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# A Medicinal-Chemistry-Guided Approach to Selective and Druglike Sigma 1 Ligands

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*Based on a medicinal-chemistry-guided approach, three novel series of druglike cycloalkyl-annelated pyrazoles were synthesized and display high affinity ($pK_i > 8$) for the σ1 receptor. Structure–affinity relationships were established, and the different scaffolds were optimized with respect to σ1 binding and selectivity versus the σ2 receptor and the hERG channel, resulting in selective compounds that have $K_i$ values (for σ1) in the subnanomolar range. Selected compounds were screened for cytochrome P450 inhibition (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4), metabolic stability (rat and human liver microsomes), and cell-membrane permeability (Caco-2). They showed favorable in vitro ADME properties as well as favorable calculated druglike and experimental physicochemical properties. Furthermore, compounds **7f** and **17a**, for example, displayed high selectivity (affinity) for the σ1 receptor against a wide range of other receptors (>60). With these valuable tool compounds in hand, we are further exploring the role of the σ1 receptor in relevant animal models corresponding to such medicinal indications as drug abuse, pain, depression, anxiety, and psychosis.*

## Introduction

The sigma (σ) receptor was first identified almost 30 years ago as a new subtype of opioid receptor in an attempt to explain the psychotomimetic effects of opiates of the benzomorphan series, particularly those of the (+) enantiomers.[1] Some years later, the σ receptor was considered to be the binding site for phencyclidine (PCP).[2] However, at present, σ receptors are known to possess specific drug selectivity patterns, differential anatomical distribution, and unique properties different from opioid, *N*-methyl-D-aspartic acid (NMDA), dopaminergic, and other known neurotransmitter and hormone receptor families.

Pharmacological data based on binding studies, anatomical distribution, and biochemical features distinguish at least two σ receptor subtypes (σ1 and σ2).[3] The σ1 subtype (but not the σ2) has been cloned in various species[4] and is better characterized at the functional and structural level. It is expressed in the brain, within neuronal perikarya and dendrites, as well as in several peripheral tissues.[5] The σ1 receptor is a protein of ≈223 amino acids that is structurally unrelated to known mammalian proteins. The predicted structure based on amino acid sequence reveals that σ1 receptors have one or two putative transmembrane domains, two hydrophobic stretches, and a double arginine endoplasmic reticulum retention signal at the N-terminus.[4a,6] Functionally, σ1 receptors do not correspond to G-protein-coupled receptors and, despite some homology (30.3% identity) with a fungal sterol C8–C7 isomerase, they do not serve this enzymatic function in mammals.[4a,7]

The unique molecular structure of the σ1 receptor has raised intriguing questions about its functional significance.[8] Interestingly, whereas σ1 receptors elicit no effects by themselves in most cases, σ1 ligands modulate signals incurred upon the stimulation of different neurotransmitter systems.[8a] Therefore, σ1 receptors are now considered to play a modulatory role in the activity of a variety of receptors and ion channels which can also act as amplifiers in signal-transduction cascades.[6b,8a] At rest, the σ1 receptor is primarily an intracellular protein anchored in the endoplasmic reticulum membrane. After acti-

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vation through ligand binding, the σ1 receptor translocates to other organelles and to the plasma membrane.[8a,9,10] At the plasma membrane, the σ1 receptor can modulate various ion channels and receptors, including potassium channels,[6b,11] calcium channels,[11b,c] NMDA receptors,[12] dopamine receptors[13] and γ-amino *n*-butyric acid (GABA) receptors.[14] As a result of its activity on ion channels and neurotransmitter responses, the σ1 receptor has been reported to modulate neural firing and the release of several neurotransmitters, including serotonin, dopamine, noradrenaline, glutamate, and GABA.[12b,15]

Owing to the broad spectrum of modulatory effects reported for σ1 ligands, a variety of pharmacological, biochemical, and behavioral studies have proposed a large number of therapeutic functions for these receptors.[8b,d,16] These include schizophrenia,[17] drug abuse,[8e,18] response to stress and depression,[8d,19] demyelinating disorders,[20] learning and memory,[21] neurodegenerative diseases,[22] modulation of opioid analgesia,[23] inflammation,[24] and others.

Numerous, structurally diverse compounds bind to σ1 receptors including benzomorphans, neuroleptics (haloperidol), antidepressants (imipramine), phencyclidine, cocaine, and others,[8c,25] but most of these compounds are not completely selective. The endogenous σ1 ligand is unknown, but there is increased evidence to suggest that steroids (neurosteroids) are the most important endogenous modulators of the σ1 receptor.[16a,26]

Few data are available for the σ2 subtype, in part because this protein has not been cloned yet. Therefore, it remains uncertain whether the two subtypes are indeed structurally related, or whether they are, in fact, two different receptor protein types, which share similar ligands and distribution patterns. Nevertheless, the σ2 receptor subtype can be distinguished based on its pharmacological profile.[3a,b,27] (+)-Pentazocine shows a very low affinity for σ2 receptors. In the same way, the steroid hormone progesterone interacts with the σ1 subtype but not with σ2 receptors. Conversely, (−)-pentazocine, haloperidol, and 1,3-di-(2-tolyl)guanidine (DTG) possess high affinity for both σ receptor subtypes. Biochemical data are also scarce, although recently σ2 receptors have been reported to regulate the activity of the dopamine transporter through the activation of protein kinase C.[28] Regarding its physiological function, it appears that this receptor subtype is involved in the regulation of cell proliferation and maintenance of cell viability, suggesting a therapeutic use of σ2 ligands as novel antineoplastic agents.[29]

Multiple pharmacological actions have been attributed to σ receptors, but a greater understanding of their physiological role and its relevance in pathophysiological processes will require the development of more potent and selective σ ligands. In the study presented herein, three novel series of druglike cycloalkyl-annelated pyrazoles have been synthesized based on a medicinal chemistry approach. These compounds display high affinity for σ receptors, with $K_i$ values in the low nanomolar to sub-nanomolar range in optimized cases. Some selected compounds show high selectivity (against $>60$ receptors) for the σ1 receptor, experimental and calculated druglike properties, and favorable in vitro ADME properties including metabolic stability, cytochrome P450 inhibition, and cell-membrane permeability.

## Results and Discussion

### Chemistry

Structurally, σ ligands are represented by a wide variety of chemical scaffolds, for example, the traditional and fairly unselective (+)-benzomorphans and phenylpiperidines, such as (+)-pentazocine and haloperidol (Figure 1).[8b,30] More selective representatives, such as siramesine[31] (a σ2 antagonist) and MS-377[15b,32] (a σ1 antagonist) have been synthesized and, in case of siramesine, tested in clinical trials. Recently, sophisticated

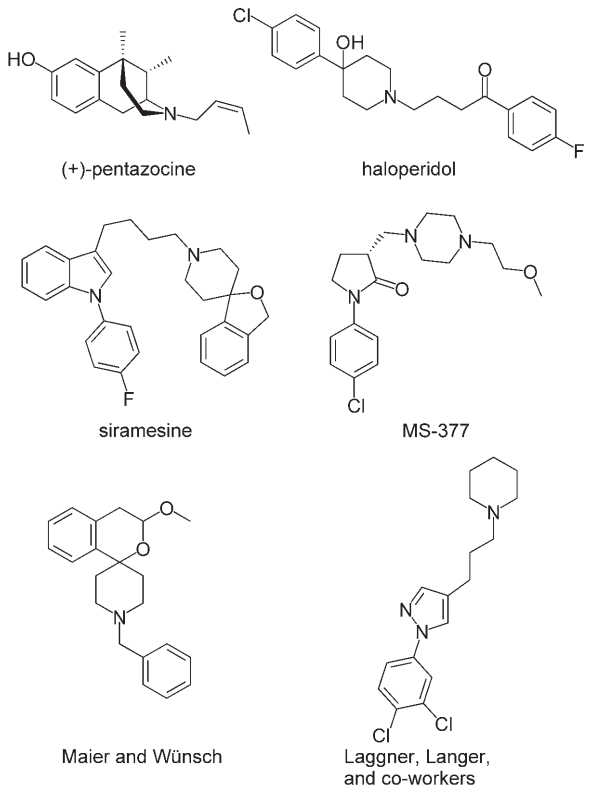
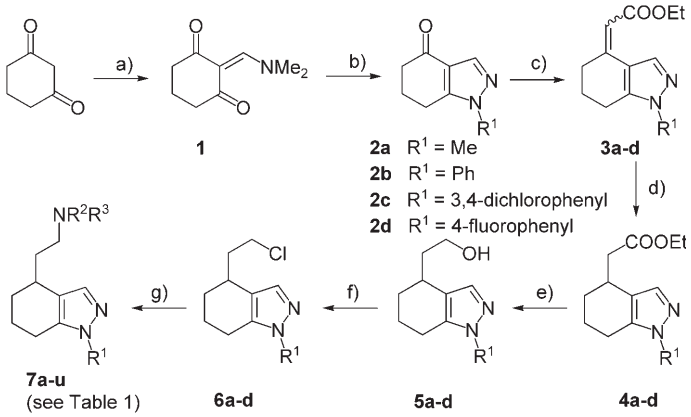



**Figure 1.** A selection of σ1 and σ2 reference ligands.

approaches such as those reported by Maier and Wünsch[25l,33] and Laggner, Langer, and co-workers[34] have resulted in ligands with even higher selectivity through the use of conformational rigidity and molecular modeling methods. Historically, the first investigations on a common σ1 pharmacophore suggested a proton donor site (amine function) linked with two hydrophobic binding sites: a primary binding site (6.0–10.0 Å distal) and a secondary binding site (2.5–3.9 Å distal).[35] Owing to the conformational flexibility of many of the available σ1 ligands, little was known about the geographical orientation of the pharmacophore and about the bulk tolerance of the linkers, for example. Recently, a more detailed pharmacophore model (based on receptor modeling) has been published.[36]

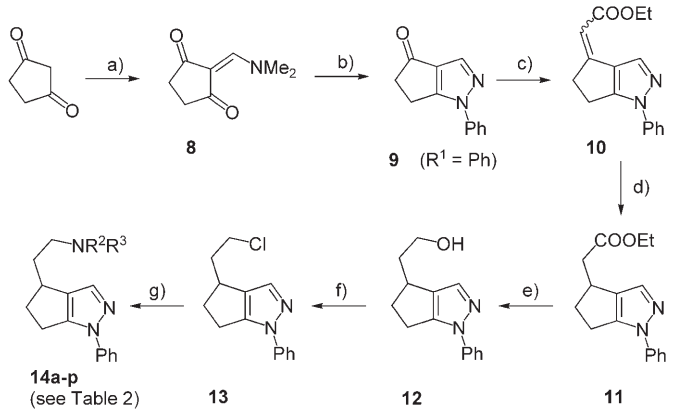
  

To design and synthesize novel, potent, selective, and drug-like σ1 ligands, we were inspired by the structures published by Laggner, Langer, and co-workers[34] (Figure 1). Our approach was to introduce more conformational constraints into the structures by annelating a carbocyclic ring system to the pyrazole moiety. This was carried out by a reaction sequence depicted in Scheme 1. Starting from 1,3-cyclohexane-dione, the

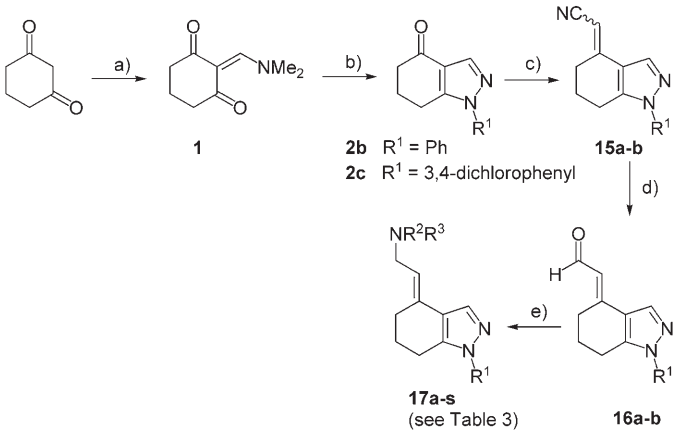



**Scheme 1.** a) Dimethylformamide dimethylacetal (DMFDMA), Δ; b) $R^1NHNH_2$, Δ; c) $EtO_2CCH_2PO(OEt)_2$, NaH; d) $H_2$, Pd-C or $H_2$, $PtO_2$; e) $LiAlH_4$; f) $SOCl_2$, toluene; g) $R^2R^3NH$, *N*,*N*-dimethylformamide (DMF).

pyrazole unit was formed through Mannich-type and ring-closure reactions with an appropriate hydrazine (compounds **2**).[37] With the second carbonyl function, racemic esters **4a**–**d** were synthesized in good yields (45–80% from 1,3-cyclohexane-dione) through a Wittig-type reaction[37] and reduction with $H_2$/Pd-C. $LiAlH_4$-mediated reduction of the ester function to the corresponding alcohol, followed by chlorination with $SOCl_2$ and $S_N2$-type introduction of different secondary amines resulted in the desired 4-aminoethyl-substituted 4,5,6,7-tetrahydroindazoles **7a**–**u**. Virtually the same strategy was used to synthesize the analogous 4-aminoethyl-substituted 1,4,5,6-tetrahydrocyclopenta[c]pyrazoles **14a**–**p** (Scheme 2) starting from 1,3-cyclopentan-dione. For the synthesis of the unsaturated 4-aminoethylidene-substituted 4,5,6,7-tetrahydroindazoles (Scheme 3), a slightly modified procedure was employed. Starting from ketones **2a** and **2b**, a methylene nitrile unit was condensed,[37] followed by reduction of the nitrile function to the corresponding aldehydes (**16a** and **16b**) and reductive amination with secondary amines to give target compounds **17a**–**s** in the *E* configuration.




**Scheme 2.** a) DMFDMA, Δ; b) $R^1NHNH_2$, Δ; c) $EtO_2CCH_2PO(OEt)_2$, NaH; d) $H_2$, Pd-C; e) $LiAlH_4$; f) $SOCl_2$, toluene; g) $R^2R^3NH$, DMF.




**Scheme 3.** a) DMFDMA, Δ; b) $R^1NHNH_2$, Δ; c) $NCCH_2PO(OEt)_2$, NaH; d) diisobutylaluminum hydride; e) $R^2R^3NH$, $NaBH_3CN$.

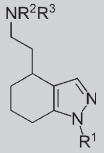
### Binding, in vitro ADME, and drug-likeness studies

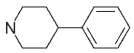
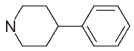
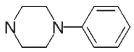
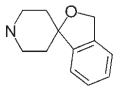
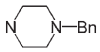
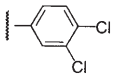
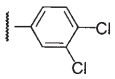
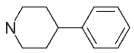
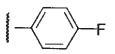
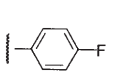
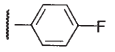
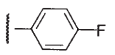
Sigma 1 binding ($K_i$) of all synthesized compounds was determined by using [$^3$H](+)-pentazocine as ligand (Experimental Section). Given the similarity of reported σ1 pharmacophore models[35,36] to those postulated[38] for human ether-a-go-go-related gene (hERG) channel interaction, all synthesized compounds were simultaneously screened for hERG channel interaction as well, with the aim of finding compounds with as little hERG inhibition as possible.[39] Selected compounds were tested for selectivity against the σ2 receptor (by using an in vitro σ2 membrane binding assay with [$^3$H]-DTG as ligand; Experimental Section) and/or were profiled in a commercially available panel of more than 60 radiolabeled-ligand-binding assays (MSD, Taiwan). These include the characterization of potential interactions at the ligand-binding site of a wide range of mostly G-protein-coupled receptors (GPCRs) and ligand-gated ion channels, plus a limited number of modulatory sites on voltage-gated ion channels.

Within the series of 4-aminoethyl-substituted 4,5,6,7-tetrahydroindazoles (compounds **7**, Table 1), four different hydrazines were used in the synthesis, leading to four different substitution patterns at $R^1$: methyl, phenyl, 3,4-dichlorophenyl, and 4-fluorophenyl. Depending on the groups $R^2$ and $R^3$ it is possible, in general, to generate compounds with very high σ1 receptor binding properties in the nanomolar or even sub-nanomolar range (for example: **7d**, $K_i$ = 3.1 nM; **7n**, $K_i$ = 0.3 nM; **7p**, $K_i$ = 4.3 nM; **7r**, $K_i$ = 2.4 nM). Interestingly, it seems that higher-affinity σ1 receptor binding is observed if at least either $R^1$ or one of the amino substituents ($R^2$ or $R^3$) consists of a larger lipophilic substituent (phenyl, benzyl, etc.). For example, com-

**Table 1.** Sigma 1, sigma 2, and hERG binding affinities of 4-aminoethyl-substituted 4,5,6,7-tetrahydroindazoles.



| Compound | $R^1$ | $NR^2R^3$ | $K_i$ [nM] (σ1) | $K_i$ [nM] (σ2) | hERG [%][a] |
|---|---|---|---|---|---|
| **7a** | Me |  | 813.7 | 39.8%[b] | ND |
| **7b** | Me |  | 9.3 | 6.3 | 18 |
| **7c** | Me | NMeBn | 110.1 | 430.9 | 0 |
| **7d** | Me |  | 3.1 | 18.8 | 0 |
| **7e** | Ph |  | 2.3 | 75.9 | 1 |
| **7f** | Ph |  | 2.8 | 474.0 | 1 |
| **7g** | Ph |  | 1.9 | 25.4 | 0 |
| **7h** | Ph |  | 7.6 | 94.6%[b] | 25 |
| **7i** | Ph |  | 1.5 | 37.5 | 68 |
| **7k** | Ph |  | 8.7 | 742 | 64 |
| **7l** | Ph |  | 0.9 | 37 | 89 |
| **7m** | Ph | NMeBn | 8.7 | 100%[b] | 34 |
| **7n** | Ph |  | 0.3 | 1.4 | 44 |
| **7o** | Ph |  | 6.1 | 70.2%[b] | 65 |
| (−)-**7f** | Ph |  | 1.3 | 60.2%[b] | 5 |
| (+)-**7f** | Ph |  | 13.4 | 79.0%[b] | 11 |
| **7p** |  |  | 4.3 | 98.1%[b] | 35 |
| **7q** |  |  | 10.6 | 316 | 83 |
| **7r** |  |  | 2.4 | ND | 4 |
| **7s** |  |  | 14.9 | 35.2 | 9 |
| **7t** |  | NMeBn | 2.6 | ND | 11 |
| **7u** |  |  | 16.2 | 14.4 | 63 |

[a] Percent inhibition at a compound concentration of 10 μM. [b] Percent inhibition at a compound concentration of 1 μM. Bn = benzyl.

pound **7a**, with morpholine as the amine function and the small and less lipophilic methyl group in the $R^1$ position, has a lower affinity for σ1 (**7a**, $K_i$ = 813.7 nM). The corresponding compound **7f** with a phenyl group in the $R^1$ position is a high-affinity ligand, with a $K_i$ value of 2.8. In general, for the series of compounds **7**, the incorporation of halogen substituents (4-fluoro and 3,4-dichloro) on the $R^1$ phenyl moiety does not significantly increase affinity (**7f** versus **7r**, $K_i$ = 2.8 and 2.4 nM, respectively). In some cases the affinity for σ1 is slightly decreased (**7f** versus **7p**, $K_i$ = 2.8 and 4.3 nM, respectively). Similar trends are observed with respect to other amines (**7i**, $K_i$ = 1.5 nM versus **7q**, $K_i$ = 10.6 nM; **7h**, $K_i$ = 7.6 nM versus **7s**, $K_i$ = 14.9 nM).

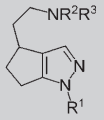
Compounds with at least two lipophilic substituents (mostly aromatic groups like phenyl and benzyl), with one at $R^1$ and the other at the $R^2$ or $R^3$ position, have high affinity for σ1 (for example, **7l**, $K_i$ = 0.9 nM and **7n**, $K_i$ = 0.3 nM). However, in agreement with postulated hERG pharmacophores,[38] such compounds significantly inhibit the hERG channel at a concentration of 10 μM (**7l**, 89% inhibition; **7n**, 44% inhibition). Therefore, these are regarded as compounds with the potential to influence the QT interval (prolongation) of the human heart. The most interesting compounds of the series **7** include compounds such as **7d**, which exhibits affinity for σ1 in the nanomolar range, some selectivity against σ2 (ca. 6-fold), and low hERG inhibition. Notably, compounds **7e**–**g** also display high (nanomolar range) affinity for σ1, high selectivity against σ2, and no hERG inhibition (Table 1). In case metabolism at the $R^1$ phenyl moiety should occur and be considered problematic, compounds **7p** and **7r**, for example, could be regarded as back-up structures for **7f**, as they have similar binding and selectivity profiles, but are likely to be less prone to metabolism at that site owing to their halogen substituents (Table 1). As expected, the two enantiomers of **7f** (obtained by resolu-

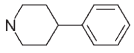
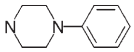
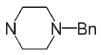
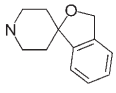
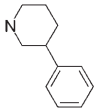
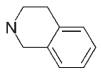
  

tion of racemic **7f** by chiral HPLC; Experimental Section) displayed different binding affinities toward σ1. The (−) enantiomer was the most potent, with an affinity for σ1 about 10-fold greater than that of the (+) enantiomer ((−)-**7f**, $K_i$ = 1.3 nM; (+)-**7f**, $K_i$ = 13.4 nM; *rac*-**7f**, $K_i$ = 2.8 nM). Confirmation of this trend is planned in future investigations by screening more enantiomer pairs.

Based on the results obtained with compound series **7**, the phenyl group was chosen as the preferred $R^1$ group in the 4-aminoethyl-substituted 1,4,5,6-tetrahydrocyclopenta[*c*]pyrazoles (compounds **14**, Table 2). Many of these compounds also show affinity for σ1 in the low nanomolar or even sub-nanomolar range (for example: **14d**, $K_i$ = 0.62 nM; **14a**, $K_i$ = 1.1 nM; **14b**, $K_i$ = 1.2 nM, **14c**; $K_i$ = 1.5 nM, and **14e**, $K_i$ = 1.8 nM). One compound, **14p**, which bears a non-basic nitrogen atom (imidazole) as the amine function, shows a significant loss of σ1 affinity ($K_i$ = 143.4 nM) which confirms the aforementioned minimum requirements for pharmacophore binding to σ1 (with respect to the proton donor site). A similar trend in hERG interaction to that observed for some members of the series of compounds **7** was detected with compounds **14** as well. Compounds with at least two lipophilic substituents (mainly aromatic groups like phenyl and benzyl), one at the $R^1$ and the other at the $R^2$ or $R^3$ position, significantly inhibit the hERG potassium channel at a concentration of 10 μM (**14g**, **14i**, and **14k**, all 100% inhibition; **14m**, 84% inhibition), in agreement with postulated hERG pharmacophores.[38] Therefore, these too are compounds with the potential to prolong the QT interval of the human heart. In slight contrast with series **7**, members of compound series **14** equipped with more lipophilic groups (compounds **14g–o**) show decreased affinity for σ1 relative to compounds **14a–f** (**14a**, $K_i$ = 1.1 nM versus **14g**, $K_i$ = 5.9 nM; **14k**, $K_i$ = 31.3 nM; **14l**, $K_i$ = 38.0; or **14m**, $K_i$ = 2.0 nM) The "introduction" of a phenyl moiety within the piperidine ring system significantly decreases σ1 affinity. The most interesting compounds of the series include **14a–d**, which bind σ1 with high affinity, have good selectivity against σ2 (10–300-fold) and low hERG inhibition (Table 2). Future activities will include investigations of the binding properties of separated enantiomers of these most interesting racemic compounds of type **14**.

**Table 2.** Sigma 1, sigma 2, and hERG binding affinities of *rac*-4-aminoethyl-substituted 1,4,5,6-tetrahydrocyclopenta[*c*]pyrazoles.



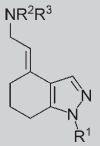
| Compound | $R^1$ | $NR^2R^3$ | $K_i$ [nM] (σ1) | $K_i$ [nM] (σ2) | hERG [%][a] |
|---|---|---|---|---|---|
| **14a** | Ph |  | 1.1 | 36.2 | 24 |
| **14b** | Ph |  | 1.2 | 17.5 | 18 |
| **14c** | Ph |  | 1.5 | 16.4 | 6 |
| **14d** | Ph |  | 0.62 | 203.4 | 2 |
| **14e** | Ph |  | 1.8 | 95.9%[b] | 35 |
| **14f** | Ph |  | 3.5 | 97.3%[b] | 7 |
| **14g** | Ph |  | 5.9 | 87.9%[b] | 100 |
| **14h** | Ph |  | 29.3 | 100.5%[b] | 78 |
| **14i** | Ph |  | 10.8 | 95.5%[b] | 100 |
| **14k** | Ph |  | 31.3 | 113%[b] | 100 |
| **14l** | Ph |  | 38.0 | 105.2%[b] | 94 |
| **14m** | Ph |  | 2.0 | 159 | 84 |
| **14n** | Ph | NMeBn | 3.9 | 104.0%[b] | 43 |
| **14o** | Ph |  | 9.3 | 26.4%[b] | 55 |
| **14p** | Ph |  | 143.4 | 22.8%[b] | 52 |

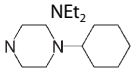
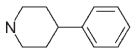
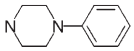
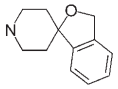
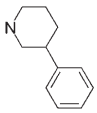
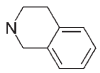
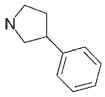
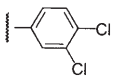
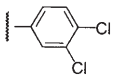
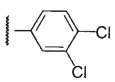
[a] Percent inhibition at a compound concentration of 10 μM. [b] Percent inhibition at a compound concentration of 1 μM. Bn = benzyl.

Based on the experiences with the compound series **7** and **14**, phenyl groups (compounds **17a–p**) and 3,4-dichlorophenyl groups (compounds **17q–s**) were chosen as the preferred substituents at the $R^1$ position for the series of the (*E*)-4-aminoethylidene-substituted 4,5,6,7-tetrahydroindazoles (compounds **17**, Table 3). Three compounds showed binding affinity for σ1 in the low nanomolar range: **17o** ($K_i$ = 1.1 nM), **17c** ($K_i$ = 1.5 nM), and **17h** ($K_i$ = 2.5 nM). The previously observed trends for compounds of type **7** and **14** were also observed for this series. Thus, compounds **17q** ($K_i$ = 6.1 nM), **17r** ($K_i$ = 59.4 nM), and **17s** ($K_i$ = 56.1 nM)—each with a 3,4-dichlorophenyl group in $R^1$ position—show similar to only slightly decreased affinity toward σ1 with respect to their phenyl-substituted counterparts **17a** ($K_i$ = 6.1 nM), **17b** ($K_i$ = 22.7 nM), and **17g** ($K_i$ = 19.5 nM). Also

**Table 3.** Sigma 1, sigma 2, and hERG binding affinities of (*E*)-4-aminoethylidene-substituted 4,5,6,7-tetrahydroindazoles.




| Compound | $R^1$ | $NR^2R^3$ | $K_i$ [nM] (σ1) | $K_i$ [nM] (σ2) | hERG [%][a] |
|---|---|---|---|---|---|
| **17a** | Ph |  | 6.1 | 495.0 | 4 |
| **17b** | Ph |  | 22.7 | 39%[b] | 4 |
| **17c** | Ph |  | 1.5 | 93%[b] | 19 |
| **17d** | Ph |  | 4.4 | 165.1 | 4 |
| **17e** | Ph | $NEt_2$ | 11.6 | ND | 10 |
| **17f** | Ph |  | 11.1 | ND | 98 |
| **17g** | Ph |  | 19.5 | ND | 6 |
| **17h** | Ph |  | 2.5 | 186.0 | 91 |
| **17i** | Ph |  | 41.7 | 56.9%[b] | 100 |
| **17k** | Ph |  | 4.9 | 478.4 | 90 |
| **17l** | Ph |  | 23.3 | 67.4%[b] | 48 |
| **17m** | Ph |  | 40.3 | 68.5%[b] | 65 |
| **17n** | Ph | NMeBn | 13.5 | ND | 37 |
| **17o** | Ph |  | 1.1 | 49.5 | 65 |
| **17p** | Ph |  | 45.9 | 278.9 | 45 |
| **17q** |  |  | 6.1 | ND | 49 |
| **17r** |  |  | 59.4 | ND | 44 |
| **17s** |  |  | 56.1 | ND | 44 |

[a] Percent inhibition at a compound concentration of 10 μM. [b] Percent inhibition at a compound concentration of 1 μM. Bn = benzyl.

analogous to the trend discussed for compounds **7**, compounds **17q–s** could potentially function as back-up structures in case greater metabolic stability at the phenyl ring is required. Again, compounds that display at least two lipophilic substituents (primarily phenyl and benzyl), one at $R^1$ and the other at the $R^2$ or $R^3$ position, significantly inhibit the hERG potassium channel at a concentration of 10 μM (**17i**, 100% inhibition; **17h**, 91% inhibition, as well as the cyclohexyl-group-containing compound, **17f**, 98% inhibition), in agreement with postulated hERG pharmacophores.[38] These compounds therefore follow suit as those with the potential to prolong the QT interval of the human heart. The most interesting compounds of series **17** include compounds **17a**, **17d**, and especially **17c**, which display high-affinity binding to σ1 ($K_i$ values in the low nanomolar range), good selectivity against σ2 (40–80-fold), and low hERG inhibition (Table 3).

Comparison of the three different series with each other reveals that for the most interesting compounds (those with high σ1 affinity, at least fair selectivity for σ1 over σ2, and no significant hERG inhibition) in which $R^1$ = Ph, the highest σ1 affinities, in general, are observed with structures **14**, followed by structures **7**, with structures **17** showing the lowest σ1 affinities of the three (**14a**, $K_i$ = 1.1 nM < **7e**, $K_i$ = 2.3 nM < **17a**, $K_i$ = 6.1; **14b**, $K_i$ = 1.2 nM < **7f**, $K_i$ = 2.8 nM < **17a**, $K_i$ = 37.2; **14c**, $K_i$ = 1.5 nM < **7g**, $K_i$ = 1.9 nM < **17d**, $K_i$ = 4.4).

Selected compounds (**7c**, **7d**, **7f**, **17a**, and **17b**) were profiled in a commercially available panel of more than 60 radiolabeled-ligand-binding assays (MSD, Taiwan). Apart from the previously characterized binding to σ1 and σ2, no displacement > 50% (at 1 μM) for any other of the investigated receptors was observed; these compounds are completely selective for the σ receptor(s).

To estimate the ADME properties and the drug-likeness of these new synthetic σ1 ligands, selected representatives were investigated for cell permeability (Table 4), metabolic stability (Table 5), cytochrome P450 inhibition (Table 6), and calculated (Tables 7–9) and experimental druglike properties (Table 10).

All investigated compounds show good permeability coefficients as determined by the Caco-2 cell permeability assay (Table 4). Permeability coefficients (Papp) range between 175 and 401 nm s$^{-1}$. With respect to their metabolic stability towards rat and human liver microsomes (Table 5), nearly all compounds investigated are sufficiently stable, with half-lives (human liver microsomes) in most cases well above 1–2 h; they are not expected to undergo extensive first-pass metabolism. The metabolic instability of compound **7c** in rat liver microsomes could be the result of metabolic cleavage of the methyl group from the amine function. A screen for inhibitory activity against a battery of different cytochrome P450 enzymes (Table 6), identified no significant inhibition except for CYP2D6. In that case, compounds **7b** and **7c** inhibited up to 71% of the original activity at a concentration of 1 μM, a concentration well above that expected to be sufficient to "block" the σ1 receptor, as determined by the binding properties. In summary, favorable ADME properties are observed in the in vitro assays for nearly all compounds investigated.

**Table 4.** Caco-2 cell-permeability coefficients for selected compounds.

| Compound | Papp [nm s$^{-1}$][a] |
|---|---|
| **7b** | 264 ± 29 |
| **7c** | 300 ± 8 |
| **7d** | 249 ± 11 |
| **7e** | 249 ± 18 |
| **7f** | 275 ± 21 |
| **7r** | 377 ± 28 |
| **7s** | 401 ± 30 |
| **14b** | 351 ± 29 |
| **17a** | 279 ± 8 |
| **17b** | 250 ± 12 |
| **17c** | 175 ± 11 |

[a] Values are mean ± SD ($n$ = 3).

**Table 5.** In vitro metabolic stability.

| Compound[a] | $t_{1/2}$ [min] (rat)[b] | $t_{1/2}$ [min] (human)[c] |
|---|---|---|
| **7b** | infinite | 165 |
| **7c** | 11 | 101 |
| **7d** | 51 | 205 |
| **7e** | 198 | 64 |
| (+)-**7f** | 47 | 62 |
| (−)-**7f** | 48 | 152 |
| **7r** | 52 | 265 |
| **7s** | 50 | infinite |
| **7t** | 24 | 41 |
| **14b** | 53 | 8188 |
| **17a** | 196 | 32 |
| **17c** | infinite | 139 |

[a] Concentration = 10 μM. [b] Determined in rat liver microsomes. [c] Determined in human liver microsomes.

With regard to drug-likeness, calculated (Tables 7, 8, and 9) and experimental (for selected compounds; Table 10) parameters were determined. The calculated parameters (Tables 7–9) reveal very few violations of Lipinski's rule-of-five. Notably, the calculated log $P$ value does not reflect the "real" situation, as its calculation is based on the nonprotonated molecule. Given the apparent p$K_a$ values at physiological pH (7.4), all molecules are present mainly in the protonated form. For that reason, the calculated log $D$ (pH 7.4) value is the more "realistic" parameter. Moreover, comparison of the calculated with the experimental log $P$ values (Table 10), shows that the experimental log $P$ values for all measured compounds (**7e**, **7f**, and **17a**) are significantly lower than those determined by calculation (**7e**, $\log P_{calcd}$ = 4.96, $\log P_{exptl}$ = 3.63; **7f**, $\log P_{calcd}$ = 3.30, $\log P_{exptl}$ = 2.41; **17a**, $\log P_{calcd}$ = 4.62, $\log P_{exptl}$ = 3.68); the experimental log $P$ values are closer to those of the druglike range. All compounds investigated are sufficiently stable and soluble under the experimental conditions chosen (Table 10). In summary, all investigated compounds are, with regard to the most important parameters, druglike and possess favorable physicochemical parameters.

## Conclusions

Three novel series of σ1 receptor ligands with high affinities and $K_i$ values in the low nanomolar and even sub-nanomolar

**Table 6.** Inhibition of cytochrome P450.[a]

| Compound | CYP1A2 (CEC) | CYP2A6 (coumarin) | CYP2B6 (EFC) | CYP2C8 (DBF) | CYP2C9 (MCF) | CYP2C19 (CEC) | CYP2D6 (AMMC) | CYP2E1 (MFC) | CYP3A (BFC) | CYP3 (DBF) |
|---|---|---|---|---|---|---|---|---|---|---|
| **7b** | −7 | −11 | −7 | 14 | 14 | 25 | 70 | 3 | 7 | −13 |
| **7c** | −4 | −11 | 4 | 12 | 13 | 13 | 71 | 4 | 14 | −12 |
| **7d** | −1 | −14 | 4 | 15 | 14 | 24 | 49 | 10 | 18 | −15 |
| **7e** | −7 | −13 | 5 | 12 | 15 | 12 | 6 | 4 | 25 | −6 |
| **7f** | −13 | ND | ND | ND | −10 | −8 | 21 | −4 | 2 | −16 |
| (+)-**7f** | −2 | −6 | 15 | 22 | 15 | 18 | 31 | 11 | 19 | 3 |
| (−)-**7f** | −6 | −10 | 0 | 1 | 11 | 10 | 41 | 1 | 12 | −6 |
| **7r** | −6 | −12 | 1 | 11 | 15 | 8 | 58 | 8 | 16 | −9 |
| **14b** | −5 | −14 | 10 | 17 | 17 | 16 | 33 | 7 | 12 | −10 |
| **17a** | −6 | −11 | 5 | 19 | 16 | 11 | 36 | 7 | 11 | −15 |
| **17b** | −9 | ND | ND | ND | 1 | −3 | 16 | −4 | 7 | −14 |

[a] All compound concentrations = 1 μM; CEC = 7-ethoxy-3-cyanocoumarin, MFC = 7-methoxy-4-(trifluoromethyl)coumarin, AMMC = 3-[2-(*N*,*N*-diethyl-*N*-methylamino)ethyl]-7-methoxy-4-methylcoumarin, BFC = 7-benzyloxy-4-(trifluoromethyl)coumarin, DBF = dibenzylfluorescein, EFC = 7-ethoxy-4-trifluoromethylcoumarin.

**Table 7.** Calculated druglike properties of σ ligands **7**.[a]

| Compound | Halogen Atoms | Rotatable Bonds | log *P* | H-bond Acceptors | Apparent p$K_a$ | log *D*[b] |
|---|---|---|---|---|---|---|
| **7a** | 0 | 4 | 1.35 | 4 | 7.63 | 0.92 |
| **7b** | 0 | 5 | 4.09 | 3 | 9.33 | 2.19 |
| **7c** | 0 | 7 | 3.66 | 3 | 8.60 | 2.44 |
| **7d** | 0 | 4 | 3.16 | 3 | 7.65 | 2.72 |
| **7e** | 0 | 4 | 4.96 | 3 | 9.69 | 2.72 |
| **7f** | 0 | 4 | 3.30 | 4 | 7.62 | 2.88 |
| **7g** | 0 | 5 | 5.45 | 3 | 9.73 | 3.19 |
| **7h** | 0 | 6 | 4.29 | 4 | 7.70 | 3.81 |
| **7i** | 0 | 5 | 6.04 | 3 | 9.31 | 4.15 |
| **7k** | 0 | 5 | 5.43 | 4 | 6.85 | 5.32 |
| **7l** | 0 | 4 | 7.50 | 4 | 9.26 | 5.67 |
| **7m** | 0 | 7 | 5.61 | 3 | 8.58 | 4.40 |
| **7n** | 0 | 4 | 5.11 | 3 | 7.63 | 4.68 |
| **7o** | 0 | 6 | 4.55 | 4 | 7.10 | 4.35 |
| **7p** | 2 | 4 | 4.37 | 4 | 7.60 | 3.95 |
| **7q** | 2 | 5 | 7.10 | 3 | 9.30 | 5.23 |
| **7r** | 1 | 4 | 3.35 | 4 | 7.61 | 2.93 |
| **7s** | 1 | 6 | 4.34 | 4 | 7.69 | 3.87 |
| **7t** | 1 | 7 | 5.66 | 3 | 8.58 | 4.46 |
| **7u** | 1 | 4 | 5.16 | 3 | 7.63 | 4.73 |

[a] Calculated values for p$K_a$, log *P* (octanol/water partition of the neutral species), and log *D* (octanol/buffer partition of ionized and unionized molecules) were determined with ACD/p$K_a$, ACD/log *P*, and ACD/log *D* software from Advanced Chemistry Development, Toronto, Canada. [b] Determined at pH 7.4.

**Table 8.** Calculated druglike properties of σ ligands **14**.[a]

| Compound | Halogen Atoms | Rotatable Bonds | log *P* | H-bond Acceptors | Apparent p$K_a$ | log *D*[b] |
|---|---|---|---|---|---|---|
| **14a** | 0 | 4 | 4.39 | 3 | 9.68 | 2.17 |
| **14b** | 0 | 4 | 2.74 | 4 | 7.61 | 2.32 |
| **14c** | 0 | 5 | 4.88 | 3 | 9.71 | 2.64 |
| **14d** | 0 | 4 | 3.73 | 3 | 7.62 | 3.30 |
| **14e** | 0 | 6 | 3.72 | 4 | 7.69 | 3.25 |
| **14f** | 0 | 4 | 3.83 | 3 | 10.35 | 1.10 |
| **14g** | 0 | 5 | 5.48 | 3 | 9.30 | 3.60 |
| **14h** | 0 | 5 | 4.87 | 4 | 6.84 | 4.75 |
| **14i** | 0 | 6 | 3.99 | 4 | 7.09 | 3.79 |
| **14k** | 0 | 4 | 6.95 | 4 | 9.25 | 5.12 |
| **14l** | 0 | 5 | 5.87 | 3 | 9.23 | 4.06 |
| **14m** | 0 | 4 | 5.14 | 3 | 8.57 | 3.94 |
| **14n** | 0 | 7 | 5.05 | 3 | 8.57 | 3.85 |
| **14o** | 0 | 4 | 4.55 | 3 | 7.62 | 4.12 |
| **14p** | 0 | 4 | 2.86 | 4 | 7.00 | 2.71 |

[a] Calculated values for p$K_a$, log *P* (octanol/water partition of the neutral species), and log *D* (octanol/buffer partition of ionized and unionized molecules) were determined with ACD/p$K_a$, ACD/log *P*, and ACD/log *D* software from Advanced Chemistry Development, Toronto, Canada. [b] Determined at pH 7.4.

range were synthesized. For each series, structure–affinity relationships (SAFIR) have been established with respect to σ1 binding and to hERG channel interaction. Furthermore, selectivity against the σ2 receptor was monitored for most of the compounds. Therefore, these ligands have been optimized for σ1 affinity and minimal inhibition of the hERG channel, and the resulting molecules match these criteria perfectly. The ligands were further profiled for their selectivity against >60 other receptors, their in vitro ADME properties, and their druglike parameters (calculated and experimental); favorable behavior is observed in each respect. For example, compounds **7f** and **17a** bind σ1 with affinity in the low nanomolar range, and are completely selective (by relative affinity) for the σ1 receptor over >60 other receptors. Virtually no hERG inhibition was observed, and cell permeability was good (Caco-2 cell assays). Both **7f** and **17a** show high metabolic stability (in rat liver microsomes) and negligible inhibition of cytochrome P450, in combination with no violation (calculated parameters and experimental log *P* values) of Lipinski's rule-of-five. With respect to experimentally determined physicochemical properties, these σ1 ligands show very good solubility and stability in solution. For these reasons, they are suitable as tool compounds for investigations of the role of the σ1 receptor in relevant in vivo models corresponding to such medicinal indications as drug abuse, pain, depression, anxiety, and psychosis. More representatives of the various scaffold classes are currently being synthesized to further optimize and understand the σ1 SAFIRs among the different lead compounds in more detail and to confirm the trends described herein.

**Table 9.** Calculated druglike properties of σ ligands **17**.[a]

| Compound | Halogen Atoms | Rotatable Bonds | log *P* | H-bond Acceptors | Apparent p$K_a$ | log *D*[b] |
|---|---|---|---|---|---|---|
| **17a** | 0 | 3 | 4.62 | 3 | 8.59 | 3.41 |
| **17b** | 0 | 3 | 3.04 | 4 | 6.67 | 2.97 |
| **17c** | 0 | 3 | 5.18 | 3 | 8.59 | 3.97 |
| **17d** | 0 | 4 | 5.11 | 3 | 8.62 | 3.87 |
| **17e** | 0 | 7 | 4.66 | 3 | 9.39 | 2.69 |
| **17f** | 0 | 4 | 4.82 | 4 | 8.16 | 3.99 |
| **17g** | 0 | 5 | 4.02 | 4 | 6.73 | 3.94 |
| **17h** | 0 | 4 | 5.70 | 3 | 8.20 | 4.83 |
| **17i** | 0 | 4 | 5.14 | 4 | 5.80 | 5.12 |
| **17k** | 0 | 3 | 7.17 | 4 | 8.15 | 6.35 |
| **17l** | 0 | 4 | 5.93 | 3 | 8.13 | 5.12 |
| **17m** | 0 | 3 | 5.22 | 3 | 7.48 | 4.87 |
| **17n** | 0 | 6 | 5.55 | 3 | 7.48 | 5.20 |
| **17o** | 0 | 4 | 5.23 | 3 | 8.88 | 3.75 |
| **17p** | 0 | 3 | 4.82 | 3 | 6.53 | 4.76 |
| **17q** | 2 | 3 | 5.68 | 3 | 8.54 | 4.52 |
| **17r** | 2 | 3 | 4.10 | 4 | 6.61 | 4.04 |
| **17s** | 2 | 5 | 5.09 | 4 | 6.68 | 5.01 |

[a] Calculated values for p$K_a$, log *P* (octanol/water partition of the neutral species), and log *D* (octanol/buffer partition of ionized and unionized molecules) were determined with ACD/p$K_a$, ACD/log *P*, and ACD/log *D* software from Advanced Chemistry Development, Toronto, Canada. [b] Determined at pH 7.4.

## Experimental Section

*General methods.* Melting points were determined on a Wagner & Munz PolyTherm D melting point apparatus and are uncorrected. Proton NMR spectra were obtained with a Varian Unity 300 MHz spectrometer. Elemental analyses for carbon, hydrogen, and nitrogen for target compounds that are critical for the interpretation of SAFIR were performed by Servei de Microanàlisi in the Consejo Superior de Investigaciones Científicas of Barcelona (CSIC) and were

**Table 10.** Experimental druglike properties for selected compounds.

| Compound | Solubility at pH 2 [μg mL$^{-1}$] | Solubility at pH 6.5 [μg mL$^{-1}$] | Solubility at pH 7.4 [μg mL$^{-1}$] | log *P* | Solution stability at pH 2 [h] | Solution stability at pH 6.5 [h] | Solution stability at pH 7.4 [h] | Solution stability at pH 6.5+*hν* [h] |
|---|---|---|---|---|---|---|---|---|
| **7b**[a] | ≥1000 | ND | ≥1000 | ND | >20 | >20 | >20 | >20 |
| **7d**[a] | ND | ND | ND | ND | >20 | >20 | >20 | >20 |
| **7e**[a] | ≥1000 | ≥1000 | ≥1000 | 3.63 | >20 | >20 | >20 | >20 |
| **7f**[a] | ≥1000 | ≥1000 | ≥1000 | 2.41 | >20 | >20 | >20 | >20 |
| **7r**[a] | ≥1000 | ND | ≥1000 | ND | >20 | >20 | >20 | >20 |
| **7s**[a] | ≥1000 | ND | 440 | ND | >20 | >20 | >20 | >20 |
| **7t** | ND | ND | ND | ND | >20 | >20 | >20 | >20 |
| **14b**[a] | ≥1000 | ND | ≥1000 | ND | >20 | >20 | >20 | >20 |
| **17a**[a] | ≥1000 | ≥1000 | ≥1000 | 3.68 | 6%[b] | >20 | >20 | >20 |
| **17b**[a] | ≥1000 | ND | ≥1000 | ND | 6%[b] | >20 | >20 | >20 |

[a] Determined as oxalate salts. [b] Percent degradation.

within ±0.4% of theory for the formulas given unless otherwise indicated (Supporting Information). Furthermore, for critical compounds, the purity was found to be >95% in each case, as determined by HPLC under the following conditions: Waters Alliance 2690 and 2695 (software: Millenium 3.20, Empower Pro) and Agilent 1100 (software: Chemstation A.06.03) equipped with Waters Symmetry C8 (particle size = 5 μm, 150 mm × 3.9 mm (∅)) and eluted with mixtures of acetonitrile and water containing heptanesulfonate (10 mM) and $KH_2PO_4$ (25 mM) at pH 2.5; Waters XTerra MS-C8 (particle size = 3.5 μm, 100 mm × 3 mm (∅)) and eluted with the following mobile phases: a) acetonitrile containing TFA (0.04%) and water containing TFA (0.05%); b) acetonitrile containing formic acid (0.04%) and water containing formic acid (0.05%); c) acetonitrile and phosphate buffer (10 mM, pH 7.0); d) acetonitrile and ammonium bicarbonate buffer (10 mM, pH 10.0); e) acetonitrile and sodium tetraborate buffer (10 mM, pH 9.2); Waters XTerra MS-C8 (particle size = 3.5 μm, 100 mm × 3.9 mm (∅)) and eluted with mixtures of acetonitrile and formic acid (10 mM) adjusted to pH 7 with ammonia or with mixtures of acetonitrile and ammonium bicarbonate buffer (10 mM, pH 7.8); Phenomenex Gemini C18 (particle size = 5 μm, 100 mm × 4.6 mm (∅)) and eluted with mixtures of acetonitrile and ammonium bicarbonate buffer (10 mM, pH 7.8 and pH 10.0, adjusted with aqueous $NH_3$). Furthermore, for selected final compounds, EI HRMS data were obtained at Lab. Espectrometría Masas y Chromatografía, Universidad de Córdoba, with a Waters VG Autospec instrument. Chiral resolution of racemic **7f** was performed by preparative chiral HPLC under the following conditions: Waters 600E pump, 486 UV detector and HP1050 injector equipped with a Daicel Chiralpak AS column particle size = 10 μm, 25 cm × 2 cm (∅)). The mobile phase used was *n*-hexane/ethanol (95:5 *v/v*) at a flow rate of 13 mL min$^{-1}$ (retention time for (+)-**7f**: ≈10 min; retention time for (−)-**7f**: ≈12 min). Analytical TLC was conducted on precoated silica gel 60 $F_{254}$ plates (Merck). Silica gel 60, 220–400 mesh, was used for flash chromatography purification. All starting materials were obtained from commercial sources and used as received. The synthesis of compounds **1**, **2a**, **2b**, **3b**, **8**, and **15a** have been described previously.[37] Compounds **2c**, **2d**, **3a**, **3c**, **3d**, **9**, **10**, and **15b** were synthesized analogously according to the same process.[37] For the analyses of solubility and C, H, and N content, some amine groups were converted into their corresponding oxalate salts by adding oxalic acid monohydrate (1.1 equiv; for **14i**, 2.2 equiv) in acetone (used as obtained without further purification), followed by filtration of the resulting solid and drying under vacuum.

*General procedure for the preparation of esters **4** and **11**: rac-ethyl 2-(4,5,6,7-tetrahydro-1-phenyl-1H-indazol-4-yl)acetate (**4b**).* Pd-C (150 mg, 10%) was added to a solution of a mixture of *E* and *Z* isomers of ethyl (1,5,6,7-tetrahydro-1-phenyl-4*H*-indazol-4-ylidene)acetate (**3b**, 1.60 g, 5.67 mmol) in EtOH (50 mL), and the resulting reaction mixture was stirred under hydrogen atmosphere (50 psi) in a Parr hydrogenator for 18 h. The reaction mixture was purged with nitrogen, filtered through Celite, and the solvent was evaporated under decreased pressure to obtain **4b** (1.60 g 99%) as a colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ = 1.29 (t, $J$ = 7.2 Hz, 3H), 1.48 (m, 1H), 1.73 (m, 1H), 1.98 (m, 2H), 2.45 (dd, $J$ = 15.4 Hz, $J'$ = 8.0 Hz, 1H), 2.68 (m, 3H), 3.26 (m, 1H), 4.20 (q, $J$ = 7.2 Hz, 2H), 7.31 (m, 1H), 7.41–7.52 ppm (m, 5H).

Compounds **4a**, **4c**, **4d** and **11** were synthesized analogously:

*rac-Ethyl 2-(1-methyl-4,5,6,7-tetrahydro-1H-indazol-4-yl)acetate (**4a**).* Colorless oil; yield 96%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.28 (t, $J$ = 7.1 Hz, 3H), 1.36 (dd, $J$ = 15.2 Hz, $J'$ = 7.8 Hz, 1H), 1.39 (m, 1H), 1.76 (m, 1H), 1.94 (m, 2H), 2.53 (m, 2H), 2.60 (dd, $J$ = 15.2 Hz, $J'$ = 7.8 Hz, 1H), 3.17 (m, 1H), 3.70 (s, 3H), 4.19 (q, $J$ = 7.1 Hz, 2H), 7.24 ppm (s, 1H).

*rac-Ethyl 2-(1-(3,4-dichlorophenyl)-4,5,6,7-tetrahydro-1H-indazol-4-yl)acetate (**4c**).* Yellowish oil; yield 81%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.29 (t, $J$ = 7.2 Hz, 3H), 1.45 (m, 1H), 1.74 (m, 1H), 1.98 (m, 2H), 2.44 (dd, $J$ = 15.4 Hz, $J'$ = 7.9 Hz, 1H), 2.61–2.73 (m, 3H), 3.24 (m, 1H), 4.20 (q, $J$ = 7.2 Hz, 2H), 7.36 (dd, $J$ = 8.8 Hz, $J'$ = 2.5 Hz, 1H), 7.50 (m, 2H), 7.64 ppm (d, $J$ = 2.5 Hz, 1H).

*rac-Ethyl 2-(1-(4-fluorophenyl)-4,5,6,7-tetrahydro-1H-indazol-4-yl)acetate (**4d**).* Colorless oil; yield 97%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.29 (t, $J$ = 7.1 Hz, 3H), 1.46 (m, 1H), 1.75 (m, 1H), 1.97 (m, 2H), 2.45 (dd, $J$ = 15.4 Hz, $J'$ = 7.9 Hz, 1H), 2.65 (m, 3H), 3.25 (m, 1H), 4.20 (q, $J$ = 7.1 Hz, 2H), 7.14 (m, 2H), 7.44 (m, 2H), 7.51 ppm (s, 1H).

*rac-Ethyl 2-(1-phenyl-1,4,5,6-tetrahydrocyclopenta[c]pyrazol-4-yl)acetate (**11**).* Colorless oil; yield 85%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.28 (t, $J$ = 7.2 Hz, 3H), 2.25 (m, 1H), 2.55 (m, 2H), 2.90 (m, 1H), 3.00 (m, 2H), 3.47 (m, 1H), 4.18 (q, $J$ = 7.2 Hz, 2H), 7.23 (m, 1H), 7.41 (m, 3H), 7.62 ppm (m, 2H).

*General procedure for the preparation of alcohols **5** and **12**: rac-2-(4,5,6,7-tetrahydro-1-phenyl-1H-indazol-4-yl)ethanol (**5b**).* A solution of *rac*-ethyl 2-(4,5,6,7-tetrahydro-1-phenyl-1*H*-indazol-4-yl)acetate (**4b**, 1.40 g, 5.79 mmol) in THF (5 mL) was added to a suspension of lithium aluminum hydride (300 mg, 7.90 mmol) in THF (20 mL), and the reaction mixture was stirred at room temperature for 18 h. After heating at reflux for 1 h, the mixture was hydrolyzed with ice and aqueous NaOH (10%), filtered through celite, and the solvent was evaporated under decreased pressure to obtain **5b** as colorless oil (1.10 g, 79%); $^1$H NMR ($CDCl_3$): $\delta$ = 1.45 (m, 1H), 1.72 (m, 2H), 1.98 (m, 3H), 2.32 (br s, 1H), 2.71 (m, 2H), 2.91 (m, 1H), 3.84 (t, $J$ = 6.7 Hz, 2H), 7.32 (m, 1H), 7.40–7.55 (m, 4H), 7.57 ppm (s, 1H).

Compounds **5a**, **5c**, **5d**, and **12** were synthesized analogously:

*rac-2-(1-Methyl-4,5,6,7-tetrahydro-1H-indazol-4-yl)ethanol (**5a**).* Colorless oil; yield 36%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.37 (m, 1H), 1.70 (m, 2H),

1.84 (m, 3H), 2.53 (m, 2H), 2.80 (m, 1H), 3.74 (s, 3H), 3.79 (t, $J$= 6.7 Hz, 2H), 7.32 ppm (s, 1H).

*rac-2-(1-(3,4-Dichlorophenyl)-4,5,6,7-tetrahydro-1H-indazol-4-yl)ethanol (**5c**).* Colorless oil; yield 56%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.43 (m, 1H), 1.70 (m, 2H), 1.90–2.18 (m, 4H), 2.69 (m, 2H), 2.87 (m, 1H), 3.79 (t, $J$ = 6.6 Hz, 2H), 7.35 (dd, $J$ = 8.7 Hz, $J'$ = 2.5 Hz, 1H), 7.49 (d, $J$ = 8.7 Hz, 1H), 7.53 (s, 1H), 7.64 ppm (d, $J$ = 2.5 Hz, 1H).

*rac-2-(1-(4-Fluorophenyl)-4,5,6,7-tetrahydro-1H-indazol-4-yl)ethanol (**5d**).* Colorless oil; yield 65%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.42 (m, 1H), 1.73 (m, 2H), 1.98 (m, 4H), 2.65 (m, 2H), 2.89 (m, 1H), 3.83 (t, $J$ = 6.7 Hz, 2H), 7.13 (t, $J$ = 8.5 Hz, 2H), 7.45 (m, 2H), 7.52 ppm (s, 1H).

*rac-2-(1-Phenyl-1,4,5,6-tetrahydrocyclopenta[c]pyrazol-4-yl)ethanol (**12**).* Colorless oil; yield 94%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.82 (m, 2H), 2.20–2.40 (m, 2H), 2.81 (m, 1H), 3.00 (m, 2H), 3.19 (m, 1H), 3.80 (t, $J$ = 6.6 Hz, 2H), 7.25 (m, 1H), 7.43 (m, 3H), 7.63 ppm (m, 2H).

*General procedure for the preparation of chlorine derivatives **6** and **13**: rac-4-(2-chloroethyl)-4,5,6,7-tetrahydro-1-phenyl-1H-indazole (**6b**).* Thionyl chloride (4 mL) was slowly added at room temperature to a solution of *rac*-2-(4,5,6,7-tetrahydro-1-phenyl-1*H*-indazol-4-yl)ethanol (**5b**, 516 mg, 2.13 mmol) in toluene (25 mL). The reaction mixture was heated at 90 °C for 2 h. The solvent was evaporated at decreased pressure, and the crude product was diluted in ethyl acetate and washed twice with saturated aqueous $NaHCO_3$. The organic phase was separated, and the solvent was evaporated at decreased pressure to obtain **6** (423 mg, 76%) as a colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ = 1.45 (m, 1H), 1.73 (m, 1H), 1.94 (m, 3H), 2.18 (m, 1H), 2.71 (m, 2H), 3.00 (m, 1H), 3.70 (t, $J$ = 6.8 Hz, 2H), 7.32 (m, 1H), 7.47 (m, 4H), 7.55 ppm (s, 1H).

Compounds **6a**, **6c**, **6d**, and **13** were synthesized analogously:

*General procedure for the preparation of amine derivatives **7** and **14**: rac-4,5,6,7-tetrahydro-4-(2-(morpholin-4-yl)ethyl)-1-phenyl-1H-indazole (**7f**).* A mixture of *rac*-4-(2-chloroethyl)-4,5,6,7-tetrahydro-1-phenyl-1*H*-indazole (**6b**, 423 mg, 1.62 mmol), $K_2CO_3$ (336 mg, 2.43 mmol), morpholine (0.420 mL, 4.87 mmol) and a catalytic amount of KI in DMF (20 mL) was heated at reflux for 18 h. The solvent was evaporated at decreased pressure, and the crude product was dissolved with ethyl acetate and washed with water. The organic phase was separated and the solvent was evaporated at decreased pressure. The resultant crude product was purified by chromatography on silica gel ($CHCl_3$/MeOH, 9:1) to obtain **7f** (384 mg, 76%) as a colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ = 1.42 (m, 1H), 1.67 (m, 2H), 1.92 (m, 3H), 2.40–2.60 (m, 6H), 2.69 (m, 2H), 2.77 (m, 1H), 3.74 (m, 4H), 7.29 (m, 1H), 7.44 (m, 4H), 7.51 ppm (s, 1H). Anal. **7f**·oxalate ($C_{19}H_{25}N_3O{\cdot}C_2H_2O_4$) C, H, N.

The following compounds were prepared analogously from the appropriate amines and the corresponding chloro derivatives **6** and **13**:

*rac-4,5,6,7-Tetrahydro-1-methyl-4-(2-(morpholin-4-yl)ethyl)-1H-indazole (**7a**).* Yellowish oil; yield 32%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.35 (m, 1H), 1.65 (m, 2H), 1.92 (m, 3H), 2.42–2.60 (m, 8H), 2.66 (m, 1H), 3.71 (s, 3H), 3.75 (m, 4H), 7.27 ppm (s, 1H). Anal. **7a**·oxalate ($C_{14}H_{23}N_3O{\cdot}C_2H_2O_4{\cdot}\frac{1}{3}H_2O$) C, H, N.

*rac-4,5,6,7-Tetrahydro-1-methyl-4-(2-(4-phenylpiperidin-1-yl)ethyl)-1H-indazole (**7b**).* Colorless oil; yield 39%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.39 (m, 1H), 1.63–2.40 (m, 11H), 2.42–2.78 (m, 6H), 3.24 (m, 2H), 3.71 (s, 3H), 7.18–7.38 ppm (m, 6H). Anal. **7b**·oxalate ($C_{21}H_{29}N_3{\cdot}C_2H_2O_4{\cdot}\frac{3}{4}H_2O$) C, H, N.

*rac-N-Benzyl-2-(4,5,6,7-tetrahydro-1-methyl-1H-indazol-4-yl)-N-methylethanamine (**7c**).* Colorless oil; yield 43%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.30 (m, 1H), 1.68 (m, 2H), 1.92 (m, 3H), 2.27 (s, 3H), 2.42–2.62 (m, 4H), 2.69 (m, 1H), 3.60 (m, 2H), 3.70 (s, 3H), 7.20–7.42 ppm (m, 6H). Anal. **7c**·oxalate ($C_{18}H_{25}N_3{\cdot}C_2H_2O_4{\cdot}\frac{1}{3}H_2O$) C, H, N.

*rac-4,5,6,7-Tetrahydro-4-(2-(isoindolin-2-yl)ethyl)-1-methyl-1H-indazole (**7d**).* Brown oil; yield 45%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.40 (m, 1H), 1.76 (m, 2H), 1.98 (m, 3H), 2.54 (br s, 2H), 2.79 (m, 1H), 2.93 (m, 2H), 3.71 (s, 3H), 4.08 (m, 4H), 7.18–7.40 ppm (m, 5H). Anal. **7d**·oxalate ($C_{18}H_{23}N_3{\cdot}C_2H_2O_4{\cdot}\frac{1}{3}H_2O$) C, H, N.

*rac-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(piperidin-1-yl)ethyl)-1H-indazole (**7e**).* Colorless oil; yield 23%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.44 (m, 3H), 1.69 (m, 6H), 1.95 (m, 3H), 2.55 (m, 6H), 2.69 (m, 2H), 2.76 (m, 1H), 7.30 (m, 1H), 7.40–7.50 (m, 4H), 7.51 ppm (s, 1H). Anal. **7e**·oxalate ($C_{20}H_{27}N_3{\cdot}C_2H_2O_4{\cdot}\frac{1}{4}H_2O$) C, H, N: calcd, 7.36; found, 7.71.

*rac-4,5,6,7-Tetrahydro-4-(2-(4-methylpiperidin-1-yl)ethyl)-1-phenyl-1H-indazole (**7g**).* Colorless oil; yield 40%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.00 (d, $J$ = 6.3 Hz, 3H), 1.40–2.25 (m, 11H), 2.50 (m, 2H), 2.69 (m, 2H), 2.80–3.00 (m, 3H), 3.40 (m, 2H), 7.33 (m, 1H), 7.46 (m, 4H), 7.49 ppm (s, 1H). Anal. **7g**·oxalate ($C_{21}H_{29}N_3{\cdot}C_2H_2O_4{\cdot}\frac{1}{4}H_2O$) C, H, N.

*rac-cis-4,5,6,7-Tetrahydro-4-(2-(2,6-dimethylmorpholin-4-yl)ethyl)-1-phenyl-1H-indazole (**7h**).* Orange oil; yield 51%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.42 (m, 1H), 1.56–1.84 (m, 4H), 1.70 (d, $J$ = 6.3 Hz, 6H), 1.94 (m, 3H), 2.51 (m, 2H), 2.62–2,90 (m, 5H), 3.74 (m, 2H), 7.30 (m, 1H), 7.40–7.52 ppm (m, 5H). Anal. **7h**·oxalate ($C_{21}H_{29}N_3O{\cdot}C_2H_2O_4$) C, H, N.

*rac-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(4-phenylpiperidin-1-yl)ethyl)-1H-indazole (**7i**).* Colorless oil; yield 28%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.44 (m, 1H), 1.70 (m, 1H), 1.79–2.11 (m, 8H), 2.23 (m, 2H), 2.48–2.85 (m, 6H), 3.22 (m, 2H), 7.16–7.35 (m, 6H), 7.40–7.52 (m, 4H), 7.54 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 385.2518; found, 385.2516.

*rac-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(4-phenylpiperazin-1-yl)ethyl)-1H-indazole (**7k**).* Colorless oil; yield 36%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.44 (m, 1H), 1.62–1.78 (m, 2H), 1.99 (m, 3H), 2.58 (m, 2H), 2.63–2.75 (m, 6H), 2.80 (m, 1H), 3.23 (m, 4H), 6.85 (t, $J$ = 7.3 Hz, 1H), 6.94 (d, $J$ = 8.8 Hz, 2H), 7.23–7.34 (m, 3H), 7.40–7.52 (m, 4H), 7.55 ppm (s, 1H). Anal. **7k**·oxalate ($C_{25}H_{30}N_4{\cdot}C_2H_2O_4{\cdot}\frac{3}{4}H_2O$) C, H, N.

*rac-4,5,6,7-Tetrahydro-1-phenyl-4-(2-[spiro{isobenzofuran-1(3H),4′-piperidin}-1′-yl]ethyl)-1H-indazole (**7l**).* Colorless oil; yield 34%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.40 (m, 1H), 1.60–1.80 (m, 4H), 1.88–2.05 (m, 5H), 2.40 (m, 2H), 2.57 (t, $J$ = 7.9 Hz, 2H), 2.67 (m, 2H), 2.74 (m, 1H), 2.90 (m, 2H), 5.04 (s, 2H), 7.10–7.30 (m, 5H), 7.37–7.48 (m, 4H), 7.51 ppm (s, 1H). Anal. **7l**·oxalate ($C_{27}H_{31}N_3O{\cdot}C_2H_2O_4{\cdot}\frac{3}{4}H_2O$) C, H, N.

*rac-N-Benzyl-2-(4,5,6,7-tetrahydro-1-phenyl-1H-indazol-4-yl)-N-methylethanamine (**7m**).* Yellowish oil; yield 45%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.39 (m, 1H), 1.67 (m, 2H), 1.93 (m, 3H), 2.29 (s, 3H), 2.60 (m, 2H), 2.69 (m, 2H), 2.81 (m, 1H), 3.59 (m, 2H), 7.20–7.53 ppm (m, 11H); EI HRMS [$M^+$]: calcd, 345.2205; found, 345.2204. Anal. **7m** ($C_{23}H_{27}N_3{\cdot}\frac{1}{2}H_2O$) C, H, N.

*rac-4,5,6,7-Tetrahydro-4-(2-(isoindolin-2-yl)ethyl)-1-phenyl-1H-indazole (**7n**).* Dark brown oil; yield 62%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.48 (m, 1H), 1.62–1.91 (m, 2H), 1.92–2.15 (m, 3H), 2.72 (br s, 2H), 2.83–3.05 (m, 3H), 4.09 (s, 4H), 7.16–7.39 (m, 5H), 7.40–7.60 ppm (m, 5H); EI HRMS [$M^+$]: calcd, 343.2048; found, 343.2048. Anal. **7n**·oxalate ($C_{23}H_{25}N_3{\cdot}C_2H_2O_4{\cdot}\frac{1}{3}H_2O$) C, H, N.

*rac-4-(2-(4-Benzylpiperazin-1-yl)ethyl)-4,5,6,7-tetrahydro-1-phenyl-1H-indazole (**7o**).* Beige solid; yield 23%; m.p. 98–101 °C; $^1$H NMR ($CDCl_3$): $\delta$ = 1.42 (m, 1H), 1.68 (m, 2H), 1.92 (m, 3H), 2.40–2.80 (m,

13H), 3.52 (s, 2H), 7.20–7.37 (m, 6H), 7.39–7.52 ppm (m, 5H); EI HRMS [$M^+$]: calcd, 400.2627; found, 400.2642. Anal. **7o** ($C_{26}H_{32}N_4 \cdot \frac{1}{2}H_2O$) C, H, N.

*(−)-4,5,6,7-Tetrahydro-4-(2-(morpholin-4-yl)ethyl)-1-phenyl-1H-indazole ((−)-**7f**).* Starting from 300 mg **7f** (0.96 mmol), 120 mg (−)-**7f** were obtained by semipreparative HPLC (as described above in general methods): colorless oil; yield 80%; $[\alpha]_D^{20} = -5.8$ ($CHCl_3$, $c = 1$); >98.4% *ee*. Anal. (−)-**7f**·oxalate ($C_{19}H_{25}N_3O \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*(+)-4,5,6,7-Tetrahydro-4-(2-(morpholin-4-yl)ethyl)-1-phenyl-1H-indazole ((+)-**7f**).* Starting from 300 mg **7f** (0.96 mmol), 119 mg (+)-**7f** were obtained by semipreparative HPLC (as described above in general methods): colorless oil; yield 80%; $[\alpha]_D^{20} = +4.3$ ($CHCl_3$, $c = 1$); >99.9% *ee*. Anal. (+)-**7f**·oxalate ($C_{19}H_{25}N_3O \cdot C_2H_2O_4$) C, H, N.

*rac-1-(3,4-Dichlorophenyl)-4,5,6,7-tetrahydro-4-(2-(morpholino-4-yl)ethyl)-1H-indazole (**7p**).* Yellowish oil; yield 23%; $^1$H NMR ($CDCl_3$): $\delta = 1.41$ (m, 1H), 1.66 (m, 2H), 1.93 (m, 3H), 2.47 (m, 6H), 2.62–2.79 (m, 3H), 3.71 (t, $J = 4.7$ Hz, 4H), 7.34 (dd, $J = 8.7$ Hz, $J' = 2.5$ Hz, 1H), 7.48 (d, $J = 8.7$ Hz, 1H), 7.49 (s, 1H), 7.63 ppm (d, $J = 2.5$ Hz, 1H); EI HRMS [$M^+$]: calcd, 379.1218; found, 379.1225.

*rac-1-(3,4-Dichlorophenyl)-4,5,6,7-tetrahydro-4-(2-(4-phenylpiperidin-1-yl)ethyl)-1H-indazole (**7q**).* Yellowish oil; yield 45%; $^1$H NMR ($CDCl_3$): $\delta = 1.36$–2.30 (m, 12H), 2.50- 2.83 (m, 6H), 3.20 (m, 2H), 7.18–7.40 (m, 6H), 7.51 (d, $J = 8.6$ Hz, 1H), 7.55 (s, 1H), 7.66 ppm (d, $J = 2.3$ Hz, 1H); EI HRMS [$M^+$]: calcd, 453.1739; found, 453.1716. Anal. **7q**·oxalate ($C_{26}H_{29}N_3Cl_2 \cdot C_2H_2O_4 \cdot \frac{3}{4}H_2O$) C, H, N.

*rac-1-(4-Fluorophenyl)-4,5,6,7-tetrahydro-4-(2-(morpholin-4-yl)ethyl)-1H-indazole (**7r**).* Orange oil; yield 46%; $^1$H NMR ($CDCl_3$): $\delta = 1.43$ (m, 1H), 1.69 (m, 2H), 1.94 (m, 3H), 2.52 (m, 6H), 2.65 (m, 2H), 2.77 (m, 1H), 3.75 (m, 4H), 7.13 (m, 2H), 7.44 (dd, $J = 9.2$ Hz, $J' = 4.8$ Hz, 2H), 7.50 ppm (s, 1H). Anal. **7r**·oxalate ($C_{19}H_{24}N_3OF \cdot C_2H_2O_4 \cdot \frac{1}{3}H_2O$) C, H, N.

*rac-cis-1-(4-Fluorophenyl)-4,5,6,7-tetrahydro-4-(2-(2,6-dimethylmorpholin-4-yl)ethyl)-1H-indazole (**7s**).* Orange oil; yield 59%; $^1$H NMR ($CDCl_3$): $\delta = 1.17$ (d, $J = 6.3$ Hz, 6H), 1.41 (m, 1H), 1.60–1.81 (m, 4H), 1.94 (m, 3H), 2.48 (t, $J = 7.7$ Hz, 2H), 2.65 (m, 2H), 2.71–2.86 (m, 3H), 3.71 (m, 2H), 7.13 (m, 2H), 7.44 (dd, $J = 9.1$ Hz, $J' = 4.8$ Hz, 2H), 7.50 ppm (s, 1H). Anal. **7s**·oxalate ($C_{21}H_{28}N_3OF \cdot C_2H_2O_4 \cdot \frac{1}{2}H_2O$) C, H, N.

*rac-N-Benzyl-2-(1-(4-fluorophenyl)-4,5,6,7-tetrahydro-1H-indazol-4-yl)-N-methylethanamine (**7t**).* Orange oil; yield 52%; $^1$H NMR ($CD_3OD$): $\delta = 1.42$ (m, 1H), 1.70 (m, 2H), 1.96 (m, 3H), 2.29 (s, 3H), 2.54–2.67 (m, 4H), 2.57 (m, 1H), 3.59 and 3.61 (AB system, $J_{AB} = 12.8$ Hz, 2H), 7.21–7.40 (m, 7H), 7.43–7.56 ppm (m, 3H); EI HRMS [$M^+$]: calcd, 363.2111; found, 363.2083.

*rac-1-(4-Fluorophenyl)-4,5,6,7-tetrahydro-4-(2-(isoindolin-2-yl)ethyl)-1H-indazole (**7u**).* Brown oil; yield 59%; $^1$H NMR ($CDCl_3$): $\delta = 1.48$ (m, 1H), 1.74 (m, 2H), 2.00 (m, 3H), 2.67 (m, 2H), 2.82–2.94 (m, 3H), 4.00 (s, 4H), 7.13 (m, 2H), 7.21 (m, 4H), 7.46 (dd, $J = 8.9$ Hz, $J' = 4.8$ Hz, 2H), 7.55 ppm (s, 1H). Anal. **7u**·oxalate ($C_{23}H_{24}N_3F \cdot C_2H_2O_4$) C, H, N.

*rac-1,4,5,6-Tetrahydro-1-phenyl-4-(2-(piperidin-1-yl)ethyl)cyclopenta[c]-pyrazole (**14a**).* Orange solid; yield 10%; m.p. 213–219 °C; $^1$H NMR ($CDCl_3$): $\delta = 1.49$ (m, 2H), 1.70 (m, 4H), 1.86 (m, 2H), 2.24 (m, 1H), 2.56 (m, 6H), 2.79 (m, 1H), 2.90–3.10 (m, 3H), 7.23 (m, 1H), 7.37 (s, 1H), 7.41 (m, 2H), 7.62 ppm (d, $J = 7.9$ Hz, 2H); EI HRMS [$M^+$]: calcd, 295.2048; found, 295.2046. Anal. **14a**·oxalate ($C_{19}H_{25}N_3 \cdot C_2H_2O_4 \cdot \frac{1}{3}H_2O$) C, H, N.

*rac-1,4,5,6-Tetrahydro-4-(2-(morpholin-4-yl)ethyl)-1-phenylcyclopenta[c]pyrazole (**14b**).* Colorless oil; yield 72%; $^1$H NMR ($CDCl_3$): $\delta = 1.82$ (m, 2H), 2.25 (m, 1H), 2.59 (m, 6H), 2.80 (m, 1H), 2.89–3.12 (m, 3H), 3.80 (br s, 4H), 7.23 (m, 1H), 7.37 (s, 1H), 7.41 (m, 2H), 7.61 ppm (d, $J = 8.2$ Hz, 2H); EI HRMS [$M^+$]: calcd, 297.1841; found, 297.1844.

*rac-1,4,5,6-Tetrahydro-4-(2-(4-methylpiperidin-1-yl)ethyl)-1-phenylcyclopenta[c]pyrazole (**14c**).* Beige solid; yield 46%; m.p. 78–82 °C; $^1$H NMR ($CDCl_3$): $\delta = 0.93$ (d, $J = 5.9$ Hz, 3H), 1.35 (m, 3H), 1.65 (m, 2H), 1.80 (m, 2H), 1.99 (m, 2H), 2.24 (m, 1H), 2.49 (m, 2H), 2.80 (m, 1H), 3.01 (m, 5H), 7.23 (m, 1H), 7.38 (s, 1H), 7.42 (m, 2H), 7.62 ppm (d, $J = 8.2$ Hz, 2H). Anal. **14c**·oxalate ($C_{20}H_{27}N_3 \cdot C_2H_2O_4 \cdot \frac{2}{3}H_2O$) C, H, N.

*rac-1,4,5,6-Tetrahydro-1-phenyl-4-[2-(thiomorpholin-4-yl)ethyl]cyclopenta[c]pyrazole (**14d**).* Brown oil; yield 56%; $^1$H NMR ($CDCl_3$): $\delta = 1.77$ (m, 2H), 2.23 (m, 1H), 2.54 (m, 2H), 2.65–2.90 (m, 9H), 3.01 (m, 3H), 7.24 (m, 1H), 7.37 (s, 1H), 7.42 (m, 2H), 7.62 ppm (d, $J = 8.5$ Hz, 2H). Anal. **14d**·oxalate ($C_{18}H_{23}N_3S \cdot C_2H_2O_4$) C, H, N.

*rac-cis-1,4,5,6-Tetrahydro-4-(2-(2,6-dimethylmorpholin-4-yl)ethyl)-1-phenylcyclopenta-[c]pyrazole (**14e**).* Orange oil; yield 64%; $^1$H NMR ($CDCl_3$): $\delta = 1.17$ (d, $J = 5.9$ Hz, 6H), 1.78 (m, 4H), 2.23 (m, 1H), 2.48 (m, 2H), 2.82 (m, 3H), 3.02 (m, 3H), 3.74 (m, 2H), 7.23 (m, 1H), 7.38 (s, 1H), 7.42 (m, 2H), 7.62 ppm (d, $J = 7.7$ Hz, 2H). Anal. **14e**·oxalate ($C_{20}H_{27}N_3O \cdot C_2H_2O_4 \cdot \frac{1}{3}H_2O$) C, H, N.

*rac-1,4,5,6-Tetrahydro-1-phenyl-4-(2-(pyrrolidin-1-yl)ethyl)cyclopenta[c]pyrazole (**14f**).* Yellowish oil; yield 38%; $^1$H NMR ($CDCl_3$): $\delta = 1.84$ (m, 6H), 3.25 (m, 1H), 2.65 (m, 6H), 2.79 (m, 1H), 2.90–3.12 (m, 3H), 7.23 (m, 1H), 7.38 (s, 1H), 7.42 (m, 2H), 7.62 ppm (d, $J = 7.8$ Hz, 2H); EI HRMS [$M^+$]: calcd, 281.1892; found, 281.1885. Anal. **14f**·oxalate ($C_{18}H_{23}N_3 \cdot \frac{7}{5}C_2H_2O_4$) C, H, N.

*rac-1,4,5,6-Tetrahydro-1-phenyl-4-(2-(4-phenylpiperidin-1-yl)ethyl)cyclopenta[c]pyrazole (**14g**).* Beige solid; yield 27%; m.p. 83–86 °C; $^1$H NMR ($CDCl_3$): $\delta = 1.63$ (m, 2H), 1.90–2.18 (m, 3H), 2.35 (m, 2H), 2.52–3.20 (m, 9H), 3.69 (m, 2H), 7.20–7.46 (m, 9H), 7.61 ppm (d, $J = 7.8$ Hz, 2H); EI HRMS [$M^+$]: calcd, 371.2361; found, 371.2366.

*rac-1,4,5,6-Tetrahydro-1-phenyl-4-(2-(4-phenylpiperazin-1-yl)ethyl)cyclopenta[c]pyrazole (**14h**).* Beige solid; yield 32%; m.p. 82–83 °C; $^1$H NMR ($CDCl_3$): $\delta = 1.82$ (m, 2H), 2.24 (m, 1H), 2.58 (m, 2H), 2.68 (m, 4H), 2.80 (m, 1H), 2.90–3.20 (m, 3H), 3.24 (m, 4H), 6.86 (m, 1H), 6.94 (d, $J = 8.1$ Hz, 2H), 7.22–7.30 (m, 3H), 7.40 (s, 1H), 7.42 (m, 2H), 7.63 ppm (d, $J = 8.1$ Hz, 2H). Anal. **14h** ($C_{24}H_{28}N_4 \cdot \frac{1}{4}H_2O$) C, H, N; EI HRMS [$M^+$]: calcd, 372.2314; found, 372.2320.

*rac-4-(2-(4-Benzylpiperazin-1-yl)ethyl)-1,4,5,6-tetrahydro-1-phenylcyclopenta[c]pyrazole (**14i**).* Orange oil; yield 62%; $^1$H NMR ($CDCl_3$): $\delta = 1.76$ (m, 2H), 2.22 (m, 1H), 2.38–2.65 (m, 10), 2.78 (m, 1H), 2.90–3,10 (m, 3H), 3.52 (s, 2H), 7.18–7.39 (m, 6H), 7.41 (s, 1H), 7.44 (m, 2H), 7.62 ppm (d, $J = 7.8$ Hz, 2H); EI HRMS [$M^+$]: calcd, 386.2470; found, 386.2446. Anal. **14i**·dioxalate ($C_{25}H_{30}N_4 \cdot 2C_2H_2O_4 \cdot 1\text{-}H_2O$) C, H, N.

*rac-1,4,5,6-Tetrahydro-1-phenyl-4-(2-[spiro[isobenzofuran-1(3H),4′-piperidin]-1′-yl]ethyl)cyclopenta[c]pyrazole (**14k**).* Yellowish oil; yield 74%; $^1$H NMR ($CDCl_3$): $\delta = 1.60$–1.92 (m, 4H), 2.00 (m, 2H), 2.24 (m, 1H), 2.43 (m, 2H), 2.57 (m, 2H), 2.70–3.12 (m, 6H), 5.06 (s, 2H), 7.05–7.30 (m, 5H), 7.40 (m, 3H), 7.62 ppm (d, $J = 7.6$ Hz, 2H); EI HRMS [$M^+$]: calcd, 329.1892; found, 329.1900. Anal. **14k**·oxalate ($C_{26}H_{29}N_3O \cdot C_2H_2O_4 \cdot \frac{3}{4}H_2O$) C, H, N.

*Mixture of diastereomers of rac-1,4,5,6-tetrahydro-1-phenyl-4-(2-(3-phenylpiperidin-1-yl)ethyl)cyclopenta[c]pyrazole (**14l**).* Colorless oil; yield 65%; $^1$H NMR ($CDCl_3$): $\delta = 1.53$ (m, 1H), 1.75–2.30 (m, 8H),

2.50–3.22 (m, 9H), 7.18–7.48 (m, 9H), 7.61 ppm (m, 2H); EI HRMS [$M^+$]: calcd, 371.2361; found, 371.2357.

*rac-1,2,3,4-Tetrahydro-2-(2-(1,4,5,6-tetrahydro-1-phenylcyclopenta[c]-pyrazol-4-yl)ethyl)isoquinoline (**14m**).* Beige amorphous oil; yield 39%; $^1$H NMR ($CDCl_3$): $\delta = 1.86$ (m, 2H), 2.28 (m, 1H), 2.65 (m, 2H), 2.70–3.18 (m, 8H), 3.66 (s, 2H), 7.03 (m, 1H), 7.11 (m, 3H), 7.23 (m, 1H), 7.41 (m, 3H), 7.63 ppm (d, $J = 7.8$ Hz, 2H); EI-HRMS [$M^+$]: calcd, 343.2048; found, 343.2035. Anal. **14m**·oxalate ($C_{23}H_{25}N_3 \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*rac-N-Benzyl-2-(1,4,5,6-tetrahydro-1-phenylcyclopenta[c]pyrazol-4-yl)-N-methylethanamine (**14n**).* Brown oil; yield 37%; $^1$H NMR ($CDCl_3$): $\delta = 1.82$ (m, 2H), 2.21 (m, 1H), 2.32 (br s, 3H), 2.61 (m, 2H), 2.77 (m, 1H), 2.97 (m, 2H), 3.08 (m, 1H), 3.64 (m, 2H), 7.20–7.45 (m, 9H), 7.61 ppm (d, $J = 7.8$ Hz, 2H); EI HRMS [$M^+$]: calcd, 331.2048; found, 331.2048.

*rac-2-(2-(1,4,5,6-Tetrahydro-1-phenylcyclopenta[c]pyrazol-4-yl)ethyl)isoindoline (**14o**).* Brown oil; yield 70%; $^1$H NMR ($CDCl_3$): $\delta = 1.97$ (m, 2H), 2.30 (m, 1H), 2.85 (m, 1H), 3.01 (m, 4H), 3.17 (m, 1H), 4.16 (s, 1H), 7.23 (s, 5H), 7.23 (s, 1H), 7.42 (m, 2H), 7.63 ppm (d, $J = 7.8$ Hz, 2H); EI HRMS [$M^+$]: calcd, 329.1892; found, 329.1900. Anal. **14o**·oxalate ($C_{22}H_{23}N_3 \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*rac-4-(2-(1H-Imidazol-1-yl)ethyl)-1,4,5,6-tetrahydro-1-phenylcyclopenta[c]pyrazole (**14p**).* Orange solid; yield 44%; m.p. 103–106 °C; $^1$H NMR ($CDCl_3$): $\delta = 2.04$ (m, 2H), 2.22 (m, 1H), 2.81 (m, 1H), 2.90–3.10 (m, 3H), 4.09 (m, 2H), 6.96 (s, 1H), 7.09 (s, 1H), 7.25 (m, 1H), 7.38 (s, 1H), 7.42 (m, 2H), 7.62 ppm (s, $J = 8.0$ Hz, 2H). Anal. **14p** ($C_{17}H_{18}N_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*General procedure for the preparation of aldehydes **16:** (E)-2-(1,5,6,7-tetrahydro-1-phenyl-4H-indazol-4-ylidene)acetaldehyde (**16a**).* Diisobutyl aluminum hydride in toluene (1 M, 0.7 mL, 0.7 mmol) was slowly added to a solution of (*E*)-2-(1,5,6,7-tetrahydro-1-phenyl-4*H*-indazol-4-ylidene)acetonitrile (**15a**, 120 mg, 0.51 mmol) in methylene chloride (5 mL), precooled at 0 °C. The mixture was stirred at 0 °C for 3 h, and the reaction was monitored by TLC. Water and a solution of HCl (0.5 N) was added dropwise consecutively, and the solids formed were filtered off. The solution was diluted in methylene chloride and washed with water and brine. The organic layer was dried over $Na_2SO_4$ and the solvent was removed at decreased pressure. The resulting crude product was purified with $SiO_2$ column chromatography ($CHCl_3$/MeOH, 9:1) to afford **16a** as a colorless oil (74.5 mg, 61%). $^1$H NMR ($CDCl_3$): $\delta = 2.02$ (m, 2H), 2.91 (m, 2H), 3.05 (m, 2H), 6.31 (d, $J = 8.1$ Hz, 1H), 7.35–7.55 (m, 5H), 7.93 ppm (s, 1H), 10.11 (d, $J = 8.1$ Hz, 1H).

Compound **16b** was synthesized analogously:

*(E)-2-(1-(3,4-dichlorophenyl)-6,7-dihydro-1H-indazol-4-ylidene)acetaldehyde (**16b**).* Yellowish oil; yield 67%; $^1$H NMR ($CDCl_3$): $\delta = 1.90$ (m, 2H), 2.97 (m, 2H), 3.32 (m, 2H), 6.34 (d, $J = 8.3$ Hz, 1H), 7.59 (m, 1H), 7.80 (m, 1H), 7.91 (d, $J = 2.3$ Hz, 1H), 8.35 (s, 1H), 10.05 ppm (d, $J = 8.3$ Hz, 1H).

*General procedure for the preparation of amines **17**: (E)-N-benzyl-2-(6,7-dihydro-1-phenyl-1H-indazol-4(5H)-yliden)-N-methylethanamine (**17n**).* A solution of (*E*)-2-(1,5,6,7-tetrahydro-1-phenyl-4*H*-indazol-4-ylidene)acetaldehyde (**16a**, 299 mg, 1.25 mmol) and *N*-benzylmethylamine (0.18 mL, 1.38 mmol) in MeOH (20 mL) was cooled at 0 °C and stirred for 20 min. Then, sodium cyanoborohydride (213 mg, 3.39 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 3 h, and the reaction was monitored by TLC. The solvent was removed at decreased pressure, and the crude product was dissolved in ethyl acetate and washed with water and brine. The organic layer was separated and dried with $Na_2SO_4$, and the solvent was removed at decreased pressure. The resulting crude product was purified with $SiO_2$ column chromatography ($CHCl_3$/MeOH, 9:1) to afford **17n** (158 mg, 36%) as a yellowish oil. $^1$H NMR ($CDCl_3$): $\delta = 1.87$ (m, 2H), 2.27 (s, 3H), 2.45 (m, 2H), 2.81 (t, $J = 6.1$ Hz, 2H), 3.23 (d, $J = 6.9$ Hz, 2H), 3.59 (s, 2H), 5.88 (m, 1H), 7.20–7.50 (m, 10H), 7.83 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 343.2048; found, 343.2029. Anal. **17n**·oxalate ($C_{23}H_{25}N_3 \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

The following compounds were prepared analogously from the corresponding aldehydes (**16**) with the appropriate amines:

*(E)-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(piperidin-1-yl)ethylidene)-1H-indazole (**17a**).* Beige solid; yield 40%; m.p. 101–104 °C; $^1$H NMR ($CDCl_3$): $\delta = 1.48$ (m, 2H), 1.68 (m, 4H), 1.87 (m, 2H), 2.37–2.66 (m, 6H), 2.81 (t, $J = 6.1$ Hz, 2H), 3.22 (d, $J = 7.3$ Hz, 2H), 5.86 (t, $J = 7.3$ Hz, 1H), 7.32 (m, 1H), 7.41–7.50 (m, 4H), 7.83 ppm (s, 1H). Anal. **17a**·oxalate ($C_{20}H_{25}N_3 \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*(E)-4,5,6,7-Tetrahydro-4-(2-(morpholin-4-yl)ethylidene)-1-phenyl-1H-indazole (**17b**).* Beige solid; yield 57%; m.p. 114–116 °C; $^1$H NMR ($CDCl_3$): $\delta = 1.87$ (m, 2H), 2.40–2.62 (m, 6H), 2.81 (t, $J = 6.1$ Hz, 2H), 3.18 (d, $J = 7.3$ Hz, 2H), 3.75 (m, 4H), 5.81 (t, $J = 7.3$ Hz, 1H), 7.33 (m, 1H), 7.47 (m, 4H), 7.81 ppm (s, 1H). Anal. **17b**·oxalate ($C_{19}H_{23}N_3O \cdot C_2H_2O_4 \cdot \frac{1}{3}H_2O$) C, H, N.

*(E)-4-(2-(Azepan-1-yl)ethylidene)-4,5,6,7-tetrahydro-1-phenyl-1H-indazole (**17c**).* Colorless oil; yield 11%; $^1$H NMR ($CDCl_3$): $\delta = 1.56$–1.80 (m, 8H), 1.85 (m, 2H), 2.47 (m, 2H), 2.73 (m, 4H), 2.81 (t, $J = 6.1$ Hz, 2H), 3.34 (d, $J = 7.1$ Hz, 2H), 5.87 (t, $J = 7.1$ Hz, 1H), 7.32 (m, 1H), 7.47 (m, 4H), 7.83 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 321.2205; found, 321.2198.

*(E)-4,5,6,7-Tetrahydro-4-(2-(4-methylpiperidin-1-yl)ethylidene)-1-phenyl-1H-indazole (**17d**).* White solid; yield 11%; m.p. 83–90 °C; $^1$H NMR ($CDCl_3$): $\delta = 0.93$ (d, $J = 6.0$ Hz, 3H), 1.28 (m, 3H), 1.65 (m, 2H), 1.82–2.03 (m, 4H), 2.47 (m, 2H), 2.81 (t, $J = 6.1$ Hz, 2H), 2.97 (m, 2H), 3.14 (d, $J = 7.2$ Hz, 2H), 5.86 (t, $J = 7.2$ Hz, 1H), 7.53 (m, 1H), 7.40–7.50 (m, 4H), 7.82 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 321.2205; found, 321.2198. Anal. **17d** ($C_{21}H_{27}N_3 \cdot \frac{1}{4}H_2O$) C, H, N.

*(E)-4-(2-(N,N-Diethylamino)ethylidene)-4,5,6,7-tetrahydro-1-phenyl-1H-indazole (**17e**).* Brown oil; yield 27%, $^1$H NMR ($CDCl_3$): $\delta = 1.14$ (t, $J = 7.2$ Hz, 6H), 1.89 (m, 2H), 2.49 (m, 2H), 2.67 (q, $J = 7.2$ Hz, 4H), 2.82 (t, $J = 6.0$ Hz, 2H), 3.36 (d, $J = 7.0$ Hz, 2H), 5.83 (t, $J = 7.0$ Hz, 1H), 7.32 (m, 1H), 7.40–7.50 (m, 4H), 7.83 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 295.2048; found, 295.2039. Anal. **17e**·oxalate ($C_{19}H_{25}N_3 \cdot \frac{13}{10}C_2H_2O_4$) C, H, N.

*(E)-4-(2-(4-Cyclohexylpiperazin-1-yl)ethylidene)-4,5,6,7-tetrahydro-1-phenyl-1H-indazole (**17f**).* Yellowish oil; yield 25%; m.p. 124–130 °C; $^1$H NMR ($CDCl_3$): $\delta = 1.05$–1.32 (m, 5H), 1.58–2.01 (m, 7H), 2,30 (m, 1H), 2.40–2.75 (m, 10H), 2.81 (t, $J = 6.0$ Hz, 2H), 3.17 (d, $J = 7.3$ Hz, 2H), 5.83 (t, $J = 7.3$ Hz, 1H), 7.33 (m, 1H), 7.47 (m, 4H), 7.81 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 390.2783; found, 390.2761.

*(E)-4,5,6,7-Tetrahydro-4-(2-(cis-2,6-dimethylmorpholin-4-yl)ethylidene)-1-phenyl-1H-indazole (**17g**).* Yellowish oil; yield 15%; $^1$H NMR ($CDCl_3$): $\delta = 1.17$ (d, $J = 6.3$ Hz, 6H), 1.80 (m, 4H), 2.48 (m, 2H), 2.84 (m, 4H), 3.19 (d, $J = 6.4$ Hz, 2H), 3.77 (m, 2H), 5.82 (m, 1H), 7.35 (m, 1H), 7.46 (m, 4H), 7.83 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 337.2154; found, 337.2138. Anal. **17g**·oxalate ($C_{21}H_{27}N_3O \cdot C_2H_2O_4 \cdot \frac{1}{3}H_2O$) C, H, N.

*(E)-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(4-phenylpiperidin-1-yl)ethylidene)-1H-indazole (**17h**).* Beige solid; yield 16%; m.p. 110–112 °C; $^1$H NMR ($CDCl_3$): $\delta = 1.90$ (m, 4H), 2.02 (m, 2H), 2.29 (m, 2H), 2.46 (m, 3H), 2.82 (t, $J = 6.1$ Hz, 2H), 3.26 (m, 2H), 3.36 (m, 2H), 5.90 (t,

$J$ = 7.1 Hz, 1H), 7.18–7.38 (m, 6H), 7.47 (m, 4H), 7.85 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 383.2361; found, 383.2339. Anal. **17h** ($C_{26}H_{29}N_3 \cdot \frac{1}{2}CH_3COCH_3$) C, H, N.

*(E)-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(4-phenylpiperazin-1-yl)ethylidene)-1H-indazole (**17i**)*. Beige solid; yield 10%; m.p. 114–116 °C; $^1$H NMR ($CDCl_3$): $\delta$ = 1.89 (m, 2H), 2.51 (m, 2H), 2.73 (m, 4H), 2.83 (t, $J$ = 6.1 Hz, 2H), 3.28 (m, 6H), 5.87 (t, $J$ = 7.2 Hz, 1H), 6.86 (t, $J$ = 7.4 Hz, 1H), 6.94 (d, $J$ = 7.9 Hz, 2H), 7.22–7.37 (m, 3H), 7.48 (m, 4H), 7.84 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 384.2314; found, 384.2307.

*(E)-4,5,6,7-Tetrahydro-1-phenyl-4-(2-[spiro[isobenzofuran-1(3H),4′-piperidin]-1′-yl]ethylidene)-1H-indazole (**17k**)*. Colorless oil; yield 24%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.78–1.97 (m, 4H), 2.20 (m, 2H), 2.48–2.70 (m, 4H), 2.82 (t, $J$ = 6.0 Hz, 2H), 3.08 (m, 2H), 3.36 (m, 2H), 5.08 (s, 2H), 5.91 (t, $J$ = 7.5 Hz, 1H), 7.18–7.38 (m, 5H), 7.47 (m, 4H), 7.86 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 411.2311; found, 411.2307.

*(E)-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(3-phenylpiperidin-1-yl)ethylidene)-1H-indazole (**17l**)*. Beige solid; yield 24%; m.p. 136–138 °C; $^1$H NMR ($CDCl_3$): $\delta$ = 1.40–2.20 (m, 9H), 2.46 (m, 2H), 2.80 (t, $J$ = 6.2 Hz, 2H), 2.85–3.38 (m, 4H), 5.87 (t, $J$ = 7.9 Hz, 1H), 7.18–7.38 (m, 6H), 7.46 (m, 4H), 7.83 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 383.2361; found, 383.2356.

*1,2,3,4-Tetrahydro-2-((E)-2-(6,7-dihydro-1-phenyl-1H-indazol-4(5H)-ylidene)ethyl)isoquinoline (**17m**)*. Colorless oil; yield 34%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.90 (m, 2H), 2.52 (m, 2H), 2.83 (m, 4H), 2.95 (m, 2H), 3.38 (d, $J$ = 7.2 Hz, 2H), 3.75 (s, 2H), 5.93 (t, $J$ = 7.2 Hz, 1H), 7.03 (m, 1H), 7.12 (m, 3H), 7.33 (m, 1H), 7.42–7.52 (m, 4H), 7.84 ppm (s, 1H). Anal. **17m**·oxalate ($C_{24}H_{25}N_3 \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*(E)-4,5,6,7-Tetrahydro-1-phenyl-4-(2-(3-phenylpyrrolidin-1-yl)ethylidene)-1H-indazole (**17o**)*. Colorless oil; yield 32%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.82 (m, 2H), 1.92 (m, 1H), 2.32 (m, 1H), 2.45 (m, 2H), 2.57 (m, 1H), 2.75 (m, 3H), 3.01 (m, 1H), 3.23 (m, 1H), 3.38 (m, 3H), 5.83 (t, $J$ = 7.5 Hz, 1H), 7.09–7.30 (m, 6H), 7.34–7.46 (m, 4H), 7.77 ppm (s, 1H). Anal. **17o**·oxalate ($C_{25}H_{27}N_3 \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*(E)-4,5,6,7-Tetrahydro-4-(2-(isoindolin-2-yl)ethylidene)-1-phenyl-1H-indazole (**17p**)*. Beige solid; yield 12%; m.p. 130–131 °C; $^1$H NMR ($CDCl_3$): $\delta$ = 1.91 (m, 2H), 2.52 (m, 2H), 2.83 (t, $J$ = 6.1 Hz, 2H), 3.70 (d, $J$ = 7.3 Hz, 2H), 4.20 (s, 4H), 5.93 (t, $J$ = 7.3 Hz, 1H), 7.20–7.50 (m, 9H), 7.83 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 341.1892; found, 341.1895.

*(E)-1-(3,4-Dichlorophenyl)-4,5,6,7-tetrahydro-4-(2-(piperidin-1-yl)ethylidene)-1H-indazole (**17q**)*. Yellowish oil; yield 48%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.45–2.00 (m, 8H), 2.40–2.90 (m, 8H), 3.56 (m, 2H), 5.91 (m, 1H), 7.36 (m, 1H), 7.52 (m, 1H), 7.63 (d, $J$ = 2.3 Hz, 1H), 7.81 ppm (s, 1H); EI HRMS [$M^+$]: calcd, 375.1269; found, 375.1264.

*(E)-1-(3,4-Dichlorophenyl)-4,5,6,7-tetrahydro-4-(2-(morpholino-4-yl)ethylidene)-1H-indazole (**17r**)*. Yellowish oil; yield 79%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.89 (m, 2H), 2.40–2.63 (m, 6H), 2.81 (t, $J$ = 6.1 Hz, 2H), 3.21 (d, $J$ = 7.2 Hz, 2H), 3.78 (m, 4H), 5.82 (t, $J$ = 6.1 Hz, 1H), 7.35 (dd, $J$ = 8.6 Hz, $J'$ = 2.2 Hz, 1H), 7.52 (d, $J$ = 8.6 Hz, 1H), 7.64 (d, $J$ = 2.2 Hz, 1H), 7.81 ppm (s, 1H). Anal. **17r**·oxalate ($C_{19}H_{21}N_3OCl_2 \cdot C_2H_2O_4 \cdot \frac{1}{4}H_2O$) C, H, N.

*(E)-1-(3,4-Dichlorophenyl)-4,5,6,7-tetrahydro-4-(2-(cis-2,6-dimethylmorpholino-4-yl)ethylidene)-1H-indazole (**17s**)*. Yellowish oil; yield 72%; $^1$H NMR ($CDCl_3$): $\delta$ = 1.15 (d, $J$ = 6.2 Hz, 6H), 1.87 (m, 4H), 2.45 (m, 2H), 2.75–2.93 (m, 4H), 3.21 (m, 2H), 3.79 (m, 2H), 5.81 (m, 1H), 7.35 (m, 1H), 7.49 (m, 1H), 7.62 (m, 1H), 7.81 ppm (s, 1H). Anal. **17s**·oxalate ($C_{21}H_{25}N_3OCl_2 \cdot C_2H_2O_4 \cdot \frac{1}{2}H_2O$) C, H, N.

*In vitro σ1 membrane binding assays*. Brain membrane preparation and binding assays for the σ1 receptor were performed as described previously[40] with some modifications. In brief, guinea pig brains were homogenized in 10 volumes (*w*/*v*) of a solution of Tris-HCl (50 mM) and sucrose (0.32 M) at pH 7.4, with a Kinematica Polytron PT 3000 at 15000 rpm for 30 s. The homogenate was centrifuged at 1000 g for 10 min at 4 °C, and the supernatants were collected and centrifuged again at 48000 g for 15 min at 4 °C. The pellet was resuspended in 10 volumes of Tris-HCl buffer (50 mM, pH 7.4), incubated at 37 °C for 30 min, and centrifuged at 48000 g for 20 min at 4 °C. Subsequently the pellet was resuspended in fresh Tris-HCl buffer (50 mM, pH 7.4) and stored on ice until use. Each assay tube contained [$^3$H](+)-pentazocine (10 μL, final concentration of 0.5 nM), tissue suspension (900 μL) to a final assay volume of 1 mL, and a final net tissue concentration of ≈30 mg mL$^{-1}$. Nonspecific binding was determined by the addition of haloperidol to a final concentration of 1 μM. All tubes were incubated at 37 °C for 150 min before termination of the reaction by rapid filtration over glass fiber filters (Schleicher & Schuell, GF 3362) previously soaked in a solution of polyethylenimine (0.5%) for at least 1 h. Filters were then washed four times with cold Tris-HCl buffer (50 mM, pH 7.4, 4 mL per wash). Following the addition of scintillation cocktail, the samples were allowed to equilibrate overnight. The amount of radioactivity bound was determined by liquid scintillation spectrometry with a Wallac Winspectral 1414 liquid scintillation counter. Protein concentrations were determined by the method of Lowry et al.[41]

*In vitro σ2 membrane binding assays*. Binding studies for the σ2 receptor were performed as described previously[42] with some modifications. In brief, brains from σ receptor type I (σ1) knockout mice[43] were homogenized in ice-cold Tris-HCl buffer (10 mM, pH 7.4) containing sucrose (320 mM) (Tris–sucrose buffer) at a volume of 10 mL g$^{-1}$ net tissue with a Potter-Elvehjem homogenizer (10 strokes at 500 rpm). The homogenates were then centrifuged at 1000 g for 10 min at 4 °C, and the supernatants were saved. The pellets were resuspended by vortexing in ice-cold Tris–sucrose buffer (2 mL g$^{-1}$) and were centrifuged again at 1000 g for 10 min. The combined 1000-g supernatants were centrifuged at 31000 g for 15 min at 4 °C. The pellets were resuspended by vortexing in Tris-HCl (10 mM, pH 7.4, 3 mL g$^{-1}$), and the suspension was kept at 25 °C for 15 min. Following centrifugation at 31000 g for 15 min, the pellets were resuspended by gentle homogenization (Potter-Elvehjem) to a volume of 1.53 mL g$^{-1}$ in Tris-HCl (10 mM, pH 7.4). The assay tubes contained [$^3$H]-DTG (10 μL, final concentration of 3 nM), tissue suspension (400 μL, 5.3 mL g$^{-1}$ in Tris-HCl, 50 mM, pH 8.0) for a final assay volume of 0.5 mL. Nonspecific binding was determined by the addition of haloperidol to a final concentration of 1 μM. All tubes were incubated at 25 °C for 120 min before termination of the reaction by rapid filtration over glass fiber filters (Schleicher & Schuell, GF 3362) previously soaked in a solution of polyethylenimine (0.5%) for at least 1 h. Filters were washed with three times with cold Tris-HCl buffer (10 mM, pH 8.0, 5 mL per wash). Following the addition of scintillation cocktail, samples were allowed to equilibrate overnight. The amount of radioactivity bound was determined by liquid scintillation spectrometry with a Wallac Winspectral 1414 liquid scintillation counter. Protein concentrations were determined by the method of Lowry et al.[41]

*Caco-2 cell-transport studies*. Caco-2 cells (obtained from Sloan-Kettering, New York, USA) were routinely maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with glucose (1 g L$^{-1}$), heat-inactivated fetal calf serum (10%), glutamine (2 mM),

penicillin (100 U ml$^{-1}$), and streptomycin (100 μg mL$^{-1}$) at 37 °C in an atmosphere of 5% $CO_2$ and 90% humidity. Cells were used at passages 55–70. Transport studies were performed in the apical to basolateral direction. Briefly, cells were seeded on polycarbonate filter inserts (96-well inserts, pores: 0.4 μm, area: 0.1 cm$^2$, Millipore) and maintained in culture medium with antibiotics for 21 days. The culture medium was removed from both sides of the monolayers, which were washed with Hank's balanced salt solution (HBSS) supplemented with glucose (10 mM) and buffered either to pH 6.5 (apical side) or to pH 7.4 (basolateral side). Apical solutions containing drug (75 μL) were added to the apical side of Caco-2 cells, whereas the receiver chamber was filled with basolateral buffer (300 μL). The appearance of the drug in the receiver side was checked at the end of the incubation time (2 h at 37 °C with shaking). The cellular integrity during transport experiments was checked by measuring the transepithelial electrical resistance (TEER) and the permeability of the paracellular marker, Lucifer Yellow. Donor solution and receiver samples were analyzed by LC–MS, and the apparent permeability coefficient (Papp) was calculated.[44]

*Metabolic stability.* In vitro metabolic stability studies were carried out with rat and human liver microsomes.[45] Compounds (10 μM) were incubated at 37 °C for up to 1 h in sodium–potassium phosphate buffer (0.05 M, pH 7.4), $MgCl_2$ (1 μM), a NADPH-generating system, and CYP (0.3 nmol mL$^{-1}$) in 96-well plates in a robotic liquid-handling system (Multiprobe II, Packard). Incubation mixtures were sampled at selected times (0, 15, 30, 45, and 60 min) and stopped with acetonitrile. Supernatants were analyzed by LC–MS. Metabolism was measured by the loss of parent drug. The biotransfomation rate ($v$) is the slope calculated from the linear fit of the ln of the percentage of parent compound remaining versus incubation time. The results were expressed as in vitro half-life ($t_{1/2}$): $t_{1/2} = \ln(2/v)$.

*CYP inhibition screening.* The inhibition potential was assessed by using cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 enzymes and the fluorescent detection method described.[46] These assays were based on CYP-catalyzed O-dealkylation reactions, which generate an easily detectable fluorescent metabolite. Two different substrates were used for CYP3A4. Tests were conducted in black, flat-bottom 96-well plates with a robotic liquid-handling system (Multiprobe II, Packard). Test compounds were incubated in duplicate at 1 μM to assess their inhibition potential. Control and blank wells contained the same percentage of organic solvent as that used in the samples.

*Experimental physicochemical properties.*[47] Solubility determinations were performed by adding the given compound (1 mg) to the buffer (0.5–1 mL) either into a Whatman vial incorporated with a PTFE membrane filter (0.45 μm) or into an HPLC vial. The solution was agitated for 4 h at 23 °C and 1250 rpm (Thermomixer Comfort, Eppendorf) and filtered through either the Whatman vial membrane or a Millipore Millex-LH membrane (0.45 μm). The filtrate was analyzed by reversed-phase HPLC using the same product dissolved in DMSO/water as standard. The buffers used were: HCl (0.01 N), phosphate buffer (68 mM, pH 6.5 and pH 7.4).

Stability determinations were performed from solutions (4 mg mL$^{-1}$) in DMSO. Five aliquots (25 μL each) were added to both 2-mL vials, and acetonitrile/buffer (1 mL $v/v$, see above) was added (two of them with acetonitrile/68 mM phosphate buffer, pH 6.5). One vial was allowed to stand at room temperature without acetonitrile/buffer added, as a control. Vials were stored at 37 °C for 20 h protected from light. One of the vials containing acetonitrile/68 mM phosphate at pH 6.5 was kept unprotected from light at room temperature. The control vial was diluted immediately before the analysis by the addition of 1 mL of acetonitrile/68 mM phosphate buffer at pH 6.5. Analysis was performed by reversed-phase HPLC using sample control as a standard. The chromatographic profiles between sample solutions and the sample control were compared.

Partition coefficients were determined by HPLC (ODP-50 column, Supelco) with a mobile phase of methanol and phosphate (10 mM, pH 11). Standards used were triphenylene (trip) and toluene (tol); values of log $P$ were calculated by the following equation ($R_t$ = retention time):

$$\log P = (R_{ttol} \times 5.49 - 2.77 \times R_{tsample} - R_{ttrip} \times 2.72)/(R_{ttol} - R_{ttrip}).$$

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