Full Paper

Synthesis and Pharmacological Evaluation of Thiazole and Isothiazole Derived Apomorphines

Attila Sipos[1], Franziska K. U. Mueller[2], Jochen Lehmann[2], Sándor Berényi[1], and Sándor Antus[1,3]

[1] Department of Organic Chemistry, University of Debrecen, Debrecen, Hungary
[2] Institut für Pharmazie, Lehrstuhl für Pharmazeutische / Medizinische Chemie, Friedrich-Schiller-Universität Jena, Jena, Germany
[3] Research Group for Carbohydrates of the Hungarian Academy of Sciences, Debrecen, Hungary

We have presented the synthesis of novel thiazolo- and isothiazolo-apomorphines **12**–**17** resulting – in part – from an unexpected isomerization step occurred during the acid-catalyzed rearrangement of precursor thiazolo-morphinandienes **3**–**5**. These 2,3-disubstituted apomorphines represent a new group of A-ring substituted aporphines. The receptor binding studies revealed that with the exception of two derivatives all the tested compounds have limited affinity for dopamine-receptor subtypes. Functional calcium assay for the most active isothiazolo-apomorphine showed higher affinities for D_1 and D_{2L} subtypes. The docking of these ligands has been modelled to human D_2 and D_3 receptors. On the basis of the predicted models, we identified an important cation-p interaction for the binding of isothiazolo-apomorphine **16**.

Keywords: Benzothiazole-benzisothiazole isomerization / Docking models / Dopamine receptor activity / Thiazole moiety

Received: May 5, 2009; accepted: June 15, 2009

DOI 10.1002/ardp.200900100

Introduction

Thiazole ring systems are widely used structural elements in medicinal chemistry. This structure, especially in case of the 2-amino substitution, has been applied in the development of drugs for the treatment of allergies, hypertension, inflammation, schizophrenia, and bacterial and HIV infections [1]. It is known to be a ligand of estrogen receptors [2] as well as of a novel class of adenosine receptors [3]. Görlitzer *et al*. prepared aminothiazole-fused morphinans in order to test the effect of this novel moiety on the affinity for opioid receptors [4]. Recently, Neumeyer's group [5] synthesized 2′-aminothiazole derivatives of morphans and morphinans to study their opioid agonist feature.

The efforts of several research groups to explore the structure-activity relationships (SAR) of apomorphine derivatives to different dopamine receptor subtypes have been summarized in a recent review [6]. It was shown that the absolute configuration of *C*-6a is a critical factor for the dopaminergic properties, since *R*-(−)-enantiomers are known to be dopamine (DA) agonist and *S*-(+)-counterparts have antagonist features. Moreover, it was also reported that C2- and C3-substituents on the A-ring have an important role in the DA-receptor binding ability. This conclusion was based on the results revealing the existence of a lipophilic cleft on DA receptors. Søndergaard *et al*. [7] reported results which revealed that a compound having large substituents such as a 4-hydroxyphenyl group in the 2-position had also high affinity for the D_2 receptor subtype. Our concept for the development of selective and potent D_2 receptor agonists was based on the fact that the 2-aminothiazole functionality was shown to be a heterocyclic bioisostere of the phenol moiety in case of the dopamine agonist pramipexole [8] and an aminothiazole-containing catechol aporphine [9] (Fig. 1).

A procedure has been elaborated for the synthesis of 2,3-thiazole-fused apomorphine derivatives. The synthesis was based on naturally occurring thebaine **1** which

Correspondence: Attila Sipos, Department of Organic Chemistry, University of Debrecen, P. O. Box 20, H-4010, Debrecen, Hungary
E-mail: asipos@puma.unideb.hu
Fax: +36 52 512-836

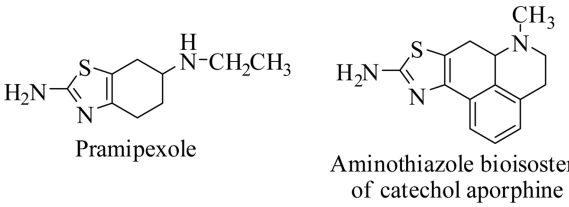


Figure 1. Structural similarity of two D_2 receptor agonists.

ensured the *R* absolute configuration of C-6a. In determining the C2 and C3 positions, the phenol-bioisostere 2-aminothiazole moiety has been inserted to mimic the 2-(4-hydroxyphenyl)-apomorphine structure (Fig. 2). The synthesis was extended for the preparation of thiazolo-aporphines containing other pharmacophore groups in the 2′-position.

Zhang *et al*. [10] recently reported the synthesis and pharmacological evaluation of a series of apomorphine derivatives privileged with a 2-aminothiazole functionality. The design of these compounds was based on fundamentally the same rationales as presented above. The 2′-aminothiazolo-apomorphine, presented in Fig. 2, was synthesized in a completely different approach involving the insertion of a 2-amino function to the protected aporphine backbone.

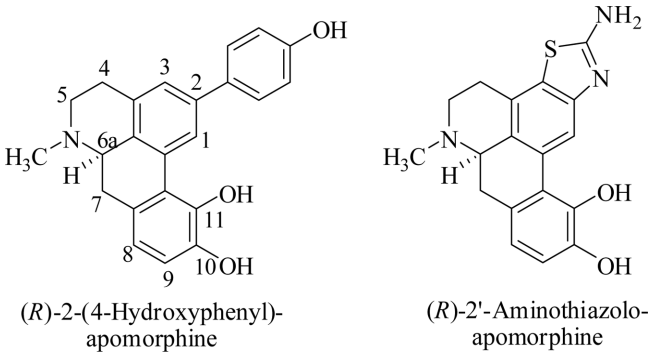


Figure 2. Bioisostere structures of apomorphine derivatives.

Results and discussion

Synthesis

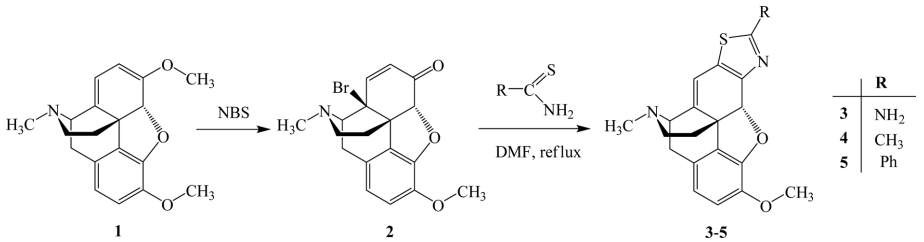
The synthesis of morphinan derivatives bearing an aminothiazole moiety at the C-ring of the morphinan skeleton was first reported by Neumeyer and his co-workers [11]. In one of our recent publications, we have reported a different approach for the synthesis of 2′-substituted thiazolo-morphinandienes [12]. In the first step, thebaine **1** is converted into 14β-bromo-codeinone **2** in a well-known reaction [13]. A Hantzsch-type thiazole synthesis is accomplished on compound **2** exploiting its ability to react as an α-haloketone [14] in the presence of an appropriate nucleophile partner (Scheme 1).

The morphinandiene structure of products **3** to **5** offers the possibility of rearrangement into aporphinoids. In the acid-catalyzed rearrangement of thiazolo-morphinandienes **3** to **5**, we observed a major difference in the behaviour of 2′-aminothiazolo-morphinandiene **3** and its 2′-methyl- (**4**) and phenyl- (**5**) congeners. This type of morphinandiene rearrangement has been studied in our laboratory for over 20 years [15] and it has been concluded that this reaction produces apocodeine derivatives almost quantitatively.

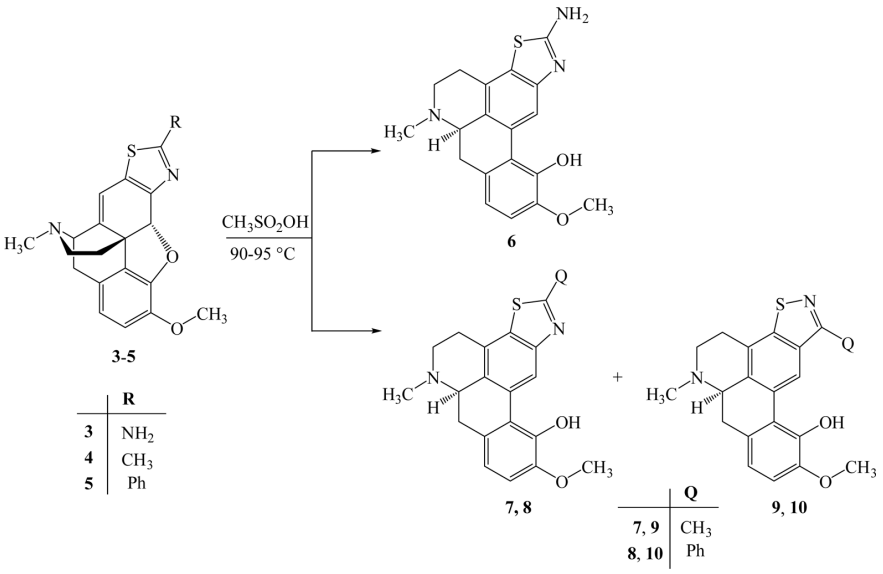
Our observations for the amino-type starting material **3** were in accordance with this general experience, however, in case of the methyl- and phenyl-derivatives **4**, **5** we found two products in approximately 1:1 ratio (Scheme 2). After isolation and full characterization of the products it was revealed that isothiazole-type compounds **9**, **10** were formed beside the previously expected thiazolo-aporphines **7**, **8**.

The explanation for the occurrence of isothiazolo-aporphines **9**, **10** is an isomerization reaction which happens simultaneous with the acid-catalyzed rearrangement. Sharp *et al*. presented the same phenomenon for ziprasidone [16]. They adopted a reversible photo-/thermal-induced isomerization mechanism as the rationalization for benzisothiazole-benzothiazole rearrangement (Scheme 3).

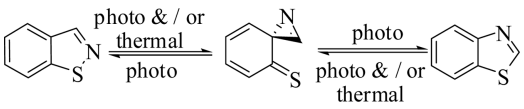
We applied 90–95°C heat, a polar solvent, and no protection from light as conditions for acid-catalyzed rearrangements. These conditions are in agreement with those used by Sharp in isomerization tests. The acid-catalyzed rearrangement was also performed in inert atmosphere (nitrogen gas) with the use of protection from light but there was no significant change observed in the ratio



Scheme 1. Synthesis route to 2′-substituted thiazolo-morphianandienes.



Scheme 2. Formation of thiazolo- and isothiazolo-apocodeines **6**–**10**.



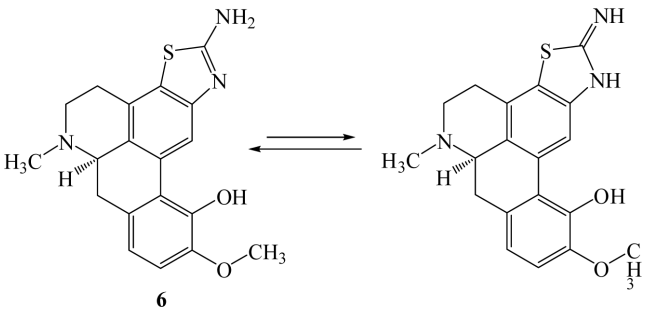
Scheme 3. Proposed mechanism for the isomerisation.

of thiazolo and isothiazolo products. A photochallenge [17] was also conducted with compounds **7** and **8** using the same solvents and concentrations as Sharp *et al*. On the basis of these results, we concluded that in this case the heat was the most important factor in the activation of the isomerization in comparison with ziprasidone [16].

D'Auria discussed the isomerization of some thiazoles into isothiazoles presenting examples for the photo-induced isomerization as well as for photo and thermal dependence of this type of rearrangements [18]. A mechanism was described for the photo- and thermal-induced isomerization of 2-Ph-thiazoles involving a Dewar 2-Ph-thiazole structure as an intermediate.

The reason for the difference in behavior of the amino-derivative and its methyl- and phenyl-congeners could be justified by the significant willingness of the 2-amino-benzothiazole moiety for tautomerization (Scheme 4). The appearance of this imine-type tautomer means an extra stabilization for the benzothiazole structure against benzisothiazole, and may be an inhibitory effect of the isomerization.

The structure of the isothiazoloaporphines was unequivocally established by NMR spectral studies. Full ^{1}H and ^{13}C resonance assignments were obtained from COSY, TOCSY, HSQC, and HMBC spectra. The main differences in



Scheme 4. Tautomer forms of 2′-aminothiazolo-apocodeine (**6**).

HMBC correlations of 2′-methylthiazolo- (**7**) and 2′-methyl-isothiazolo-derivatives (**9**) are presented in Fig. 3.

In order to expand the range of pharmacophores in the 2′-position and to facilitate further functionalization (*e.g*. by palladium-catalyzed cross-coupling), the 2′-amino group was replaced in product **6** with a chloro substituent in a one-pot method using isoamyl-nitrite-effected (*i*-Am-ONO) diazotation and halogenations with $CuCl_2$ in acetonitrile (Scheme 5) [19].

The conversion of 10-methoxyaporphines **6**–**11** into catechol-aporphines **12**–**17** was carried out by means of the methanesulfonic acid/methionine reagent-combination which was successfully used earlier by our laboratory (Scheme 6) [20].

As discussed above, the isomerization from thiazole derivatives to isothiazoles was primarily induced by thermal effects. To inhibit further isomerization of apocodeines **7**–**10** during the *O*-demethylation step, the classic conditions were modified and a microwave-assisted procedure was established on the basis of previous studies

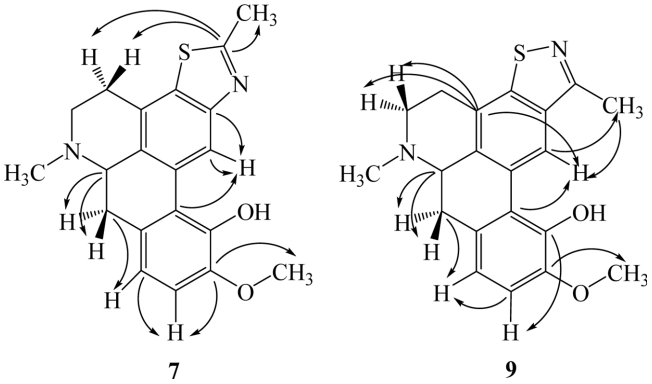
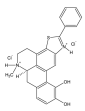
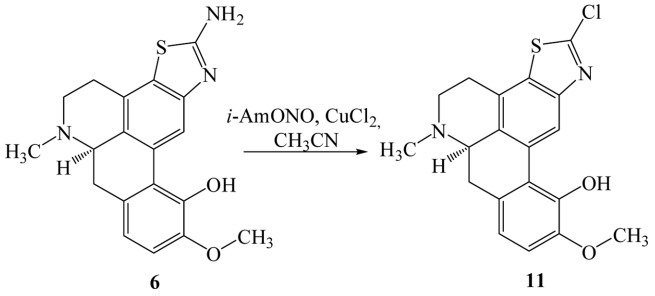


Figure 3. Important HMBC correlations for benzothiazole- and benzisothiazole-type compounds.

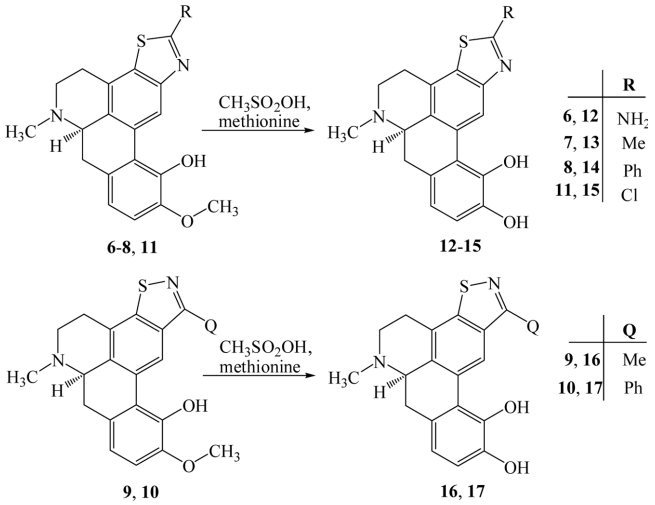
Table 1. Affinities for dopamine receptor subtypes measured by radioligand-binding studies.

Compounds		K_i (nM) Average ± SD or SEM (experiments in triplicate)	
		hD_1	hD_{2L}
Apomorphine · HCl*		101	32
12		2113 ± 203 (2)	2883 ± 664 (2)
13		2753± 61 (2)	5192 ± 684 (2)
14		1812 ± 49 (2)	2548 ± 544 (2)
15		>10 000	>10 000
16		1027 ± 137 (2)	197 ± 83 (2)
17		>10 000	5362 ± 2172 (2)

SD = Standard deviation; SEM = Standard error of the mean; the SEM was used, when the number of values was less than three; * data from Ref. [7].



Scheme 5. Synthesis of 2′-chlorothiazolo-apocodeine **11**.



Scheme 6. Formation of apomorphine derivatives **12**–**17**.

on the application of microwave-promoted rearrangements of morphinans [21]. The temperature was decreased from 90–95°C to 75°C, and inert atmosphere was applied. The reaction time was sharply reduced from an average of 120 min to 5 min. No significant isomerization was observed during the *O*-demethylation of benzothiazolo- and benzisothiazolo-type apocodeines **6**–**11**. Apomorphine derivatives **12**–**17** were precipitated from ether in HCl salt form. These salts were found to be appropriately water-soluble for pharmacological testing.

Pharmacology

All compounds were screened for their binding affinities for the human cloned D_1 and D_{2L} receptor subtypes by *in vitro* radioligand-binding studies. The results are summarized in Table 1. With the exception of **15**, all of the compounds display low affinities reaching the maximum for the isothiazole derivative **16** at the D_2 binding site. It could also be concluded from the presented data that the binding activities for both D_1 and D_{2L} subtypes

were largely affected by the spatial size and lipophilicity of the substitutent at the 2′-position of the thiazole ring. The affinities of compound **14**, containing a large and a lipophilic phenyl moiety at 2′-position, were found to be superior in comparison to ligands **12**, **13**, and **15** bearing either a small and lipophilic subtituent (compound **13**, R = CH_3) or small and hydrophilic moieties (compounds **13** and **15**, R = NH_3^+ and Cl). In the case of compound **12**, the obtained binding affinities for the human cloned D_1 and D_{2L} were found to be in good agreement with the trends observed by Zhang *et al.* [10].

In the case of isothiazole-derived apomorphines, the spatial size of the 3′-subtituent was found to be determining. For isothiazole-fused backbone it was observed that it tolerated much smaller deviation in spatial size in the proximity of the 2-position of the aporphine skeleton. This observation contradicts the generally accepted view that the binding affinity of 2-substituted apomorphines are not predominantly determined by the size of the moiety introduced at the 2-position (referring to the high affinities of both 2-methoxy- and 2-(4-hydroxyphenyl)-apomorphines) [6, 7]. This phenomenon was associated with the additional electrostatic effects of the isothiazole moiety being introduced on the aporphine backbone. However, it was only possible to draw further conclusions after the establishment of binding models for the studied dopamine-receptor subtypes (see Section 2.3: Binding models and predicting ligand interactions).

In order to obtain a detailed pharmacological profile for the most active compound, the binding affinities of compound **16** were extended to all five human dopamine-receptor subtypes and the results are summarized in Table 2.

These tests revealed that 3′-methylisothiazolo-apomorphine **16** had a significantly stronger affinity for the D_{2L} subtype referring to the calculated specificities. It is also remarkable that there is a considerable difference in the binding activity compared to the two subtypes of the D_2-like receptor family.

For functional studies, hD_1 and hD_{2L} receptors were chosen as characteristic representatives of each of the two dopamine-receptor subtype groups allowing a comparison of functional and binding data. The inhibition by compound **16** of agonist-induced changes in intracellular [Ca^{2+}] in intact HEK293 cells was estimated (Table 3 and Fig. 4). In the functional assay, compound **16** was of agonistic activity and it was almost equally active on the D_1 and D_2. The functional assay is dependent on the fact that stimulation of the dopamine receptors by agonists, for example, quinpirole for D_2-like receptors and SKF 38393 for D_1 will cause an increase in intracellular [Ca^{2+}] which appears to represent a universal second messenger signal for a majority of recombinant GPCRs. Assay of the produced [Ca^{2+}] can be performed using Oregon Green 488 BAPTA-1/AM and a microplate reader. Dopamine agonists or antagonists are expected to alter the response to the standard agonists. The microplate reader-based calcium assay is a very useful method for simply and quickly selecting the active compounds. Comparing radioligand- and functional-study data, it can be seen that there are differences in the K_i values obtained, as the calcium assay monitors a fast calcium signal, these results represent non-equilibrium data, whereas radioligand-binding studies were performed under equilibrium conditions.

Table 2. Affinities for dopamine receptor subtypes measured by radioligand-binding studies.

Compounds		K_i (nM) Average ± SD or SEM (experiments in triplicate)	hD_{2L}/hD_x specificity fold
Apomorphine · HCl*	hD_1	101	3.2
	hD_{2L}	32	–
	hD_3	26	0.8
	$hD_{4.4}$	2.6	0.08
	hD_5	10	0.3
16	hD_1	1027± 137 (2)	5.2
	hD_{2L}	197± 83 (2)	–
	hD_3	1874 (1)	9.5
	$hD_{4.4}$	1142± 603 (2)	5.8
	hD_5	2361 (1)	12.0

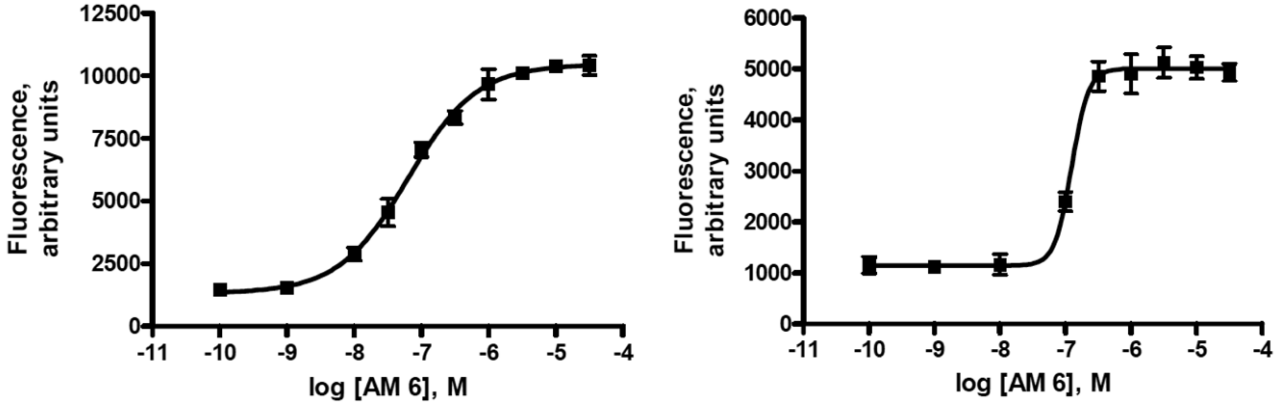
SD = Standard deviation; SEM = Standard error of the mean; SEM was used, when the number of values was less than three; *data from Ref. [7].

Table 3. Functional calcium assay results for compound **16**.

Compound	Functional Ca^{2+} assay	
	Change% of the agonist induced fluorescence by a 10-μM solution of the compound	K_i-values (nM) Average ± SEM
16	D_1 88.1%	D_1 62.8 ± 2.3
	D_{2L} 79.2%	D_{2L} 124 ± 36

Binding models and predicting ligand interactions

As a part of a larger project devoted to the modelling and understand of highly dopamine-active aporphinoids, our group established models for the binding of substituted apomorphines to human D_1-D_5 receptor subtypes. To have a further insight into the binding characteristics of above-described thiazolo- and isothiazolo-apomorphines **12** – **17**, we applied the available models for these ligands with special emphasis on D_2-like receptors and compound **16**. The reason for this focusing on D_2-like recep-



AM6 is the in-house code for compound **16**. Data are shown as average ± SEM ($n = 4$).

Figure 4. Functional characterization of the affinity of compound **16** for D_1 and D_{2L} subtypes.

Table 4. Determining hD_{2L} residues in the formation of binding complex with compound **16** upon predicted model.

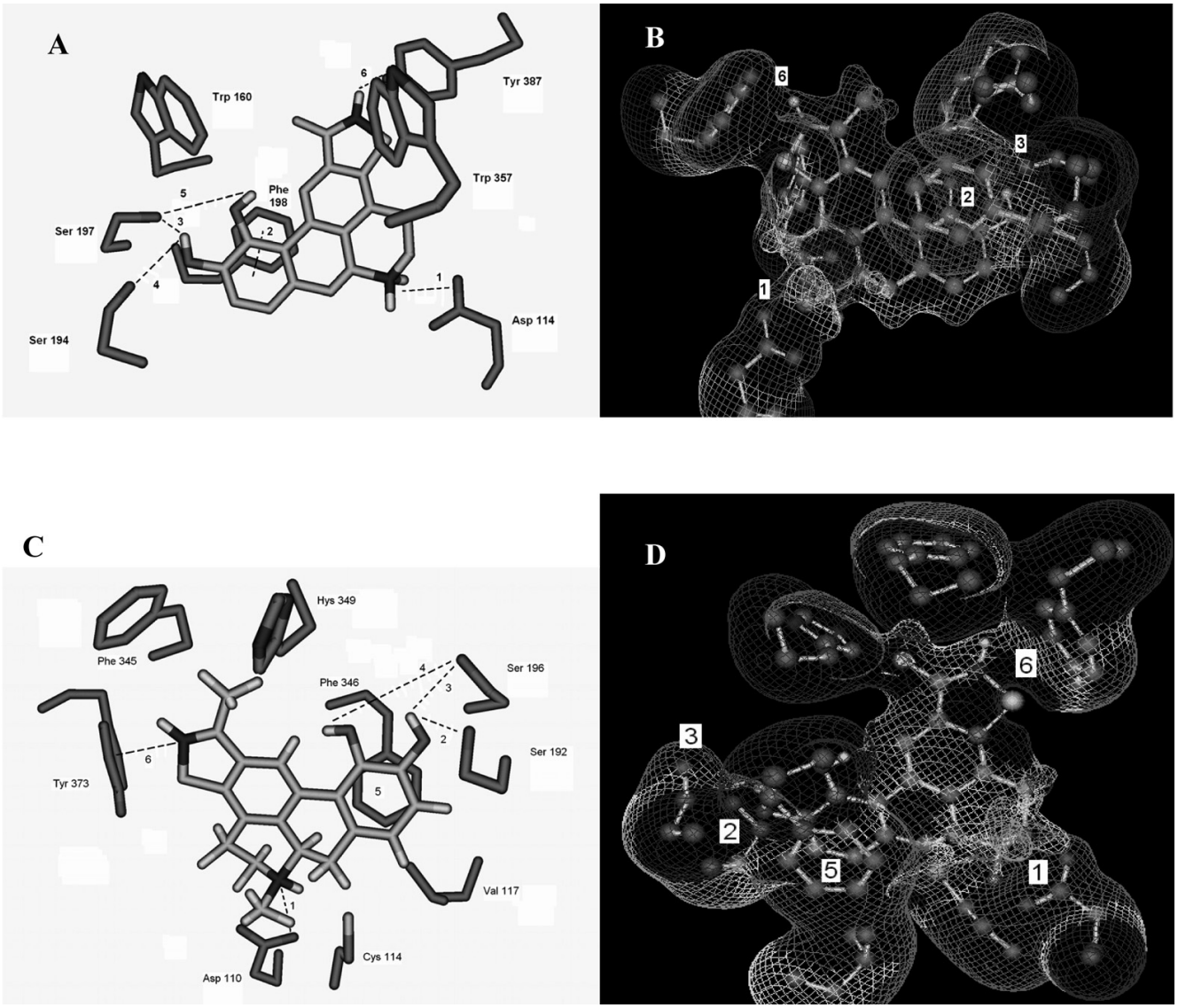
Interaction	Pharmacophore of compound 16	D_2 residue	Helix	Type of interaction	Distance (Å)
1	N17	Asp 114	TM3	Salt bridge	2.11
2	Ring D	Phe 198	TM5	Hydrophobic (p-p)	3.75
3	C10-OH	Ser 197	TM6	Hydrogen bonding	2.56
4	C10-OH	Ser 194	TM6	Hydrogen bonding	1.37
5	C11-OH	Ser 197	TM6	Hydrogen bonding	3.72
6	N2′	Tyr 387	TM7	Cation-p	2.68

Table 5. Determining hD_3 residues in the formation of binding complex with compound **16** upon predicted model.

Interaction	Pharmacophore of compound 16	D_3 residue	Helix	Type of interaction	Distance (Å)
1	N17	Asp 110	TM3	Salt bridge	2.61
2	C10-OH	Ser 192	TM5	Hydrogen bonding	2.65
3	C10-OH	Ser 196	TM5	Hydrogen bonding	2.70
4	C11-OH	Ser 196	TM5	Hydrogen bonding	3.34
5	Ring D	Phe 346	TM6	Hydrophobic (p-p)	2.49
6	N2′	Tyr 373	TM7	Cation-p	2.82

tors is that they are generally considered as main pharmacological targets of A-ring-substituted apomorphines.

The predicted D_{2L} and D_3 binding models for this compound are shown in Fig. 5. In the case of the D_2 receptor ligand, **16** occupies the binding site formed by TM3, TM5, and TM6 [22]. Residues Asp 114, Ser 194, Ser 197, and Tyr 387 in the receptor were found to interact with ligand **16** and with isothiazolo-apomorphine **17**. These residues interact also with thiazolo-aporphines **12**–**15**, however, the role of Tyr 387 changes dramatically. Residues Phe 198, Trp 160, and Trp 357 make contact with some but not all of the ligands examined and their function is estimated to be less important. Our D_2 docking studies suggest that there are two determining features in the pharmacophore: a salt bridge with N17, a cation-π interaction and there are three further important factors: a hydrophobic π-π interaction and donors/acceptors for two hydrogen bonds (Table 3). This model suggests that Tyr 387 has a significant impact on the properties of the receptor-ligand complexes. The cation-π interaction formed with the protonated isothiazole ring proved more significant than the weaker hydrophobic (-CH_3–Ar in the binding of compound **13**) or aromatic π-π interactions (-Ph–Ar in the binding of compound **14**). Interest-



Electrostatic surface calculations were extended for the major residues of the binding site to highlight interactions.

Figure 5. Conformations and significant interactions of the D_2 (Panels A & B) and the D_3 (Panels C & D) binding site complexes with compound **16**.

ingly, we found the formation of a stable hydrogen bridge between 2′-chloro substituent of compound **15** and Tyr 387 incompatible with maintaining the position of higher priority interactions (*i.e.* salt bridge between N17 and Asp 114 and hydrogen bridges of catecholic hydroxyls as it is described in Section 4.3.5: Computational background).

Regarding the model for D_3 receptor binding, it is important that ligand **16** occupies the binding site formed by TM3, TM5, and TM6 [23]. Residues Asp 110, Ser 192, Ser 196, Phe 346, and Tyr 373 in the receptor were identified as the most significant ones interacting with ligand **16** and with isothiazolo-apomorphine **17** (Table 4). Residues Cys 114, Val 117, Phe 345, and Hys 349 were found to interact with all the ligands examined but their function is estimated to be less important. The same function of Tyr 373 of helix 7 was identified in the binding complexes of receptor D_3 as Tyr 387 in D_2 receptor binding.

Comparing the predicted interactions for ligand **16** (Tables 3 and 4) it can be concluded that it was possible to position this compound in a more favorable position at the hD_{2L} binding site with respect to the predicted distances of the most important complex-forming interactions.

Conclusion

We hereby present the synthesis of novel thiazolo- and isothiazolo-apomorphines **12**–**17**. During the synthesis of the aimed thiazolo derivatives, an unexpected isomerization step was identified allowing the preparation and characterization of isothiazolo-apomorphines. With the exception of two derivatives, all the studied ligands showed low affinities for dopamine-receptor subtypes in comparison to apomorphine. Compound **16** was found to possess moderate affinity to D_{2L} subtype with approximately 10-fold selectivity over D_3 affinity. This derivative was studied in a conventional functional assay in order to determine its agonistic properties to D_1-like and D_2-like subtypes. These tests revealed similar agonistic properties to both receptor families and suggest higher affinities in comparison to the radioligand-binding assays. To be able to give a detailed interpretation, binding models were established for D_2-like receptors which were generally considered as main pharmacological targets of A-ring-substituted apomorphines. On the basis of the predicted models, we identified an important cation-π interaction for the binding of isothiazolo-apomorphine **16**.

The authors are grateful for the financial support to the National Science Foundation (Grant OTKA reg. No. NI61336). We thank the Péter Pazmány Programme (NKTH, RET006/2004) to allow us the use of microwave reactor and Szabolcs Fekete for valuable technical help. A. S. is indebted to the Eötvös Scholarship of the Hungarian State. We thank Bärbel Schmalwasser and Petra Wiecha for skilful technical assistance.

The authors have declared no conflict of interest.

Experimental

General

Melting points were determined with a Kofler hot-stage apparatus (C. Reichert, Vienna, Austria) and are uncorrected. Thin layer chromatography was performed on precoated Merck 5554 Kieselgel 60 F_{254} foils (Merck, Germany) using chloroform/methanol, 8:2 mobile phase. The spots were visualized with Dragendorff's reagent. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM 360 spectrometer (Bruker Bioscience,USA), chemical shifts are reported in ppm (δ) from internal TMS and coupling constants (*J*) are measured in Hz. ^{1}H- and ^{13}C-NMR assignments were based on 2D-NMR studies of the compounds. High resolution mass spectral measurements were performed with a Bruker micrOTOF-Q instrument (Bruker) in the ESI mode. Optical rotation was determined with a Perkin Elmer Model 241 polarimeter (Perkin-Elmer, Norwalk, CT, USA).

The microwave-induced reactions were carried out in a Discover model microwave reactor manufactured by CEM Corporation, USA. Controlled temperature, power, pressure, and time settings were used for all reactions.

Chemistry

Acid-catalyzed rearrangement of morphinandienes

A mixture of the diene (1 g) and methanesulfonic acid (5 mL) was stirred for 20 min at 0°C. Then, the reaction mixture was added dropwise, with stirring and external ice-cooling, to a solution of potassium hydrogen carbonate (10 g) in water (50 mL). After extraction with chloroform (3 × 15 mL), the combined extracts were washed with saturated brine, dried ($MgSO_4$), and concentrated *in vacuo*. In case of necessity, the residue was submitted to purification by means of column chromatography (Kieselgel 40, chloroform/methanol, 1:1) to yield the appropriate apocodeines.

*(–)-R-2′-Amino-11-hydroxy-10-methoxy-2,3:4′,5′-thiazolo-aporphine **6***

Pale yellow, cubic crystals; m.p.: 109–111°C; yield: 488 mg (89%); $[\alpha]_D^{25}$ = −203 (c = 0.1, methanol); R_f (CH_2Cl_2/CH_3OH, 8:2): 0.34; ^{1}H-NMR (360 MHz, DMSO-d_6) δ: 8.87 (s, 1H, 11-OH), 8.29 (s, 1H, H1), 7.41 (s, 2H, 2′-NH_2), 6.82 (dd, $J_{8,9}$ = 8.4 Hz, 2H, H8-9), 3.84 (s, 3H, 10-OCH_3), 3.31-2.78 (m, 4H, $H4_a$, $H5_a$, $H5_b$, $H6_a$), 2.47 (s, 3H, NCH_3), 2.66–2.09 (m, 3H, $H4_b$, $H7_a$, $H7_b$); ^{13}C-NMR (90 MHz, DMSO-d_6) δ: 162.87 (C2′), 148.32 (C2), 148.10 (C10), 143.41 (C11), 135.21–114.51 (Ar, 9C), 60.33 ($C6_a$), 56.41 (OCH_3), 52.41 (C5), 42.41 (=NCH_3), 35.75 (C7), 28.82 (C4); HRMS (ESI) *m/z* (%) found: 354.1261 [M^+ + 1] (100); calculated: 354.1274 [M^+ + 1].

*(–)-R-11-Hydroxy-2′-methyl-10-methoxy-2,3:4′,5′-thiazolo-aporphine **7***

Column chromatography was applied to separate compounds **7** and **9**. Compound **7** was the first eluted fraction. Grey, cubic crystals; m.p.: 170–173°C; yield: 216 mg (44%); $[\alpha]_D^{25}$ = −109 (c = 0.1, chloroform); R_f (CH_2Cl_2/CH_3OH, 8:2): 0.47; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 8.95 (s, 1H, H1), 6.77 (dd, $J_{8,9}$ = 7.2 Hz, 2H, H8-9), 6.71 (s, 1H, 11-OH), 3.92 (s, 3H, 10-OCH_3), 3.52–2.92 (m, 4H, $H4_a$, $H5_a$, $H5_b$, $H6_a$), 2.85 (s, 3H, 2′-CH_3), 2.81–2.51 (m, 6H, $H4_b$, $H7_a$, $H7_b$, NCH_3); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 169.60 (C2′), 152.87 (C2), 148.48 (C10), 142.60 (C11), 136.72–114.38 (Ar, 9C), 60.41 (OCH_3), 58.63 ($C6_a$), 53.10 (C5), 40.72 (=NCH_3), 35.11 (C7), 24.21 (C4), 22.22 (C-**C**H_3); HRMS (ESI) *m/z* (%) found: 353.1297 [M^+ + 1] (100); calculated: 353.1304 [M^+ + 1].

*(–)-R-11-Hydroxy-10-methoxy-2′-phenyl-2,3:4′,5′-thiazolo-aporphine **8***

Column chromatography was applied to separate compounds **8** and **10**. Compound **8** was the first eluted fraction. Off-white, cubic crystals, m.p.: 162–164°C; yield: 188 mg (39%); $[\alpha]_D^{25}$ = −206 (c = 0.1, chloroform); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.56; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 7.91 (s, 1H, H1), 7.77–7.19 (m, 5H, 2′-Ph), 6.68 (dd, $J_{8,9}$ = 7.7 Hz, 2H, H8-9), 6.12 (br s, 1H, 11-OH), 3.83 (s, 3H, 10-OCH_3), 3.09–2.41 (m, 4H, $H4_a$, $H5_a$, $H5_b$, $H6_a$), 2.38 (s, 3H, NCH_3), 2.30–2.07 (m, 3H, $H4_b$, $H7_a$, $H7_b$); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 166.66 (C2′), 154.24 (C2), 149.14 (C10), 142.69 (C11), 135.42–112.11 (Ar, 15C), 59.01 (OCH_3), 56.53 ($C6_a$), 52.79 (C5), 41.21 (=NCH_3), 35.10 (C7), 27.44 (C4); HRMS (ESI) *m/z* (%) found: 415.1459 [M^+ + 1] (100); calculated: 415.1484 [M^+ + 1].

*(–)-R-11-Hydroxy-3′-methyl-10-methoxy-2,3:4′,5′-isothiazolo-aporphine **9***

Column chromatography was applied to separate compounds **7** and **9**. Compound **9** was the second eluted fraction. Grey, cubic crystals, m.p.: 142 – 147°C; yield: 167 mg (44%); $[\alpha]_D^{25}$ = +16 (c = 0.1, chloroform); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.22; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 7.98 (s, 1H, H1), 6.77 (dd, $J_{8,9}$ = 7.9 Hz, 2H, H8-9), 6.44 (s, 1H, 11-OH), 3.92 (s, 3H, 10-OCH_3), 3.52 – 2.83 (m, 5H, $H4_a$, $H4_b$, $H5_b$, $H6_a$, $H7_b$,), 2.60 (s, 3H, 3′-CH_3), 2.55 (m, 1H, $H7_a$), 2.51 (s, 3H, NCH_3), 2.41 (m, 1H, $H5_a$); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 158.69 (C2′), 147.91 (C10), 142.88 (C11), 142.71 ($C3_a$), 138.21 (C3), 133.80 – 114.58 (Ar, 8C), 60.63 ($C6_a$), 56.38 (OCH_3), 52.07 (C5), 41.21 (=NCH_3), 33.98 (C7), 23.80 (C4), 19.47 (C-**C**H_3); HRMS (ESI) m/z (%) found: 353.1315 [M^+ + 1] (100); calculated: 353.1321 [M^+ + 1].

*(–)-R-11-Hydroxy-10-methoxy-3′-phenyl-2,3:4′,5′-isothiazolo-aporphine **10***

Column chromatography was applied to separate compounds **8** and **10**. Compound **10** was the second eluted fraction. White, cubic crystals; m.p.: 179 – 183°C; yield: 159 mg (33%); $[\alpha]_D^{25}$ = +15 (c = 0.1, chloroform); R_f (CH_2Cl_2/CH_3OH, 8:2): 0.37; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 9.23 (s, 1H, H1), 8.11 – 7.43 (m, 5H, 3′-Ph), 6.70 (dd, $J_{8,9}$ = 7.9 Hz, 2H, H8-9), 3.59 (s, 3H, 10-OCH_3), 3.31 – 2.77 (m, 5H, $H4_a$, $H4_b$, $H5_b$, $H6_a$, $H7_b$), 2.63 – 2.51 (m, 5H, $H5_b$, $H7_a$, NCH_3); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 157.01 (C2′), 149.21 (C10), 144.42 (C3), 142.36 (C11), 141.20 ($C3_a$), 133.81–113.34 (Ar, 14C), 60.23 (OCH_3), 56.93 ($C6_a$), 52.09 (C5), 40.87 (=NCH_3), 35.42 (C7), 29.31 (C4); HRMS (ESI) *m/z* (%) found: 415.1499 [M^+ + 1] (100); calculated: 415.1485 [M^+ + 1].

*(–)-R-2′-Chloro-11-hydroxy-10-methoxy-2,3:4′,5′-thiazolo-aporphine **11***

100 mg (0.28 mmol) of compound **6** were dissolved and mixed in 2 mL of acetonitrile and cooled to 0°C. 45 mg (0.34 mmol) $CuCl_2$ were added followed by a dropwise addition of isoamyl nitrite (49 mg, 0.41 mmol). The mixture was stirred for 2 h. The solvent was removed by evaporation under reduced pressure and the residue was purified by flash chromatography (EtOAc/MeOH, 8:2). Pale brown powder, m.p.: 111 – 114°C; yield: 63 mg (69%); $[\alpha]_D^{25}$ = – 217 (c = 0.1, chloroform); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.31; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 7.82 (s, 1H, H1), 6.62 (dd, $J_{8.9}$ = 7.9 Hz, 2H, H8-9), 3.91 (s, 3H, 10-OCH_3), 3.17–2.52 (m, 3H, $H4_a$, $H5_b$, $H6_a$), 2.39 (s, 3H, NCH_3), 2.44 – 2.03 (m, 4H, $H4_b$, $H5_a$, $H7_a$, $H7_b$); ^{13} C-NMR (90 MHz, $CDCl_3$) δ: 154.77 (C2′), 150.32 (C2), 149.52 (C10), 142.61 (C11), 135.55 – 113.38 (Ar, 9C), 59.73 ($C6_a$), 56.22 (OCH_3), 53.32 (C5), 40.88 (=NCH_3), 35.10 (C7), 24.52 (C4); HRMS (ESI) *m/z* (%) found: 373.0802 [M^+ + 1] (100); calculated: 373.0782 [M^+ + 1].

O-Demethylation of apocodeines by MW irradiation

A mixture of the apocodeine (4.56 mmol), methionine (1 g, 6.70 mmol) and methanesulfonic acid (4 mL) was stirred in the pressurized glass vial, equipped with a magnetic stirring bar, for 20 min at 0°C carefully protected from sunlight under nitrogen atmosphere. The vial was inserted into the microwave cavity of the microwave reactor, irradiated at the target temperature 75°C for 5 min hold time and subsequently cooled by rapid gas-jet cooling. The product mixture was allowed to cool to room temperature in the microwave cavity. After cooling, the pH of the mixture was set to 10 by concentrated NH_3 solution and extracted with chloroform (3 × 15 mL). The organic layers were collected, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to silica gel column chromatography. Elution with chloroform/methanol, 1:1 gave the corresponding apomorphines. The crystalline product is precipitated by addition of abs. diethylether and converted into hydrochloride salt with 1 M HCl/ethanol.

*(–)-R-2′-Amino-10,11-dihydroxy-2,3:4′,5′-thiazolo-aporphine trihydrochloride **12** · 3 HCl*

Yellow, plate shape crystals; m.p.: 194 – 196°C; yield: 750 mg (92%); $[\alpha]_D^{25}$ = – 119 (c = 0.1, DMSO); R_f (CH_2Cl_2/CH_3OH, 8:2): 0.27; ^{1}H-NMR (360 MHz, DMSO-d_6) δ: 8.20 (s, 1H, H1), 6.76 (dd, $J_{8,9}$ = 7.9 Hz, 2H, H8-9), 6.34 (br s, 2H, 10-OH, 11-OH), 3.11 – 2.51 (m, 3H, $H4_a$, $H5_b$, $H6_a$), 2,47 (s, 3H, NCH_3), 2.40 – 2.09 (m, 4H, $H4_b$, $H5_a$, $H7_a$, $H7_b$), no NH_2 sign was observed; ^{13}C-NMR (90 MHz, DMSO-d_6) δ: 160.68 (C2′), 145.10 (C10), 144.62 (C11), 140.32 (C2), 132.79 – 116.11 (Ar, 9C), 59.93 ($C6_a$), 51.14 (C5), 39.92 (=NCH_3), 36.64 (C7), 27.44 (C4); HRMS (ESI) *m/z* (%) found: 340.1121 [M^+ + 1] for free base (100); calculated: 340.1114 [M^+ + 1].

*(–)-R-10,11-Dihydroxy-2′-methyl-2,3:4′,5′-thiazolo-aporphine dihydrochloride **13** · 2 HCl*

Off-white, plate shape crystals; m.p.: >250°C; yield: 199 mg (79%); $[\alpha]_D^{25}$ = – 93 (c = 0.025, DMSO); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.21); ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 7.68 (s, 1H, H1), 6.88 (dd, $J_{8,9}$ = 7.6 Hz, 2H, H8-9), 6.61 (br s, 2H, 10-OH, 11-OH), 3.44 – 2.87 (m, 3H, $H4_a$, $H5_b$, $H6_a$), 2.85 (s, 3H, 2′-CH_3), 2.74–2.37 (m, 7H, $H4_b$, $H5_a$, $H7_a$, $H7_b$, NCH_3); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 166.42 (C2′), 145.48 (C10), 144.22 (C11), 142.63 (C2), 132.11 – 116.32 (Ar, 9C), 58.63 ($C6_a$), 51.18 (C5), 39.82 (=NCH_3), 36.42 (C7), 26.58 (C4), 24.37 (C-**C**H_3); HRMS (ESI) *m/z* (%) found: 339.1149 [M^+ + 1] (100); calculated: 339.1162 [M^+ + 1].

*(–)-R-10,11-Dihydroxy-2′-phenyl-2,3:4′,5′-thiazolo-aporphine dihydrochloride **14** · 2 HCl*

Off-white, plate shaped crystals, m.p.: >250°C; yield: 176 mg (82%); $[\alpha]_D^{25}$ = – 188 (c = 0.1, DMSO); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.19; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 8.02 (s, 1H, H1), 7.82 – 7.29 (m, 5H, 2′-Ph), 6.72 (dd, $J_{8.9}$ = 7.6 Hz, 2H, H8-9), 6.54 (br s, 2H, 10-OH, 11-OH), 3.14–2.37 (m, 6H, $H4_a$, $H5_b$, $H6_a$, NCH_3), 2.23 – 1.99 (m, 4H, $H4_b$, $H5_a$, $H7_a$, $H7_b$); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 160.36 (C2′), 145.21 (C10), 145.63 (C11), 140.28 (C2), 135.42 – 112.11 (Ar, 15C), 56.53 ($C6_a$), 52.79 (C5), 41.21 (=NCH_3), 36.42 (C7), 27.01 (C4); HRMS (ESI) *m/z* (%) found: 401.1342 [M^+ + 1] (100); calculated: 401.1333 [M^+ + 1].

*(–)-R-2′-Chloro-10,11-dihydroxy-2,3:4′,5′-thiazolo-aporphine dihydrochloride **15** · 2 HCl*

Greenish yellow, shiny, plate shape crystals, m.p.: 203 – 206°C; yield: 64 mg (87%); $[\alpha]_D^{25}$ = – 198 (c = 0.1, DMSO); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.20; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 7.92 (s, 1H, H1), 6.68 (dd, $J_{8,9}$ = 8.0 Hz, 2H, H8-9), 6.56 (br s, 2H, 10-OH, 11-OH), 3.24 – 2.32 (m, 6H, $H4_a$, $H5_b$, $H6_a$, NCH_3), 2.30 – 1.92 (m, 4H, $H4_b$, $H5_a$, $H7_a$, $H7_b$); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 158.37 (C2′), 145.32 (C10), 144.69 (C11), 140.31 (C2), 132.30 – 116.25 (Ar, 9C), 59.82 ($C6_a$), 51.19 (C5), 39.98 (=NCH_3), 36.33 (C7), 26.15 (C4); HRMS (ESI) *m/z* (%) found: 359.0610 [M^+ + 1] (100); calculated: 359.0616 [M^+ + 1].

*(–)-R-10,11-Dihydroxy-3′-methyl-2,3:4′,5′-isothiazolo-aporphine dihydrochloride **16** · 2 HCl*

Off-white, plate shape crystals, m.p.: 208–210°C; yield: 175 mg (90%); $[\alpha]_D^{25}$ = +33 (c = 0.1, DMSO); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.24; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 8.02 (s, 1H, H1), 6.77 (dd, $J_{8,9}$ = 7.9 Hz, 2H, H8-9), 6.44 (s, 1H, 11-OH), 3.92 (s, 3H, 10-OCH_3), 3.52–2.92 (m, 4H, $H4_a$, $H5_a$, $H5_b$, $H6_a$), 2.85 (s, 3H, 3′-CH_3), 2.81–2.51 (m, 6H, $H4_b$, $H7_a$, $H7_b$, NCH_3); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 159.12 (C3′), 145.81 (C10), 144.78 (C11), 140.33 (C2), 132.24–116.04 (Ar, 9C), 59.80 ($C6_a$), 51.69 (C5), 40.09 (=NCH_3), 36.33 (C7), 26.82 (C4), 18.98 (C-**C**H_3); HRMS (ESI) *m/z* (%) found: 339.1181 [M^+ + 1] (100); calculated: 339.1162 [M^+ + 1].

*(–)-R-10,11-Dihydroxy-3′-phenyl-2,3:4′,5′-isothiazolo-aporphine dihydrochloride **17** · 2 HCl*

White, plate shape crystals; m.p.: 179–183°C; yield: 160 mg (88%); $[\alpha]_D^{25}$ = +23 (c = 0.1, DMSO); R_f (CH_2Cl_2/CH_3OH, 8:2) 0.31; ^{1}H-NMR (360 MHz, $CDCl_3$) δ: 7.91 (s, 1H, H1), 7.77–7.19 (m, 5H, 3′-Ph), 6.68 (dd, $J_{8,9}$ = 7.7 Hz, 2H, H8-9), 3.83 (s, 3H, 10-OCH_3), 3.09–2.41 (m, 4H, $H4_a$, $H5_a$, $H5_b$, $H6_a$), 2.38 (s, 3H, NCH_3), 2.30–2.07 (m, 3H, $H4_b$, $H7_a$, $H7_b$); ^{13}C-NMR (90 MHz, $CDCl_3$) δ: 159.58 (C3'), 145.72 (C10), 144.63 (C11), 140. 27 (C2), 132.87–116.21 (Ar, 15C), 59.75 ($C6_a$), 51.30 (C5), 39.97 (=NCH_3), 37.29 (C7), 26.44 (C4); HRMS (ESI) *m/z* (%) found: 401.1338 [M^+ + 1] (100); calculated: 401.1318 [M^+ + 1].

Pharmacology

Cell culture

Human D_1, D_{2L}, or D_5 receptors were stably expressed in human embryonic kidney cells (HEK293). Stable cell lines of HEK293 cells were generated by transfecting the plasmids coding for hD_3 using Polyfect® transfection reagent (Qiagen, Hilden, Germany) according to manufacturer's instructions and were selected using G-418 (400 μg/mL medium). The human $D_{4.4}$ receptor was stably expressed in CHO cells. The density of D_1-like receptors measured with [^{3}H]-SCH23390 was 3139 fmol/mg protein. For D_2-like receptors the density of receptors was 186.53 fmol/mg protein measured with [^{3}H]-spiperone. Cells were grown at 37°C under a humidified atmosphere of 5% CO_2/95% air in Dulbecco's modified Eagles Medium Nutrient mixture F-12 Ham, supplemented with 10% fetal bovine serum, 1 mM L-glutamine and 0.2 μg/mL G 418 (all by Sigma-Aldrich, Germany).

Preparation of whole-cell-suspension

Human D_1-like and D_2-like receptor cell lines were grown on T 175 culture dishes (Greiner Bio-One, Frickenhausen, Germany) to 85% confluency, the medium was removed and the cells were incubated with 3 mL trypsine-EDTA-solution (Sigma-Aldrich) to remove the cells from the culture dish. After incubation cells were suspended in 3–6 mL added medium in order to stop the effect of trypsine-EDTA-solution. The resulting suspension was centrifuged (1800–2400 rpm, 4°C, 4 min), the pellet resuspended in 10 mL PBS (ice-cooled, calcium- and magnesium-free), pelleted, and this procedure repeated. The resulting pellet was then resuspended in 12 mL buffer (5 mM magnesium chloride, 50 mM TRIS-HCl, pH 7.4) and the resulting suspension was directly used for the radioligand-binding assay.

Radioligand-binding assay

The binding studies were performed following the protocol previously described but in 96- well format [24]. The assays with the whole-cell-suspension were carried out in triplicate in a volume of 550 μL (final concentration): TRIS-Mg^{2+}-buffer (345 μL), [^{3}H]-ligand (50 μL), whole-cell-suspension (100 μL), and appropriate drugs (55 μL). Non-specific binding was determined using fluphenazine (100 μM) in hD_1 tests and haloperidol (10 μM) in hD_{2L} tests. The incubation was initiated by addition of the radioligand [^{3}H]SCH23390 for hD_1-like receptors and [^{3}H]spiperone for hD_{2L}-like receptors (both Amersham Biosiences, Little Chalfront, UK). It was carried out in 96-deep well plates (Greiner Bio-One) using a Thermocycler (Eppendorf, Wessling, Germany) at 27°C. The incubation was terminated after 90 min by rapid filtration with a PerkinElmer Mach III Harvester™ using a PerkinElmer Filtermat A, previously treated with a 0.25% polyethyleneimine-solution (Sigma-Aldrich) and washed once with water. The filtermat was dried for 3 min with 400 W using a microwave (MW 21, Clatronic, Kempen, Germany). The dry filtermat was placed in a filter plate (Omni filter plates, PerkinElmer Life Sciences) and each field of the filtermat moistened with 50 μL Microscint 20™ scintillation cocktail. The radioactivity retained on the filters was counted using a Top Count NXT™ microplate scintillation counter (Packard Instruments, USA). For determining the K_i values at least two independent experiments each in triplicate were performed.

The competition binding data were analysed by the software GraphPad Prism™ using nonlinear least squares fit. For calculating the mean, standard deviation and standard error of the mean the software Microsoft Excel™ was used. K_i values were calculated from IC_{50} values applying the equation of Cheng and Prusoff [25].

Measurement of changes in intracellular [Ca^{2+}] in HEK293 cells

The functional calcium assay based on dopamine-receptor agonist induced intracellular calcium decrease. Intracellular calcium is measured using the calcium binding fluorescent dye Oregon Green [26]. Human embryonic kidney cells (HEK293) stably expressing human D_1 or D_{2L} receptors were grown at 37°C under a humidified atmosphere of 5% CO_2, 95% air in Dulbecco's modified Eagles Medium Nutrient mixture F-12 Ham, supplemented with 10% fetal bovine serum, 1 mM L-glutamine and 0.2 μg/mL G 418 (all by Sigma-Aldrich). Human D_1 and D_{2L} receptor cell lines were grown on T 175 culture dishes (Greiner Bio-One) to 85–90% confluence. The medium was removed and cells rinsed twice with 6 mL Krebs-HEPES buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 11.7 mM D-Glucose, 1.3 mM $CaCl_2$, 10 mM HEPES, pH 7.4) each time. After two washes, cells were loaded with 3 μL of a 0.5 M Oregon Green 488 BAPTA-1/AM-solution (Molecular Probes, Eugene, OR, USA) (in DMSO) in 6 mL of the same buffer containing 3 μL of a 20% Pluronic F-127-solution (Sigma Aldrich) (in DMSO) for 45 min at 37°C. After 35 min incubation, the culture dish was rapped slightly in order to remove all cells from the dish for further incubation. Then, 5 mL of Krebs-HEPES buffer were added and cells were suspended. The resulting suspension was separated in ten vials (1.5 mL) and centrifuged (10 000 rpm, 10 s), the pellets were resuspended in 1 mL Krebs-HEPES buffer twice per five pellets and centrifuged again. The pellets were resuspended 18 mL Krebs-HEPES buffer. And plated into 96-well plates (OptiPlate HTRF-96, Packard Instruments, Meriden, CT, USA; Cellstar,

Tissue Culture Plate, 96W, Greiner Bio-One) Microplates were kept at 37°C under a humidified atmosphere of 5% CO_2, 95% air for 30 min. Screening for agonistic and antagonistic activity was performed using a NOVOstar microplate reader (BMG LabTechnologies) with a pipettor system. All compounds were dissolved in Krebs-HEPES buffer and experiments were performed at 37°C. SKF 38393 was used as standard agonist for D_1 receptors and Quinpirole for D_{2L} receptors (final concentration: 1 μM). Dose response curves in presence of an agonist were received after 30 min incubation of the plated cell suspension, by injecting 20 μL of different compound dilutions (final concentrations: 50, 10, 5, 1 μM, 500 nM, 100, 50, 10, 1, 0.1 nM) into separate wells. To get control values for every experiment, 20 μL of buffer alone and standard agonist were injected.

Determination of antagonistic activity was conducted by preincubating the cells with 20 μL of the compound dilutions (final concentrations: 50, 10, 5, 1 μM, 500 nM, 100, 50, 10, 1, 0.1 nM) 30 min prior to injection of 20 μL standard agonist. Starting with injection of buffer or agonist, fluorescence intensity was measured separately for each well during 30 s at 0.4 s intervals. Concentration-response curves were obtained by determination of the maximum fluorescence intensity of each data set and nonlinear regression on GraphPadPrism™ 3.0. K_i values were then calculated applying a modified Cheng-Prusoff equation [25]:

$$K_i = \frac{IC_{50}}{1 + \frac{L}{EC_{50}}} \quad (1)$$

L: Concentration of standard agonist (M); EC_{50}: effective concentration 50% of the standard agonists (M); IC_{50}: inhibitory concentration 50% of test compounds at the given experimental conditions, that is, standard agonist concentration.

Computational background

Applied receptor and ligand structures

Amino acid sequencing and receptor structure modelling were based on the procedure established by Xhaard *et al*. in their study performed on human catecholamine-binding GPCRs [27]. The coordinates of the complete models for human D_2 and D_3 receptors were applied for building receptor structures with Maestro™, Version 8.0.308. The 3D structure of the studied compound **16** were constructed and optimized with Gaussian 98 program [28] with the standard 6-31G* basis set. We carried out these calculations by means of the hybrid functional developed by Becke and also Lee, Yang and Parr, which is customarily denoted as B3LYP [29].

Docking and receptor-ligands complexes

The optimized structure of ligand **16** was automatically docked to the binding cavities of receptor model structures using Version 3.0.5 of AutoDock [30]. AutoDock allows flexibility of the ligand while it keeps the binding site rigid. Standard parameters were used with the exception that during the docking procedure a 3.5 Å distance restraint was used to enforce interactions between (i) an oxygen atom from the carboxylate group of Asp 114/Asp 110 (D_2 or D_3 receptor models, respectively) and the N17 protonated amine; and (ii) the catecholic hydroxyl, C10-OH, with oxygen atoms of Ser 194/196 and Ser 192/194 (D_2 and D_3 models, respectively). There is compelling evidence that these interactions do take place (for D_2-like receptors) [31].

Grid maps of $90 \times 90 \times 90$ points with a grid-point spacing of 0.375 Å were generated using the AutoGrid tool. 250 Genetic Algorithm (GA) runs were performed with the following parameters: population size of 50, maximum number of 2.5×10^5 energy evaluations, maximum number of 27 000 generations, an elitism of 1, a mutation rate of 0.02, and a crossover rate of 0.8.

References

[1] K. D. Hargrave, F. K. Hess, J. T. Oliver, *J. Med. Chem.* **1983**, *26*, 1158 – 1163; W. C. Patt, H. W. Hamilton, M. D. Taylor, M. J. Ryan, *et al.*, *J. Med. Chem.* **1992**, *35*, 2562 – 1163; F. Haviv, J. D. Ratajczyk, R. W. DeNet, F. A. Kerdesky, *et al.*, *J. Med. Chem.* **1988**, *31*, 1719 – 1728; F. Clemence, O. L. Marter, F. Delevalle, J. Benzoni, *et al.*, *J. Med. Chem.* **1988**, *31*, 1453 – 1462; J. C. Jaen, L. D. Wise, B. W. Caprathe, H. Tecle, *et al.*, *J. Med. Chem.* **1990**, *33*, 311 – 317; K. Tsuji, H. Ishikawa, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1601 – 1606; N. Bailey, A. W. Dean, D. B. Judd, D. Middlemiss, *et al.*, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1409 – 1414; F. W. Bell, A. S. Cantrell, M. Hoberg, S. R. Jaskunas, *et al.*, *J. Med. Chem.* **1995**, *38*, 4929 – 4936.

[2] B. A. Fink, D. S. Mortensen, S. R. Stauffer, Z. D. Aron, J. A. Katzenellenbogen, *Chem. Biol.* **1999**, *6*, 205 – 219.

[3] J. E. Van Muijlwijk-Koezen, H. Timmerman, R. C. Vollinga, J. F. Von Drabbe *et al.*, *J. Med. Chem.* **2001**, *44*, 749 – 762; M. D. Chordia, L. J. Murphree, T. L. Macdonald, J. Linden, R. A. Olsson, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1563 – 1566.

[4] K. Görlitzer, R. Schumann, *Pharmazie* **1992**, *47*, 893 – 897; K. Görlitzer, R. Schumann, *Pharmazie* **1993**, *48*, 31 – 33.

[5] A. Zhang, S. van Vilet, J. L. Neumeyer, *Tetrahedron Lett.* **2003**, *44*, 6459 – 6462; A. Zhang, W. Xiong, J. E. Hilbert, E. K. DeVita, *et al.*, *J. Med. Chem.* **2004**, *47*, 1886 – 1888.

[6] A. Zhang, Y. Zhang, A. R. Branfman, R. J. Baldessarini, *et al.*, *J. Med. Chem.* **2007**, *50*, 171 – 181.

[7] K. Søndergaard, J. L. Kristensen, M. Palner, N. Gillings, *et al.*, *Org. Biomol. Chem.* **2005**, *3*, 4077 – 4081.

[8] J. Mierau, F. J. Schneider, H. A. Ensinger, C. L. Chio, *et al.*, *Eur. J. Pharmacol.* **1995**, *290*, 29 – 36.

[9] C. S. Schneider, J. Mierau, *J. Med. Chem.* **1987**, *30*, 494 – 498.

[10] Z. Liu, X. Chen, L. Yu, X. Zhen, *et al.*, *Bioorg. Med. Chem.* **2008**, *16*, 6675 – 6681.

[11] A. Zhang, F. Li, C. Ding, Q. Yao, *et al.*, *J. Med. Chem.* **2007**, *50*, 2747 – 2751.

[12] M. Tóth, S. Gyulai, S. Berényi, A. Sipos, *Lett. Org. Chem.* **2007**, *4*, 539 – 543.

[13] H. Conroy, *J. Am. Chem. Soc.* **1955**, *77*, 5960 – 5966.

[14] S. Berényi, S. Makleit, L. Szilágyi, *Acta Chim. Hung.* **1984**, *117*, 307 – 369; C. Simon, S. Berényi, S. Makleit, V. Fekete, *Acta Chim. Hung.* **1987**, *124*, 497 – 503.

[15] S. Berényi, S. Hosztafi, S. Makleit, I. Szeifert, *Acta Chim. Acad. Sci. Hung.* **1982**, *110*, 363 – 369; S. Berényi, S. Makleit, F. Rantal, *Acta Chim. Acad. Sci. Hung.* **1985**, *120*, 201 – 2005; C. Csutorás, S. Berényi, S. Makleit, *Synth. Commun.* **1996**, *26*, 2251 – 2256.

[16] R. T. Sharp, K. R. Leeman, D. E. Bryant, G. J. Horan, *Tetrahedron Lett.* **2003**, *44*, 1559 – 1561.

[17] A. Sipos, S. Berényi, *Mon. Chem.* **2009**, *140*, 387–396.

[18] M. D'Auria, *Tetrahedron* **2002**, *58*, 8037.

[19] J. F. Okonya, F. Al-Obeidi, *Tetrahedron Lett*. **2002**, *43*, 7051–7053.

[20] S. Berényi, C. Csutorás, Z. Gyulai, G. Rusznyák, *Synth. Commun*. **2001**, *31*, 1987–1992; S. Berényi, A. Sipos, I. Szabó, T. Kálai, *Synth. Commun.* **2007**, *37*, 467–471.

[21] C. Csutorás, S. Berényi, J. L. Neumeyer, *Lett. Org. Chem.* **2007**, *4*, 409–413; C. Csutoras, *Acta Acad. Paed. Agriensis, Sectio Pericemonologica* **2007**, *34*, 105.

[22] M. Y. S. Kalani, N. Vaidehi, S. E. Hall, R. J. Trabanino, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3815–3820.

[23] J. Varady Hobrath, S. Wang, *J. Med. Chem.* **2006**, *49*, 4470–4476.

[24] M. Decker, J. Lehmann, *Arch. Pharm. Pharm. Med. Chem.* **2003**, *336*, 466–476.

[25] Y. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

[26] M. U. Kassack, B. Hofgen, J. Lehmann, N. Eckstein, *et al.*, *J. Biomol. Screen.* **2002**, *7*, 233–246.

[27] H. Xhaard, V.-V. Rantanen, T. Nyrönen, M. S. Johnson, *J. Med. Chem.* **2006**, *49*, 1706–1719. The data of the Supporting Information file and the on-line source described in the article itself were both applied.

[28] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, *et al.*, Gaussian *98, Revision A.3*, Gaussian, Inc., Pittsburgh, PA **1998**.

[29] A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648–5652; A. D. Becke, *Phys. Rev. A* **1998**, *38*, 3098–3100; C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1980**, *37*, 785; S. H. Vosko, L. Wilk, M. Nusair, *Can. J. Phys.* **1980**, *58*, 1200–1211.

[30] D. S. Goodsell, A. J. Olson, *Proteins* **1990**, *8*, 195–202; G. M. Morris, D. S. Goodsell, R. Huey, A. J. Olson, *J. Comput. Aided Mol. Des.* **1996**, *10*, 293–304; G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, *et al.*, *J. Comput. Chem.* **1998**, *19*, 1639–1662.

[31] B. A. Cox, R. A. Henningsen, A. Spanoyannis, R. L. Neve, K. A. Neve, *J. Neurochem.* **1992**, *59*, 627–635; R. Woodward, C. Coley, S. Daniell, L. H. Naylor, P. G. Strange, *J. Neurochem.* **1996**, *66*, 394–402; C. Coley, R. Woodward, A. M. Johansson, P. G. Strange, L. H. Naylor, *J. Neurochem.* **2000**, *74*, 358–366; N. Sartania, P. G. Strange, *J. Neurochem.* **1992**, *72*, 2621–2624.

