# *N*-(ω-(4-(2-Methoxyphenyl)piperazin-1-yl)alkyl)carboxamides as Dopamine $D_2$ and $D_3$ Receptor Ligands

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The dopamine $D_3$ receptor is recognized as a potential therapeutic target for the treatment of various neurological and psychiatric disorders. Targetting high affinity and $D_3$ versus $D_2$ receptor-preferring ligands, the partial agonist BP 897 was taken as a lead structure. Variations in the spacer and the aryl moiety led to *N*-alkylated 1-(2-methyoxyphenyl)piperazines with markedly improved affinity and selectivity. Molecular modeling studies supported the structural development. Pharmacophore models for dopamine $D_2$ and $D_3$ receptor ligands were developed from their potentially bioactive conformation and were compared in order to get insight into molecular properties of importance for $D_2/D_3$ receptor selectivity. For the 72 compounds presented here, an extended and more linear conformation in the aliphatic or aryl spacers turned out to be crucial for dopamine $D_3$ receptor selectivity. Structural diversity in the aryl moiety (benzamides, heteroarylamides, arylimides) had a major influence on (sub)nanomolar $D_3$ receptor affinity, which was optimized with more rigid aryl acrylamide derivatives. Compound **38** (**ST 280**, (*E*)-4-iodo-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide) displayed a most promising pharmacological profile ($K_i$ ($hD_3$) = 0.5 nM; $K_i$ ($hD_{2L}$) = 76.4 nM; selectivity ratio of 153), and above that, compound **38** offered the prospect of a novel radioligand as a pharmacological tool for various $D_3$ receptor-related in vitro and in vivo investigation.

## Introduction

The neurotransmitter dopamine is implicated in various physiological and pathophysiological processes. The dopaminergic system regulates brain functions such as motion, emotion, and cognition. Its effects are mediated by dopamine receptors, which belong to the family of G protein-coupled receptors and share the characteristic of seven transmembrane domains. Dopamine receptors can be divided into five different receptor subtypes and are further classified into two families, the $D_1$-like ($D_1$ and $D_5$) and the $D_2$-like ($D_2$, $D_3$, and $D_4$), with related pharmacological, structural, and genetic properties, respectively.[1,2] An imbalance within the dopaminergic system is related to several psychiatric and neurological disorders, e.g., Parkinson's disease, Huntington's disease, and schizophrenia. Medical treatment of dopamine related disorders is often limited by side effects as a consequence of binding to various dopamine subreceptors or other related monoamine receptors. Therefore, effective therapy calls for selective dopamine subreceptor ligands.[3] Dopamine $D_3$ receptors are relatively few in number but display a discrete localization in special limbic areas of the central nervous system, which are thought to control emotional and cognitive but not locomotor functions.[4,5] For a long time dopamine $D_2$-like receptor antagonists have been used to treat schizophrenia and related psychiatric disorders.[6] A suitably selective dopamine $D_3$ receptor antagonist may provide antipsychotic properties in the relative absence of limiting extrapyramidal side effects.[7] Recent findings suggest that dopamine $D_3$ receptor agonists may have beneficial effects for Parkinson's patients.[8,9] Furthermore, dopamine $D_3$ receptor partial agonists are supposed to be beneficial in the treatment of l-DOPA-induced dyskinesia in Parkinson's patients. It has been impressively demonstrated in MPTP (1-**m**ethyl-4-**p**henyl-1,2,3,6-**t**etrahydro**p**yridine)-treated monkeys, a primate model for longtime Parkinson's disease treatment, that $D_3$ receptor partial agonists normalize exacerbated $D_3$ receptor function.[10,11] Moreover, the treatment of drug addiction has been considered a relevant therapeutic target for dopamine $D_3$ receptor partial agonists.[12] These ligands are thought to modulate the long-term effects caused by the reuptake-inhibitor cocaine (second-order schedule of reinforcement).[13] At the receptor site partial agonists act as agonists or antagonists, depending on dopaminergic activation and on receptor state. Partial agonists are supposed to regulate receptor activation within physiological limits. Consequently they should not exhibit any rewarding activity themselves. In a recent review,

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[⊥] Unité de Neurobiology et Pharmacologie Moléculaire.

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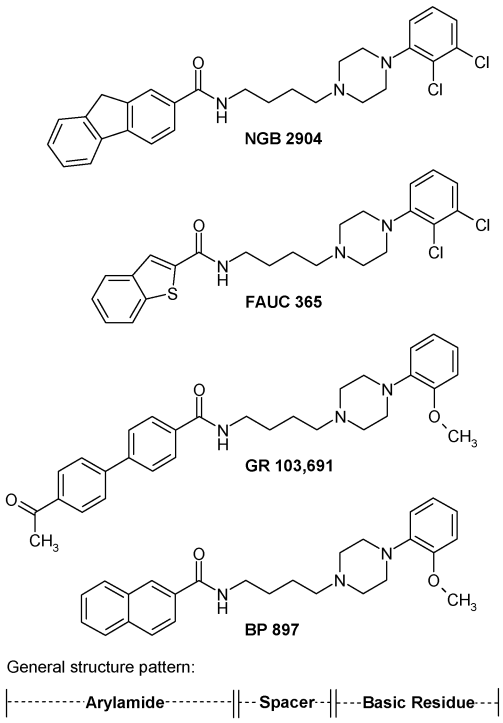
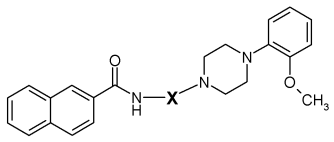
**Chart 1**

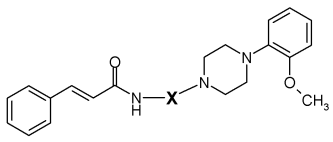



structure−activity relationships for dopamine $D_3$ receptor partial agonists and antagonists and their potential therapeutic application have been comprehensively discussed.[14] For many dopamine $D_3$ receptor ligands with antagonist properties, a general structure pattern can be applied, which is divided into three subunits: an aryl moiety, which is connected to an amide (first subunit) and further linked via an alkyl or aryl spacer (second subunit) to a basic residue with aryl substituent (third subunit) (Chart 1). Among the structural development, compounds with a basic 4-phenylpiperazino residue have turned out to provide prominent selectivity for dopamine $D_3$ receptors (Chart 1). Compounds with 4-(2,3-dichlorophenyl)piperazino residue, e.g., NGB 2904[15] or FAUC 365,[16] mostly provided an antagonist profile, while compounds with 4-(2-methoxyphenyl)piperazino residue showed antagonism, e.g., GR 103 691,[17] or partial agonism, e.g., BP 897.[18] The acknowledged *n*-butyl spacer has been exchanged to a cyclohexylethyl spacer in some compounds.[19,20] In many cases the spacer has been linked to an aryl moiety via an amide bond or has been coupled directly,[21] but ether bridges have been built up as well.[22,23] The lipophilic residue on the arylamide moiety allowed diverse modifications including aryl, biphenyl, heteroaryl, or cycloalkyl substituents.[16,17,19,24] Our lead structure BP 897 combined high affinity with pronounced preference for the human dopamine $D_3$ receptor compared to that for the human $D_2$ receptor[25] (cf. Table 2) and has shown inhibition of cocaine-seeking behavior in animal models.[18] In this study we present a pharmacophore model for dopamine $D_3$ receptor ligands and one for dopamine $D_2$ receptor

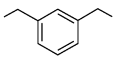
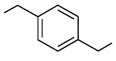
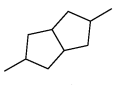
**Table 1.** Structures, Physical Data, and Pharmacological Screening Results of Compounds with Spacer Variations



**1 – 4**

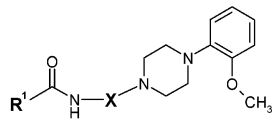


**5**

| No. | X | formula | $M_r$ | mp (°C) | $D_3$ [a] $K_i$ (nM) ± SEM | $D_2$ [a] $K_i$ (nM) ± SEM | ratio [b] $D_2/D_3$ |
|---|---|---|---|---|---|---|---|
| **1** |  | $C_{30}H_{31}N_3O_2 \cdot C_2H_2O_4$ | 555.6 | 219.0 [c] | 750 ±190 | 28.4 ±7.4 | 0.04 |
| **2** |  | $C_{30}H_{31}N_3O_2 \cdot C_2H_2O_4$ | 555.6 | 183.0 [c] | ≈100 | 45 ±17 | 0.5 |
| **3** |  | $C_{30}H_{31}N_3O_2 \cdot 1.25C_2H_2O_4$ | 578.1 | 183-184 [c] | 40.0 ±6 | 200 ±14 | 5.0 |
| **4** |  | $C_{30}H_{31}N_3O_2 \cdot C_2H_2O_4 \cdot 0.75H_2O$ | 483.1 | 156-158 [d] | 37.2 ±1.9 | 178 ±12 | 4.8 |
| **5** |  | $C_{28}H_{31}N_3O_2$ | 441.6 | 188.0 [d] | ≈325 | 18 ±4 | 0.06 |
| **6**[e] | | $C_{30}H_{42}N_4O_2 \cdot 2C_2H_2O_4$ | 670.8 | 190-191 [c] | 700 ±85 | 630 ±115 | 0.9 |
| **7**[e] | | $C_{38}H_{55}N_5O_2 \cdot 3C_2H_2O_4 \cdot 2H_2O$ | 922.0 | >215 [f] | 358 ±10 | 103 ±6 | 0.3 |

[a] Binding assays using [$^{125}$I]iodosulpiride, in CHO cells expressing $hD_{2L}$ and $hD_3$ receptors. [b] Ratio $K_i$ ($D_2$)/$K_i$ ($D_3$). [c] Crystallized from EtOH. [d] Crystallized from 2-propanol. [e] For structure see Scheme 2. [f] Crystallized from EtOH/$Et_2O$.

**Table 2.** Structures, Physical Data, and Pharmacological Screening Results of Aryl, Heteroaryl, and Arylalkyl Carboxylic Acid Derivatives



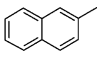
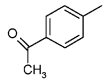
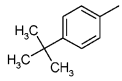
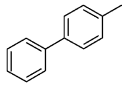
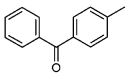
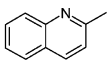
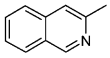
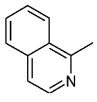
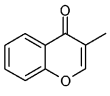
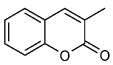
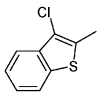
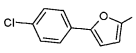
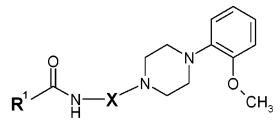
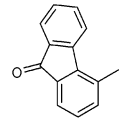
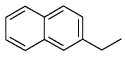
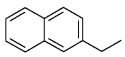
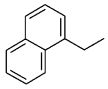
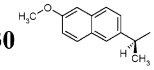
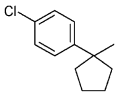
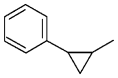
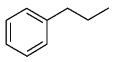
| No. | $R^1$ | X | formula | $M_r$ | mp (°C) | $D_3$ [a] $K_i$ (nM) ± SEM | $D_2$ [a] $K_i$ (nM) ± SEM | ratio [b] $D_2/D_3$ |
|---|---|---|---|---|---|---|---|---|
| BP 897 |  | $(CH_2)_4$ | $C_{26}H_{31}N_3O_2$ | 417.6 | 121 | 0.92 ±0.2 | 61 ±0.2 | 66 |
| **8**[c] |  | $(CH_2)_4$ | $C_{22}H_{28}ClN_3O_2 \cdot C_2H_2O_4$ | 492.0 | 161.5 [d] | 0.50 ±0.09 | 19.5 ±1.2 | 39 |
| **9**[c] |  | $(CH_2)_4$ | $C_{22}H_{28}IN_3O_2$ | 493.4 | 167-168 [e] | 3.9 ±0.2 | 146 ±15 | 37 |
| **10** |  | $(CH_2)_3$ | $C_{21}H_{26}IN_3O_2$ | 479.4 | 134-135 [f] | 396 ±48 | 117 ±12 | 0.3 |
| **11** |  | $(CH_2)_4$ | $C_{24}H_{31}N_3O_3$ | 409.5 | 143.5 [g] | 1 ±0.08 | 17.5 ±1 | 18 |
| **12** |  | $(CH_2)_4$ | $C_{26}H_{37}N_3O_2$ | 423.6 | 136 [h] | 7.99 ±0.94 | 26.5 ±3.5 | 3.3 |
| **13** |  | $(CH_2)_4$ | $C_{28}H_{33}N_3O_2 \cdot C_2H_2O_4$ | 533.6 | 167.5 [j] | 2.85 ±0.33 | 75 ±4 | 26 |
| **14** |  | $(CH_2)_4$ | $C_{29}H_{33}N_3O_3 \cdot C_2H_2O_4 \cdot H_2O$ | 579.7 | 127-128 [k] | 2.5 ±0.2 | 63 ±2.7 | 25 |
| **15** |  | $(CH_2)_4$ | $C_{22}H_{28}IN_3O_2 \cdot C_2H_2O_4$ | 583.4 | 146.5 [d] | 9.2 ±1 | 68 ±10 | 7.4 |
| **16** |  | $(CH_2)_3$ | $C_{21}H_{26}IN_3O_2 \cdot C_4H_4O_4$ | 595.4 | 94-95 [d] | 300 ±32 | 235 ±16 | 0.8 |
| **17** |  | $(CH_2)_4$ | $C_{22}H_{28}IN_3O_2$ | 493.4 | 117.5 [f] | 7.8 ±2 | 15.2 ±1.2 | 1.9 |
| **18** |  | $(CH_2)_3$ | $C_{21}H_{26}IN_3O_2 \cdot C_2H_2O_4$ | 569.4 | 185-186 [j] | 213 ±13 | 126 ±8.9 | 0.6 |
| **19** |  | $(CH_2)_4$ | $C_{25}H_{30}N_4O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$ | 517.6 | 170.5 [l] | 6.6 ±0.98 | 53.2 ±4.0 | 8.1 |
| **20** |  | $(CH_2)_4$ | $C_{25}H_{30}N_4O_2 \cdot C_2H_2O_4 \cdot 0.3H_2O$ | 514.6 | 172-173 [h] | 1.48 ±0.19 | 27.5 ±2 | 19 |
| **21** |  | $(CH_2)_4$ | $C_{25}H_{30}N_4O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$ | 517.6 | 143-144 [l] | 9.62 ±0.69 | 20.9 ±2 | 2.2 |
| **22** |  | $(CH_2)_4$ | $C_{25}H_{29}N_3O_4 \cdot C_2H_2O_4 \cdot 0.5H_2O$ | 534.6 | 178-179 [d] | 3.3 ±0.9 | 47 ±5 | 14 |
| **23** |  | $(CH_2)_4$ | $C_{25}H_{29}N_3O_4$ | 435.5 | 135-136 [m] | 1.8 ±0.3 | 23 ±4 | 13 |
| **24** |  | $(CH_2)_4$ | $C_{24}H_{28}ClN_3O_2S$ | 458.0 | 109-110 [h] | 6.7 ±1.2 | 71.9 ±9.3 | 11 |
| **25** |  | $(CH_2)_4$ | $C_{26}H_{30}ClN_3O_3 \cdot 2C_2H_2O_4$ | 648.1 | 142.5 [n] | 6.3 ±0.5 | 200 ±30 | 32 |

**Table 2.** (Continued)



| No. | $R^1$ | X | formula | $M_r$ | mp (°C) | $D_3$ [a] $K_i$ (nM) ± SEM | $D_2$ [a] $K_i$ (nM) ± SEM | ratio [b] $D_2/D_3$ |
|---|---|---|---|---|---|---|---|---|
| **26** |  | $(CH_2)_4$ | $C_{29}H_{31}N_3O_3$ | 469.6 | 181.5 [j] | 2.96 ±0.18 | 18 ±0.6 | 6.1 |
| **27** |  | $(CH_2)_4$ | $C_{27}H_{33}N_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$ | 530.6 | 77-78 [d] | 32 ±2.4 | 35 ±1.9 | 1.1 |
| **28** |  | $(CH_2)_3$ | $C_{26}H_{31}N_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$ | 516.6 | 173.5 [i] | 140 ±6 | 56 ±8 | 0.4 |
| **29** |  | $(CH_2)_3$ | $C_{26}H_{31}N_3O_2 \cdot C_2H_2O_4 \cdot 1.25H_2O$ | 530.1 | 125.5 [i] | 108 ±6.5 | 44 ±2.9 | 0.4 |
| **30** |  | $(CH_2)_3$ | $C_{28}H_{35}N_3O_3 \cdot C_2H_2O_4$ | 551.6 | 133-134 [m] | 262 ±52 | 94.6 ±10.5 | 0.4 |
| **31** |  | $(CH_2)_4$ | $C_{27}H_{36}ClN_3O_2 \cdot C_2H_2O$ | 560.1 | 143.5 [n] | 42 ±3 | 56 ±3 | 1.3 |
| **32** |  | $(CH_2)_4$ | $C_{25}H_{33}N_3O_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$ | 502.1 | 94-95 [o] | 15.5 ±1.6 | 81 ±10 | 5.2 |
| **33** |  | $(CH_2)_4$ | $C_{24}H_{33}N_3O_2 \cdot C_2H_2O_4$ | 485.6 | 116-118 [l] | 4.8 ±0.2 | 48.7 ±2.5 | 10 |

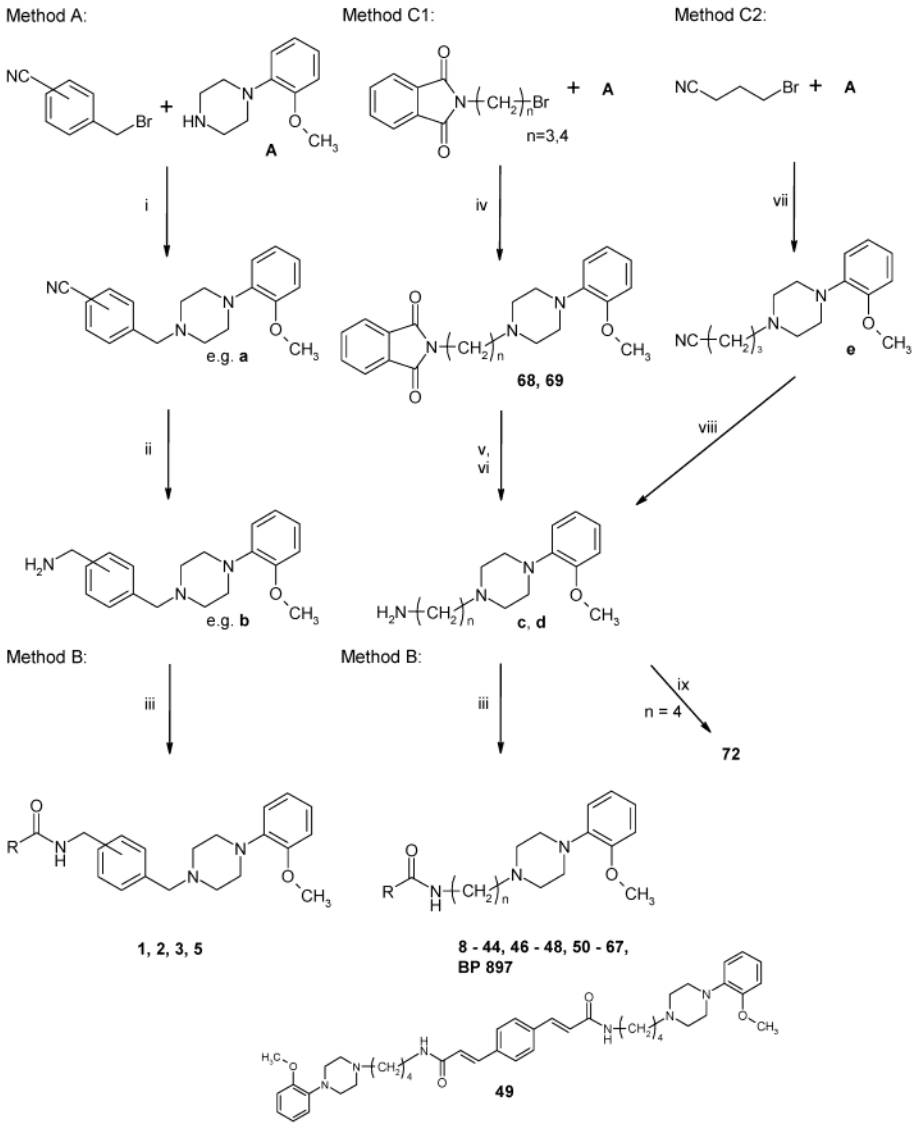
[a] Binding assays using [$^{125}$I]iodosulpiride, in CHO cells expressing $hD_{2L}$ and $hD_3$ receptors. [b] Ratio $K_i$ ($D_2$)/$K_i$ ($D_3$). [c] Ref 54. [d] Crystallized from EtOH/$Et_2O$. [e] Crystallized from MeOH/EtOH. [f] Crystallized from EtOH/$H_2O$. [g] Crystallized from ethyl acetate. [h] Crystallized from 2-propanol. [i] Crystallized from EtOH. [k] Crystallized from EtOH/2-propanol. [l] Crystallized from 2-propanol/$Et_2O$. [m] Crystallized from 2-propanol/EtOH. [n] Crystallized from acetone/$Et_2O$. [o] Crystallized from acetone.

ligands, as well as a dopamine $D_3$ receptor model pointing out crucial binding regions between ligand and receptor. In strong correlation to the modeling studies, we developed 72 compounds, which displayed structural modifications in the spacer group and in the aryl moiety. Most of the compounds share the general structure pattern mentioned previously and the 4-(2-methoxyphenyl)piperazino element. To a large extent $D_3$ receptor affinity was influenced by structural variations in the aryl moiety and selectivity versus the $D_2$ receptor by structural variations in the spacer group. The iodinated compound **38** (**ST 280**) provides a promising pharmacological profile and may serve, after further evaluation, as a potential novel radioligand.

## Chemistry

All compounds described share a 4-(2-methoxyphenyl)piperazino group. The general preparation pathway is outlined in Scheme 1: divergent methods led to the primary alkanamines (e.g., **b**, **c**, **d**), which were condensed with an activated carboxylic acid to give a carboxamide as final product. According to method A, 1-(2-methoxyphenyl)piperazine was alkylated with isomeric (bromomethyl)benzonitriles by means of procedures known in the literature.[26] Subsequent reduction of the benzonitrile (e.g., **a**) to a benzylamine (e.g., **b**) was achieved by treatment with lithium aluminum hydride. Acylation of the amine with 2-naphthoyl chloride or cinnamoyl chloride provided compounds **1**−**3** and **5** (method B). For amines with trimethylene or tetramethylene spacers, two methods were applied. For method C1, preparation started with the alkylation of 1-(2-methoxyphenyl)piperazine with *N*-(*ω*-bromoalkyl)-phthalimide. As an advantage of method C1, the intermediates **68** (NAN 190)[27] and **69**[28] could be obtained for pharmacological testing. The phthalimides were cleaved by hydrazinolysis to afford the desired alkanamines (**c**, **d**).[29] This provided a facile way for the first compounds but was not practical for a greater number of compounds. The alternative and more economic method C2 provided the butanamine derivatives through alkylation of 1-(2-methoxyphenyl)piperazine with 4-bromobutyronitrile and subsequent catalytic reduction of the cyano functionality (**e**).[29] This preparative way allowed scaling up as well as reducing time and expenditure of work. The ensuing method B employed the alkanamines, obtained by method C1 or C2, and diverse acid chlorides to afford compounds **8**−**44**, **46**−**67**, and BP 897. Furthermore, according to standard procedures the butanamine derivative was added to 3-phenylfuran-2,5-dione and delivered compound **72**. Scheme 2 depicts the reaction way for bicyclo derivatives, which resulted in the additional formation of valuable bisamination byproducts. In the first reaction step, reductive alkylation of 1-(2-methoxyphenyl)piperazine with *cis*-bicyclo[3.3.0]-octane-3,7-dione was performed.[30−32] The major product

**Scheme 1.** Synthesis of Compounds **1**−**3**, **5**, **8**−**44**, **46**−**69**, **72**, BP 897[a]




[a] Reagents and conditions. Method A: (i) acetone, $K_2CO_3$, reflux, 6 h; (ii) dry THF, $LiAlH_4$, reflux, 3 h. Method B: (iii) acyl chloride, dry $CH_2Cl_2$, $Et_3N$, ambient temperature, 3−24 h. Method C1: (iv) acetonitrile or acetone, $K_2CO_3$, reflux, 14 h; (v) $H_2N-NH_2$, MeOH, reflux, 2 h; (vi) 2 N HCl, reflux, 1 h. Method C2: (vii) acetonitrile, $K_2CO_3$, reflux, 6 h; (viii) *Ra*−*Ni*, 25 bar $H_2$, 24 h; (ix) glacial acetic acid, 3-phenylfuran-2,5-dione, reflux, 3 h.

was the desired aminoketone (**f**), accompanied by the octahydropentalene product of bisamination (**6**). A second reductive amination, with ammonia, delivered the primary amine (**g**) and another product of bisamination (**7**). The bisamination products were favorably separated as their salts by crystallization. The primary amine (**g**) was treated with 2-naphthoyl chloride according to method B to give compound **4**. A versatile synthesis for imide derivatives is described in Scheme 3. The reaction of 1-(2-methoxyphenyl)piperazine with 1,4-dibromobutane led to a quaternary spiro structure (**B**), which was obtained in almost quantitative yields by performing the reaction in *n*-butanol.[33,34] This suitable synthon was treated with imides to provide compounds **70** and **71**.[35,36]

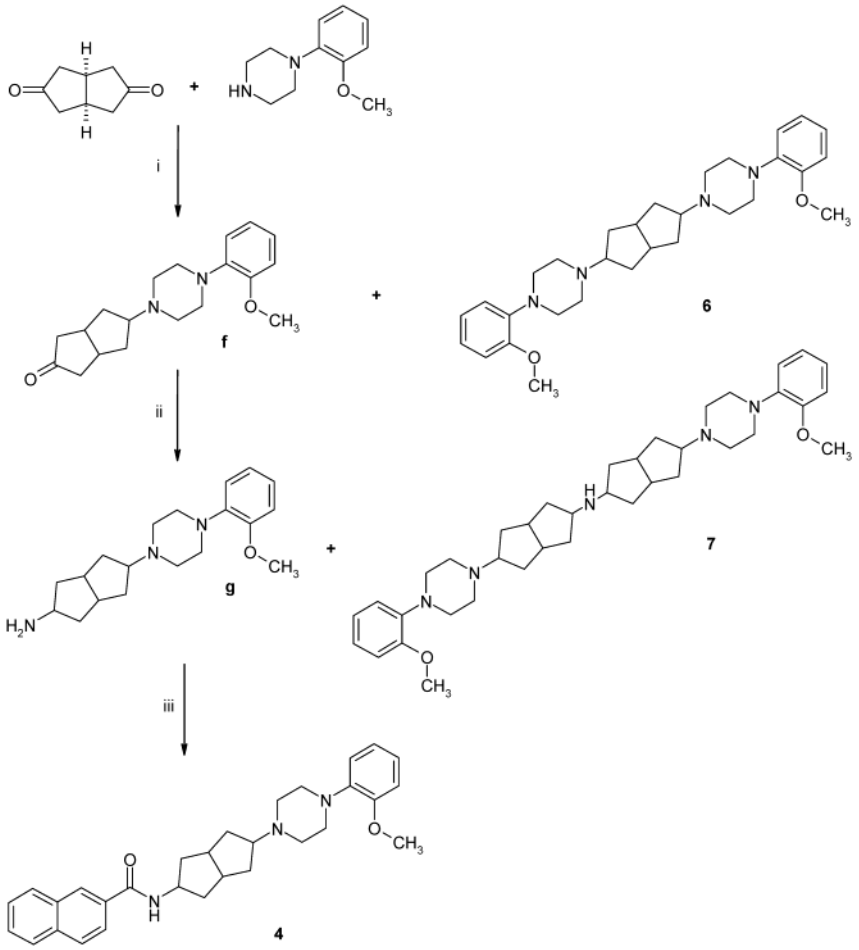
While establishing a method for radiolabeling, phenylstannane compound **45** was obtained as a stable intermediate through cleavage of distannanes by iodine-substituted compound **9** in the presence of palladium catalysts[37] (not shown). Compound **45** was reconverted into aryliodide **9** using molecular iodine, which was generated in situ from NaI and chloramin T in good yield.[38] If [$^{125}$I]NaI were used for this regioselective electrophile *ipso*-substitution, this method would enable facile radiolabeling.

## Computational Methods

**Ligand Construction.** Molecular structures were generated using the SYBYL program.[39] Assuming physiological conditions, the basic aliphatic nitrogen atom of the piperazine was taken as protonated. The geometry was optimized using the Tripos force field applying the conjugate gradient method until the energy difference between successive cycles was below 0.01 kcal $mol^{-1}$. Partial atomic charges were calculated with the Gasteiger−Hückel method, and the dielectric function was set to a constant value of 80.[40]

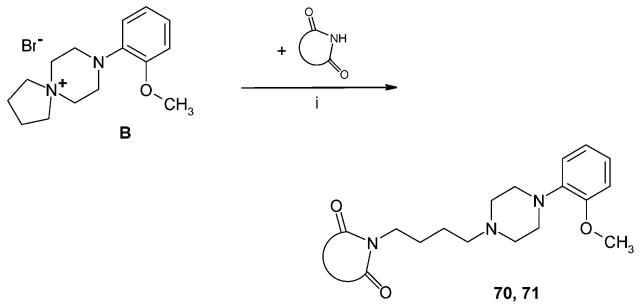
**Pharmacophore Generation.** The steric and electrostatic information coming from partially rigid, highly potent ligands was used to generate the pharmacophore

**Scheme 2.** Synthesis of Compounds **4**, **6**, and **7**[a]



[a] Reagents and conditions: (i) 1,2-dichloroethane, $NaBH(OCOCH_3)_3$, glacial acetic acid, ambient temperature, 2 h; (ii) $NH_3$ in MeOH, Pd/C, $H_2$, 12 h. Method B: (iii) 2-naphthoyl chloride, dry $CH_2Cl_2$, $Et_3N$, ambient temperature, 10 h.

**Scheme 3.** Synthesis of Compounds **70** and **71**[a]



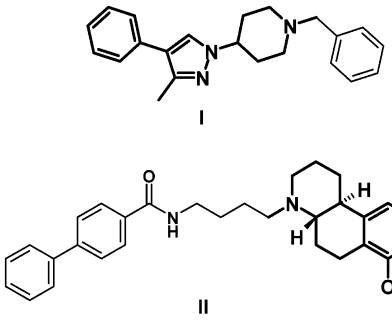
[a] Reagents and conditions (for **B**, see refs 28 and 29): (i) xylene, $K_2CO_3$, 18-crown-6, reflux, 24 h.

models. To explain the receptor selectivity of the studied ligands, pharmacophore models were developed for dopamine $D_2$ and dopamine $D_3$ receptor ligands. The investigated potent dopamine $D_3$ receptor ligands are characterized by two lipophilic/aromatic moieties, a hydrogen bond acceptor, and a basic aliphatic nitrogen atom.

The molecular alignments were carried out using the FLEXS program.[41] FLEXS approaches flexible superpositioning on the basis of a combinatorial matching procedure. Pairs of molecules are aligned, one of which is treated as rigid and the other one is flexibly fitted. The strategy is to decompose the flexible structure into relatively rigid portions, to start the placement using a manually selected portion, and to add the remaining portions in an iterative incremental procedure. The scoring functions used to choose appropriate conformations comprise energy-like matching terms for paired intermolecular interactions and overlap terms using Gaussian functions. The Gaussian functions are used to describe different field properties. Therefore, applying the molecular comparison procedure within FLEXS, it is a priori not necessary to define the pharmacophoric features for superimpositioning molecules. This has been demonstrated by Lemmen et al.[41] in an extensive evaluation of the approach based on experimental data.

To determine the $D_3$ pharmacophore, the following compounds were examined in detail: compound **4**, compound **I**[42], and compound **II**[43] (Chart 2). Critical features for the selected compounds were high binding affinity for the dopamine $D_3$ receptor and rigid molecule structure, but not necessarily $D_3$ receptor preference (compound **I** $K_i$ ($D_3$) = 28 nM, $K_i$ ($D_2$) = 6.1 nM, $K_i$ ($D_4$) = 0.39 nM; compound **II** $K_i$ ($D_3$) = 1 nM, $K_i$ ($D_2$) = 25 nM). Since none of the ligands examined is completely rigid but some are partially rigid, these three molecules were initially decomposed into fragments (Chart 2), and the conformational space of the restricted fragments were then examined individually. The ring conformations (i.e., octahydropentalene in **4** and 4-methyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolin-7-ol in **II**) were generated applying the simulated annealing pro-

**Chart 2**




cedure within SYBYL. The fragments were heated fifty times to 1000 K and were subsequently annealed to 0 K. The generated conformations were compared with corresponding fragments from the Cambridge Structural Database.[44] Similar conformations were detected by both approaches. The comparison of the 4-(2-methoxyphenyl)piperazino fragment, which is part of compound **4** and all other ligands under study, and the conformationally restricted 4-methyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolin-7-ol fragment of compound **II** determined the common structural properties in the basic residue of the ligands. The distal aryl moiety, which contains the naphthamidic part, was assigned to be planar, referring to known potent imidic ligands, e.g., compound **68**.[27] Taking these conformational restrictions into account, a conformational analysis was performed for the whole structure of **4**. The rotatable bonds were scanned using a 10° increment within a 0−350° interval. Using the Tripos force field, 992 energetically favorable conformations were calculated for compound **4** within an energy range of 15 kcal/mol. The conformational space of compounds **I**, **II**, **4**, and **71** was compared in pairs using the FLEXS program. In such a way we succeeded in identifying at least one conformer for each active ligand, which could be superimposed onto at least one conformer of the other ligands. The final superimposition of the selected conformers allowed us to extract the geometric requirements in terms of distances between pharmacophoric points (Figure 1).

Due to their preference for the dopamine $D_2$ receptor subtype, compounds **1** and **5** were examined in detail to determine the bioactive conformation of $D_2$ receptor ligands. The four rotatable bonds in the alkyl−aryl spacer, which link the basic and the aryl/acryl moiety, were scanned using a 10° increment within a 0−350° interval. Thus, 417 energetically favorable conformations were obtained, from which the bioactive form had to be determined. Compound **2**, which also possesses affinity for the $D_2$ receptor, was superimposed onto each possible conformation of **1** and **5** using program FLEXS. The putative bioactive conformation of compound **1** was derived from the top scored superimposition calculated by FLEXS. All the remaining compounds were superimposed onto the predicted bioactive conformation of compound **4** for the dopamine $D_3$ receptor and onto the predicted bioactive conformation of compound **1** for the $D_2$ receptor. This procedure was performed using the flexible fitting procedure within FLEXS. In the following step, SYBYL's multifit routine was used for the refinement of the molecular alignment. Each structure was relaxed using the steepest descent method up to a gradient of below 0.1 kcal $mol^{-1}$.

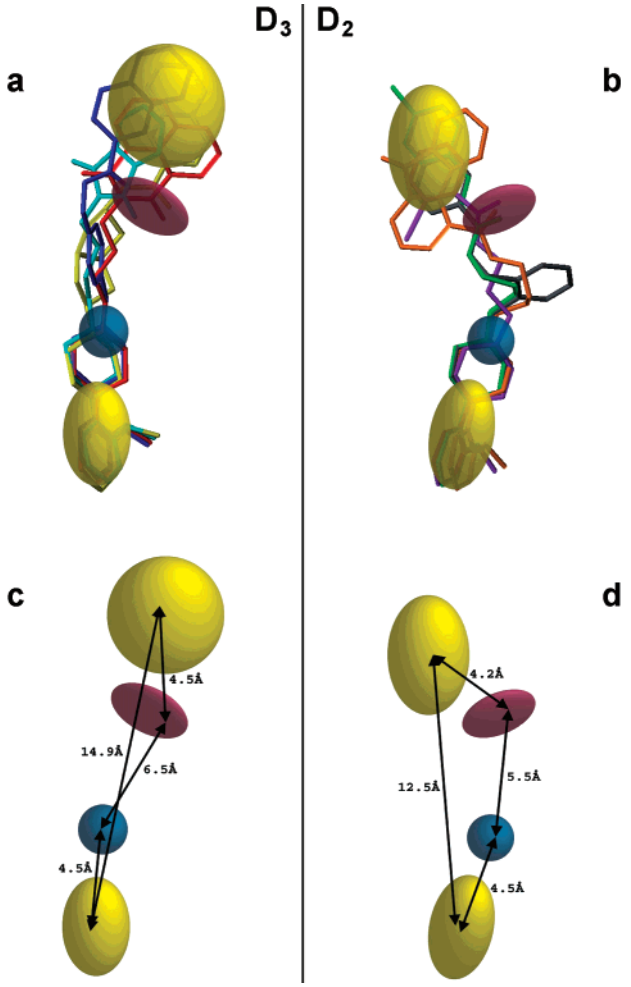



**Figure 1.** Pharmacophore model for dopamine $D_3$ and $D_2$ receptor ligands. Superimposition of ligands **3**, **68**, **71**, and **4** for the $D_3$ receptor (a) and of **18**, **26**, **37**, and **1** for the $D_2$ receptor (b). Pharmacophoric points (lipophilic/aromatic moieties, yellow; hydrogen bond acceptor, violet; basic aliphatic nitrogen atom, blue) and their distances within the pharmacophore for dopamine $D_3$ (c) and $D_2$ receptor ligands (d). Compounds are colored as follows: (a) **3** blue, **4** yellow, **68** cyan, **71** red; (b) **1** grey, **18** violet, **26** orange, **37** green.

The derived molecular alignments for dopamine $D_3$ and the $D_2$ receptor ligands are shown in Figure 1. The superimposition of the potent ligands **3**, **68**, **71**, and **4** onto the $D_3$ pharmacophore illustrates that the crucial structural features, e.g., positively charged nitrogen atoms, amidic parts, and aryl moieties, occupy a similar area in space. The same is true for the $D_2$ receptor. The superimposition of ligands **18**, **26**, **37**, and **1** onto the $D_2$ pharmacophore shows that the crucial features are positioned in a similar area in space. The projection of the $D_3$ receptor ligands onto the pharmacophore shows clearly that they adopt an extended and more linear conformation. Gmeiner and co-workers very recently confirmed linearity as a crucial structural feature for good $D_3$ receptor binding properties.[16] The distances between the chemical features are recapitulated in Figure 1. In contrast to the bioactive conformation calculated for the $D_3$ receptor, ligands at the $D_2$ receptor adopt a more bent conformation. The corresponding distances between the pharmacophoric features are different compared to those measured in the $D_3$ pharmacophore. Comparing both pharmacophore models, it

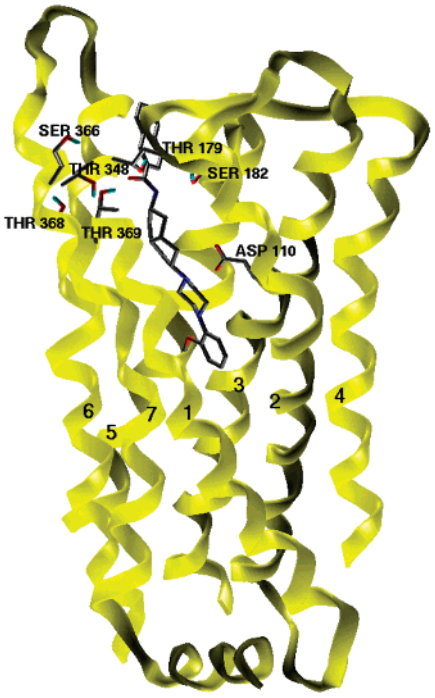



**Figure 2.** Compound **4** positioned in the dopamine $D_3$ receptor. The receptor is restricted to its binding relevant fragments. Putative transmembrane domains are numbered 1−7. Additionally, several the ligand surrounding threonine and serine molecules are pointed out, which allow alternative orientations for the amide oxygen. Asp 110, the counterion to the basic part, is shown for clarity.

can be observed that bulky substitutions in the spacer region are not tolerated with $D_3$ selective ligands.

**GRID Field Calculations.** After definition of the arrangement of the pharmacophoric descriptors, the models were validated by closer examination of molecular fields that are produced by the ligands superimposed onto each other in their bioactive conformation. The GRID program[45] is an approach to predict noncovalent interactions between a molecule of known three-dimensional structure, i.e., the ligand, and a small group as a probe, representing chemical features of corresponding amino acid residues. On a cube enclosing the complete ligand, a series of calculations was performed, searching for potential binding sites complementary to the functional groups. The SYBYL program subsequently showed the contour maps superimposed on the receptor model.

**Receptor Modeling.** The coordinates of the bovine rhodopsin crystal structure[46] and a structure−function analysis of rhodopsin-like receptors[47] delivered the basis for building and optimizing the human dopamine $D_3$ receptor using the Insight II/Discover modeling package.[48] The receptor was structurally minimized with the steepest descent method until the RMS value was below 0.1 kcal $mol^{-1}$ $Å^{-1}$ and subsequently minimized with the conjugate gradient method until the RMS value was below 0.01 kcal $mol^{-1}$ $Å^{-1}$. Minimizations were performed with the Consistent Valence force field, implemented in Insight II/Discover, and a distance depending dielectric constant of 4. In a first approach, compound **4** was docked manually into the binding pocket located between the transmembrane helices 3, 5, 6, and 7. The dock procedure within SYBYL was used for this purpose. The resulting complex was minimized as described above (Figure 2). To further analyze the properties of the binding pocket, the molecular interaction fields for compound **4** were calculated using program GRID and were compared with the amino acid residues of the ligand binding site. The hydrophilic OH probe and the lipophilic DRY probe were chosen to examine favorable corresponding interaction regions. As depicted in Figure 3, lipophilic regions coincided with positions of lipophilic amino acids Met 113, Trp 342, Phe 345, and Tyr 365, whereas hydrophilic areas coincided with positions of hydrophilic amino acids Asp 110, Thr 179, and Thr 369. The FLEXX program,[49] which was designed to place flexible molecules into active sites of proteins considering their physicochemical properties, was used to dock the ligands into the binding pocket. Since Asp 110 of the dopamine $D_3$ receptor most probably represented the counterion to the basic part,[4] a salt-bridge was enabled toward the protonated nitrogen of the ligands. In the aryl moiety, large and bulky substituents, e.g., **48**, **49**, **70**, and **71**, were tolerated, irrespective of amidic or imidic binding. This slight effect on affinity may be due to interactions with various amino acids near the extracellular space (Figure 2), which allow the amide or imide oxygens to point to various directions. Since a number of surrounding amino acids were able to form hydrogen bonds, the carbonyl group was able to undergo several interactions.

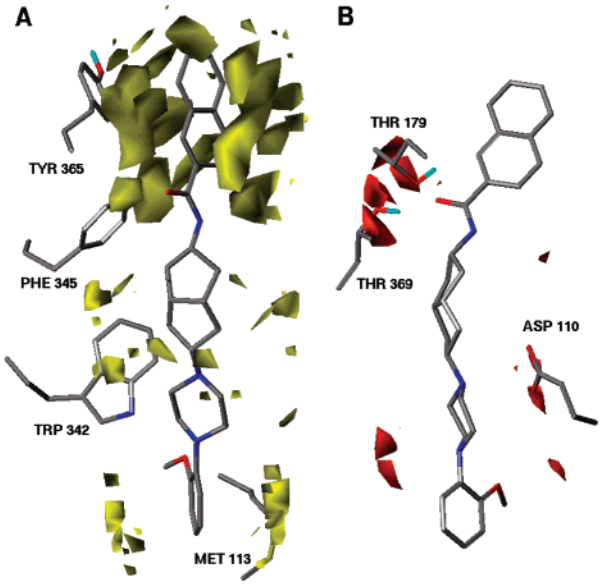



**Figure 3.** Lipophilic (A) and hydrophilic (B) interactions of compound **4** with discrete amino acids of the dopamine $D_3$ receptor. Calculations were performed with the GRID DRY-probe (A) displaying interaction zones in yellow, contoured at an energy level of −0.45 kcal $mol^{-1}$, and with the GRID OH-probe (B) displaying interaction zones in red, contoured at an energy level of −4 kcal $mol^{-1}$.

## Pharmacological Results and Discussion

**Binding Studies.** Chinese hamster ovary (CHO) cells were stably transfected with cDNA of human $D_{2L}$ and $D_3$ receptors and cloned.[50,51] With the cell lines obtained, binding was determined using [$^{125}$I]iodosulpiride in order to measure radioactivity. $K_i$ values were calculated from the $IC_{50}$ values according to the Cheng−Prusoff equation.[52] All novel compounds showed moder-

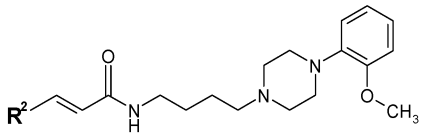
ate to high binding affinities for the dopamine $D_2$ receptor or for the dopamine $D_3$ receptor ($K_i$ values of 750−0.33 nM) (Tables 1−4). Throughout the whole series an important impact of the chain length and linearity of the spacer was observed. As expected from the modeling study results, flexible spacers showed a good concordance between a tetramethylene chain ($C_4$) and high dopamine $D_3$ receptor affinity, whereas a trimethylene spacer ($C_3$) was favorable for dopamine $D_2$ receptor binding. Contrary to the shorter trimethylene spacer, the tetramethylene spacer was able to span the distance of about 6.5 Å between the pharmacophoric features basic aliphatic nitrogen and hydrogen bond acceptor (Figure 1). Within pairs of compounds, which only diverged in the length of the chain, the $C_4$ bridge effected generally lower $K_i$ values than the $C_3$ analogue and was simultaneously favoring the $D_3$ receptor (**9** → **10**, **15** → **16**, **17** → **18**, **27** → **28**, **68** → **69**). These results were transferred to compounds with rigid xylene (**1**−**3**, **5**) or octahydropentalene (**4**) spacers (Table 1). $D_3$ receptor binding was favored by the linear molecules **3** and **4**, and $D_2$ receptor binding by the angled ones (**1**, **2**, **5**). Obviously the aliphatic structure of the chain was not essential for dopamine $D_2$-like receptor affinity. Since compounds with $C_4$ spacer were able to adopt both bioactive conformations that were determined for the $D_2$ and $D_3$ receptor and since affinity for both receptors was nanomolar, selectivity toward one or the other receptor could not be determined exclusively by the conformational requirements of these compounds. Detailed comparative molecular field analyses are therefore needed to examine the effects of different $C_4$ compounds, which cause a change of binding affinity and selectivity toward one receptor. Nevertheless, the qualitative difference in both receptor pharmacophores stressed important points for drug optimization.

Among the three synthesized dimers (compounds with two identical elements in their structure), compound **6** showed low affinity, compound **7** bound moderately, and compound **49** showed improved affinity for both receptors. As outlined in Figure 2, the ligands bound along the helices of the receptor protein with a moiety positioned near the extracellular site. Assuming physiological conditions, the dimers appeared to be 2-fold (**6**, **49**) or 3-fold (**7**) protonated. This induced a strong shift of the physicochemical properties compared to other monoprotonated compounds in this series. Thus, binding properties of compound **6** and **7** may be deteriorated because their protonated nitrogens occupy an area which requires an aryl moiety in the pharmacophore model. Compound **49** comprised all required pharmacophoric features, but the additional, protonated dimeric residue reaches out of the pharmacophore area. This elongated part of the molecule had no negative effect on $D_2$ and $D_3$ receptor binding. According to our receptor model, binding deterioration was reduced in correlation to a greater distance between the second proton and the binding pocket. For nonsymmetrical dimeric $D_3$ receptor ligands with 4-phenylpiperazino and alkylamine residues, a second proximal binding site has been postulated.[53]

Variations of the naphthoyl residue were performed with benzamides and analogues (Table 2). Divergent benzamide substituents with different requirements in space and interaction possibilities were accepted (**8**−**18**) and the $D_3$ receptor affinity was maintained below 8 nM for para-substituted compounds with $C_4$ spacer. The *p*-chlorobenzoyl derivative **8**[54] displayed significant higher dopamine $D_3$ receptor affinity than that of the lead compound BP 897 and showed considerable $D_3$ receptor preference. The low nanomolar affinity values of the three iodobenzoyl structures declined in a sequence of *m*-, *o*,- and *p*-substitution, the latter (**9**)[54] also giving an enhanced selectivity ratio for the dopamine $D_3$ receptor. Introduction of voluminous phenyl (**13**) and benzoyl substituents (**14**) was well tolerated for binding and selectivity. Replacement of the aryl moiety into quinoline (**19**), isoquinolines (**20**, **21**), chromone (**22**), and cumarine (**23**) moieties delivered improved binding properties for the isoquinoline **20**. Introduction of 3-chlorobenzo[*b*]thiophene (**24**), related to FAUC 365, or the more bulky fluorenone (**26**) as a potential bioisosteric group led to low nanomolar binding values. A comparable fluorenone moiety was already presented in another series[15] and very recently an analogue, which only diverged in the 4-phenylpiperazino group.[55] Above that, substituted furan **25** gave an improved selectivity ratio compared to the other heteroaryl compounds **19**−**24** and **26**. Naphthyl acetic acid derivatives **27**−**30** showed no improvement in $D_3$ receptor affinity, with the $C_3$ spacer compounds having certain $D_2$ receptor preference. Compounds selective for the $D_2$ receptor were not optimized in this investigation. A consequent favorable development of binding properties and selectivity was observed in the series of benzyl compound with steric hindering substituent (**31**), rigid cyclopropane phenyl acetic acid derivative **32**, and the 3-phenylpropionic acid derivative **33.**

Following these results and the modeling study, molecules were established bearing the amide in a certain distance to the steric fixed aromatic structure (Table 3). Parallel to our development of *E*-cinnamide as a valuable structural basis for further compounds (**34**), some potent *E*-cinnamoyl derivatives have been published.[56] Isomeric *Z*-derivatives have been reported to possess lower affinities.[56] The group of mono-, di-, and trisubstituted cinnamoyl amides (**35**−**61**) and analogues (**62**−**67**) consisted of compounds with moderate to enhanced selectivity ratios and partly high affinity. The unsubstituted molecule **34** reached an excellent binding affinity for the dopamine $D_3$ receptor ($K_i$ ($D_3$) = 0.33 nM), superior to that of lead BP 897, and considerable $D_3$ receptor preference. *p*-Fluoro (**35**) or -chloro substitution (**36**) did not lead to further improvement in affinity but increased selectivity for the chloro derivative ($ratio_{(D2/D3)}$ 51), equivalent to that of the *p*-bromo derivative **37**. Best results in this series were obtained with the *p*-iodo derivative **38** (**ST 280**). Selectivity ratio was raised to a value of 153 combined with subnanomolar binding affinity. Substitution with iodine in *o*- (**55**) or *m*-position (**51**) also resulted in low nanomolar $D_3$ receptor binding and high selectivity ratios but did not exceed those of **38**. Halogen monosubstitution (**36**) was preferable to disubstitution (**57**, **58**) for affinity and selectivity scores. Electron-withdrawing as well as electron-delivering substituents (**43**, **56**) were introduced without great deterioration in affinity. In contrast to the benzamides, space-demanding substituents (**44**−**48**, **53**,

**Table 3.** Structures, Physical Data, and Pharmacological Screening Results of α,β-Unsaturated Carboxylic Acid Derivatives



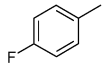
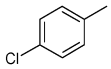
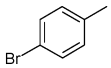
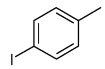
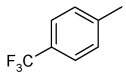
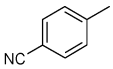
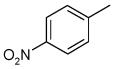
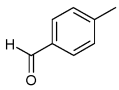
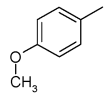
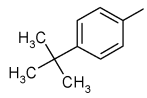
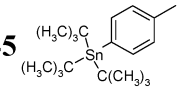
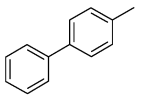
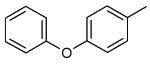
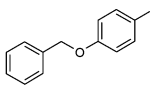
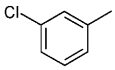
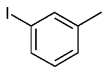
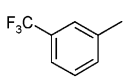
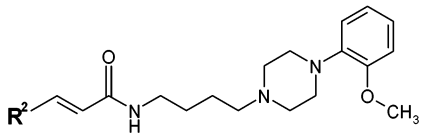
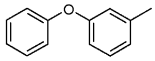
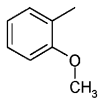
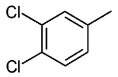
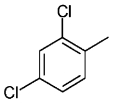
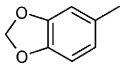
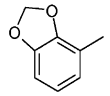
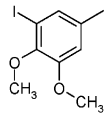
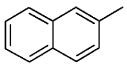
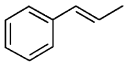
| No. | $R^2$ | formula | $M_r$ | mp (°C) | $D_3$ [a] $K_i$ (nM) ± SEM | | $D_2$ [a] $K_i$ (nM) ± SEM | | ratio [b] $D_2/D_3$ |
|---|---|---|---|---|---|---|---|---|---|
| **34** |  | $C_{24}H_{31}N_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$ | 492.6 | 128.5 [d] | 0.33 | ±0.1 | 13.54 | ±1.2 | 41 |
| **35** |  | $C_{24}H_{30}FN_3O_2$ | 411.5 | 149-150 [j] | 0.69 | ±0.11 | 20.7 | ±0.8 | 30 |
| **36** |  | $C_{24}H_{30}ClN_3O_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$ | 522.5 | 138.5 [d] | 0.44 | ±0.09 | 22.6 | ±1.0 | 51 |
| **37** |  | $C_{24}H_{30}BrN_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$ | 571.5 | 138.5 [d] | 0.43 | ±0.13 | 23.7 | | 55 |
| **38** |  | $C_{24}H_{30}IN_3O_2$ | 519.4 | 161-162 [h] | 0.50 | ±0.13 | 76.4 | | 153 |
| **39** |  | $C_{25}H_{30}F_3N_3O_2$ | 461.5 | 158.5 [i] | 2.14 | ±0.2 | 36.79 | ±6.5 | 17 |
| **40** |  | $C_{25}H_{30}N_4O_2 \cdot 2C_2H_2O_4$ | 598.6 | 181-183 [l] | 0.46 | ±0.03 | 10 | ±0.7 | 22 |
| **41** |  | $C_{24}H_{30}N_4O_4 \cdot C_2H_2O_4 \cdot H_2O$ | 546.6 | 117-118 [l] | 0.74 | ±0.1 | 14 | ±3 | 19 |
| **42** |  | $C_{25}H_{31}N_3O_3 \cdot 2C_2H_2O_4 \cdot 0.5H_2O$ | 610.6 | 128-129 [l] | 0.73 | ±0.1 | 11.5 | ±1.9 | 16 |
| **43** |  | $C_{25}H_{33}N_3O_3$ | 423.6 | 137 [i] | 0.38 | ±0.1 | 12 | ±1 | 32 |
| **44** |  | $C_{28}H_{39}N_3O_2 \cdot C_2H_2O_4$ | 539.7 | 88.5 [d] | 10.2 | ±0.94 | 128 | ±31.6 | 13 |
| **45** |  | $C_{36}H_{57}N_3O_2Sn$ | 682.6 | oily | 92 | ±6 | 729 | ±104 | 8 |
| **46** |  | $C_{30}H_{35}N_3O_2$ | 469.6 | 185-186 [p] | 16 | ±3 | 37 | ±15 | 2.3 |
| **47** |  | $C_{30}H_{35}N_3O_3$ | 485.6 | 158-159 [h] | 10.1 | ±0.9 | 65.2 | ±29.7 | 6 |
| **48** |  | $C_{31}H_{37}N_3O_3$ | 499.7 | 159-160 [h] | 19.5 | ±1.5 | 32 | ±4 | 1.6 |
| **49** [q] | | $C_{42}H_{56}N_6O_4$ | 708.9 | 207-208 [r] | 3.6 | ±0.6 | 7.2 | ±0.4 | 2 |
| **50** |  | $C_{24}H_{30}ClN_3O_2 \cdot C_2H_2O_4 \cdot H_2O$ | 536.0 | 125-127 [d] | 0.46 | ±0.1 | 20 | ±4 | 43 |
| **51** |  | $C_{24}H_{30}IN_3O_2$ | 519.4 | 119-120 [h] | 1.2 | ±0.3 | 161 | ±1 | 134 |
| **52** |  | $C_{25}H_{30}F_3N_3O_2 \cdot 1.5C_2H_2O_4$ | 596.6 | 120-121 [h] | 4.1 | ±0.4 | 50.2 | ±9.3 | 12 |

**Table 3.** (Continued)



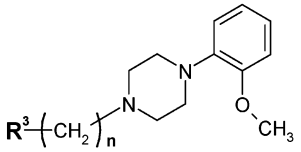
| No. | $R^2$ | formula | $M_r$ | mp (°C) | $D_3$ [a] $K_i$ (nM) ± SEM | | $D_2$ [a] $K_i$ (nM) ± SEM | | ratio [b] $D_2/D_3$ |
|---|---|---|---|---|---|---|---|---|---|
| **53** |  | $C_{30}H_{35}N_3O_3 \cdot C_2H_2O_4 \cdot 0.25H_2O$ | 580.2 | 106-107 [l] | 19.5 | ±1.5 | 114 | ±27 | 6 |
| **54** |  | $C_{24}H_{30}ClN_3O_2 \cdot 2C_2H_2O_4 \cdot 0.5H_2O$ | 617.1 | 86-88 [i] | 0.52 | ±0.03 | 15 | ±6 | 29 |
| **55** |  | $C_{24}H_{30}IN_3O_2$ | 519.4 | 143-144 [i] | 0.75 | ±0.34 | 63 | ±8 | 84 |
| **56** |  | $C_{25}H_{33}N_3O_3$ | 423.6 | 136 [i] | 0.56 | ±0.1 | 14 | ±2 | 25 |
| **57** |  | $C_{24}H_{29}Cl_2N_3O_2 \cdot C_2H_2O_4 \cdot H_2O$ | 570.5 | 132-134 [d] | 1.31 | ±0.1 | 23 | ±4 | 18 |
| **58** |  | $C_{24}H_{29}Cl_2N_3O_2 \cdot C_2H_2O_4 \cdot 1.5H_2O$ | 579.5 | 115-117 [d] | 1.28 | ±0.04 | 21 | ±2 | 16 |
| **59** |  | $C_{25}H_{31}N_3O_4$ | 437.5 | 133-134 [h] | 1 | ±0.2 | 10.3 | ±1.3 | 10 |
| **60** |  | $C_{25}H_{31}N_3O_4$ | 437.5 | 139 [h] | 0.55 | | 8 | | 15 |
| **61** |  | $C_{26}H_{34}IN_3O_4 \cdot C_2H_2O_4$ | 669.5 | 189-190 [l] | 14.9 | ±3.9 | 38.5 | ±7.4 | 2.6 |
| **62** |  | $C_{22}H_{29}N_3O_2S \cdot C_2H_2O_4 \cdot 0.25H_2O$ | 494.1 | 144-145 [h] | 0.59 | ±0.04 | 9.5 | ±0.7 | 16 |
| **63** |  | $C_{22}H_{29}N_3O_2S \cdot 0.25H_2O$ | 404.1 | 134-135 [h] | 0.64 | ±0.04 | 13 | ±3 | 20 |
| **64** |  | $C_{22}H_{29}N_3O_3 \cdot C_2H_2O_4 \cdot C_2H_5OH$ | 519.6 | 86 [d] | 0.6 | | 11.1 | | 19 |
| **65** |  | $C_{23}H_{30}N_4O_2 \cdot 2C_2H_2O_4$ | 574.6 | 154-155 [h] | 0.5 | ±0.02 | 14.5 | ±0.4 | 29 |
| **66** |  | $C_{28}H_{33}N_3O_2$ | 443.6 | 131 [i] | 0.68 | ±0.1 | 32 | ±2 | 47 |
| **67** |  | $C_{26}H_{33}N_3O_2 \cdot 0.25H_2O$ | 424.1 | 138-139 [h] | 1.5 | ±0.1 | 19 | ±2 | 13 |

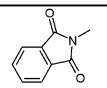
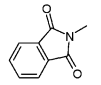
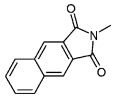
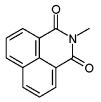
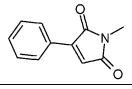
[a,b,d,g,h,i,l] See corresponding footnotes in Table 2. [p] Free base crystallized while drying in vacuo. [q] For structure, see Scheme 1. [r] Crystallized from MeOH.

**61**) only moderately deteriorated in binding properties. Interesting exceptions were the benzo[1,3]dioxoles **59** and **60**, which showed structural similarity to the addictive drug MDMA (3,4-**m**ethylene**d**ioxy-*N*-**m**ethyl-**a**mphetamine).[57] All cinnamoyl analogues with aryl heterocycles (**62**−**65**), naphthyl (**66**), and conjugated phenylalkadiene structure (**67**) showed high affinity below 1.5 nM, but unfortunately no improvement in selectivity.

With the amide BP 897 as lead on one hand and with steric demands of the pharmacophore concerning the binding pocket on the other hand, it seemed reasonable to integrate an imide as structural feature (Table 4). But change from amides to imides did not lead to improved binding or selectivity properties. Analogous imides **68** and **71** showed similar binding affinities despite bearing differently bulky aromatic residues. Only **70** revealed slight selectivity for the dopamine $D_3$

**Table 4.** Structures, Physical Data, and Pharmacological Screening Results of Imide Compounds



| No. | $R^3$ | n | formula | $M_r$ | mp (°C) | $D_3$ [a] $K_i$ (nm) ± SEM | $D_2$ [a] $K_i$ (nm) ± SEM | ratio [b] $D_2/D_3$ |
|---|---|---|---|---|---|---|---|---|
| **68**[s] |  | 4 | $C_{23}H_{27}N_3O_3 \cdot C_2H_2O_4$ | 483.5 | 150.5[i] | 38 ±5.7 | 50 ±6 | 1.3 |
| **69**[t] |  | 3 | $C_{22}H_{25}N_3O_3 \cdot 1.25C_2H_2O_4$ | 541.5 | 212-213[m] | ≈560 | ≈300 | 0.5 |
| **70** |  | 4 | $C_{27}H_{29}N_3O_3$ | 443.6 | 121-122[u] | 23.3 ±2 | 145 ±6.5 | 6.2 |
| **71** |  | 4 | $C_{27}H_{29}N_3O_3$ | 443.6 | 133.5[u] | 29 ±4 | 40 ±3 | 1.4 |
| **72** |  | 4 | $C_{25}H_{29}N_3O_3 \cdot C_2H_2O_4$ | 509.6 | 131-132[l] | 320 ±40 | 330 ±80 | 1.0 |

[b,c,i,m,l] See corresponding footnotes in Table 2. [s] NAN 190.[27] [t] Ref 28. [u] Crystallized from $Et_2O$.

receptor. Similar to analogue compounds with $C_3$ spacer, **69** displayed remarkably high dopamine $D_3$ receptor $K_i$ values and a dopamine $D_2$ receptor preference. These results indicated that the related aromatic or lipophilic binding regions of the receptor were tolerant to volume and that the linearity increased the affinity to both receptor subtypes.

Compound **68**[27] and other structurally related compounds[58] have also been described as 5-$HT_{1A}$ receptor and $\alpha_1$ receptor ligands.[54] Thus, further pharmacological investigations are required to clarify the ligands' confined cross affinity for related receptors.

**Functional Receptor Tests.** Dopamine $D_3$ receptor-based treatment of cocaine abuse with the partial agonist BP 897 was described for monkeys.[18] It cannot be totally excluded that these effects may possibly be related to antagonist properties of BP 897, which have been published for different in vitro tests.[59] Also for other dopamine $D_3$ receptor antagonists, inhibition of cocaine-seeking behavior has been reported.[60] The needed optimal value of intrinsic activity of a partial agonist for the treatment of human cocaine abusers is not known. Therefore, compounds with different intrinsic activity are required for pharmacological screening and evaluation. A mitogenesis test was performed on NG 108-15 cells expressing the dopamine $D_3$ receptor, measuring [$^3$H]thymidine incorporation.[61] From the most promising compounds with an affinity below 4 nM and a selectivity ratio exceeding 18, structural representatives of comparable affinity and selectivity scores were selected for the mitogenesis test. Lead compound BP 897 displayed an intrinsic activity ($\alpha$) of 0.6 for the dopamine $D_3$ receptor (dopamine: $\alpha = 1.0$) and an $EC_{50}$ value of 3 nM.[18] The *p*-iodo-substituted benzamide **9** revealed an intrinsic activity of 0.5 with $EC_{50}$-value of $1.2 \pm 0.4$ nM. The same intrinsic activity was obtained for benzamide derivative **13**, although bearing a more bulky phenyl substituent. Due to its high lipophilicity this molecule was supposed to pass the blood-brain barrier rapidly. For heterocyclic compound **20**, partial $D_3$ receptor agonism was found with diminished intrinsic activity of value 0.3 ($EC_{50} = 2.5 \pm 0.8$ nM). In the cinnamoyl group, compound **36** was tested and showed with an intrinsic activity of 0.7 ($EC_{50} = 10 \pm 3.5$ nM) comparable values to BP 897.

## Conclusions

Binding and selectivity properties of lead compound BP 897 were improved remarkably by means of variations in distinct structural moieties of the molecule. The development of ligands was supported by molecular modeling methods, based on two pharmacophore models, one for dopamine $D_3$ receptor and one for dopamine $D_2$ receptor ligands. The built model for the dopamine $D_3$ receptor depicted the putative amino acids responsible for binding to the pharmacophore. The modeling results clearly showed a perpendicular positioning of ligands at the $D_3$ receptor site. According to computational calculations, a stretched dopamine $D_3$ receptor binding pocket offered ideal binding facilities for a linear ligand with a spacer length of approximately 6.5 Å. This positive effect was determined for both aliphatic chains (tetramethylene or octahydropentalene) and aromatic linkers (*p*-xylene). Steric inflexibility of the aromatic amidic residue resulted in advantageous affinity and selectivity scores. Acryl amides met all these requirements and delivered a structural basis for new dopamine $D_3$ receptor partial agonists with high affinity and selectivity. These results led to highly potent *p*-substituted 3-phenylacryl amides with tetramethylene spacer, of which the iodo derivative **38** displayed an improved dopamine $D_3$ receptor selectivity up to a value of 153 combined with a subnanomolar binding affinity. This compound gives perspective for a desired new radio-

ligand, possibly enabling direct visualization of dopamine $D_3$ receptors in brain. For potential therapeutic application, the selected compounds tested in the functional mitogenesis test showed the pharmacologically desired divergent partial agonism with intrinsic activities of 0.3 (**20**), 0.5 (**9**, **13**), and 0.7 (**36**).

## Experimental Section

**Chemistry. General Procedures.** Melting points were determined on an Electrothermal IA 9000 digital or a Büchi 512 melting point apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Bruker DPX 400 Avance (400 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal $Me_4Si$ as reference. $^1$H NMR data are reported in the following order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet); approximate coupling constants in Hertz (Hz); number of protons; *, exchangeable by $D_2O$; Naph, naphthyl; Xyl, xylenyl; MeOPh, 2-methoxyphenyl; Pipera, piperazinyl; Ph, phenyl; Isoq, isoquinolinyl; Phth, phthalimidyl; Mal, maleic acid. Elemental analyses (C, H, N) were measured on Perkin-Elmer 240 B or Perkin-Elmer 240 C instruments and were within ±0.4% of theoretical values for all compounds. Preparative, centrifugally accelerated, rotatory chromatography was performed using a Chromatotron 7924T (Harrison Research) and glass rotors with 4 mm layers of silica gel 60 $PF_{254}$ containing gypsum (Merck). Thin-layer chromatography (TLC) was performed on silica gel $PF_{254}$ plates (Merck). The spots were visualized with fast blue salt B, ninhydrin or by UV absorption at 254 nm. Spectral data and elemental analyses are shown for intermediates (**a**−**g**, **68**, **69**, **B**) and parent compounds, which were obtained by different reactions or methods, and additionally the most potent compounds (**1**, **3**, **4**, **8**, **9**, **11**, **13**, **14**, **20**, **22**, **25**, **34**, **37**, **38**, **40**, **51**, **55**, **57**, **58**, **60**, **66**, **70**).

**General Procedure for Preparation of ((Aminomethyl)-phenyl)methyl Derivatives 1−3, 5. Method A.** 1-(2-Methoxyphenyl)piperazine·HCl (**A**) (5.5 mmol, 1.26 g) and $K_2CO_3$ (11 mmol, 1.52 g) were added under ice-cooling to a solution of bromomethyl-benzonitrile (5 mmol, 1 g) in 30 mL of acetone and then heated to reflux for 6 h. After cooling to ambient temperature, the solid was removed by filtration, the residue washed with acetone, and the filtrate was concentrated in vacuo. The residue (4-(4-(2-methoxyphenyl)piperazin-1-ylmethyl)benzonitrile, e.g., (a) was dissolved in 15 mL of freshly distilled THF and slowly added under ice-cooling to a suspension of $LiAlH_4$ (95%) (10 mmol, 0.38 g) in 15 mL of freshly distilled THF. After stirring at room temperature for 30 min, the mixture was heated to reflux for 3 h. After cooling again to ambient temperature, saturated $NH_4Cl$ solution was added to the mixture to perform hydrolization. The organic layer was separated and the aqueous phase extracted with ethyl acetate twice. The combined organic phases were evaporated to dryness. The product obtained 4-(4-(2-methoxyphenyl)-piperazin-1-ylmethyl)benzylamine, e.g., (b) was pure enough to be used in the following reaction step (method B) without further purification.

**4-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)benzonitrile (a).** Method A. Yield: 87%. $^1$H NMR (DMSO-$d_6$) $\delta$ 7.88 (d, $J$ = 8.0 Hz, 2H, NC−Ph−3H, NC−Ph−5H), 7.63 (d, $J$ = 8.0 Hz, 2H, NC−Ph−2H, NC−Ph−6H), 7.00 (m, 2H, 2 MeOPh−H), 6.89 (m, 2H, 2 MeOPh−H), 3.92 (s, 2H, 1-Pipera−$CH_2$), 3.77 (s, 3H, $OCH_3$), 3.05 (br m, 4H, 4 Pipera−H), 2.80 (br m, 4H, 4 Pipera−H). Anal. ($C_{19}H_{21}N_3O\cdot C_2H_2O_4$) C, H, N.

**4-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)benzylamine (b).** Method A. Yield: 83%. $^1$H NMR (DMSO-$d_6$) $\delta$ 7.36 (m, 4H, 4 Xyl−H), 6.96 (m, 2H, 2 MeOPh−H), 6.87 (m, 2H, 2 MeOPh−H), 3.87 (s, 2H, 1-Pipera−$CH_2$), 3.81 (s, 3H, $OCH_3$), 3.51 (s, 2H, $NH_2-CH_2$), 2.95 (br m, 4H, 4 Pipera−H), 2.63 (br m, 4H, 4 Pipera−H). $C_{19}H_{25}N_3O$.

**General Procedure for Preparation of Amides 1−3, 5, 8−44, and 46−67. Method B.** A mixture of the amine (2 mmol), triethylamine (6 mmol, 0.84 mL), and 10 mL of dry $CH_2Cl_2$ was stirred under argon for 10 min. Then the corresponding carboxylic acid chloride (2.1 mmol) was added and the stirring continued for another 3−24 h. An additional amount of carboxylic acid chloride (0.5−1 mmol) was added if any residual amine was detected by TLC control (ninhydrin). The solvent was removed under reduced pressure and the mixture stirred with water. Water was decanted from semisolids and the procedure repeated to give solid crystals. Oily products were extracted with $CH_2Cl_2$ or ethyl acetate. If necessary, the product was purified by rotatory chromatography and crystallized as salt of oxalic acid.

***N*-(2-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)-phenylmethyl)-2-naphthalenecarboxamide (1).** Method A, method B. Yield for last reaction step: 77%. $^1$H NMR (DMSO-$d_6$) $\delta$ 9.35 (m, 1H*, NH), 8.47 (s, 1H, Naph−1H), 8.00 (m, 4H, 4 Naph−H), 7.59 (m, 2H, 2 Naph−H), 7.48 (m, 1H, Xyl−H), 7.39 (m, 2H, 2 Xyl−H), 7.31 (m, 1H, Xyl−H), 6.94 (m, 2H, 2 MeOPh−H), 6.84 (m, 2H, 2 MeOPh−H), 4.70 (d, $J$ = 5.5 Hz, 2H, NH−$CH_2$), 4.05 (br m, 2H, 1-Pipera−$CH_2$), 3.89 (s, 3H, $OCH_3$), 3.17 (br m, 4H, 4 Pipera−H), 2.92 (br m, 4H, 4 Pipera−H). Anal. ($C_{30}H_{31}N_3O_2\cdot C_2H_2O_4$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)-phenylmethyl)-2-naphthalenecarboxamide (3).** Method A, method B. Yield for last reaction step: 20%. $^1$H NMR (DMSO-$d_6$) $\delta$ 9.23 (t, $J$ = 5.6 Hz, 1H*, NH), 8.52 (s, 1H, Naph−1H), 8.00 (m, 4H, 4 Naph−H), 7.61 (m, 2H, 2 Naph−H), 7.38 (m, 4H, 4 Xyl−H), 6.95 (m, 2H, 2 MeOPh−H), 6.86 (m, 2H, 2 MeOPh−H), 4.57 (d, $J$ = 5.6 Hz, 2H, NH−$CH_2$), 3.97 (br m, 2H, 1-Pipera−$CH_2$), 3.75 (s, 3H, $OCH_3$), 3.06 (br m, 4H, 4 Pipera−H), 2.92 (br m, 4H, 4 Pipera−H). Anal. ($C_{30}H_{31}N_3O_2\cdot 1.25C_2H_2O_4$) C, H, N.

**General Procedure for Preparation of Amine Precursors for 8−44, 46−67, and 72. Method C1.** A mixture of 1-(2-methoxyphenyl)piperazine·HCl (**A**) (20 mmol, 4.57 g), *N*-($\omega$-bromoalkyl)phthalimide (20 mmol), and $K_2CO_3$ (80 mmol, 11.06 g) in 60 mL of acetonitrile or acetone was heated to reflux for 6 h. After further addition of *N*-($\omega$-bromoalkyl)phthalimide (2 mmol), the mixture was heated for another 8 h. To achieve higher reaction temperatures, the use of dimethylformamide was recommended for less reactive components. The hot suspension was filtrated and the residue washed with acetone several times. The filtrates were concentrated under reduced pressure to give the phthalimide intermediate (**68**, **69**).

***N*-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)phthalimide (68).** Method C1. Yield: 98%. $^1$H NMR (DMSO-$d_6$) $\delta$ 7.88 (m, 4H, 4 Phth−H), 6.97 (m, 4H, 4 MeOPh−H), 3.76 (s, 3H, $OCH_3$), 3.66 (t, $J$ = 6.0 Hz, 2H, Phth−$CH_2$), 3.28−2.74 (m, 10H, 8 Pipera−H, 1-Pipera−$CH_2$), 1.98 (m, 2H, $CH_2-CH_2-CH_2$). Anal. ($C_{22}H_{25}N_3O_3\cdot 1.5C_2H_2O_4$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)phthalimide (69).** Method C1. Yield: 75%. $^1$H NMR (DMSO-$d_6$) $\delta$ 7.88 (m, 4H, 4 Phth−H), 7.00 (m, 4H, 4 MeOPh−H), 3.79 (s, 3H, $OCH_3$), 3.63 (t, $J$ = 6.3 Hz, 2H, Phth−$CH_2$), 3.28−2.74 (m, 10H, 8 Pipera−H, 1-Pipera−$CH_2$), 1.65 (m, 4H, $CH_2-CH_2-CH_2-CH_2$). Anal. ($C_{23}H_{27}N_3O_3\cdot C_2H_2O_4$) C, H, N.

The *N*-($\omega$-(4-(2-methoxyphenyl)piperazin-1-yl)alkyl)phthalimide (18 mmol) and hydrazine hydrate (22 mmol, 1.07 g) in 30 mL of methanol were heated to reflux for 2 h. To the hot solution was added 20 mL of 2N HCl, and reflux was continued for one more hour. After cooling to ambient temperature, the mixture was filtrated, the residue washed with methanol, and the filtrate evaporated to dryness. This residue was suspended in water and alkalized with 2N NaOH. Extraction with ethyl acetate (or $CH_2Cl_2$) delivered an oily product (**c**, **d**), which was pure enough for the following reaction step (method B).

**3-(4-(2-Methoxyphenyl)piperazin-1-yl)propylamine (c).**[29] Method C1. Yield: 91%. $^1$H NMR (DMSO-$d_6$) $\delta$ 11.25 (s, 1H*, $Pipera^+$−H), 8.18 (s, 1H*, $H_3N^+$), 7.03 (m, 4H, 4 MeOPh−H), 3.78 (s, 3H, $OCH_3$), 3.51 (m, 4H, 4 Pipera−H), 3.22 (m, 2H, 1-Pipera−$CH_2$), 3.14 (m, 4H, 4 Pipera−H), 2.94 (m, 2H, $H_3N^+CH_2$), 2.12 (m, 2H, $CH_2-CH_2-CH_2$). $C_{14}H_{23}N_3O\cdot 2HCl$.

**4-(4-(2-Methoxyphenyl)piperazin-1-yl)butylamine (d).**[29] Method C1. Yield: 93%. $^1$H NMR (DMSO-$d_6$) $\delta$ 6.98 (m, 4H, 4 MeOPh−H), 6.05 (s, 4H, 4 Mal), 3.79 (s, 3H, $OCH_3$), 3.35 (m, 8H, 8 Pipera−H), 2.92 (t, $J$ = 7.0 Hz, 2H, 1-Pipera−$CH_2$), 2.84

(t, $J = 7.1$ Hz, 2H, $H_3N^+CH_2$), 1.70 (m, 2H, $CH_2$), 1.58 (m, 2H, $CH_2$). Anal. ($C_{15}H_{25}N_3O \cdot 2C_2H_4O_4$) C, H, N.

**Method C2.** A mixture of 1-(2-methoxyphenyl)piperazine·HCl (**A**) (20 mmol, 4.57 g), 4-bromobutyronitrile (25 mmol, 2.5 mL), and $K_2CO_3$ (44 mmol, 6.14 g) in 50 mL of acetonitrile was heated to reflux for 6 h. After further addition of 10 mmol (1 mL) of 4-bromobutyronitrile, the mixture was heated for another 6 h. The cooled mixture was filtrated, the residue washed with acetone, and the combined filtrates evaporated to dryness to afford a yellow oil, which was pure enough for the following reaction step. The 4-(4-(2-methoxyphenyl)piperazin-1-yl)butyronitrile (**e**)[29] obtained (17.4 mmol, 4.5 g) was subjected to catalytic hydrogenation using freshly prepared Raney nickel (from 5 g of aluminum−nickel alloy, according to standard procedures) in 150 mL of aqueous solution of ammonia and 20 bar hydrogen for at least 24 h. Cautious filtration and concentration under reduced pressure delivered an oily product (**d**), which crystallized after some time.

**4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyronitrile (e).**[29] Method C2. Yield: 93%. $^1$H NMR (DMSO-$d_6$) $\delta$ 6.99 (m, 4H, 4 MeOPh−H), 3.78 (s, 3H, $OCH_3$), 3.02 (t, $J = 4.7$ Hz, 4H, 4 Pipera−H), 2.54 (t, $J = 4.6$ Hz, 4H, 4 Pipera−H), 2.49 (m, partially covered by DMSO 2H, NC−$CH_2$), 2.43 (t, $J = 6.9$ Hz, 2H, 1-Pipera−$CH_2$), 1.79 (m, 2H, $CH_2$−$CH_2$−$CH_2$). $C_{15}H_{21}N_3O$.

**4-Chloro-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)benzamide (8).** Method C, method B. Yield for last reaction step: 39%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.60 (m, 1H*, NH), 7.87 (d, $J = 7.9$ Hz, 2H, Cl−Ph−2H, Cl−Ph−6H), 7.54 (d, 2H, Cl−Ph−3H, Cl−Ph−5H), 6.96 (m, 4H, 4 MeOPh−H), 3.79 (s, 3H, $OCH_3$), 3.29 (m, 2H, CONH−$CH_2$), 3.19−2.90 (m, 10H, 8 Pipera−H, 1-Pipera−$CH_2$), 1.69 (m, 2H, CONH−$CH_2$−$CH_2$), 1.57 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{22}H_{28}ClN_3O_2 \cdot C_2H_2O_4$) C, H, N.

**4-Iodo-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-benzamide (9).** Method C, method B. Yield for last reaction step: 39%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.49 (m, 1H*, NH), 7.82 (d, $J = 7.0$ Hz, 2H, I−Ph−2H, I−Ph−6H), 7.60 (d, $J = 7.0$ Hz, 2H, I−Ph−3H, I−Ph−5H), 6.90 (m, 2H, 2 MeOPh−H), 6.85 (m, 2H, 2 MeOPh−H), 3.74 (s, 3H, $OCH_3$), 3.29 (br m, 2H, CONH−$CH_2$), 2.92 (br m, 4H, PhN($CH_2$)$_2$), 2.5 (hidden by DMSO 4H, PhN($CH_2$−$CH_2$)$_2$), 2.32 (m, 2H, 1-Pipera−$CH_2$), 1.50 (m, 4H, CONH−$CH_2$−$CH_2$−$CH_2$). Anal. ($C_{22}H_{28}IN_3O_2$) C, H, N.

**4-Acetyl-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)benzamide (11).** Method C, method B. Yield for last reaction step: 26%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.49 (t, $J = 5.4$ Hz, 1H*, NH), 8.03 (d, $J = 8.4$ Hz, 2H, Ph−2H, Ph−6H), 7.06 (d, $J = 8.4$ Hz, 2H, Ph−3H, Ph−5H), 6.87 (m, 4H, 4 MeOPh−H), 3.77 (s, 3H, $OCH_3$), 3.31 (m, 2H, CONH−$CH_2$), 2.94 (br m, 4H, PhN($CH_2$)$_2$), 2.62 (s, 3H, $COCH_3$), 2,5 (hidden by DMSO 4H, PhN($CH_2$−$CH_2$)$_2$), 2.35 (t, $J = 7.1$ Hz, 2H, 1-Pipera−$CH_2$), 1.57 (m, 2H, CONH−$CH_2$−$CH_2$), 1.53 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{24}H_{31}N_3O_3$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)biphenyl-4-carboxamide (13).** Method C, method B. Yield for last reaction step: 44%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.57 (m, 1H*, NH), 7.95 (d, $J = 8.2$ Hz, 2H, NCOPh−2H, NCOPh−6H), 7.77 (d, $J = 8.2$ Hz, 2H, NCOPh−3H, NCOPh−5H), 7.72 (m, 2H, OCPhPh−2H, OCPhPh−6H), 7.48 (m, 2H, OCPhPh−3H, OCPhPh−5H), 7.41 (m, 1H, OCPhPh−4H), 6.97 (m, 4H, 4 MeOPh−H), 3.79 (s, 3H, $OCH_3$), 3.35 (m, 12H, CONH−$CH_2$, 8 Pipera−H, 1-Pipera−$CH_2$), 1.72 (m, 2H, CONH−$CH_2$−$CH_2$), 1.60 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{28}H_{33}N_3O_2 \cdot C_2H_2O_4$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)benzophenone-4-carboxamide (14).** Method C, method B. Yield for last reaction step: 10%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.75 (t, $J = 5.3$ Hz, 1H*, NH), 8.01 (d, $J = 8.2$ Hz, 2H, NCOPh−2H, NCOPh−6H), 7.81 (d, $J = 8.2$ Hz, 2H, NCOPh−3H, NCOPh−5H), 7.76 (d, $J = 7.4$ Hz, 2H, PhCOPh−2H, PhCOPh−6H), 7.72 (m, 1H, PhCOPh−4H), 7.59 (m, 2H, PhCOPh−3H, PhCOPh−5H), 6.97 (m, 4H, 4 MeOPh−H), 3.79 (s, 3H, $OCH_3$), 3.35 (m, 2H, CONH−$CH_2$), 3.25−2.82 (10H, 8 Pipera−H, 1-Pipera−$CH_2$), 1.70 (m, 2H, CONH−$CH_2$−$CH_2$), 1.58 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{29}H_{33}N_3O_3 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)isoquinoline-3-carboxamide (20).** Method C, method B. Yield for last reaction step: 25%. $^1$H NMR (DMSO-$d_6$) $\delta$ 9.38 (s, 1H, Isoq−4H), 9.01 (t, $J = 6.0$ Hz, 1H*, NH), 8.50 (s, 1H, Isoq−1H), 8.24 (d, $J = 8.1$ Hz, 1H, Isoq−H), 8.18 (d, $J = 8.1$ Hz, 1H, Isoq−H), 7.87 (m, 1H, Isoq−H), 7.80 (m, 1H, Isoq−H), 6.96 (m, 4H, 4 MeOPh−H), 3.77 (s, 3H, $OCH_3$), 3.40 (m, 2H, CONH−$CH_2$), 3.38−3.02 (m, 10H, 8 Pipera−H, 1-Pipera−$CH_2$), 1.65 (m, 4H, Pipera−$CH_2$−$CH_2$−$CH_2$). Anal. ($C_{25}H_{30}N_4O_2 \cdot C_2H_2O_4 \cdot 0.3H_2O$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-4-oxo-4*H*-chromene-3-carboxamide (22).** Method C, method B. Yield for last reaction step: 29%. $^1$H NMR (DMSO-$d_6$) $\delta$ 9.23 (t, $J = 5.7$ Hz, 1H*, NH), 8.09 (m, 1H, Chromene−H), 7.93 (m, 1H, Chromene−H), 7.77 (m, 1H, Chromene−H), 7.58 (m, 1H, Chromene−H), 6.97 (m, 4H, 4 MeOPh−H), 6.87 (s, 1H, Chromene−2H), 3.81 (s, 3H, $OCH_3$), 3.44 (m, 2H, CONH−$CH_2$), 3.30 (m, 10H, 8 Pipera−H, 1-Pipera−$CH_2$), 1.72 (m, 2H, CONH−$CH_2$−$CH_2$), 1.64 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{25}H_{29}N_3O_4 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**5-(4-Chlorophenyl)-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)furan-2-carboxamide (25).** Method C, method B. Yield for last reaction step: 9%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.61 (t, $J = 5.4$ Hz, 1H*, NH), 7.96 (d, $J = 8.4$ Hz, 2H, ClPh−2H, ClPh−6H), 7.54 (d, $J = 8.4$ Hz, 2H, ClPh−3H, ClPh−5H), 7.16 (m, 2H, 2 Furan−H), 6.97 (m, 4H, 4 MeOPh−H), 3.78 (s, 3H, $OCH_3$), 3.41 (m, 12H, CONH−$CH_2$, 8 Pipera−H, 1-Pipera−$CH_2$), 1.72 (m, 2H, CONH−$CH_2$−$CH_2$), 1.58 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{26}H_{30}ClN_3O_3 \cdot 2C_2H_2O_4$) C, H, N.

**(*E*)-*N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-cinnamoylamide (34).** Method C, method B. Yield for last reaction step: 20%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.21 (t, $J = 5.5$ Hz, 1H*, NH), 7.56 (m, 2H, 2 Ph−H), 7.41 (m, 4H, 3 Ph−H, Ph−CH), 6.96 (m, 4H, 4 MeOPh−H), 6.64 (d, $J = 15.9$ Hz, 1H, CO−CH), 3.79 (s, 3H, $OCH_3$), 3.40 (m, 12H, CONH−$CH_2$, 8 Pipera−H, 1-Pipera−$CH_2$), 1.68 (m, 2H, CONH−$CH_2$−$CH_2$), 1.51 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{24}H_{31}N_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**(*E*)-4-Bromo-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (37).** Method C, method B. Yield for last reaction step: 12%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.22 (t, $J = 5.4$ Hz, 1H*, NH), 7.61 (d, $J = 8.4$ Hz, 2H, Ph−2H, Ph−6H), 7.52 (d, $J = 8.4$ Hz, 2H, Ph−3H, Ph−5H), 7.39 (d, $J = 15.8$ Hz, 1H, Ph−CH), 6.98 (m, 2H, 2 MeOPh−H), 6.89 (m, 2H, 2 MeOPh−H), 6.65 (d, $J = 15.8$ Hz, 1H, CO−CH), 3.79 (s, 3H, $OCH_3$), 3.22 (br m, 10H, CONH−$CH_2$, 8 Pipera−H), 3.01 (br m, 2H, 1-Pipera−$CH_2$), 1.67 (m, 2H, CONH−$CH_2$−$CH_2$), 1.50 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{24}H_{30}BrN_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**(*E*)-4-Iodo-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)cinnamoylamide (38).** Method C, method B. Yield for last reaction step: 56%. $^1$H NMR ($CDCl_3$) $\delta$ 7.67 (d, $J = 7.9$ Hz, 2H, I−Ph−2H, I−Ph−6H), 7.55 (d, $J = 15.6$ Hz, 1H, Ph−CH), 7.21 (d, $J = 7.9$ Hz, 2H, I−Ph−3H, I−Ph−5H), 7.02 (s, 1H, NH), 6.92 (m, 4H, 4 MeOPh−H), 6.48 (d, $J = 15.6$ Hz, 1H, CO−CH), 3.86 (s, 3H, $OCH_3$), 3.43 (s, 2H, CONH−$CH_2$), 3.20 (s, 4H, PhN($CH_2$)$_2$), 2.80 (s, 4H, PhN($CH_2$−$CH_2$)$_2$), 2.57 (s, 2H, 1-Pipera−$CH_2$), 1.69 (s, 4H, CONH−$CH_2$−$CH_2$−$CH_2$). Anal. ($C_{24}H_{30}IN_3O_2$) C, H, N.

**(*E*)-4-Cyano-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)cinnamoylamide (40).** Method C, method B. Yield for last reaction step: 3%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.31 (t, $J = 5.4$ Hz, 1H*, NH), 7.87 (d, $J = 7.8$ Hz, 2H, Ph−2H, Ph−6H), 7.46 (d, $J = 7.8$ Hz, 2H, Ph−3H, Ph−5H), 7.48 (d, $J = 15.8$ Hz, 1H, Ph−CH), 6.98 (m, 2H, 2 MeOPh−H), 6.89 (m, 2H, 2 MeOPh−H), 6.77 (d, $J = 15.8$ Hz, 1H, CO−CH), 3.79 (s, 3H, $OCH_3$), 3.24 (br m, 10H, CONH−$CH_2$, 8 Pipera−H), 3.08 (br m, 2H, 1-Pipera−$CH_2$), 1.68 (m, 2H, CONH−$CH_2$−$CH_2$), 1.52 (m, 2H, 1-Pipera−$CH_2$−$CH_2$). Anal. ($C_{25}H_{30}N_4O_2 \cdot 2C_2H_2O_4$) C, H, N.

**(*E*)-3-(3-Iodophenyl)-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)acrylamide (51).** Method C, method B. Yield for last reaction step: 30%. $^1$H NMR ($CDCl_3$) $\delta$ 7.85 (s, 1H,

I−Ph−2H), 7.66 (d, $J$ = 7.9 Hz, 1H, I−Ph−H), 7.53 (d, $J$ = 15.6 Hz, 1H, Ph−CH), 7.45 (d, $J$ = 7.8 Hz, 1H, I−Ph−H), 7.09 (br m, 6H, I−Ph−H, NH, 4 MeOPh−H), 6.49 (d, $J$ = 15.6 Hz, 1H, CO−CH), 3.86 (s, 3H, $OCH_3$), 3.46 (m, 2H, CONH−C$H_2$), 3.26 (s, 4H, PhN($CH_2$)$_2$), 2.84 (s, 4H, PhN($CH_2$−C$H_2$)$_2$), 2.67 (s, 2H, 1-Pipera−$CH_2$), 1.81 (m, 4H, CONH−$CH_2$−C$H_2$−C$H_2$). Anal. ($C_{24}H_{30}IN_3O_2$) C, H, N.

**(*E*)-3-(2-Iodophenyl)-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)acrylamide (55).** Method C, method B. Yield for last reaction step: 39%. $^1$H NMR ($CDCl_3$) $\delta$ 7.88 (d, $J$ = 7.9 Hz, 1H, I−Ph−H), 7.82 (d, $J$ = 15.4 Hz, 1H, Ph−CH), 7.54 (d, $J$ = 7.8 Hz, 1H, I−Ph−H), 7.32 (m, 2H, I−Ph−H), 7.05 (m, 2H, 2 MeOPh−H), 6.93 (m, 3H, 2 MeOPh−H, NH), 6.43 (d, $J$ = 15.4 Hz, 1H, CO−CH), 3.86 (s, 3H, $OCH_3$), 3.48 (m, 2H, CONH−C$H_2$), 3.28 (s, 4H, PhN($CH_2$)$_2$), 2.97 (s, 4H, PhN($CH_2$−C$H_2$)$_2$), 2.73 (s, 2H, 1-Pipera−$CH_2$), 1.85 (m, 2H, CONH−$CH_2$−C$H_2$), 1.74 (m, 2H, Pipera−$CH_2$−C$H_2$). Anal. ($C_{24}H_{30}IN_3O_2$) C, H, N.

**(*E*)-3,4-Dichloro-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (57).** Method C, method B. Yield for last reaction step: 5%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.19 (m, 1H*, NH), 7.85 (s, 1H, Ph−2H), 7.67 (d, $J$ = 8.4 Hz, 1H, Ph−6H), 7.57 (d, $J$ = 8.4 Hz, 1H, Ph−5H), 7.40 (d, $J$ = 15.8 Hz, 1H, Ph−CH), 6.99 (m, 2H, 2 MeOPh−H), 6.89 (m, 2H, 2 MeOPh−H), 6.70 (d, $J$ = 15.7 Hz, 1H, CO−CH), 3.79 (s, 3H, $OCH_3$), 3.22 (m, 12H, CONH−C$H_2$, 8 Pipera−H, 1-Pipera−$CH_2$), 1.66 (m, 2H, CONH−$CH_2$−C$H_2$), 1.54 (m, 2H, 1-Pipera−$CH_2$−C$H_2$). Anal. ($C_{24}H_{29}Cl_2N_3O_2$·$C_2H_2O_4$) C, H, N.

**(*E*)-2,4-Dichloro-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (58).** Method C, method B. Yield for last reaction step: 3%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.32 (m, 1H*, NH), 7.70 (m, 3H, 2 Ph−2H, Ph−CH), 7.50 (d, $J$ = 7.9 Hz, 1H, Ph−H), 6.99 (m, 2H, 2 MeOPh−H), 6.89 (m, 2H, 2 MeOPh−H), 6.70 (d, $J$ = 15.7 Hz, 1H, CO−CH), 3.79 (s, 3H, $OCH_3$), 3.22 (m, 12H, CONH−C$H_2$, 8 Pipera−H, 1-Pipera−$CH_2$), 1.68 (m, 2H, CONH−$CH_2$−C$H_2$), 1.52 (m, 2H, 1-Pipera−$CH_2$−C$H_2$). Anal. ($C_{24}H_{29}Cl_2N_3O_2$·$C_2H_2O_4$·1.5$H_2O$) C, H, N.

**(*E*)-3-(Benzo[1,3]dioxol-4-yl-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)acrylamide (60).** Method C, method B. Yield for last reaction step: 37%. $^1$H NMR ($CDCl_3$) $\delta$ 7.55 (d, $J$ = 15.7 Hz, 1H, Ph−CH), 7.05 (m, 2H, ($CH_2O_2$)Ph−*H*, NH), 6.94 (m, 4H, 2 ($CH_2O_2$)Ph−*H*, 2 MeOPh−H), 6.84 (m, 2H, 2 MeOPh−H), 6.73 (d, $J$ = 15.8 Hz, 1H, CO−CH), 5.88 (s, 2H, ($CH_2O_2$)Ph), 3.87 (s, 3H, $OCH_3$), 3.46 (m, 2H, CONH−C$H_2$), 3.27 (s, 4H, PhN($CH_2$)$_2$), 2.93 (s, 4H, PhN($CH_2$−C$H_2$)$_2$), 2.71 (s, 2H, 1-Pipera−$CH_2$), 1.83 (m, 2H, CONH−$CH_2$−C$H_2$), 1.72 (m, 2H, Pipera−$CH_2$−C$H_2$). Anal. ($C_{25}H_{31}N_3O_4$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-3-(2-naphthyl)acrylamide (66).** Method C, method B. Yield for last reaction step: 10%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.14 (t, $J$ = 5.4 Hz, 1H*, NH), 8.06 (s, 1H, Naph−1H), 7.93 (m, 3H, 3 Naph−H), 7.72 (d, $J$ = 8.6 Hz, 1H, Naph−H), 7.56 (m, 3H, 2 Naph−H, Naph−CH), 6.92 (m, 4H, 4 MeOPh−H), 6.76 (d, $J$ = 15.7 Hz, 1H, CO−CH), 3.76 (s, 3H, $OCH_3$), 3.23 (m, 2H, CONH−C$H_2$), 2.95 (m, 4H, PhN($CH_2$)$_2$), 2.5 (hidden by DMSO 4H, PhN($CH_2$−C$H_2$)$_2$), 2.34 (m, 2H, 1-Pipera−$CH_2$), 1.51 (m, 4H, CONH−$CH_2$−C$H_2$−C$H_2$). Anal. ($C_{28}H_{33}N_3O_2$) C, H, N.

**Preparation of Bicyclo Derivatives 4, 6, and 7.** Reductive alkylation of 1-(2-methoxyphenyl)piperazine·HCl (5 mmol, 0.96 g) was performed with *cis*-bicyclo[3.3.0]octane-3,7-dione (5 mmol, 0.69 g), triacetoxyborohydride (5 mmol, 1.06 g), and 1 mL of glacial acetic acid in 80 mL of dichloroethane. The mixture was stirred at ambient temperature for 2 h. Then, a half-saturated solution of $NaHCO_3$ was added, followed by extraction with dichloromethane. The unified organic phases were washed, dried, and concentrated in vacuo. The oily product was dissolved in ethanol. Addition of oxalic acid led to fractionated crystallization. In minor yields the dimer 3,7-bis(4-(2-methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane (**6**) was obtained, but the major product was 7-(4-(2-methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane-3-one (**f**). This ketone (1.4 mmol) was dissolved in 50 mL of a methanolic solution of $NH_3$ with a catalytic amount of palladium on activated charcoal (200 mg). The mixture was hydrogenated with 1 bar $H_2$ for 12 h under TLC control. The catalyst was removed by filtration and the filtrate evaporated in vacuo. The residue was suspended in water and extracted with dichloromethane. Again fractionated crystallization with oxalic acid delivered a bis-adduct (bis(7-(4-(2-methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]oct-3-yl)amine, **7**) and a distinct major product (7-(4-(2-metoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane-3-amine, **g**). The free base (ca. 0.5 mmol, 0.15 g) of this amine was treated according to method B with 2-naphthoyl chloride (0.53 mmol, 0.1 g) to provide the amide **4**. For compound **4**, absolute conformation has not been confirmed yet, and minor percentage of diastereomeres cannot be excluded.

**7-(4-(2-Methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]-octane-3-one (f).** Yield: 32%. $^1$H NMR (DMSO-$d_6$) $\delta$ 6.98 (m, 2H, 2 MeOPh−H), 6.90 (m, 2H, 2 MeOPh−H), 3.79 (s, 3H, $OCH_3$), 3.51 (br m, 1H, NCH), 3.21 (br m, 8H, 8 Pipera−H), 2.68 (m, 2H), 2.46 (m, partially covered by DMSO 2H), 2.39 (m, 2H), 2.13 (m, 1H), 2.08 (m, 1H), 1.63 (m, 2H). Anal. ($C_{15}H_{26}N_2O_2$·$C_2H_4O_4$·$H_2O$) C, H, N.

**7-(4-(2-Methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]-octane-3-amine (g).** Yield: 49%. $^1$H NMR (DMSO-$d_6$) $\delta$ 6.97 (m, 2H, 2 MeOPh−H), 6.93 (m, 2H, 2 MeOPh−H), 3.79 (m, 4H, 3 $OCH_3$, NCH), 3.10 (m, 1H), 2.95 (m, 4H, 4 Pipera−H), 2.54 (m, partially covered by DMSO 4H, 4 Pipera−H), 2.29 (m, 2H), 2.10 (m, 2H), 2.03 (m, 2H), 1.57 (m, 1H), 1.38 (m, 1H), 1.21 (m, 2H). $C_{19}H_{29}N_3O$.

***N*-(7-(4-(2-Methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]-octane-3-yl)-2-naphthalenecarboxamide (4).** Yield for last reaction step: 26%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.52+8.43 (d, $J$ = 7.3 Hz, 1H, NH), 7.43 (s, 1H, Naph−1H), 7.96 (m, 4H, 4 Naph−H), 7.60 (m, 2H, 2 Naph−H), 6.93 (m, 4H, 4 MeOPh−H), 6.87 (m, 4H, 4 MeOPh−H), 4.47+4.31 (m, 1H, NH−C*H*) 3.77 (s, 6H, 2 $OCH_3$), 2.95 (br m, 4H, 4Pipera−H), 2.5 (hidden by DMSO 4H, 4 Pipera−H), 2.39 (m, 1H, 1-Pipera−CH), 2.19 (m, 4H, 4 C*H*H), 1.72 (m, 2H, HC−CH), 1.45−1.00 (4H, 4 CH*H*). Anal. ($C_{30}H_{35}N_3O_2$·0.75$H_2O$) C, H, N.

**General Procedure for Preparation of *N*-Alkylated Imides 70, 71.** A mixture of 1-(2-methoxyphenyl)piperazine·HCl (10 mmol, 2.29 g), $K_2CO_3$ (20 mmol, 2.76 g), and 1,4-dibromobutane (15 mmol, 1.79 mL) in 100 mL *n*-butanol was heated to reflux for 3 h. The hot suspension was filtrated and concentrated under reduced pressure to deliver 8-(2-methoxyphenyl)-8-aza-5-azoniaspiro[4,5]decane-bromide (**B**). This intermediate (4 mmol, 1.31 g), $K_2CO_3$ (5 mmol, 0.69 g), the dicarboximide (4 mmol), and a catalytic amount of 18-crown-6 were dissolved in 20 mL of xylene and heated to reflux under argon for 24 h. After filtration of the hot mixture, the filtrate was evaporated to dryness. The obtained colored oil was stirred in diethyl ether and crystallized.

**8-(2-Methoxyphenyl)-8-aza-5-azoniaspiro[4,5]decane-bromide (B).** Yield: 70%. $^1$H NMR (DMSO-$d_6$) $\delta$ 6.99 (m, 4H, 4 MeOPh−H), 3.81 (s, 3H, $OCH_3$), 3.63 (m, 4H, $N^+$($CH_2$)$_2$), 3.59 (m, 4H, $N^+$($CH_2$)$_2$), 3.30 (br m, 4H, Ph−N($CH_2$)$_2$), 2.10 (m, 4H, $N^+$($CH_2$−C$H_2$)$_2$. Anal. ($C_{15}H_{23}BrN_2O$·0.75$H_2O$) C, H, N.

***N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-2,3-naphthalenedicarboximide (70).** Yield for last reaction step: 59%. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.52 (s, 2H, Naph−1H, Naph−4H), 8.27 (m, 2H, Naph−5H, Naph−8H), 7.79 (m, 2H, Naph−6H, Naph−7H), 6.87 (m, 4H, 4 MeOPh−H), 3.75 (s, 3H, $OCH_3$), 3.69 (t, $J$ = 6.7 Hz, 2H, Phth−N−$CH_2$), 2.92 (m, 4H, Ph−N($CH_2$)$_2$), 2.47 (partially hidden by DMSO 4H, Ph−N($CH_2$−C$H_2$)$_2$), 2.34 (t, $J$ = 6.7 Hz, 2H, 1-Pipera−$CH_2$), 1.68 (m, 2H, Phth−N−$CH_2$−C$H_2$), 1.49 (m, 2H, 1-Pipera−$CH_2$−C$H_2$). Anal. ($C_{27}H_{29}N_3O_3$) C, H, N.

**Pharmacological Testing. Binding Studies.**[62] Human $D_{2L}$ and $D_3$ receptors were expressed in stably transfected Chinese hamster ovary (CHO) cells.[50,51] These cell lines were cultured in Dulbecco's Modified Eagle Medium supplemented in 10% fetal calf serum in an atmosphere of 5% $CO_2$. Cells were harvested from culture dishes in the presence of 0.2% trypsin, centrifuged at 2000*g* for 5 min and homogenized in 10 mM Tris-HCl, pH 7.4, containing 5 mM $MgCl_2$ using a Polytron. The homogenate was centrifuged at 20 000*g* for 15 min at 4°C, and the pellet was resuspended by sonication in

50 mM Tris-HCl, pH 7.4, containing (in milimolar): NaCl, 120; KCl, 5; $CaCl_2$, 2; and $MgCl_2$, 8 (incubation buffer). Membranes were used either immediately or after storage at −70 °C. A membrane volume of 200 $\mu$L, diluted in incubation buffer supplemented with 0.2% bovine serum albumin, was added to polystyrene tubes containing (in 100 $\mu$L) 0.1 nM [$^{125}$I]iodosulpiride and drug diluted in 100 $\mu$L of incubation buffer. Nonspecific binding was determined in the presence of 1 $\mu$M enomapride. Incubations were run at 30°C for 30 min. Reactions were stopped by vacuum filtration through Whatman GF/B glass-fiber filters coated in 0.3% polyethylenimine with automated cell harvester (Brandel-Beckman, Gaithersburg, MD). Filters were rinsed three times with 5 mL of ice-cold incubation buffer and counted by liquid scintillation in 5 mL of ACS II (Amersham).

**Functional Receptor Tests.**[62] NG 108-15 cells expressing the human $D_3$ receptor were cultured in Dulbecco's Modified Eagle Medium supplemented in 10% fetal calf serum in an atmosphere of 5% $CO_2$ and plated in collagen-coated 96-well plates. After a 24-hour culture, cells were washed twice with culture medium without feral calf serum and incubated for 16 h with 1 $\mu$M forskoline and quinpirole in increasing concentrations, in the absence or presence of compounds at 1.5, 3, 30, or 300 nM. Then, [$^3$H]thymidine (1 $\mu$Ci/well) was added for 2 h and cells were harvested by vacuum filtration through Whatman GF/C glass-fiber filters using an automated cell harvester. The filters were rinsed 15 times with 200 $\mu$L of phosphate buffered saline. Radioactivity was counted by liquid scintigraphy in 5 mL of ACS (Amersham).

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