 5.6 Hz), 6.51–6.55 (m, 2H, ArH), 6.75–6.79 (m, 2H, ArH), 6.87–6.94 (m, 2H, ArH), 7.20 (t, 1H, J = 7.8 Hz, ArH), 10.39 (s, 1H, NH), 10.52 (s, 1H, NH). MS: *m/e* 372.134. Anal.: $C_{19}H_{21}ClN_4O_2$ (C, H, N).

*7.3.6.8. 5-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethoxy}-1,3-dihydro-2H-benzimidazole-2-one **(40)**.* Yield: 60%; m.p. 181 °C; IR (cm^{-1}): 1168, 1233, 1699, 1751, 2819; ^{1}H NMR (d_6DMSO): δ 2.63 (t, 4H, J = 4.8 Hz), 2.74 (t, 2H, J = 5.6 Hz), 3.14 (t, 4H, J = 4.6 Hz), 4.05 (t, 2H, J = 5.6 Hz), 6.51–6.55 (m, 2H, ArH), 6.80 (d, 1H, J = 9.4 Hz, ArH), 6.93 (d, 2H, J = 9 Hz, ArH), 7.22 (d, 2H, J = 8.8 Hz, ArH), 10.39 (s, 1H, NH), 10.52 (s, 1H, NH). MS: *m/e* 372.134. Anal.: $C_{19}H_{21}ClN_4O_2$ (C, H, N).

7.3.7. General procedure for the synthesis of 1H-benzotriazoles

Cold sodium nitrite solution (0.24 g, 3.47 mmol) and 0.36 ml water were poured in a cooled mixture (0 °C) of diamine **19** (0.8 g, 3.1 mmol), 0.7 ml acetic acid and 1.4 ml water. Upon the addition was completed, the mixture was heated at 70 °C for additional 10–15 min, cooled to ambient temperature, neutralized with 10% sodium carbonate solution, extracted with CH_2Cl_2 and concentrated in vacuo. The resulting benzotriazoles were purified by chromatography or recrystallized from hot EtOH.

*7.3.7.1. 5-[2-(4-Phenylpiperazin-1-yl)ethoxy]-1H-benzotriazole **(41)**.* Yield: 58%; m.p. 91 °C; IR (cm^{-1}): 1209, 1452, 1598, 1627, 2780, 2952; ^{1}H NMR (d_6DMSO): δ 2.66 (t, 4H, J = 4.6 Hz), 2.82 (t, 2H, J = 5.6 Hz), 3.14 (t, 4H, J = 4.8 Hz), 4.23 (t, 2H, J = 5.6 Hz), 6.77 (t, 1H, J = 7.2 Hz, ArH), 6.92 (d, 2H, J = 7.8 Hz, ArH), 7.04 (dd, 1H, J = 2.2 Hz, J = 6.8 Hz, ArH), 7.17–7.26 (m, 3H, ArH), 7.84 (d, 1H, J = 9.2 Hz, ArH). MS: *m/e* 323.175. Anal.: $C_{18}H_{21}N_5O$ (C, H, N).

*7.3.7.2. 5-(2-{4-[3-(Trifluoromethyl)phenyl]piperazin-1-yl}-ethoxy)-1H-benzotriazole **(42)**.* Yield: 59%; m.p. oil; IR (cm^{-1}): 1121, 1165, 1452, 1621, 2831; ^{1}H NMR: δ 2.81 (t, 4H, J = 4.6 Hz), 2.93 (t, 2H, J = 5.2 Hz), 3.28 (t, 4H, J = 5 Hz), 4.16 (t, 2H, J = 5 Hz), 6.93 (dd, 1H, J = 2 Hz, J = 7.2 Hz, ArH), 7.01–7.09 (m, 4H, ArH), 7.78 (d, 1H, J = 9.2 Hz, ArH), 11.63 (s, 1H, NH). MS: *m/e* 391.163. Anal.: $C_{19}H_{20}F_3N_5O$ (C, H, N).

*7.3.7.3. 5-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy}-1H-benzotriazole **(43)**.* Yield: 59%; m.p. 82 °C; IR (cm^{-1}): 1217, 1242, 1455, 1500, 1628, 2831; ^{1}H NMR (d_6DMSO): δ 2.89 (m, 6H), 3.18 (m, 4H), 3.82 (s, 3H, OCH_3), 4.13 (t, 2H, J = 4.8 Hz), 6.84–7.04 (m, 5H, ArH), 7.28 (s, 1H, ArH), 7.74 (d, 1H, J = 8.8 Hz, ArH), 12.27 (s, 1H, NH). MS: *m/e* 353.187. Anal.: $C_{19}H_{23}N_5O_2$ (C, H, N).

7.4. Pharmacology

7.4.1. Membrane preparation, radioligand binding assays and data analysis

Synaptosomal membranes from fresh bovine caudate nuclei and hippocampi for radioligand binding assays were prepared as previously described [25]. [^{3}H]SCH 23390 (spec. act. 80 Ci $mmol^{-1}$), [^{3}H]spiperone (spec. act. 70.5 Ci $mmol^{-1}$) and 8-OH-[^{3}H]DPAT (spec. act. 223 Ci $mmol^{-1}$) used to label D_1, D_2 and 5-HT_{1A} receptors, respectively, were purchased from Amersham Buchler GmbH (Braunschweig, Germany).

7.4.1.1. [^{3}H]Spiperone receptor binding assay. [^{3}H]Spiperone binding was assayed in 1.0 mM EDTA, 4 mmol $MgCl_2$, 1.5 mM $CaCl_2$, 5 mmol KCl, 120 mM NaCl, 25 mM Tris–HCl solution, pH 7.4, at membrane protein concentration of 0.7 mg ml^{-1} at 37 °C for 20 min in a total volume of incubation mixture of 1.0 ml. Binding of the radioligand to 5-HT_2 receptors was prevented by 50 mM ketanserin. The K_i values of the tested compounds were determined by competition binding at 0.2 nmol of the radioligand and eight to 10 different concentrations of each compound (10^{-4}–10^{-10} M). Non-specific binding was measured in the presence of 1.0 mmol (+)-butaclamol. The reaction was terminated by rapid filtration through Whatman GF/C filters, which were further washed three times with 5.0 ml of ice-cold incubation buffer. Each point was determined in triplicate. Retained radioactivity was measured by introducing dry filters into 10 ml of toluene-based scintillation liquid and counting in a 1219 Rackbeta Wallac scintillation counter at an efficiency of 51–55% for tritium.

7.4.1.2. [^{3}H]SCH 23390 receptor binding assay. Binding of [^{3}H]SCH 23390 was examined by the same rapid filtration assay discussed for [^{3}H]spiperone, in the absence of ketanserin.

7.4.1.3. 8-OH-[^{3}H]DPAT receptor binding assay. Each tube contained 1.0 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris–HCl, 10 μmol nialamide and 0.1% ascorbic acid, pH 7.4, membrane protein concentration of 0.7 mg ml^{-1}, 0.6 nmol 8-OH-[^{3}H]DPAT and various concentrations (10^{-4}–10^{-10} M) of the tested compounds in a final volume of 0.5 ml. The tubes were incubated (20 min, 37 °C) and the reaction terminated by vacuum filtration through Whatman GF/B filters. The filters were washed three times with 5 ml of ice-cold 25 mM Tris–HCl buffer, pH 7.4, and bound radioactivity measured by liquid scintillation spec-

trometry. 5-HT_{1A} specific binding was defined as the difference between the binding in the absence and in the presence of 10 μM 5-hydroxytryptamine.

Competition binding data were analyzed by the iterative non-linear least-squares curve-fitting program LIGAND [26].

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