Bioorganic & Medicinal Chemistry 21 (2013) 4143–4150

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# 1-substituted apomorphines as potent dopamine agonists

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ARTICLE INFO

*Article history:*
Received 17 February 2013
Revised 5 May 2013
Accepted 8 May 2013
Available online 16 May 2013

*Keywords:*
Apomorphine
Acid-catalyzed rearrangement
Dopamine agonist
Dopamine $D_1$ and $D_2$ receptors
Morphinandiene

ABSTRACT

A novel set of 1-substituted apomorphines as dopaminergic agonists were synthesized according to our new strategy employing the acid-catalyzed rearrangement of diversely functionalized 5β-substituted-6-demethoxythebaines. The activities of new compounds for dopamine receptors subtypes were evaluated using HEK293 based stable cell lines expressing $D_1$, $D_{2L}$ or $D_3$ receptor subtypes. All studied compounds had affinities in nanomolar range for $D_{2L}$ and $D_3$ receptors and the change of the nature of substituent in position 1 had only moderate effect. $D_1$ receptors were sensitive to the introduction of the 4-OH-benzyl function resulting in an increased affinity. The small hydrophilic group (hydroxymethyl) highly reduced the agonist affinity and potency thereby increasing subtype selectivity. This strategy for selective modulation of affinities and potencies of 1-substituted apomorphines gives essential hints for future design of subtype selective dopaminergic ligands.



## 1. Introduction

Dopamine plays crucial regulatory functions in the brain. Malfunctions in the dopaminergic system, and especially disturbances related to the dopamine $D_2$ receptor function, are connected to many different neurological and psychiatric disorders, including Parkinson's disease, Huntington's chorea, schizophrenia, attention deficit-hyperactivity disorder, Tourette's syndrome, restless leg syndrome, erectile dysfunction and addiction.[1,2] Therefore diverse approaches towards the synthesis and characterization of selective and potent $D_2$ agonists are considered very important.[3,4] Dopamine $D_1$ and $D_2$ receptors are both ubiquitous in the brain and are connected to different effector systems. The $D_2$ receptor is highly homologous to the dopamine $D_3$ receptor, which is present at lower densities and has more restricted distribution in the brain, and also a different function.[5] Most dopamine $D_2$ receptor ligands have similar affinity also for $D_3$ receptors, which has to be considered when developing subtype selective ligands.

The chemical modification of the substitution pattern of ring A of apomorphine (**1**, Fig. 1), the non-selective dopamine agonist clinically used for the management of Parkinson's disease and erectile dysfunction, led to several potent and selective $D_2$ agonist derivatives, for example 2-fluoroapomorphine (**2**), 2-hydroxyapomorphine (**3**) and 2-(4-hydroxyphenyl)apomorphine (**4**).[6–9]

These examples suggest that aporphines modified in position 2 are the most important derivatives of this family of alkaloids, however it has been already confirmed that other substitution patterns in ring A may also lead to potent ligands.[10–12] On the basis of these results and the classic model for the interactions of aporphinoids with $D_2$ receptor binding site identifying a lipophilic cleft in the proximity of position 2 of the aporphine skeleton, the synthesis and pharmacological characterization of a novel set of 1-substituted apomorphines was targeted.[13,14] It was presumed that results on these uniquely substituted aporphines might remarkably contribute to the existing structure–activity relationships and help further explore the electronic and steric interactions in the region of ring A of this scaffold.

## 2. Results and discussion

The synthesis of the aimed 1-substituted apomorphine derivatives was based on the previous observation that 6-demethoxythebaine (**5**) can be selectively alkylated in the 5β-position to yield precursors to ring A derivatized aporphines (Scheme 1).[15,16]

The preparation of compound **5** was achieved in accordance with the original procedure published by Berényi et al.,[17] however there were other alternative routes reported to this morphinandiene.[18] In brief, neopine, a minor opium alkaloid, was mesylated in position 6 and the mesyloxy group was eliminated in the presence of tetrabutylammonium fluoride. The strategy for versatile 5β-substitution of thebaine was elaborated by Gates et al.[19,20] and was successfully extended by many other groups.[18] The procedure

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http://dx.doi.org/10.1016/j.bmc.2013.05.014

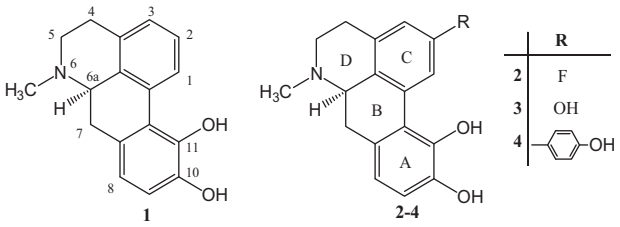


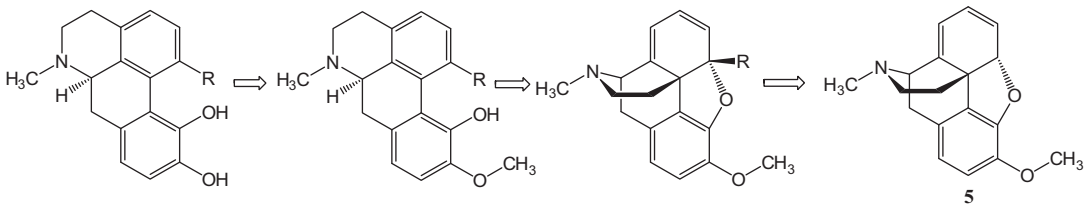

**Figure 1.** Apomorphine (**1**) and some of its potent and selective ring A modified congeners.

was performed in accordance with the modified methodology of Gates et al. described earlier for the benzylation of 6-demethoxythebaine (**5**).[20] The carefully dried compound **5** was dissolved in anhydrous THF, cooled to −78 °C and treated with *t*-BuLi affording the corresponding anion. This intermediate was reacted with the electrophile partner. In order to be able to extensively examine the targeted structure–activity relationships, the electrophile partners were chosen in a wide range with respect to their spatial and electronic properties (Scheme 2).

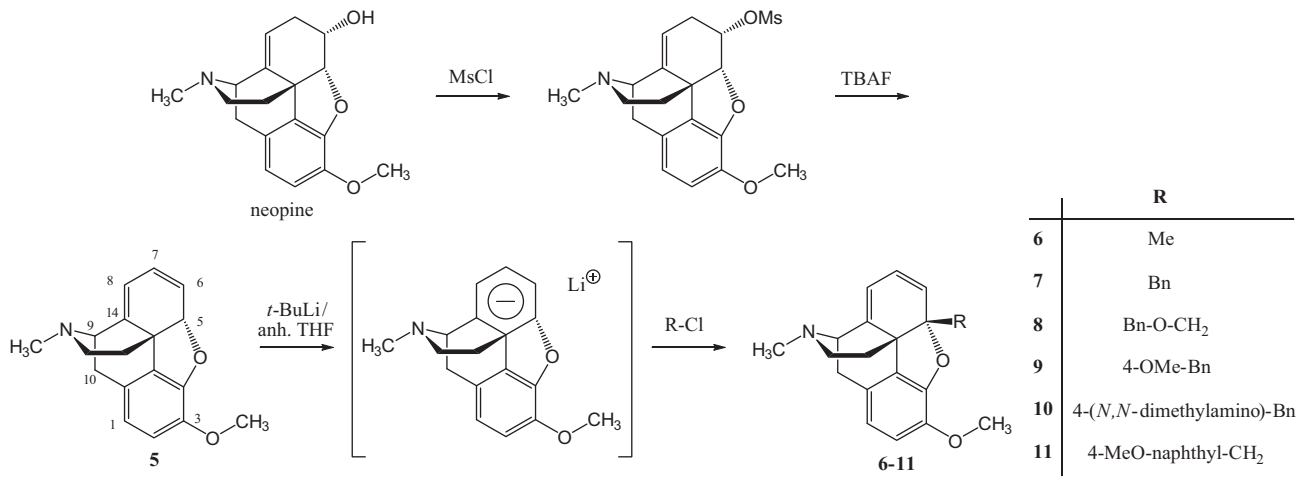
It was decided to insert also hydroxyl functions in the proximity of ring A of the aporphine skeleton to be able to test the effect of this moiety and to compare it with the superior binding activity and agonist efficacy of reference compound **4**. According to the nature of the 5β-substitution of morphinandiene **5** in the presence of *t*-BuLi, it was critical to choose adequately protected alcohols and phenols. On the other hand, it was also an important aspect to choose protective groups that could be removed as a one-pot procedure during the closing step of our strategy (10-*O*-demethylation of the intermediate apocodeines) without the need for additional synthesis steps. Therefore phenolic OH groups were introduced in the form of methyl ethers and alcoholic hydroxyls were protected with benzyl group, because it is known to be stable under such harsh conditions. As it was reported previously,[16] the reaction between the morphinandiene anion and electrophiles results two main products, namely the 5β- and 7-substituted morphinans. In the course of the preparation of compounds **8**–**11** the crude product mixtures were found to be more complex. The isolation of the targeted compounds was based on the observation that the first eluted fraction resulted by column chromatography was the 5β-derivatized morphinandiene. Unfortunately, it was not possible to isolate 7-substituted morphinandines in pure form; therefore we were unable to access to 3-substituted aporphines in this way. The yields for 5β-substituted morphinans were in the range of 15–30%. The next step of the synthesis was the acid-catalyzed rearrangement of compounds **6**–**11** into apocodeines **12**–**17** (Scheme 3).

The conventional explanation[21,22] for the acid-catalyzed rearrangement of the thebaine-like molecules involves the proton-promoted cleavage of the C5–O ether bond as the first step, resulting in a cation at ring C. Meanwhile the bridgehead bond of ring N at the fusion of rings B and C relocates from 13-position to 14-position. As the next steps of the mechanism, a series of electron pair shifts take place at ring C in order to form the aromatic structure; these rearrangements are terminated with a further relocation of ring N from 14-position to 8-position of the late morphinan backbone. As a results of the configuration of C9 of the morphinan structure, the configuration of the only resided chiral centre (C6a) is predetermined. The rearrangement was achieved by methanesulfonic acid (99.5% purity) and yielded the aimed compounds in good yields (67–77%). The closing step of this synthetic route was the $BCl_3$-mediated 10-*O*-demethylation at −10 °C in $CH_2Cl_2$ (Scheme 4).

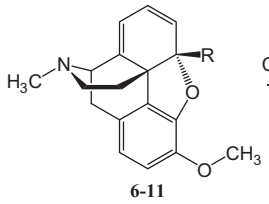
This procedure was applied successfully for the efficient preparation of derivatized apomorphines.[23] In the case of apocodeines **14**, **15** and **17** it was aimed to perform a double deprotection of both 10-*O*-methyl function and the substituent in position 1.




**Scheme 1.** Retrosynthetic analysis of our route to 1-substituted apomorphines.

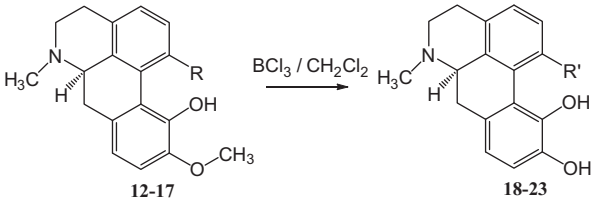



**Scheme 2.** Synthesis of 5β-substituted-6-demethoxythebaines **6**–**11**.




| | R |
|---|---|
| 6, 12 | Me |
| 7, 13 | Bn |
| 8, 14 | Bn-O-$CH_2$ |
| 9, 15 | 4-OMe-Bn |
| 10, 16 | 4-(N,N-dimethylamino)-Bn |
| 11, 17 | 4-MeO-naphthyl-$CH_2$ |

**Scheme 3.** Synthesis of apocodeines **12–17**.




| | R | R' | |
|---|---|---|---|
| 12 | Me | Me | 18 |
| 13 | Bn | Bn | 19 |
| 14 | Bn-O-$CH_2$ | OH-$CH_2$ | 20 |
| 15 | 4-OMe-Bn | 4-OH-Bn | 21 |
| 16 | 4-(N,N-dimethylamino)-Bn | 4-(N,N-dimethylamino)-Bn | 22 |
| 17 | 4-MeO-naphthyl-$CH_2$ | 4-OH-naphthyl-$CH_2$ | 23 |

**Scheme 4.** Synthesis of targeted apomorphines **18–23**.

According to the literature, it was found that the benzyl deprotection of 1-hydroxymethyl function was also possible with this reagent.[24] The one-pot deprotections of compounds **14**, **15** and **17** gave rise to the desired apomorphines in lower yields (36%, 41% and 51%, respectively) in comparison to the simple 10-*O*-demethylated compounds **12**, **13** and **16** (66%, 70% and 54%, respectively). The apomorphines **18–23** were isolated in the stable hydrochloride salt form.

For testing the new compounds three stable cell lines expressing the human dopamine receptor subtypes $D_1$, $D_{2L}$ and $D_3$ were generated in human embryonic kidney cell line (HEK293). The HEK293 cell line is easy to transfect has high protein expression levels as well as a proper set of the signaling pathway components of many GPCRs.[25] The obtained stable lines were characterized by radioligand binding and compared to tritium-labeled control compounds. The dopamine $D_1$ receptor expression was determined by the specific binding of [$^3$H]SCH23390 with its $K_D$ = 0.48 ± 0.06 nM and $B_{max}$ = 570 ± 20 fmol/$10^6$ cells. The expression of dopamine $D_{2L}$ and $D_3$ receptors was determined by the specific binding of a non-selective $D_2$-family radioligand, [$^3$H]raclopride with a $K_D$ = 0.66 ± 0.07 nM, $B_{max}$ = 164 ± 4 fmol/$10^6$ cells, and $K_D$ = 0.83 ± 0.13 nM, $B_{max}$ = 111 ± 4 fmol/$10^6$ cells, for $D_{2L}$ and $D_3$ receptors, respectively. These affinities are in agreement with data published earlier for these radioligands and receptor subtypes.[26–28]

Subtype identity was further confirmed by displacement experiments with different dopaminergic ligands. A representative example for the selectivity of ligands for the $D_1$, $D_{2L}$ and $D_3$ receptors is their endogenous ligand, dopamine, which reportedly has the highest affinity for the $D_3$ subtype and the lowest for the $D_1$ subtype.[29] The potency of dopamine to displace the binding of radioligand was characterized with $K_i$ values of 12 μM, 2.1 μM and 15 nM for the $D_1$R, $D_2$R and $D_3$R, respectively. Additionally more than 200-fold binding selectivity for the $D_3$ over the $D_2$ subtype was recorded with known dopaminergic compounds, 7-OH-PIPAT and NGB2904, confirming the distinct characteristics of the receptor subtypes (data not shown).

In addition to the receptor binding affinity, the ability to modulate downstream signaling cascade is also an important characteristic of a drug. Most direct approach is to follow activation of receptor-coupled G proteins by measuring its GTPase activity or [$^{35}$S]GTPγS binding, what we also used for characterization of ligands' potencies with $D_2$ receptors.[30] An alternative approach is to measure modulation of the level of second messengers ($Ca^{2+}$, $IP_3$, cAMP). In the case of dopamine receptors, the inhibition and activation of adenylate cyclase and measuring the corresponding change in cAMP levels are widely used for the characterization of receptor activation.[31] The latter method is widely applied for $G_i$-coupled receptors, but for other G-protein subtypes the results are contradictory.[32] The reason for choosing the cAMP accumulation assay for comparing the properties of ligands at different receptor subtypes was the fact that it allows the measurement of the $G_s$ and $G_i$ protein coupled receptor signaling at the same downstream effector level.

With the generated cell lines the cyclic AMP accumulation assay was successfully established for the dopamine $D_1$ and $D_{2L}$ receptors, while several problems were encountered in obtaining reliable and consistent results with the cells expressing the dopamine $D_3$ receptors. To confirm the existence of a functional receptor-G protein complex which is required for effective signal transduction, the extent of GTP-dependent high affinity agonist binding[33] was measured in membrane preparations of the generated cell lines. With both $D_1$ and $D_{2L}$ receptors a clear effect of nucleotides on dopamine affinity was achieved, but for the $D_3$ receptors only a negligible effect was detected (data not shown). The guanine nucleotide insensitivity on agonist binding to the dopamine $D_3$ receptors has been reported several times earlier for various cell lines.[34,35] It has been, however, under debate whether the observation is connected with the lack of appropriate subtypes of $G_{i/o}$ proteins or their regulators in these cells or even with the probability of the preference of $D_3$ receptor to a different signaling pathway. Zaworski et al. have shown that the major factors limiting proper $D_3$ receptor signaling in HEK293 cells are the lack of AC-V isoform and also N-type $Ca^{2+}$ channels, whereas $G_o$ proteins necessary for signaling are present in sufficient amount.[36] As the results of cAMP accumulation assay for the $D_3$ receptors were controversial, only the data for the dopamine $D_1$ and $D_{2L}$

**Table 1**
Binding affinities of synthesized compounds for human dopamine $D_1$, $D_{2L}$ and $D_3$ receptors and their subtype selectivities

| | $K_i$ (nM) | | | Selectivity | |
|---|---|---|---|---|---|
| | $D_1$ versus [$^3$H]SCH23390 | $D_{2L}$ versus [$^3$H]raclopride | $D_3$ versus [$^3$H]raclopride | $D_1/D_{2L}$ | $D_{2L}/D_3$ |
| Dopamine | 12,000 | 2100 | 15 | 5.7 | 140 |
| **1** | 492 ± 49 | 53 ± 15 | 17 ± 3 | 9.3 | 3.2 |
| **18** | 187 ± 46 | 54 ± 10 | 23 ± 5 | 3.4 | 2.4 |
| **19** | 168 ± 34 | 77 ± 20 | 55 ± 9 | 2.2 | 1.4 |
| **20** | 980 ± 160 | 43 ± 13 | 17 ± 4 | 22 | 2.6 |
| **21** | 40 ± 11 | 47 ± 9 | 28 ± 6 | 0.8 | 1.7 |
| **22** | 135 ± 39 | 79 ± 31 | 53 ± 12 | 1.7 | 1.5 |
| **23** | 437 ± 83 | 170 ± 64 | 130 ± 30 | 2.6 | 1.3 |

$K_i$ characterizes the ability of a compound to inhibit [$^3$H]SCH23390 ($D_1$) or [$^3$H]raclopride ($D_2$ and $D_3$) binding to the corresponding human dopamine receptor subtypes in membrane preparations of HEK293 cells. Values are represented as mean ± SEM of the apparent inhibition constant ($K_i$) from at least three independent experiments carried out in duplicate or triplicate. Selectivity toward corresponding subtype is the ratio of the compound's affinity constants.

**Table 2**
Potencies and efficacies of synthesized compounds in cAMP accumulation assay using HEK293 cells expressing the human dopamine $D_1$ and $D_{2L}$ receptors

| | $EC_{50}$, nM (efficacy, %) | | Selectivity |
|---|---|---|---|
| | $D_1$ | $D_{2L}$ | $D_1/D_{2L}$ |
| **1** | 25.7 ± 4.8 (100 ± 5) | 4.0 ± 0.9 (100 ± 8) | 6.5 |
| **18** | 21.9 ± 6.7 (109 ± 9) | 5.1 ± 1.2 (97 ± 8) | 4.3 |
| **19** | 78 ± 17 (97 ± 6) | 49 ± 11 (98 ± 8) | 1.6 |
| **20** | 117 ± 20 (102 ± 8) | 3.4 ± 0.9 (95 ± 8) | 34.7 |
| **21** | 10.5 ± 3.7 (95 ± 11) | 2.7 ± 0.5 (102 ± 13) | 3.9 |
| **22** | 741 ± 138 (106 ± 7) | 174 ± 33 (104 ± 12) | 4.3 |
| **23** | 316 ± 44 (101 ± 5) | 76 ± 15 (100 ± 12) | 4.2 |

$EC_{50}$ characterizes the concentration of a compound to generate 50% of the receptor-dependent change in cAMP concentration. Efficacy represents the compound's maximal response in comparison with the effect of apomorphine (**1**). Values are represented as mean ± SEM from at least three independent experiments carried out in duplicate or triplicate. Selectivity toward the $D_{2L}$ receptors is the ratio of the compound's $D_1$ and $D_{2L}$ potency values.

receptor activation has been reported herein for the new compounds (Table 2).

All 1-substituted apomorphines **18–23** tested had at least submicromolar affinity for the three abovementioned dopamine receptor subtypes (Table 1). The compounds **18–23** behaved as full agonists in our model systems (Table 2). The substitutions made at position 1 had only a slight effect on the affinities for the $D_2$ and $D_3$ receptors and the main changes were connected with the binding to $D_1$ receptors. The highest subtype selectivity was achieved with compound **20**, having a 1-hydroxymethyl function, as the selectivities for $D_2$ and $D_3$ over $D_1$ receptors were more than 20- and 50-fold, respectively. The increase in selectivity was connected with the loss of affinity for $D_1$ receptors, while the affinity for the $D_2$ and $D_3$ receptors remained comparable to the reference compound, apomorphine (**1**). Small hydrophilic substituent in position 1, as in compound **20**, might be disrupting the hydrogen bonding of catechol hydroxyl groups with serine residues in TM5 region that are shown to be important for ligand binding to the $D_1$, but affects less the binding to the $D_2$-type receptors.[23,37] The above is supported also by the results from functional assays—the potency for the $D_1$ receptor decreased fivefold with practically no difference on the potency for $D_2$ receptors in comparison to the data of compound **1**. The presence of 4-hydroxybenzyl moiety (compound **21**) caused significant (10-fold) increase in the affinity for the $D_1$ receptor compared to reference **1**. It can be proposed that this group no significant effect on the interactions between the TM5 serines (Ser198, Ser199 and Ser202) of the receptor and the catechol moiety, and the additional hydroxyl group generates hydrogen bonds with neighboring amino acid residues. Besides that it has also been proposed that the presence of hydrophobic substituents in the proximity of position 2 of the aporphine skeleton leads increased binding affinity.[3,4] However, the subtype selectivity of the compound was completely lost by this 4-hydroxybenzyl substitution (Tables 1 and 2). It seems likely that the 1-methyl and 1-benzyl substituents, as for example in compounds **18** and **19**, form a certain scaffold for a better interaction with the $D_1$ receptor, whereas addition of the hydroxyl to the benzyl group, as in compound **21**, fixes the compound into an active conformation, which results in a superior binding affinity and increased potency compared to apomorphine (**1**). The largest difference in the potency and binding affinity to $D_1$ receptors appeared with compound **22**, in which case the addition of a basic dimethylaminobenzylic moiety decreased the potency in activation of cAMP synthesis up to 30-fold, while its binding affinity, on the contrary, was found to be slightly increased (Tables 1 and 2). Introducing and modifying basic moieties in position 1 might therefore be useful for developing more subtype selective ligands. The bicyclic 1-(4-hydroxynaphthyl) function of compound **23** reduced potency for the $D_1$ receptors and it also reduced the affinity and potency for the $D_2$-type receptors. Loss in receptor function with no or minor loss in ligand affinity has been related to the loss of interaction with the Ser198 in TM5 region,[38,39] which would be the reason for the observed decrease in potencies of compounds **19**, **22** and **23**.

Generally it is difficult to draw exact conclusions from the comparison of binding and cellular profile data; however major trends with the change of the substitution pattern could be properly followed up. It was found in previous studies that, in contrast to dopamine antagonists, for the agonist compounds, both efficacy and affinity drive the functional effects.[40] In fact, the affinity, an intrinsic property of the molecule, can easily be determined by binding assays, but the efficacy depends on the environment of the receptor, expression level of receptors and coupling proteins and the method of detection. It was confirmed that the cellular response to a pharmacological agonists depended on the efficacy of the agonist and the sensitivity of the system.[41]

## 3. Conclusions

In summary, a set of novel 1-substituted apomorphines was synthesized and characterized for their activity toward human dopamine $D_1$, $D_{2L}$ and $D_3$ receptors. Newly developed HEK293 based stable cell lines expressing the dopamine $D_1$, $D_{2L}$ and $D_3$ receptors enabled to assess binding and functional activity in the same cell type. The substitution in position 1 of the apomorphine backbone mainly decreased the interactions with the dopamine $D_1$ receptor and thus increased selectivity for the $D_2$ and $D_3$ receptors. The most characteristic effect was noticed in case of hydroxymethyl group, which probably disrupts the interactions between the catechol moiety and the Ser202 of the receptor TM5 region. 4-OH-benzyl group in the same position, on the other hand, in-

creased the affinity for the $D_1$ receptors and led to the loss of the subtype selectivity of the compounds. The basic function (e.g., dimethylaminobenzyl) caused significant loss in the ligand potency in modulation of AC for both subtypes studied, but only a slight influence on the affinity. In summary, the results show that substitutions at position 1 of apomorphine remarkably may affect the affinity for $D_1$ receptor subtype, while the other subtypes studied were only slightly affected. The modulation in affinities and potencies connected with 1-substituted apomorphines suggests possibilities for future design of selective dopaminergic ligands in the aporphine series.

## 4. Experimental

Fine chemicals used in the synthesis were purchased from Sigma–Aldrich and UkrOrgSynthesis Ltd. and were used without any purification. Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Thin layer chromatography was performed on pre-coated Merck 5554 Kieselgel 60 $F_{254}$ foils. The spots were visualized with Dragendorff's reagent. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance 360 spectrometer, at 360 MHz and 90 MHz, respectively; using $CDCl_3$ as a solvent for morphinans and apocodeines and DMSO-$d_6$ for apomorphine salts. Chemical shifts are reported in parts per million ($\delta$) from internal TMS and coupling constants ($J$) are measured in hertz. LC–MS spectra were recorded on an Agilent 1100 Series LC/MSD instrument, including quaternary gradient pump, automatic injector, column oven, DAD detector and MSD Trap XCT ion trap mass spectrometer (equipped with electrospray ionization source). Applied chromatographic parameters—sample size: 100 μL; column: Agilent TC-$C_{18}$ (4.6 mm × 250 mm, 5 μm); column temperature: 30 °C; rate: 1.0 mL min$^{-1}$; detection wavelength: 272 nm; mobile phase: acetonitrile (A)—0.01% aqueous formic acid (B). Gradient elution: eluent A was 2% in 0–10 min and increased from 2% to 16% in 10–40 min. Applied detector settings—MS: ESI, inspection method: positive ion; atomization pressure: 40 kPa; flow rate of dry gas: 10 L min$^{-1}$; temperature of dry gas: 350 °C; spray voltage: 4 kV; multi-level scanning collision gas: nitrogen. Optical rotation was determined with a Perkin Elmer Model 241 polarimeter. Elemental analyses (C, H and N) were obtained on a Carlo Erba EA1108 analyzer.

### 4.1. 5β-Substitution of 6-demethoxythebaine (5)

To a stirred solution of 1 g (3 mmol) of 6-demethoxythebaine (**5**)[19] in 80 mL of dry THF, cooled to −78 °C, was added 1.7 M *tert*-BuLi (in pentane, 1.2 equiv). The mixture was stirred for 30 min before the corresponding chloride (1.2 equiv) was added. The solution was stirred at −78 °C for 1 h before it was allowed to come to room temperature over 2 h and stirred at ambient temperature for further 2 h. The workup was performed in line with Gates' method,[20] products were separated by means of column chromatography (dichloromethane:methanol:concentrated ammonium hydroxide 90:9:1).

### 4.2. 5β-Methyl-6-demethoxythebaine (6)

Yield: 30%. The physical and spectral characteristics of the product were in accordance with previously published data.[15]

### 4.3. 5β-Benzyl-6-demethoxythebaine (7)

Yield: 27%. The physical and spectral characteristics of the product were in accordance with previously published data.[16]

### 4.4. 5β-(Benzyloxymethyl)-6-demethoxythebaine (8)

Yield: 27%. Off-white solid. Mp: 99–101 °C; calculated for free base $C_{26}H_{27}NO_3$: C, 77.78; H, 6.78; N, 3.49; found: C, 77.69; H, 6.91; N, 3.52; $^1$H NMR ($CDCl_3$) $\delta$ = 7.21–7.42 (m, 5H, Ph), 6.69 (d, 1H, H1, $J_{1-2}$ = 7.8), 6.63 (d, 1H, H2, $J_{1-2}$ = 7.8), 5.91–5.74 (m, 3H, H6, H7, H8), 4.88 (s, 2H, OC$H_2$-Ph), 3.87 (s, 3H, $OCH_3$), 3.88–3.61 (m, 2H, C5-C$H_2$), 3.31 (1H, d, H10α, $J_{10\alpha-10\beta}$ = 16.4), 2.82–2.52 (m, 3H, H10β, H16α, H16β), 2.33 (s, 3H, $NCH_3$), 2.21 (ddd, 1H, H15β, $J$ = 5.4, >2, >2), 1.79 (ddd, 1H, H15α $J$ = 5.4, >2, >2). $^{13}$C NMR ($CDCl_3$) $\delta$ = 144.6, 141.4, 138.6, 136.9, 135.4, 131.9, 128.6, 128.1, 127.0, 126.6, 126.4, 126.3, 125.1, 121.3, 114.7, 108.7, 90.4, 74.8, 73.4, 63.5, 57.1, 48.9, 47.6, 42.7, 37.2, 29.1.

### 4.5. 5β-(4-Methoxybenzyl)-6-demethoxythebaine (9)

Yield: 30%. Pale yellow foam. Calculated for free base $C_{26}H_{27}NO_3$: C, 77.78; H, 6.78; N, 3.49; found: C, 77.48; H, 6.83; N, 3.50; $^1$H NMR ($CDCl_3$) $\delta$ = 7.04 (d, 2H, Ar, $J$ = 6.1), 6.81 (d, 2H, Ar, $J_{1-2}$ = 6.1), 6.61 (d, 1H, H1, $J_{1-2}$ = 8.0), 6.59 (d, 1H, H2, $J_{1-2}$ = 8.0), 5.81–5.69 (m, 3H, H6, H7, H8), 3.88 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 3.29 (1H, d, H10α, $J_{10\alpha-10\beta}$ = 16.1), 2.84–2.37 (m, 5H, H10β, H16α, H16β, C5-C$H_2$), 2.28 (s, 3H, $NCH_3$), 2.14 (ddd, 1H, H15β, $J$ = 5.4, >2, >2), 1.87 (ddd, 1H, H15α $J$ = 5.2, >2, >2). $^{13}$C NMR ($CDCl_3$) $\delta$ = 158.4, 144.0, 142.7, 136.7, 132.6, 132.4, 129.7, 129.4, 127.5, 125.4, 124.6, 121.3, 120.4, 114.9, 114.7, 113.9, 91.4, 63.8, 59.7, 56.3, 50.8, 44.1, 42.9, 35.9, 28.1.

### 4.6. 5β-[4-(*N,N*-Dimethylamino)-benzyl]-6-demethoxythebaine (10)

Yield: 15%. Pale brown foam. Calculated for free base $C_{27}H_{30}N_2O_2$: C, 78.23; H, 7.29; N, 6.76; found: C, 78.11; H, 7.41; N, 6.89; $^1$H NMR ($CDCl_3$) $\delta$ = 6.70 (d, 1H, H1, $J_{1-2}$ = 7.9), 6.63–6.51 (m, 5H, H2, Ar), 5.72-5.54 (m, 3H, H6, H7, H8), 3.90 (s, 3H, $OCH_3$), 3.28 (1H, m, H10α), 3.12 (s, 6H, 2 × NC$H_3$), 3.04–2.55 (m, 5H, H10β, H16α, H16β, C5-C$H_2$), 2.31 (s, 3H, $NCH_3$), 2.07 (ddd, 1H, H15β, $J$ = 5.4, >2, >2), 1.76 (ddd, 1H, H15α $J$ = 5.2, >2, >2). $^{13}$C NMR ($CDCl_3$) $\delta$ = 148.9, 143.9, 142.6, 136.2, 134.6, 132.1, 130.0, 129.7, 127.5, 126.7, 125.0, 124.9, 124.7, 124.6, 114.3, 110.3, 91.6, 63.4, 56.1, 50.3, 48.6, 43.7, 42.4, 40.7, 35.9, 28.1.

### 4.7. 5β-(4-Methoxynaphthylmethyl)-6-demethoxythebaine (11)

Yield: 17%. Pale brown solid. Mp: 123–126 °C, calculated for free base $C_{30}H_{29}NO_3$: C, 79.80; H, 6.47; N, 3.10; found: C, 79.59; H, 6.58; N, 3.20; $^1$H NMR ($CDCl_3$) $\delta$ = 8.06–7.98 (m, 2H, Ar), 7.56–7.43 (m, 2H, Ar), 6.78–6.49 (m, 4H, H1, H2, Ar), 5.68–5.45 (m, 3H, H6, H7, H8), 3.94 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 3.29–3.12 (3H, m, H10α, C5-C$H_2$), 2.88–2.39 (m, 3H, H10β, H16α, H16β), 2.37 (s, 3H, $NCH_3$), 2.04 (ddd, 1H, H15β, $J$ = 5.6, >2, >2), 1.80 (ddd, 1H, H15α $J$ = 5.6, >2, >2). $^{13}$C NMR ($CDCl_3$) $\delta$ = 156.2, 145.0, 143.3, 136.7, 135.5, 134.6, 133.5, 133.0, 128.9, 127.5, 126.8, 125.5, 124.9, 124.7, 124.0, 123.9, 121.1, 114.3, 112.2, 104.9, 92.2, 63.3, 56.9, 55.9, 52.6, 48.7, 42.3, 40.9, 35.3, 28.2.

### 4.8. Acid-catalyzed rearrangement of 5β-substituted-6-demethoxythebaines (6–11) into 1-substituted-apocodeines (12–17)

A mixture of the diene (250 mg) and 99.5% methanesulfonic acid ( mL) was stirred for  min at 0 °C and then for 30 min at 90–95 °C (the progress of the reaction was followed by TLC). After completion the reaction mixture was added dropwise, with stirring and external ice cooling, to a solution of potassium hydrogen carbonate (2 g) in water (12.5 mL). After extraction with chloroform

(3 × 15 mL), the combined organic extracts were washed with saturated brine, dried ($MgSO_4$) and concentrated under vacuum to yield crude apocodeine. Purification was realized by means of column chromatography (dichloromethane:methanol 8:2).

### 4.9. 1-Methylapocodeine (12)

Yield: 77%. Off-white solid. $[\alpha]_D^{25}$ − 76 (*c* 0.10, dichloromethane); mp: 123 °C (dec.); calculated for free base $C_{19}H_{21}NO_2$: C, 77.27; H, 7.17; N, 4.74; found: C, 77.01; H, 7.28; N, 4.80; $^1$H NMR ($CDCl_3$) $\delta$ = 7.09 (dd, 2H, H2, H3, $J_{2-3}$ = 7.4), 6.78 (d, 1H, H8, $J_{8-9}$ = 7.6), 6.70 (d, 1H, H9, $J_{8-9}$ = 7.6), 3.94–3.92 (m, 1H, H6α), 3.88 (s, 3H, $OCH_3$), 3.02–2.44 (6H, m, H4α, H4β, H5α, H5β, H7α, H7β), 2.40 (s, 3H, $NCH_3$), 2.29 (s, 3H, C1-$CH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ = 148.1, 144.3, 138.4, 135.4, 133.2, 130.0, 128.5, 127.9, 127.1, 126.7, 121.6, 119.4, 66.7, 57.1, 54.9, 43.2, 30.5, 27.1, 23.9.

### 4.10. 1-Benzylapocodeine (13)

Yield: 69%. The physical and spectral characteristics of the product were in accordance with previously published data.[16]

### 4.11. 1-(Benzyloxymethyl)apocodeine (14)

Yield: 71%. Off-white foam. $[\alpha]_D^{25}$ − 80 (*c* 0.10, dichloromethane); Calculated for free base $C_{26}H_{27}NO_3$: C, 77.78; H, 6.78; N, 3.49; found: C, 77.61; H, 6.89; N, 3.50; $^1$H NMR ($CDCl_3$) $\delta$ = 7.39–7.29 (m, 5H, Ar), 7.24 (d, 1H, H2, $J_{2-3}$ = 6.8), 7.21 (d, 1H, H3, $J_{2-3}$ = 6.8), 6.89 (d, 1H, H8, $J_{8-9}$ = 7.4), 6.81 (d, 1H, H9, $J_{8-9}$=7.3), 4.87 (s, 2H, C5-C$H_2$), 4.33 (s, 2H, O-C$H_2$-Ph), 3.99–3.97 (m, 1H, H6α), 3.83 (s, 3H, $OCH_3$), 2.84–2.31 (9H, m, H4α, H4β, H5α, H5β, H7α, H7β, $NCH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ = 147.8, 143.9, 136.4, 136.2, 133.1, 131.3, 130.7, 130.4, 129.4, 128.6, 128.4, 128.3, 127.8, 126.1, 124.9, 124.7, 120.8, 111.6, 73.9, 72.7, 65.7, 56.6, 56.4, 43.7, 30.1, 23.7.

### 4.12. 1-(4-Methoxybenzyl)apocodeine (15)

Yield: 67%. Pale yellow solid. $[\alpha]_D^{25}$ − 69 (*c* 0.10, dichloromethane); Mp: 114 °C (dec.); calculated for free base $C_{26}H_{27}NO_3$: C, 77.68; H, 6.78; N, 3.49; found: C, 77.80; H, 6.66; N, 3.41; $^1$H NMR ($CDCl_3$) $\delta$ = 7.07 (d, 1H, H2, $J_{2-3}$ = 7.2), 7.02 (d, 1H, H3, $J_{2-3}$ = 7.2), 7.00–6.85 (m, 4H, Ar), 6.78 (d, 1H, H8, $J_{8-9}$ = 7.8), 6.74 (d, 1H, H9, $J_{8-9}$ = 7.8), 3.99 (s, 2H, C6-C$H_2$), 3.95–3.92 (m, 1H, H6α), 3.87 (s, 3H, $OCH_3$), 3.81(s, 3H, $OCH_3$), 2.91–2.44 (6H, m, H4α, H4β, H5α, H5β, H7α, H7β), 2.38 (s, 3H, $NCH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ = 159.8, 148.1, 144.9, 143.7, 141.5, 137.6, 135.5, 134.6, 132.7, 130.5, 128.5, 128.3, 126.9, 126.4, 122.4, 117.8, 112.4, 112.3, 64.9, 57.3, 53.8, 43.4, 39.9, 36.7, 30.5, 23.9.

### 4.13. 1-[4-(*N,N*-Dimethylamino)-benzyl]-apocodeine (16)

Yield: 70%. Pale yellow foam. [$[\alpha]_D^{25}$ − 73 (*c* 0.10, dichloromethane); Calculated for free base $C_{27}H_{30}N_2O_2$: C, 78.23; H, 7.29; N, 6.76; found: C, 78.12; H, 7.40; N, 6.89; $^1$H NMR ($CDCl_3$) $\delta$ = 7.10–7.01 (m, 6H, H2, H3, Ar), 6.78 (d, 1H, H8, $J_{8-9}$ = 8.0), 6.69 (d, 1H, H9, $J_{8-9}$ = 8.0), 3.99 (s, 2H, C6-C$H_2$), 3.90 (s, 3H, $OCH_3$), 3.89–3.83 (m, 1H, H6α), 3.03 (s, 3H, N-$CH_3$), 3.00 (s, 3H, N-$CH_3$), 2.84–2.44 (6H, m, H4α, H4β, H5α, H5β, H7α, H7β), 2.36 (s, 3H, $NCH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ = 149.8, 147.8, 144.4, 144.3, 142.1, 141.3, 137.5, 135.7, 133.2, 130.9, 129.9, 129.6, 128.9, 125.7, 120.5, 113.6, 113.4, 111.7, 65.3, 56.9, 56.8, 44.2, 41.4, 37.8, 33.2, 29.4, 23.7.

### 4.14. 1-(4-Methoxynaphthylmethyl)-apocodeine (17)

Yield: 66%. Yellow foam. $[\alpha]_D^{25}$ − 87 (*c* 0.10, dichloromethane); Calculated for free base $C_{30}H_{29}NO_3$: C, 79.80; H, 6.47; N, 3.10; found: C, 79.61; H, 6.55; N, 3.23; $^1$H NMR ($CDCl_3$) $\delta$ = 8.28–8.20 (m, 2H, Ar), 7.65–7.59 (m, 2H, Ar), 7.09 (d, 1H, H3, $J_{2-3}$ = 7.6), 7.04 (d, 1H, H2, $J_{2-3}$ = 7.6), 6.90–6.78 (m, 4H, H8, H9, Ar), 4.21 (s, 2H, C6-C$H_2$), 3.90–3.87 (m, 4H, H6α, $OCH_3$), 3.82 (s, 3H, $OCH_3$), 3.13–2.39 (6H, m, H4α, H4β, H5α, H5β, H7α, H7β), 2.36 (s, 3H, $NCH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ = 155.1, 147.3, 143.9, 142.7, 141.6, 137.8, 135.5, 131.7, 130.9, 129.3, 128.4, 127.5, 126.8, 126.7, 126.5, 125.6, 124.3, 123.9, 123.5, 120.3, 111.3, 108.9, 65.8, 56.9, 56.7, 54.6, 45.7, 43.7, 30.2, 23.1.

### 4.15. *O*-Deprotection of aporphines 12–17

A suspension of the apocodeine (0.5 mmol) in 10 mL of abs $CH_2Cl_2$ was cooled to −10 °C and treated with cooled solution of $BCl_3$ (1 mL, 1 M in $CH_2Cl_2$) by stirring for 6 h under argon atmosphere. The reaction mixture was quenched with 2 mL of methanol. After evaporation of the solvent, the residue was treated with aqueous $NaHCO_3$ and extracted with $CH_2Cl_2$ (3 × 15 mL). The extract was dried over $Na_2SO_4$, filtered and treated with methanol saturated with HCl and then with ether to give rise to stable HCl salt.

### 4.16. 1-Methylapomorphine·HCl (18)

Yield: 66%. Off-white solid. Mp: 149–150 °C; calculated for salt $C_{18}H_{20}ClNO_2$: C, 68.03; H, 6.34; N, 4.41; found: C, 68.17; H, 6.48; N, 4.50; HPLC–MS (*m*/*z*) for free base: 282.2 [M+1$^+$] base peak. HPLC purity: >97.4% (rt 14.14 min). $^1$H NMR (DMSO-$d_6$) $\delta$ = 10.91 (br s, 1H, NH$^+$), 9.73 (br s, 2H, OH), 7.10 (d, 1H, H2, $J_{2-3}$ = 7.9), 7.12 (d, 1H, H3, $J_{2-3}$ = 7.9), 6.76 (d, 1H, H9, $J_{8-9}$ = 7.4), 6.72 (d, 1H, H8, $J_{8-9}$ = 7.4), 3.98–3.96 (m, 1H, H6α), 2.89–2.52 (9H, m, H4α, H4β, H5α, H5β, H7α, H7β, $NCH_3$), 2.49 (s, 3H, C1-$CH_3$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ = 144.7, 141.8, 132.1, 131.5, 128.7, 127.8, 127.4, 126.7, 124.9, 124.5, 120.9, 113.4, 66.3, 54.3, 44.5, 29.1, 26.1, 22.8.

### 4.17. 1-Benzylapomorphine·HCl (19)

Yield: 70%. Pale grey solid. Mp: 137–139 °C; calculated for salt $C_{24}H_{24}ClNO_2$: C, 73.18; H, 6.14; N, 3.56; found: C, 73.32; H, 6.31; N, 3.43; HPLC–MS (*m*/*z*) for free base: 358.2 [M+1$^+$] base peak. HPLC purity: >96.3% (rt 12.23 min). $^1$H NMR (DMSO-$d_6$) $\delta$ = 11.13 (br s, 1H, NH$^+$), 9.66 (br s, 2H, OH), 7.28–7.21 (m, 5H, Ar), 7.08 (dd, 2H, H2, H3, $J_{2-3}$ = 7.8), 6.82 (d, 1H, H9, $J_{8-9}$ = 7.6), 6.77 (d, 1H, H8, $J_{8-9}$ = 7.6), 3.95–3.92 (m, 1H, H6α), 3.91 (s, 2H, C5-C$H_2$), 2.86–2.44 (9H, m, H4α, H4β, H5α, H5β, H7α, H7β, $NCH_3$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ = 145.0, 144.0, 142.8, 137.1, 135.1, 131.1, 130.7, 129.4, 129.2, 128.3, 128.1, 127.5, 126.7, 126.4, 126.3, 124.5, 121.2, 114.0, 65.4, 57.1, 43.5, 38.9, 29.7, 23.4.

### 4.18. 1-(Hydroxymethyl)-apomorphine·HCl (20)

Yield: 36%. Off-white solid. Mp: 121–123 °C; calculated for salt $C_{18}H_{20}ClNO_3$: C, 64.77; H, 6.04; N, 4.20; found: C, 64.61; H, 6.27; N, 4.29; HPLC–MS (*m*/*z*) for free base: 298.1 [M+1$^+$] base peak. HPLC purity: >96.0% (rt 12.20 min). $^1$H NMR (DMSO-$d_6$) $\delta$ = 11.31 (br s, 1H, NH$^+$), 9.88 (br s, 2H, OH), 7.21 (d, 1H, H2, $J_{2-3}$ = 8.0), 7.12 (d, 1H, H3, $J_{2-3}$ = 8.0), 6.82 (d, 1H, H9, $J_{8-9}$ = 7.7), 6.80 (d, 1H, H8, $J_{8-9}$ = 7.7), 4.51 (s, 2H, C5-C$H_2$), 4.08–4.05 (m, 1H, H6α), 3.12–2.69 (9H, m, H4α, H4β, H5α, H5β, H7α, H7β, $NCH_3$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ = 144.3, 142.9, 137.4, 135.1, 131.5, 128.7, 127.8, 127.4, 124.9, 124.5, 121.6, 115.0, 66.1, 55.8, 44.1, 29.7, 24.7.

### 4.19. 1-(4-Hydroxybenzyl)-apomorphine·HCl (21)

Yield: 41%. Pale green solid. Mp: 133–135 °C; calculated for salt $C_{24}H_{24}ClNO_3$: C, 70.32; H, 5.90; N, 3.42; found: C, 70.44; H, 6.01; N,

3.37; HPLC–MS (*m*/*z*) for free base: 374.2 [M+1$^+$] base peak. HPLC purity: >95.4% (rt 14.23 min). $^1$H NMR (DMSO-$d_6$) $\delta$ = 10.98 (br s, 1H, NH$^+$), 9.47 (br s, 3H, OH), 7.14–7.04 (m, 4H, H2, H3, Ar), 6.75 (d, 1H, H9, $J_{8-9}$ = 8.0), 6.72 (d, 1H, H8, $J_{8-9}$ = 8.0), 6.59 (d, 2H, Ar, $J$ = 7.2), 4.21–4.18 (m, 1H, H6α), 3.91 (s, 2H, C5-C$H_2$), 3.19–2.44 (9H, m, H4α, H4β, H5α, H5β, H7α, H7β, NCH$_3$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ = 153.6, 144.4, 142.8, 142.4, 137.2, 135.7, 134.2, 131.9, 130.7, 129.3, 128.1, 127.5, 126.7, 126.4, 125.8, 124.5, 122.3, 114.7, 65.7, 56.8, 43.2, 39.7, 29.1, 23.7.

### 4.20. 1-[4-(*N*,*N*-Dimethylamino)-benzyl]-apomorphine·2HCl (22)

Yield: 54%. Pale green solid. Mp: 148 °C (dec); calculated for salt $C_{26}H_{30}Cl_2N_2O_2$: C, 65.96; H, 6.36; N, 5.92; found: C, 66.09; H, 6.43; N, 5.81; HPLC–MS (*m*/*z*) for free base: 401.2 [M+1$^+$] base peak. c $^1$H NMR (DMSO-$d_6$) $\delta$ = 10.40 (br s, 1H, NH$^+$), 10.01 (br s, 1H, NH$^+$), 9.25 (br s, 2H, OH), 7.80–7.62 (m, 4H, Ar), 7.10 (d, 1H, H3, $J_{2-3}$ = 7.7), 7.13 (d, 1H, H2, $J_{2-3}$ = 7.7), 6.85 (d, 1H, H9, $J_{8-9}$ = 8.0), 6.80 (d, 1H, H8, $J_{8-9}$ = 8.0), 4.19–4.09 (m, 1H, H6α), 3.87 (s, 2H, C5-C$H_2$), 3.22 (s, 6H, 2 × NC$H_3$), 3.21–2.36 (9H, m, H4α, H4β, H5α, H5β, H7α, H7β, NCH$_3$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ = 150.6, 144.9, 142.9, 142.6, 142.5, 137.6, 135.1, 131.9, 129.3, 128.3, 128.2, 126.7, 126.4, 125.8, 124.5, 121.6, 121.3, 114.9, 66.0, 57.4, 47.9, 43.1, 37.8, 29.7, 23.6.

### 4.21. 1-(4-Hydroxynaphthylmethyl)-apomorphine·HCl (23)

Yield: 51%. Grey solid. Mp: 135 °C (dec.); Calculated for salt $C_{28}H_{26}ClNO_3$: C, 73.11; H, 5.70; N, 3.05; found: C, 73.31; H, 5.83; N, 3.20; HPLC–MS (*m*/*z*) for free base: 424.2 [M+1$^+$] base peak. HPLC purity: >96.8% (rt 13.29 min). $^1$H NMR (DMSO-$d_6$) $\delta$ = 10.32 (br s, 1H, NH$^+$), 9.64 (br s, 2H, 2 × OH), 8.20–8.15 (m, 2H, Ar), 7.51–7.47 (m, 2H, Ar), 7.06 (d, 1H, H3, $J_{2-3}$ = 8.0), 7.01 (d, 1H, H2, $J_{2-3}$ = 8.0), 6.80–6.61 (m, 4H, H8, H9, Ar), 4.67 (s, 2H, C6-C$H_2$), 4.14–4.06 (m, 1H, H6α), 3.23–2.56 (9H, m, H4α, H4β, H5α, H5β, H7α, H7β, NCH$_3$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ = 151.4, 149.7, 142.9, 142.0, 141.3, 137.1, 135.0, 132.2, 131.4, 129.8, 128.5, 127.2, 126.4, 126.7, 125.7, 124.4, 124.0, 123.5, 123.0, 122.0, 113.8, 113.7, 65.3, 56.8, 44.9, 43.5, 30.1, 23.4.

## 5. Detailed description of pharmacological methods

The human embryonic kidney cell line (HEK293) used as a host for dopamine receptors in pharmacological assays was obtained from American Type Culture Collection, Boras, Sweden. The pcDNA3.1+ expression vectors (Invitrogen) containing the desired gene of human wild type dopamine receptor (hD$_1$, hD$_{2L}$ and hD$_3$) was purchased from the Missouri S&T cDNA Resource Center (http://www.cdna.org). Lipofectamine™ 2000, fetal bovine serum (FBS, Gibco®) were from Invitrogen Life Technologies, Paisley, UK. Dulbecco's Modified Eagle's Medium (DMEM), Dulbecco's Phosphate Buffered Saline (DPBS), geneticin (G418) and penicillin/streptomycin were from PAA Laboratories GmbH, Pasching, Austria. Cell culture plates (Nunc™) were from Thermo Fisher Scientific, Roskilde, Denmark. Dopamine, (+)-butaclamol and 3-isobutyl-1-methylxanthine (IBMX) were from Sigma–Aldrich Co., Helsinki, Finland. Tris(hydroxymethyl)aminomethane (Tris), $MgCl_2$, KOH, NaCl, KCl, dithiotreitol (DTT), potassium phosphate were from AppliChem GmbH, Darmstadt, Germany. Perchloric acid was from Reakhim, Moscow, Russia and 2,2′,2″,2‴-(ethane-1,2-diyldinitrilo)tetraacetic acid (EDTA) from Merck, Munich, Germany. Cyclic adenosine monophosphate (cAMP) was from Tocris (http://www.tocris.com) and tritium labeled cAMP from BIOTREND Chemikalien GmbH, Köln, Germany. [$^3$H]SCH23390 and [$^3$H]raclopride were from PerkinElmer, MA, USA, as well as all the equipment and materials used for counting radioactivity.

## 6. Generation of stably transfected HEK293 cells expressing dopamine D$_1$, D$_{2L}$ and D$_3$ receptors

HEK293 cells were cultured on Petri dishes in DMEM supplemented with 10% FBS, 100 μg/mL streptomycin, 100 U/mL penicillin and maintained at 37 °C and 5% $CO_2$ in a humidified incubator. For transfection cells were seeded on 6-well plates, cultured 24 h to reach ~90% confluence and transfected with 4 μg of DNA (pcDNA3.1.(+) with a gene encoding for either hD$_1$, hD$_{2L}$ or hD$_3$ receptors) per well using Lipofectamine™ 2000 according to manufacturer's instructions. To obtain stable lines the cells were maintained and passed for 2 weeks in the presence of 800 μg/mL G418. Four to six G418 resistant colonies per each receptor subtype were selected and after five further passages the clonal cultures were tested for receptor expression by radioligand binding. Henceforth the cells were maintained in the presence of 400 μg/mL of G418. The clones with sufficient receptor densities were further tested for their binding of known dopaminergic ligands to confirm their identity (data not shown) and then used in experiments with the novel test compounds.

## 7. Membrane preparation

All ligand binding experiments were performed on membrane suspensions, prepared as follows. The cells were centrifuged at 800*g* at room temperature and the pellet stored at −80 °C. The frozen pellets were melted on ice and washed by homogenization with a tissue homogenizer (Coleparmer Labgen 125) for 30 s in ice-cold DPBS and centrifugation at 800*g* for 5 min at 4 °C. The pellet was re-homogenized in 50 mM Tris–HCl buffer, (pH 7.4) and centrifuged at 30,000*g* for 20 min followed by a second resuspension and homogenization step. The latter homogenization and centrifugation steps were repeated once more and the final pellet was homogenized in incubation buffer (IB: 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.4) typically at a concentration of 1 × 10$^7$ cells/mL (corresponding to ~0.5 mg protein/mL quantified using Bio-Rad protein assay Kit (Bio-Rad Laboratories) with BSA as a standard. The membrane preparations were stored at −80 °C until further testing.

## 8. Radioligand competition experiments with 1-substituted apomorphines

Receptor expression and affinity of radioligands was determined as described earlier[39,42] with some modifications. Reactions were carried out in U-bottom 96-well plates in a final volume of 250 μL in IB and incubated at 25 °C for 90 min. In all assays 150 μL of membrane suspension was added to wells containing 50 μL of radioligand and 50 μL of competing unlabeled ligand (or buffer) at their 5× final concentration. Receptor-positive clones were identified by one-point assay at a saturating concentration of [$^3$H]SCH23390 or [$^3$H]raclopride with or without an unlabeled dopaminergic antagonist, 1 μM (+)-butaclamol (data not shown). In saturation binding experiments the concentration of radioligands was varied from 0.2 to 10 nM and non-specific binding was determined in the presence of 1 μM (+)-butaclamol. For 8-point competition assay the concentration of radioligand was fixed to near $K_d$ value (0.9 ± 0.2 nM) and concentration of the test compounds was varied from 0.1 nM to 100 μM. Reactions were initiated by addition of membrane suspension (app 5 × 10$^4$–5 × 10$^5$ cells/assay for different receptor subtypes).

All the reactions were stopped by fast filtration through thick GF/B glass fiber filters (Whatham) using a FilterMate Harvester (Model D961962, Perkin Elmer). After five washes with ice-cold phosphate buffer (20 mM K-phosphate, 100 mM NaCl, pH 7.4), filter mats were dried in a microwave oven and impregnated with a MeltiLex™ B/HS scintillant using a MeltiLex® Heatsealer. Filter-bound radioactivity was counted using a PerkinElmer/Wallac MicroBeta TriLux 1450 LSC Luminescence Counter.

All pharmacological data were analyzed by means of non-linear least squares regression analysis using the commercial program GRAPHPAD PRISM™ 4.03 (GraphPad Software Inc., CA). Data were fit to one-site binding curve and inhibition constant values ($K_i$) calculated according to the Cheng–Prusoff equation.[43]

## 9. cAMP accumulation assay

Two to three days before the assay cells were seeded on 96-well cell culture plates at a density of 2–5 × $10^4$ cells/well in 100 μL of DMEM without G418. On the day of assay DMEM was replaced by 50 μL DPBS w/o $Ca^{2+}$ and $Mg^{2+}$ prewarmed to 37 °C. Thereafter 50 μL of increasing concentrations of 2× ligand solutions in DPBS with 2 mM $MgCl_2$ and 200 μM IBMX, also prewarmed to 37 °C, were added to cells and incubated for 15 min at 37 °C. In case of the $D_{2L}$ and $D_3$ receptors, the added mixture contained also Forskolin at a final concentration of 24 μM.

Reaction was stopped by addition of 50 μL of ice cold 1.2 M perchloric acid and incubation on ice for 1 h. The mixture in wells was neutralized by addition of 50 μL of 1.2 M KOH and the plates were then stored at −20 °C until the cAMP assay.

cAMP concentration was assayed as described earlier by Vonk et al.[44] with modifications. In brief, the samples were thawed and diluted 2- to 10-fold, depending on the amount of cAMP in samples, in Brown's Buffer containing 50 mM Tris–HCl (pH 7.4 at 4 °C), 8 mM theophyllin, 10 mM EDTA, 100 mM NaCl and 6 mM DTT. To a 50 μL of diluted sample a 50 μL of 30 nM tritium labeled cAMP (specific activity 25 Ci/mmol) and 50 μL of cAMP-binding protein (stock solution diluted 10-fold), prepared as described by Brown et al.,[45] were added and incubated at 2–8 °C for 1 h. After incubation the samples were filtrated and further treated in essence as described above under radioligand binding experiments.

## Acknowledgments

The authors are grateful to the National Science Foundation (Grant OTKA Reg. No. K81701), Estonian Science Foundation (8314, 8011), Estonian Ministry of Education and Science (SF0180032s12) and the European Regional Development Fund (TK114, 30020) for the financial supports.

## References and notes

1. Bozzi, Y.; Borelli, E. *Trends Neurosci.* **2006**, *29*, 167.
2. Marsden, C. A. *Br. J. Pharmacol.* **2006**, *147*, S136.
3. (a) Zhang, A.; Neumeyer, J. L.; Baldessarini, R. *J. Chem. Rev.* **2007**, *107*, 274; (b) Finnema, S. F.; Bang-Andersen, B.; Wikstrom, H. V.; Halldin, C. *Curr. Top. Med. Chem.* **2010**, *10*, 1477.
4. Booth, R. G.; Neumeyer, J. L.; Baldessarini, R. J. Therapeutic and diagnostic agents for the Parkinson's disease *Burger's Medicinal Chemistry and Drug Discovery*, 7th edition; John Wiley and Sons, Inc., 2012.
5. Cho, D. I.; Zheng, M.; Kim, K.-M. *Arch. Pharm. Res.* **2010**, *33*, 1521.
6. Zhang, A.; Zhang, Y.; Branfman, A. R.; Baldessarini, R. J.; Neumeyer, J. L. *J. Med. Chem.* **2007**, *50*, 171.
7. Søndergaard, K.; Kristensen, J. L.; Palner, M.; Gillings, N.; Knudsen, G. M.; Roth, B. L.; Begtrup, M. *Org. Biomol. Chem.* **2005**, *3*, 4077.
8. Sipos, A.; Kiss, B.; Schmidt, E.; Greiner, I.; Berényi, S. *Bioorg. Med. Chem.* **2008**, *16*, 3773.
9. Herm, L.; Berényi, S.; Vonk, A.; Rinken, A.; Sipos, A. *Bioorg. Med. Chem.* **2009**, *17*, 4756.
10. Liu, Z.; Chen, X.; Yu, L.; Zhen, X.; Zhang, A. *Bioorg. Med. Chem.* **2008**, *16*, 6675.
11. Sipos, A.; Mueller, F. K. U.; Lehmann, J.; Berényi, S.; Antus, S. *Arch. Pharm.* **2009**, *342*, 557.
12. Sipos, A.; Tóth, M.; Mueller, F.; Lehmann, J.; Berényi, S. *Monatsh. Chem.* **2009**, *140*, 473.
13. Kalani, M. Y.; Vaidehi, N.; Hall, S. E.; Trabanino, R. J.; Freddolino, P. L.; Kalani, M. A.; Floriano, W. B.; Kam, V. W.; Goddard, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3815.
14. Neumeyer, J. L. Synthesis and structure–activity relationships of aporphines as dopamine receptor agonists and antagonists *The Chemistry and Biology of Isoquinoline Alkaloids*; Spinger-Verlag, 1985. pp. 146–170.
15. Woudenberg, R. H.; Piet, D. P.; Sinnema, A.; Lie, T. S.; Maat, L. *Rec. Trav. Chim. Pays-Bas* **1991**, *110*, 405.
16. Sipos, A.; Berényi, S. *Tetrahedron* **2008**, *64*, 5851.
17. Berényi, S.; Makleit, S.; Hosztafi, S.; Szeifert, I. *Acta Chim. Acad. Sci. Hung.* **1982**, *110*, 363.
18. Berényi, S.; Csutorás, Cs.; Sipos, A. *Curr. Med. Chem.* **2009**, *16*, 3215.
19. Boden, R. M.; Gates, M.; Ho, S. P.; Sundararaman, P. *J. Org. Chem.* **1982**, *47*, 1347.
20. Gates, M.; Boden, R. M.; Sundararaman, P. *J. Org. Chem.* **1989**, *54*, 972.
21. Grancelli, F. E.; Filer, C. N.; Soloway, A. H.; Neumeyer, J. L. *J. Org. Chem.* **1980**, *45*, 2275.
22. Berényi, S.; Czirják, M.; Makleit, S. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2137.
23. Reinart, R.; Gyulai, Zs.; Berényi, S.; Antus, S.; Vonk, A.; Rinken, A.; Sipos, A. *Eur. J. Med. Chem.* **2011**, *46*, 2992.
24. Williams, D. R.; Brown, D. L.; Benbow, J. W. *J. Am. Chem. Soc.* **1923**, *1989*, 111.
25. Thomas, P.; Smart, T. G. *J. Pharmacol. Toxicol. Meth.* **2005**, *51*, 187.
26. Cao, Y.; Sun, W.-C.; Jin, L.; Xie, K.-Q.; Zhu, X.-Z. *Eur. J. Pharmacol.* **2006**, *548*, 29.
27. Häidkind, R.; Eller, M.; Harro, M.; Kask, A.; Rinken, A.; Oreland, L.; Harro, J. *Eur. Neuropsychopharmacol.* **2003**, *13*, 19.
28. Hidaka, K.; Tada, S.; Matsumoto, M.; Ohmori, J.; Tasaki, Y.; Nomura, T.; Usuda, S.; Yamaguchi, T. *Br. J. Pharmacol.* **1996**, *117*, 1625.
29. Freedman, S. B.; Patel, S.; Marwood, R.; Emms, F.; Seabrook, G. R.; Knowles, M. R.; McAllister, G. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 417.
30. Sipos, A.; Csutorás, C.; Berényi, S.; Uustare, A.; Rinken, A. *Bioorg. Med. Chem.* **2008**, *16*, 4563.
31. McAllister, G.; Knowles, M. R.; Ward-Booth, S. M.; Sinclair, H. A.; Patel, S.; Marwood, R.; Emms, F.; Patel, S.; Smith, A.; Seabrook, G. R.; Freedman, S. B. *J. Recept. Signal. Transd.* **1995**, *15*, 267.
32. Milligan, G. *Trends Pharmacol. Sci.* **2003**, *24*, 87.
33. Rinken, A.; Terasmaa, A.; Raidaru, G.; Fuxe, K. *Neurosci. Lett.* **2001**, *302*, 5.
34. Vanhauwe, J. F.; Fraeyman, N.; Francken, B. J.; Luyten, W. H.; Leysen, J. E. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 908.
35. Skinbjerg, M.; Namkung, Y.; Halldin, C.; Innis, R. B.; Sibley, D. R. *Synapse (New York, N.Y.)* **2009**, *63*, 462.
36. Zaworski, P. G.; Alberts, G. L.; Pregenzer, J. F.; Im, W. B.; Slightom, J. L.; Gill, G. S. *Br. J. Pharmacol.* **1999**, *128*, 1181.
37. Chemel, B. R.; Bonner, L. A.; Watts, V. J.; Nichols, D. E. *Mol. Pharmacol.* **2012**, *81*, 729.
38. Hwa, J.; Perez, D. M. *J. Biol. Chem.* **1996**, *271*, 6322.
39. Harro, J.; Terasmaa, A.; Eller, M.; Rinken, A. *Brain Res.* **2003**, *976*, 209.
40. Fouchaq, B.; Wang, S. R.; Piwinca, D.; Coussy, L.; Jolas, T.; Néliat, G. Comparison of Binding and Cellular profiles of Multi-target G Protein-Coupled Receptors. In: 12th Society for Biomolecular Sciences Annual Conference and Exhibition—Seattle, WA (USA), 17–21/09/2006.
41. Kenakin, T. *Nat. Rev. Drug Disc.* **2003**, *2*, 429.
42. Uustare, A.; Reinart, R.; Rinken, A. *Neurosci. Lett.* **2006**, *406*, 169.
43. Cheng, Y.-C.; Prusoff, W. H.; Yung-Chi, C. *Biochem. Pharmacol.* **1973**, *22*, 3099.
44. Vonk, A.; Reinart, R.; Rinken, A. *J. Pharm. Sci.* **2008**, *108*, 63.
45. Brown, D. L.; Albano, J. D.; Ekins, R. P.; Sgherzi, A. M. *Biochem. J.* **1971**, *121*, 561.