Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 10 May 2017

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Hydroxy-Substituted Heteroarylpiperazines: Novel Scaffolds for β-Arrestin-Biased D_2R Agonists

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ABSTRACT

By means of a formal structural hybridization of the antipsychotic drug aripiprazole and the heterocyclic catecholamine surrogates present in the β_2-adrenoceptor agonists procaterol and BI-167107 (**4**), we designed and synthesized a collection of novel hydroxy-substituted heteroarylpiperazines and heteroarylhomopiperazines with high dopamine D_2 receptor (D_2R) affinity. In contrast to the weak agonistic behavior of aripiprazole, these ligands are capable of effectively mimicking those interactions of dopamine and the D_2R that are crucial for an active state, leading to the recruitment of β-arrestin-2. Interestingly, some ligands show considerably lower intrinsic activity in guanine nucleotide exchange experiments at D_2R and consequently represent biased agonists favoring β-arrestin-2 recruitment over canonical G protein activation. The ligands' agonistic properties are substantially driven by the presence of an endocyclic H-bond donor.

INTRODUCTION

The five different dopamine receptor subtypes (D_1R-D_5R) are widely distributed throughout the body, especially within the central nervous system (CNS). These G protein coupled receptors (GPCRs) mediate the physiological functions of dopamine, a neurotransmitter and hormone with a catecholamine structure.[1] Non-surprisingly, malfunctions of the dopaminergic neurotransmission are involved in a number of pathophysiological conditions including schizophrenia, addiction and Parkinson's disease (PD). PD is characterized by severe locomotor deficits, which are mainly caused by the loss of dopaminergic neurons in the *substantia nigra pars compacta*. Besides the cardinal symptoms akinesia, rigidity and tremor at rest, patients also exhibit cognitive and other non-motor symptoms in both, advanced and early stages of the disease.[2] Despite considerable efforts to develop novel therapeutic agents, L-DOPA (L-3,4-dihydroxyphenylalanine) applied together with a peripheral decarboxylase inhibitor remains the 'gold standard' in symptomatic antiparkinsonian therapy. However, the prolonged use of this dopamine precursor results in a loss of efficacy over time, resulting first in "off-phases" and concomitantly occurring L-DOPA-induced dyskinesias (LID) during the "on-phases" at later stages of the disease.[3] Attempts to improve fluctuations or to delay the onset of fluctuations and LIDs with D_2R agonists as monotherapy or in combination with a lower L-DOPA dose did not prove successful, as the onset of LID is not dependent on the duration of therapy, but rather the duration of the disease.[4, 5] The exact mechanisms underlying LIDs remain a matter of debate and probably depend on the overall context of denervation and compensatory processes in the CNS. However, animal models of PD suggest LIDs may be associated with uncontrolled neuronal excitability and dopamine receptor supersensitivity.[6, 7]

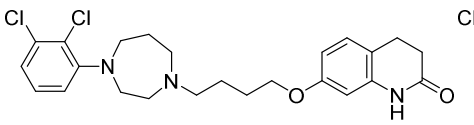
Activation of D_2Rs, as for a plethora of other GPCRs, leads to conformational changes transmitted from the ligand-binding pocket towards intracellular receptor domains. Distinct receptor conformations are thought to guide the intracellular signaling events by interaction of the receptor with

different signal transducers, namely G proteins and β-arrestin-2.[8] Using evolutionary trace for the generation of D_2R mutants specifically interacting with the G protein or β-arrestin signaling pathway, it was demonstrated that G protein- and β-arrestin-mediated signaling at the D_2R is dissociable and has distinct physiological outcomes. Although the generated mutant receptors differed in only two residues compared to wild-type, only the β-arrestin-specific D_2R mutant was able to promote significant amphetamine-induced forward locomotion in mice.[9] Preferential activation of one signaling pathway over the other does not only occur in response to genetically modified receptors but also in a ligand-dependent fashion. This ligand-directed signaling is known as 'functional selectivity', 'ligand bias' or 'biased agonism' and has gained considerable interest over the past decades.[10, 11]

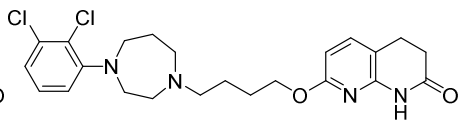
Very recently, it was demonstrated in various animal models of PD, that dopamine receptor-mediated activation of β-arrestin-2 is required for the therapeutic effects of L-DOPA. In contrast, genetic deletion of the signal transducer molecule markedly enhanced dyskinesia-like effects of both, acute and chronic L-DOPA treatment and significantly reduced forward locomotion.[12] Similar results were obtained when GRK6, a kinase upstream in the β-arrestin-2 signaling cascade, was deleted.[13] Noteworthy, overexpression of β-arrestin-2 in PD animal models enhanced the therapeutic effects of L-DOPA, while alleviating dyskinesia.[12] Specifically targeting the D_2R-mediated activation of β-arrestin-2 might therefore be a valuable approach for the design of novel antiparkinsonian drugs. Here we report on structural modifications of aripiprazole, a weak D_2R partial agonist, leading to novel β-arrestin-biased D_2R agonists. Previously, a number of β-arrestin-2 biased partial agonists for D_2Rs including prototypic ligands **1** (UNC0006)[14], **2** (UNC9975)[14] and **3** (UNC9994)[14] have been described as putative novel antipsychotics (Chart 1). However, these ligands displayed relatively weak intrinsic activities, and none of them exhibited dopamine-like properties for the recruitment of β-arrestin-2. Unlike these partial agonists, our results show hydroxy-substituted heteroarylpiperazines capable to

fully activate the D_2R-promoted recruitment of β-arrestin-2, while displaying considerably lower tendency to stimulate G protein mediated signaling.

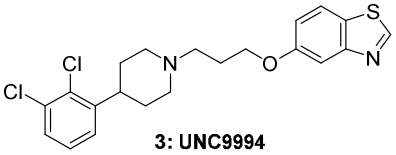
Chart 1. Previously described β-arrestin-biased agonists **1-3** with partial agonist characteristics at D_2R.



1: UNC0006



2: UNC9975



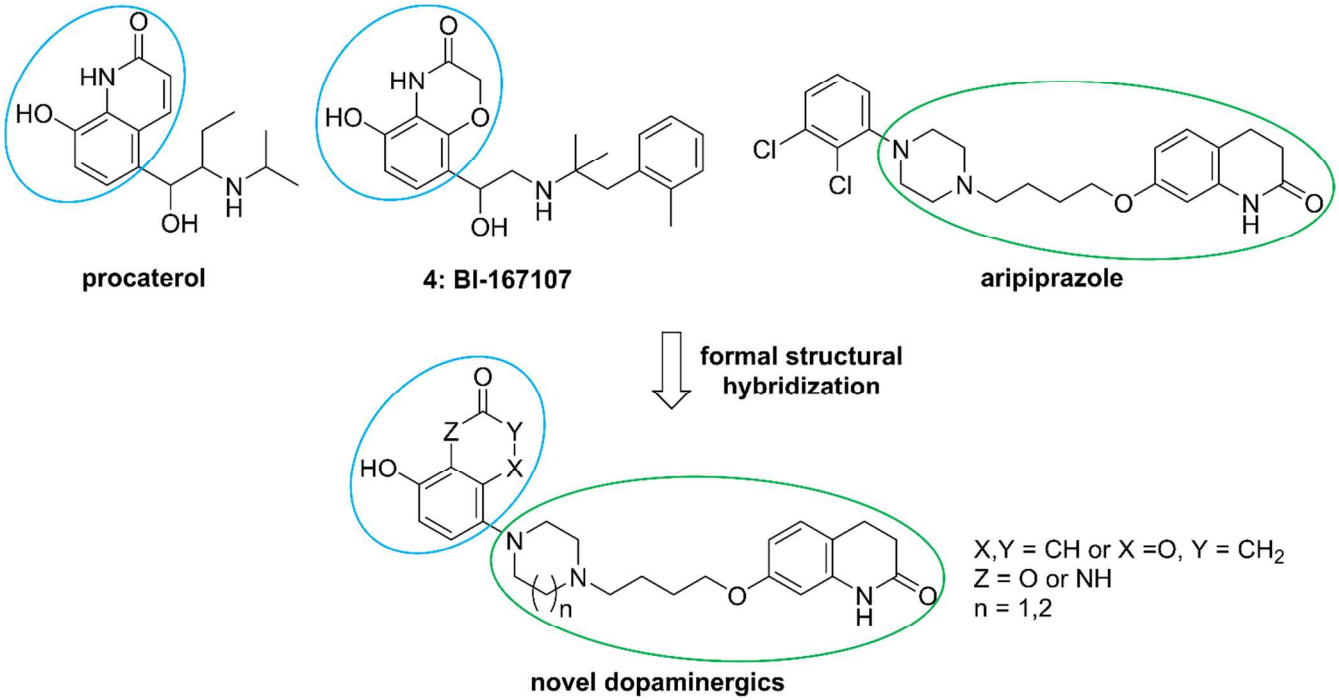
3: UNC9994

RESULTS AND DISCUSSION

Ligand design. When aiming to develop functionally selective agonists, a ligand-based strategy suggests starting from a balanced agonist and trying to replace or remove molecule parts associated with receptor activation. If such a modification disrupts one or the other signaling pathway more effectively, ligand bias will be observed. A complementary design embarks on an antagonist scaffold, and depends on the inclusion of agonist-type structural moieties. Thus, biased agonists may be accessed by designing hybrid-structures containing agonist and antagonist moieties. Starting from the typical 1,4-disubstituted aromatic piperazine (1,4-DAP) aripiprazole, a typical weak D_2R partial agonist, we envisioned incorporation of typical catechol-like agonist substructures in order to generate putatively biased D_2R agonists. The head groups of typical 1,4-DAPs are not appropriately substituted to form hydrogen bonds with the side chains of $Ser^{5.42}$, $Ser^{5.43}$ and $Ser^{5.46}$ within transmembrane helix 5, which are crucial for the activation of aminergic GPCRs by catecholamines and heterocyclic bioisosteres thereof.[15-18] Inspired by such H-bond forming headgroups,[19] we conceived a formal structural hybridization of the 1,4-DAP substructure of ariprazole and the heterocyclic neurotransmitter surrogates present in the high affinity β_2-AR agonists indacaterol,[20] procaterol,[21, 22] olodaterol[23] and BI-167107 (**4**),[17, 24] leading to a novel chemotype of dopaminergics (Chart 2). Both, the 8-hydroxyquinolin-2(1*H*)-one and the 5-hydroxy-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one substructures have been shown to mimic the phenolic hydroxyls of the endogenous β_2-AR agonists norepinephrine.[17, 25]

Besides these two heterocycles, we included the respective oxygen-analog 8-hydroxycoumarine, an 8-hydroxy-substituted quinoline and a formally reduced 1,4-benzodioxane. Although benzodioxane-substructures have been employed previously within heterocyclic 1,4-DAPs,[26] these ligands were lacking the additional hydroxyl functionality. In addition to the variation of the aromatic head group, we intended to replace the typical 1,4-piperazine by a homologous 1,4-diazepane (1,4-homopiperazine), since this modification has been previously reported to foster D_2R activation in a putatively β-arrestin-biased manner.[14, 27] As lipophilic appendages, we either employed the 3,4-dihydroquinolin-2(1*H*)-one substructure of aripiprazole, or used a pyrazolo[1,5-*a*]pyridine, a moiety that has been previously shown to convey high dopamine receptor affinity.[28, 29] In both cases, we connected the lipophilic moiety to the piperazine in analogy to the lead structure aripiprazole, using a flexible butoxy-spacer.

Chart 2. Formal structural hybridization of the D_2R partial agonist aripiprazole and the known β_2-AR agonists procaterol and **4**, leading to novel dopaminergics.



Synthesis. Synthesis of heteroarylpiperazines comprising a 3,4-dihydroquinolin-2(1*H*)-one or pyrazolo[1,5-*a*]pyridine moiety and a butoxy spacer was established based on a convergent synthesis

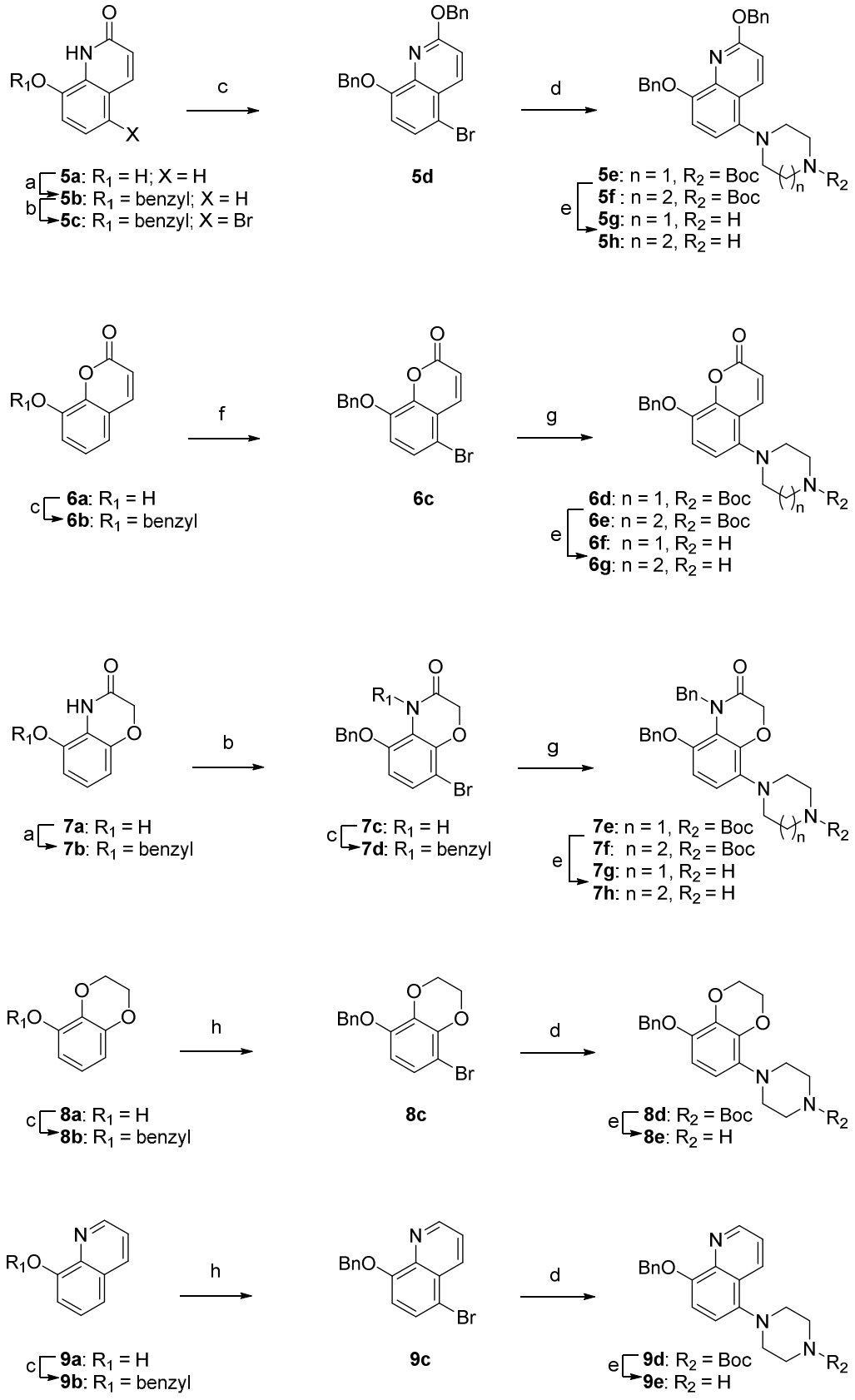
strategy. In general, heteroarylpiperazines and their homopiperazine analogs were synthesized starting from the unsaturated heterocycles, which were benzyl-protected and brominated to allow introduction of *N*-boc-protected 1,4-piperazine or 1,4-diazepane building blocks by Buchwald-Hartwig amination reactions (Scheme 1). These head groups were then reacted with the 4-bromobutoxy-substituted 3,4-dihydroquinolin-2(1*H*)-one or pyrazolo[1,5-*a*]pyridine moieties to give the target compounds (Scheme 2). In detail, the synthesis of the 8-(benzyloxy)-5-bromoquinolin-2(1*H*)-one building block **5c**[25, 30] was performed starting from commercially available 8-hydroxyquinolin-2-(1*H*)-one which was reacted with benzyl bromide using DBU as a base followed by bromination of the aromatic core in the *para*-position of the benzyl-protected hydroxy group yielding the intermediates **5b** and **5c**, respectively. Unfortunately, reaction of **5c** and boc-protected piperazine was not successful. Therefore, a second *O*-benzylation was performed, yielding the 2,8-bis(benzyloxy)-5-bromoquinoline **5d**. The synthesis of 8-hydroxycoumarine-based ligands was established in analogy to described procedures,[31, 32] starting from 2,3-dihydroxybenzaldehyde, which was subjected to a Wittig reaction and intramolecular cyclization with carbethoxycarbonylmethylene triphenylphosphorane in diethylaniline. The phenol **6a** was subsequently converted into the benzyl ether **6b** and reaction with bromine in THF yielded the central building block 8-(benzyloxy)-5-bromo-2*H*-chromen-2-one (**6c**). The 5-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one **7a** was prepared following a previously described three step procedure. First, 2-nitroresorcinol was converted to 2-aminoresorcinol by catalytic hydrogenation. The primary aromatic amine was reacted with chloroacetyl chloride, before the final cyclization under basic conditions afforded **7a**.[19] Subsequently, the phenol group was reacted with benzyl bromide using DBU as a base and the aromatic core was brominated in the *para*-position of the benzyl-protected hydroxyl, giving access to the intermediates **7b** and **7c**.[19] The lactam nitrogen required protection by addition of a second benzyl group, which was introduced by reaction with benzyl bromide, using elevated temperatures and potassium carbonate as a base to afford the intermediate **7d**. Synthesis of the 8-hydroxybenzo-1,4-dioxane head group started from pyrogallol which was converted to 8-

hydroxybenzo-1,4-dioxane (**8a**) by reaction with 1,2-dibromoethane following a previously described protocol.[33] Subsequently, the phenolic hydroxyl group was benzyl-protected (**8b**) and a bromine residue was introduced into the *para*-position of the benzyl-protected hydroxyl, yielding intermediate **8c**. Benzyl protection[34] followed by bromination of commercially available 8-hydroxyquinoline afforded **9c**,[35] which served as precursor for 8-hydroxyquinoline-derived ligands. The five different bromo-substituted heterocycles were subsequently reacted with boc-protected 1,4-piperazine in Buchwald-Hartwig cross-coupling reactions, using conditions described for the reaction of **9c** with piperidine.[35] All reactions were carried out in toluene using either Cs_2CO_3 or NaO^tBu as a base. As palladium species, $Pd(OAc)_2$ or $Pd_2(dba)_3$ together with racemic BINAP were used. Using the reaction sequence elaborated for the preparation of the arylpiperazines **5e**, **6d**, **7e**, **8d** and **9d**, reaction of the bromo-substituted precursors **5d**, **6c** and **7d** with boc-protected 1,4-diazepane yielded the homopiperazine analogs **5f**, **6e** and **7f**. Finally, cleavage of the tert-butyloxycarbonyl groups with TFA in dichloromethane resulted in formation of five 1,4-heteroarylpiperazines (**5g**, **6f**, **7g**, **8e**, **9e**) and three 1,4-heteroarylhomopiperazines (**5h**, **6g**, **7h**). For the synthesis of the final compounds, the secondary amines of the heteroarylpiperazines (**5g**, **6f**, **7g**, **8e**, **9e**) and -homopiperazines (**5h**, **6g**, **7h**) were subjected to nucleophilic displacement reactions with 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one[36] or 5-(4-bromobutoxy)-pyrazolo[1,5-*a*]pyridine[28, 37] (Scheme 2). In both cases, the alkylbromides were activated by refluxing with sodium iodide.[28, 36] Finally, the remaining benzyl protecting groups were cleaved by hydrogenation or under acidic conditions, affording the heteroarylpiperazines **12a-e**, **16a-e** and heteroarylhomopiperazines **13a-c** and **17a**.

In addition to the collection of ligands bearing a secondary moiety attached via a butoxy-spacer, we intended to complement our investigations with a different lipophilic appendage (Scheme 3). Very recently, we have developed a vanillin-derived scaffold linked to an amidopropyl or aminobutyl spacer as a useful moiety for specific molecular tools,[38, 39] and functionally selective D_2R ligands.[30] Thus, we ligated the homopiperazine-8-hydroxyquinolin-2(1*H*)-one **5h** as a representative head group with the

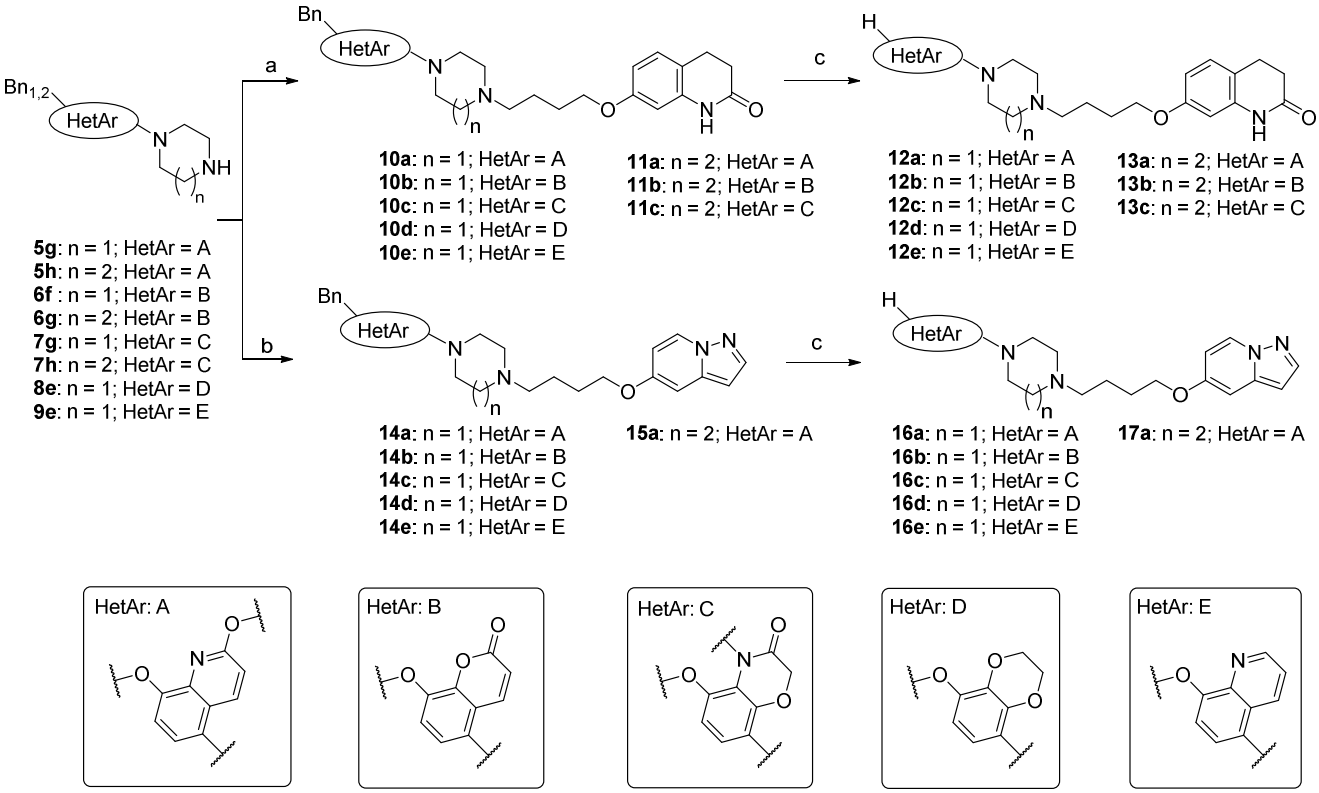
carbaldehyde function of the vanillin-type appendages by reductive amination.[30, 38] The intermediates **18a**,**b** were converted to the final compounds **19a**,**b** by cleavage of the benzyl protection groups with methanesulfonic acid in toluene.

Scheme 1.[a] Synthesis of heterocyclic aromatic piperazine and homopiperazine head groups.



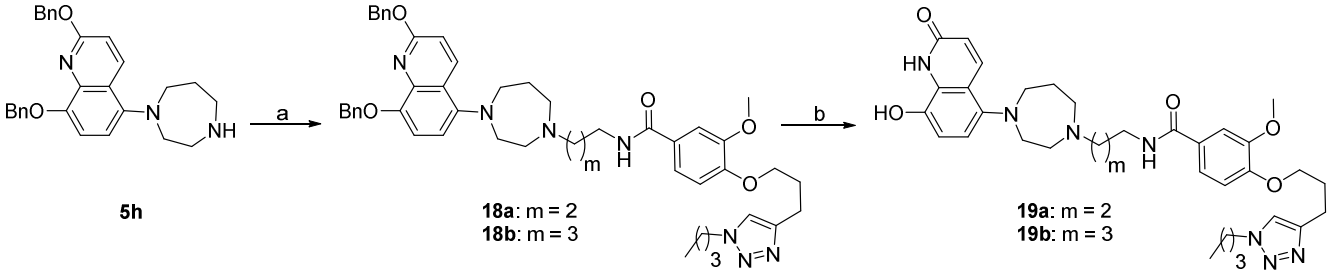
[a]Reagents and conditions: (a) DBU, benzyl bromide, isopropyl alcohol, rt, 1.5 h (70-88 %); (b) Br_2, AcOH, rt, 4 h (53-84 %); (c) K_2CO_3, benzyl bromide, DMF, 50°C, 24 h or rt, 2 h (57-91 %); (d) $Pd_2(dba)_3$ or $Pd(OAc)_2$, *rac*-BINAP, NaO^{t}Bu, 1-boc-piperazine or 1-boc-homopiperazine, toluene, 85°C, 115°C or reflux, 24-48 h (63-73 %); (e) TFA, CH_2Cl_2, rt, 2 h (51-97 %); (f) Br_2, THF, 0°C, 2 h, then rt, 16 h (47 %); (g) $Pd_2(dba)_3$ or $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, 1-boc-piperazine or 1-boc-homopiperazine, toluene, 85°C or reflux, 24-72 h (38-66 %); (h) Br_2, CH_2Cl_2, rt, 5 h (55-75 %).

Scheme 2.[a] Synthesis of full length dopaminergics.



[a]Reagents and conditions: (a) 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one, NaI, acetonitrile, reflux 30 min, then addition of triethylamine in acetonitrile, 80°C, 16h (45-74 %); (b) 5-(4-bromobutoxy)-pyrazolo[1,5-*a*]pyridine, NaI, acetonitrile, reflux 30 min, then addition of triethylamine in acetonitrile, 80°C, 16h (65-77 %); (c) 10% Pd/C in acetic acid/methanol, rt, 4 h (63-79 %) or TFA in toluene, reflux, 8 h (57-79 %) or MsOH in toluene, reflux, 3 h (48-59 %).

Scheme 3.[a] Synthesis of heteroarylpiperazines with alternate appendages.



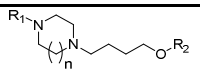
[a]Reagents and conditions: (a) 4-(3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propoxy)-*N*-(3-oxopropyl)-3-methoxybenzamide or 4-(3-(1-butyl-1H-1,2,3-triazol-4-yl)propoxy)-*N*-(4-oxobutyl)-3-methoxybenzamide, $NaBH(OAc)_3$, dichloroethane, rt, 6 h (80-89 %); (b) MsOH in toluene, reflux, 3 h (43-74 %).

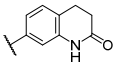
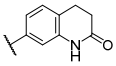
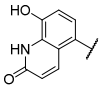
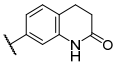
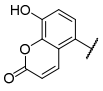
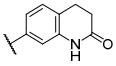
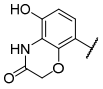
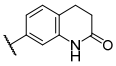
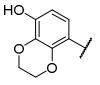
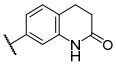
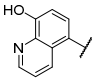
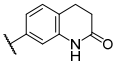
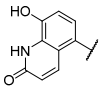
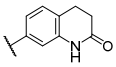
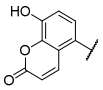
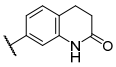
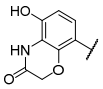
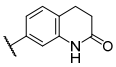
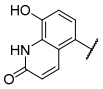
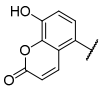
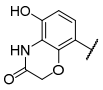
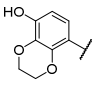
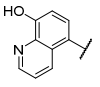
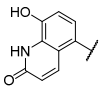
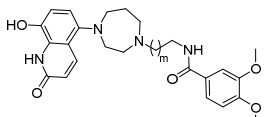
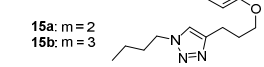
Binding affinities. In order to determine the impact of the structural modifications on dopamine receptor recognition, we performed radioligand displacement studies with the dopamine receptor subtypes D_1R, $D_{2L}R$, $D_{2S}R$, D_3R, D_4R and D_5R. The resulting affinity and selectivity profiles were compared to those of the chemically similar reference ligands aripiprazole and **1** (Table 1). Binding affinities were obtained by measuring the ability of the target compounds to compete with the radioligands [^{3}H]SCH23990 or [^{3}H]spiperone and membranes from cells expressing the respective receptor subtypes. In general, the series of target compounds showed only weak affinity for the D_1R and D_5R subtypes, while they bound to the D_2R-like family with low nanomolar to even subnanomolar K_i values. Indeed, heteroarylpiperazines comprising a 3,4-dihydroquinolin-2(1*H*)-one appendage (**12a-e**) were highly similar to the reference agents and displayed binding affinities ranging from 0.87-4.8, 0.67-2.2 and 1.0-12 nM at $D_{2L}R$, $D_{2S}R$ and D_3R, respectively. Interestingly, the incorporation of a methylene unit into the 6-membered cyclic diamine (piperazine vs. homopiperazine) did not significantly alter the binding affinities for ligands with 8-hydroxyquinolinone (**13a**) and 5-hydroxybenzoxazinone (**13c**) head groups (K_i 0.81-2.0 nM for $D_{2S/L}R$ and D_3R), but induced a 15- to 40-fold loss of affinity for the homopiperazine **13b** (K_i 37-240 nM) comprising an 8-hydroxycoumarine head group. When the 3,4-dihydroquinolin-2(1*H*)-one appendage was replaced by a pyrazolo[1,5-*a*]pyridine moiety (**16a-e**), binding affinities for $D_{2L}R$ and $D_{2S}R$ were decreased. Interestingly, the extent of this effect was found to depend on the employed heteroaryl pharmacophore. While the quinolinone **16a** and the benzoxazine **16c** still showed high affinity towards $D_{2L}R$ and $D_{2S}R$ (K_i 0.86-8.3 nM), derivatives lacking an the endocyclic hydrogen bond donor at the heterocyclic head group showed substantially lower binding affinities for $D_{2L}R/D_{2S}R$ (K_i 19-110 nM). The homopiperazine **17a** showed lower D_2R binding affinity (K_i 78 and 46 nM for $D_{2L}R$ and $D_{2S}R$, respectively). Notably, affinities for the closely related D_3R subtype did not follow the same trend, as the pyrazolo[1,5-*a*]pyridine derivatives **16a-c** and **17a** displayed low nanomolar or even sub-nanomolar binding affinities (K_i 0.71-8.5 nM) that were highly similar to those of the reference agents aripiprazole and **1**

(K_i 1.9 and 2.9 nM). At the D_4R subtype, only the heteroarylpiperazines **12c** and **16c** sharing the benzoxazinone head group displayed low nanomolar affinity (8.3 and 4.8 nM).

Additional radioligand binding studies were performed to determine the compounds' affinities towards related serotonergic (5-$HT_{1A}R$, 5-$HT_{2A}R$) and adrenergic (α_1-AR) receptors using the radioligands [^{3}H]WAY100635, [^{3}H]ketanserin or [^{3}H]prazosin. In comparison to the reference agents aripiprazole and **1**, our newly developed compounds showed substantially altered binding properties. At the 5-$HT_{1A}R$, the test compounds' affinities ranged from 12 nM to 1,200 nM, with the highest binding affinities observed for **12d**, **12e** and **16d** containing an 8-hydroxybenzo-1,4-dioxane or an 8-hydroxyquinoline head group. 5-$HT_{2A}R$ affinities were substantially lower than for aripiprazole or **1**, with the highest affinities observed for the 8-hydroxyquinolines **12e** and **16e** and the 8-hydroxybenzo-1,4-dioxane **12d**. Thus the vast majority of novel target compounds displayed high selectivity for D_2R over 5-$HT_{2A}R$, while maintaining moderate affinity for the 5-$HT_{1A}R$, a receptor that has been identified as additional target in the treatment of PD.[40] Compared to the typical 1,4-DAP structures, inclusion of a propylamido- or butyramido-vanillin-type appendage (**19a**,**b**) led to decreased binding affinities at $D_{2L}R$, $D_{2S}R$ and D_4R. Notably, **19a**,**b** displayed one-digit nanomolar *K*i values at D_3R (1.6 and 3.2 nM) leading to an approximately 100-fold selectivity for D_3R over D_2R.

Table 1. Binding affinities of the heteroarylpiperazines towards dopaminergic, serotonergic and α-adrenergic receptors.



comp.	R_1	n	R_2	$K_i \pm$ SD [nM][a]								
				hD_1R[b]	$hD_{2L}R$[c]	$hD_{2S}R$[c]	hD_3R[c]	hD_4R[c]	hD_5R[b]	p5-$HT_{1A}R$[d]	h5-$HT_{2A}R$[e]	$p\alpha_1R$[f]
aripip.		1		310 ± 140	0.52 ± 0.17	0.38 ± 0.16	2.9 ± 0.66	63 ± 17	1600 ± 500	88 ± 7	3.6 ± 1.1	17 ± 1
1		2		340 ± 40	0.53 ± 0.37	0.20 ± 0.06	1.9 ± 0.52	76 ± 22	1,100 ± 140	8.7 ± 4.8	2.0 ± 0.4	16 ± 3
12a		1		5,400 ± 1,700	0.89 ± 0.71	1.0 ± 0.8	12 ± 7	66 ± 20	9,300 ± 1,600	48 ± 11	2,200 ± 500	96 ± 11
12b		1		>20,000	4.8 ± 3.6	2.0 ± 1.0	7.7 ± 3.9	870 ± 380	>20,000	230 ± 190	>20,000	1,400 ± 700
12c		1		12,000 ± 3,200	0.87 ± 0.81	0.67 ± 0.24	1.0 ± 0.8	8.3 ± 2.9	4,200 ± 2,100	15 ± 1	430 ± 60	20 ± 2
12d		1		4,500 ± 700	2.9 ± 0.6	2.2 ± 0.4	38 ± 10	300 ± 70	16,000 ± 5,000	12 ± 11	160 ± 40	130 ± 60
12e		1		1,500 ± 1,000	3.4 ± 1.5	1.5 ± 0.4	27 ± 16	94 ± 64	3,400 ± 920	12 ± 4	39 ± 24	32 ± 7
13a		2		11,000 ± 8,000	1.6 ± 0.7	0.81 ± 0.48	1.9 ± 0.4	75 ± 37	>20,000	150 ± 60	3,400 ± 1,600	61 ± 25
13b		2		>20,000	100 ± 40	37 ± 20	240 ± 90	5,000 ± 100	>20,000	800 ± 430	2,300 ± 100	900 ± 60
13c		2		2,700 ± 2,600	1.6 ± 1.1	1.6 ± 0.9	2.0 ± 0.9	60 ± 30	15,000 ± 12,000	280 ± 170	2,000 ± 1,600	200 ± 80
16a		1		4,700 ± 4,200	8.3 ± 2.7	3.7 ± 0.4	3.8 ± 1.3	31 ± 10	13,000 ± 9,000	63 ± 25	3,600 ± 3,500	87 ± 23
16b		1		>20,000	40 ± 38	19 ± 6	8.5 ± 5.1	270 ± 70	>20,000	17 ± 4	2,800 ± 600	160 ± 10
16c		1		2,300 ± 300	2.0 ± 1.4	0.86 ± 0.44	0.71 ± 0.57	4.8 ± 3.0	16,000 ± 15,000	25 ± 4	1,300 ± 1,200	15 ± 1
16d		1		5,300 ± 5,200	110 ± 30	68 ± 13	40 ± 14	160 ± 50	19,000 ± 1,000	13 ± 0	480 ± 420	110 ± 20
16e		1		1,100 ± 500	33 ± 7	25 ± 6	50 ± 10	55 ± 6	5,800 ± 3,500	28 ± 4	200 ± 20	93 ± 5
17a		2		13,000 ± 2,000	78 ± 52	46 ± 17	3.4 ± 2.5	190 ± 70	>20,000	1,200 ± 400	9,000 ± 4,300	430 ± 40
19a				17,000 ± 14,000	350 ± 170	440 ± 250	1.6 ± 1.0	3,200 ± 600	>20,000	3,300 ± 500	4,200 ± 700	1,000 ± 150
19b				4,000 ± 3,100	200 ± 120	220 ± 160	3.2 ± 1.6	2,100 ± 500	>20,000	1,400 ± 600	1,500 ± 1,000	810 ± 50

[a]Data represent mean ± S.D. of two to fifteen independent experiments, each performed in triplicate. Radioligands: [b][^{3}H]SCH23390, [c][^{3}H]spiperone, [d][^{3}H]WAY100635, [e][^{3}H]ketanserin, [f][^{3}H]prazosin.

Functional activity at D_2R. The influence of the individual structural modifications on the ligands' ability to activate D_2R was evaluated in a β-arrestin-2 recruitment assay. The arrestin receptor interaction was initially discovered to attenuate or terminate G protein-mediated signaling.[41] There is now broad consensus that β-arrestins also represent signal transducers on their own.[42] D_2R activation mediated through β-arrestin-2 leads to the G protein-independent activation of GSK3β, an enzyme which has been implicated in hyperdopaminergic pathophysiological conditions of neuropsychiatric disorders such as schizophrenia.[43] In contrast, studies with animal models of PD suggest that enhanced stimulation of β-arrestin-2 signaling may have beneficial effects on the control of locomotion while reducing dyskinesia frequently occurring in PD treatment.[12] We measured the recruitment of β-arrestin-2 to ligand-activated $D_{2S}R$ employing the DiscoverX PathHunter technology in HEK293 cells as described previously,[28] and compared the results of our target compounds to those of known D_2R agonists and the 1,4-DAPs aripiprazole and **1** (Table 2). When the reference agonist quinpirole, the endogenous agonist dopamine and the antiparkinsonian drugs rotigotine, pramipexole and ropinirole were examined for their ability to induce β-arrestin-2 recruitment, sigmoid dose response curves were observed in all cases (Supplementary Figure S1). The ligands displayed similar efficacies ranging from full agonist behavior to very strong partial agonism observed for ropinirole (E_{max} 83 %). The highest potency was observed for rotigotine (EC_{50} 4.1 nM), while about 100-fold higher concentrations were necessary to activate $D_{2S}R$ with the endogenous agonist dopamine (EC_{50} 390 nM). In comparison to these relatively similar effects of the reference ligands, the novel heteroarylpiperazines and their homopiperazine analogs sampled a wide range of efficacies (Supplementary Figure S2). Indeed, the maximum efficacy was found to depend on two main factors: the nature of the orthosteric heteroaryl moiety and the ring size of the central diamine. More specifically, piperazines **12b**,**d**,**e** and **16b**,**d**,**e** comprising an 8-hydroxybenzo-1,4-dioxane, an 8-hydroxyquinoline or an 8-hydroxycoumarine head group were devoid of agonistic properties in the β-arrestin-2 recruitment assay, similar to the results

obtained for the antipsychotic drug aripiprazole. In contrast, ligands **12a**,**c** and **16a**,**c** sharing an 5-hydroxybenzoxazin-3-one or 8-hydroxyquinolin-2(1*H*)-one substructure showed partial agonist activities with E_{max} values between 21 and 44 % and potencies comparable to quinpirole and dopamine (EC_{50} 62-240 nM). Notably, enlargement of the cyclic diamine moiety from a 6-membered (piperazine) to a 7-membered ring (homopiperazine) increased the intrinsic efficacy of the compounds. Thus, the β-arrestin-2 recruitment assay revealed an E_{max} value of 75% for the homopiperazine **13c** comprising a 5-hydroxybenzoxazin-3-one head group. Moreover, the 8-hydroxyquinolin-2(1*H*)-one-homopiperazines **13a** and **17a** behaved as full agonists (E_{max} 90-102 %, Figure 1a,c). These findings are in accordance with earlier reports on an increase in intrinsic efficacy for the diazepane/piperazine replacement in classical 1,4-DAP scaffolds.[27] However, the presence of the homopiperazine subunit does not lead to pronounced recruitment of β-arrestin-2 on its own, since the reference ligand **1** only induced marginal recruitment of β-arrestin-2 under these assay conditions (Supplementary Figure S1). The main difference between **1**, its closely related analogs **2** and **3** (Chart 1)[14, 27] and our newly synthesized ligands can be found in the heterocyclic moiety, were these ligands lack functional groups capable to interact with the polar serine residues in the orthosteric binding pocket of the receptor. Similar to the rank-order in efficacies observed among the piperazines **12a-e**, the 8-hydroxycoumarine-homopiperazine **13b** was less effective (E_{max} 21 %, Figure 1b) than **13a** or **13c**. These findings underline the importance of the presence of an additional hydrogen bond donor rather than an H-bond acceptor within the aromatic heterocycle, since **13b** and results from **13a** in a formal NH to O exchange. These results are in excellent agreement with the binding pose of the high-affinity agonist **4** within the active-state structure of the β_2-AR.[17] Moreover, studies on the β_1-AR in complex with different agonists[18] and the β_2-AR crystal structure in complex with a covalent agonist[25] highlighted the importance of the hydrogen bond network stabilized by catecholamine surrogates for receptor activation. All homopiperazines investigated (**13a-c**, **17a**) displayed potencies similar to the endogenous agonist dopamine (EC_{50} 190-470 nM). In agreement with previous results pointing towards

a profitable effect of the vanillin-type appendage for the recruitment of β-arrestin-2,[30] incorporation of this lipophilic moiety in combination with the 8-hydroxyquinolin-2(1*H*)-one-substituted homopiperazine head group led to pronounced partial agonist properties for the recruitment of β-arrestin-2 at $D_{2S}R$. However, in accordance with the reduced D_2R affinities, ligands **19a**,**b** displayed relatively weak potencies (EC_{50} 680 and 1,600 nM).

Table 2. Ligand-induced recruitment of β-arrestin-2 at $D_{2S}R$.

β-arrrestin-2 recruitment at $D_{2S}R$[a]		
compound	EC_{50} ± SEM [nM]	E_{max} ± SEM [%][b]
quinpirole	52 ± 6	100 ± 1
dopamine	390 ± 20	96 ± 2
rotigotine	4.1 ± 1.0	101 ± 4
ropinirole	89 ± 5	83 ± 4
pramipexole	27 ± 4	90 ± 6
aripiprazole	1100 ± 600	13 ± 3
1	n.d.[c]	≤ 10
12a	93 ± 44	31 ± 4
12b	n.d.[c]	≤ 10
12c	92 ± 36	44 ± 4
12d	n.d.[c]	≤ 10
12e	n.d.[c]	≤ 10
13a	190 ± 40	102 ± 4
13b	220 ± 30	21 ± 2
13c	470 ± 80	75 ± 2
16a	240 ± 60	21 ± 1
16b	n.d.[c]	≤ 10
16c	62 ± 9	43 ± 3
16d	n.d.[c]	≤ 10
16e	n.d.[c]	≤ 10
17a	370 ± 100	90 ± 3
19a	680 ± 70	90 ± 2
19b	1600 ± 600	76 ± 5

[a]Data represent mean ± S.E.M. of four to ten independent experiments, each performed in duplicate. [b]E_{max} relative to the effect of the reference agonist quinpirole. [c]n.d., not determined

In an attempt to identify β-arrestin-biased D_2R agonists, a subset of the target compounds was investigated for their intrinsic activity towards the activation of canonical signaling at $D_{2S}R$. Thus, we studied ligand-promoted nucleotide exchange in membrane preparations from HEK293T cells co-expressing $D_{2S}R$ and a pertussis toxin insensitive variant of the $G\alpha_{o1}$ subunit[44] as a proximal measure of G protein activation.[45] Although D_2R is known to promiscuously couple to all members of the $G\alpha_{i/o}$ family,[46] $G\alpha_o$ proteins have been identified as predominant mediators of D_2R-signaling in the central nervous system (CNS).[47] Incubation of the membranes with the endogenous agonist dopamine and the antiparkinsonian drugs rotigotine, ropinirole and pramipexole led to a similar degree of nucleotide exchange compared to the reference agonist quinpirole (Table 3). While quinpirole, dopamine, pramipexole and ropinirole showed relatively similar potencies (EC_{50} 100-330 nM), rotigotine was effective at 30- to 100-fold lower concentration (EC_{50} 3.2 nM). These results are in good agreement with the recruitment of β-arrestin-2, when rotigotine was also found to be the most potent agonist (EC_{50} 4.1 nM). Relative to these high intrinsic efficacies, aripiprazole and **1** were relatively weak partial agonists (E_{max} 14-17 %). In particular, very high concentrations of **1** were necessary to elicit guanine nucleotide exchange at $D_{2S}R$. These findings are in good agreement with literature reports indicating a low tendency of **1** to inhibit cAMP formation.[14] Compared to 2,3-dichlorophenyl-substituted piperazines or homopiperazines, hydroxylated heteroarylhomopiperazines **13a-c** and **17a** showed substantially higher intrinsic efficacies for the induction of canonical $D_{2S}R$ signaling (E_{max} 26-55 %). Potencies of **13a**, **13c** and **17a** were found to be slightly higher than those of dopamine or quinpirole (EC_{50} 67-90 nM), while **13b** only elicited significant G protein activation at concentrations above 1 µM (Figure 1b). The target compounds **13a**,**c** and **17a** were about 50 % less efficacious in promoting nucleotide exchange compared to the recruitment of β-arrestin-2 (E_{max} 26-55 % and 75-102 %, respectively). Although relative efficacies suggest a preferential engagement β-arrestins over G

proteins, a quantitative bias analysis employing the operational model of agonism did not reveal significantly biased agonists among these target compounds, most likely due to the increased potencies for G protein activation. In contrast, the lactone **13b**, renouncing an endocyclic hydrogen bond donor at the heterocyclic head group, was identified as a β-arrestin-biased ligand, when the endogenous agonist dopamine was used as a reference (Supplementary Figure S3a). Bias was also detected for the very weak partial agonist **1**.

Table 3. Ligand-induced nucleotide exchange determined with membranes from HEK293T cells coexpressing $D_{2S}R$ and $G\alpha_{o1}$ and β-arrestin-2 recruitment promoted by $D_{2S}R$ in presence of GRK2.

	[^{35}S]GTPγS ($D_{2S}R + G\alpha_{o1}$)[a]		β-arrrestin-2 recruitment ($D_{2S}R$ + GRK2)[a]		bias factor[d] ($D_{2S}R$)
compound	EC_{50} ± SEM [nM]	E_{max} ± SEM [%][b]	EC_{50} ± SEM [nM]	E_{max} ± SEM [%][b]	$\Delta\Delta log\left(\frac{\tau}{K_A}\right)$
quinpirole	270 ± 30	100 ± 1	16 ± 1	100 ± 1	1.00 ± 0.07[e]
dopamine	200 ± 20	93 ± 2	160 ± 20	97 ± 2	0.00 ± 0.08
rotigotine	3.2 ± 0.5	91 ± 2	4.4 ± 1.1	106 ± 4	0.15 ± 0.10
ropinirole	330 ± 60	92 ± 2	30 ± 6	104 ± 3	0.99 ± 0.08[e]
pramipexole	100 ± 30	102 ± 3	9.6 ± 1.4	100 ± 3	0.79 ± 0.09
aripiprazole	130 ± 60	14 ± 2	740 ± 180	43 ± 5	-0.14 ± 0.35
1	> 5 μM[c]	17 ± 7[c]	270 ± 80	19 ± 3	2.42 ± 0.48[e]
13a	67 ± 20	55 ± 4	200 ± 20	110 ± 6	-0.48 ± 0.10
13b	> 5μM[c]	36 ± 6[c]	130 ± 20	51 ± 5	1.91 ± 0.19[e]
13c	78 ± 25	26 ± 2	630 ± 110	94 ± 5	-0.58 ± 0.20
17a	90 ± 16	56 ± 4	250 ± 40	103 ± 2	-0.19 ± 0.10

[a]Data represent mean ± S.E.M. of five to fifteen independent experiments, each performed in triplicate. [b]E_{max} relative to the effect of the reference agonist quinpirole. [c]No complete dose response curves obtained, E_{max} determined at 30 μM. [d]Ligand bias quantified by the operational model, values < 0 indicate preference for GTP binding, values > 0 preferential recruitment of β-arrestin-2. [e]Significant bias determined by one-way ANOVA, followed by Dunnet's post hoc test, $p < 0.05$.

Since it is known that recruitment of β-arrestins is greatly facilitated upon adequate receptor phosphorylation,[48] we performed additional β-arrestin-2 recruitment assays in the presence of GRK2, a kinase that has been reported to be relevant for the interaction of β-arrestin-2 and D_2R.[26, 49, 50] Dose response curves of the endogenous agonist dopamine and the reference agonists quinpirole, rotigotine,

ropinirole and pramipexole revealed highly similar maximum efficacies (E_{max} 97-104 %) under these conditions. While the potency of rotigotine was unaffected by the presence of GRK2 (EC_{50} 4.4 nM), a 2- to 3-fold increase was observed for the four other agonists including the endogenous neurotransmitter dopamine (EC_{50} 9.6-160 nM). In good agreement with earlier reports, an increase in efficacy was observed for the weak partial agonists aripiprazole and **1** (E_{max} 43 and 19 %, respectively). Among our newly developed hydroxylated heterocyclic homopiperazines, coexpression of GRK2 substantially increased the efficacy of **13b** (E_{max} 51 %, Figure 1b) and **13c** (E_{max} 94 %), comprising an 8-hydroxycoumarine or a 5-hydroxybenzoxazin-3-one head group, respectively. For the 8-hydroxyquinolin-2(1*H*)-ones **13a** and **17a** only minor changes in efficacy or potency were observed (Figure 1a,c), probably indicating a minor contribution of GRK2 to the signaling panorama of these two ligands. A quantitative comparison of the signaling profiles confirmed the preference for β-arrestin-2 recruitment for the 8-hydroxycoumarine **13b** and the reference agent **1** ($\Delta\Delta\log(\tau/K_A)$ 1.91 and 2.42, Figure 1d). No such bias was observed for the ligands **13a,c** and **17a**, although the signaling profiles of these two ligands are substantially different from those of typical antiparkinsonian drugs (e.g. rotigotine) or the endogenous agonist dopamine (Supplementary Figures S1-3). These results are most likely caused by the detrimental changes of potency and efficacy observed for **13a**,**c** and **17a** between the different test systems, and the relative importance attributed to these influencing factors in the operational model of agonism. In order to relate these distinct signaling profiles to pharmacological outcomes, and thus to estimate the therapeutic value of such compounds future studies, potentially also involving β-arrestin knock-out animals, will be required.

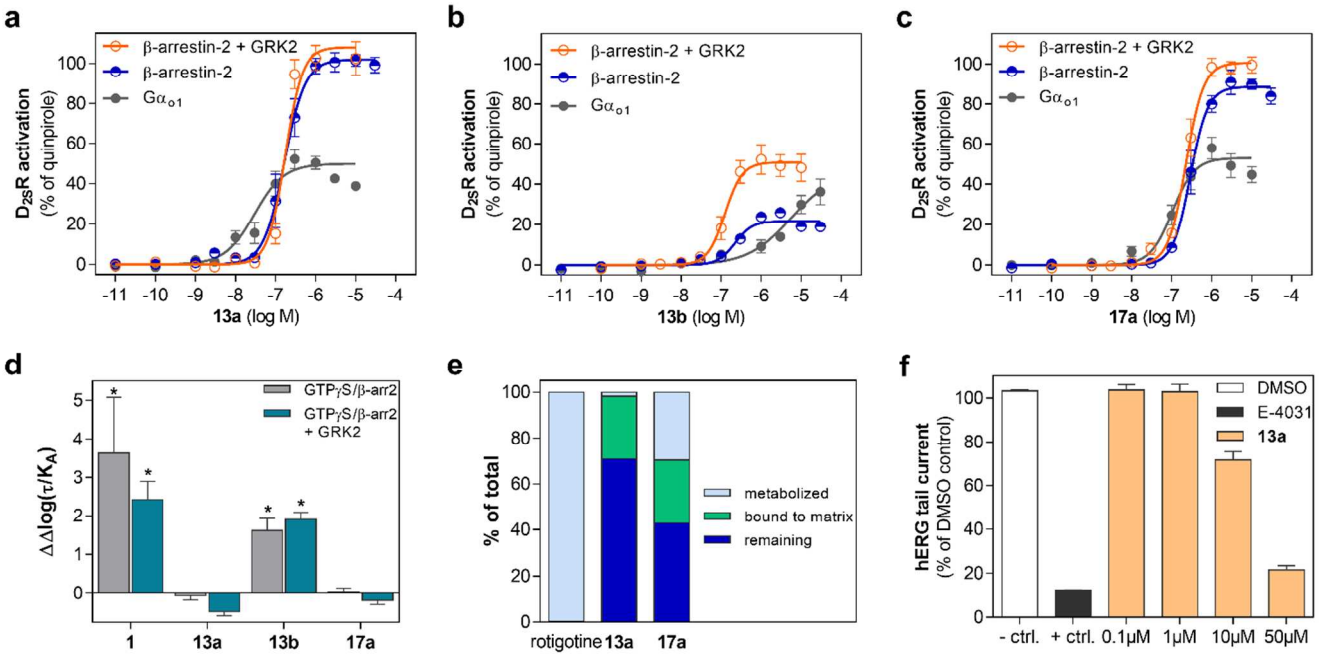


Figure 1. Dose-response curves at $D_{2S}R$ and metabolic stability of target compounds **13a**,**b** and **17a**. (**a-c**) Dose-response curves for $D_{2S}R$-mediated activation of $G\alpha_{o1}$ (grey) and the recruitment of β-arrestin-2 in absence (blue) or presence (orange) of GRK2. (**d**) Bias factors for $D_{2S}R$ activation were calculated using the operational model of agonism. Positive values for $\Delta\Delta\log(\tau/K_A)$ indicate a preference for β-arrestin-2 recruitment. (**e**) Metabolic stability of **13a** and **17a** assessed in rat liver microsomes: distribution between metabolized substrate, substrate nonspecifically bound to matrix and the remaining fraction after 60 min of incubation. (**f**) Inhibition of hERG tail currents by **13a** was measured to estimate susceptibility of cardiotoxicity. Data represent mean ± SEM of at least three independent experiments.

Metabolism and inhibition of hERG tail currents. In order to assess the drug-like properties of our target compounds we calculated octanol/water partition coefficients as described previously (Supplementary Table S1).[28] Compared to the typical 1,4-DAP aripiprazole, hydroxy-substituted heteroarylpiperazines and their homopiperazine analogs were predicted to be less lipophilic. With the exception of the most hydrophilic compounds **12c**, **13c** and **16c** sharing the 5-hydroxybenzoxazinone head group, the ligands' lipophilicity was predicted to be similar to the range covered by the approved D_2R agonists ropinirole, pramipexole and rotigotine. Furthermore, with the exception of **19a**,**b**, none of the compounds hit constraints predicting poor solubility or permeability as indicated by Lipinski's rule of five.[51] Metal chelating properties for various divalent cations are well known characteristics of 8-

hydroxyquinoline substructures and have been associated to undesired side effects.[52] Although chelating properties are likely for some of the presented ligands, no toxic effects were observed under the conditions of our functional experiments.

Besides adequate physicochemical properties, sufficient metabolic stability is a general requirement in drug development. Thus, to determine the metabolic stability of the novel heteroarylpiperazines, we explored the representative ligands **13a** and **17a** for their propensity to undergo phase I transformations using rat liver microsomes in comparison to the antiparkinsonian drug rotigotine. In agreement with previous findings,[53] rotigotine was extensively metabolized within 15 minutes of incubation, and a number of known metabolites were identified using mass spectrometry (Supplementary Figures S4, S5). The half life and intrinsic clearance for rotigotine were determined to be 5.8 min and 240 µL/min/mg protein, respectively (Supplementary Figure S4).[54, 55] In contrast, only minor metabolization of the pyrazolo[1,5-*a*]pyridine **17a** was observed ($T_{1/2}$ 51 min, CL_{int} 27 µL/min/mg protein), while the 3,4-dihydroquinolin-2(1*H*)-one **13a** was hardly metabolized at all ($T_{1/2}$ 130 min, CL_{int} 11 µL/min/mg protein). Based on the intrinsic clearance, **17a** can be regarded as intermediately metabolized, while **13a** falls into the low clearance category.[54, 55] Both ligands revealed binding to matrix protein (~27 %) in control experiments when the enzymatic transformation was prevented by the absence of the cofactor NADPH (Figure 1e, Supplementary Figures S4, S5).

QTc-interval prolongation leading to the very rare but potentially lethal *Torsades de Pointes* (TdP) arrhythmia is a serious complication associated with pharmacotherapy.[56, 57] Although not the only cause, inhibition of the delayed rectifier potassium I_{Kr} current mediated by hERG (human-ether-à-go-go related gene) channels is predictive for QTc prolongation and the liability of a drug-like molecule to cause TdP.[58] Drug-induced arrhythmia has been observed for a number of dopaminergic drugs, including the phenylpiperidine haloperidol and the aromatic piperazine ziprasidone.[59] In order to assess the susceptibility to induce arrhythmia, and hence estimate cardiotoxicity, the influence of a

representative hydroxy-substituted heterocyclic phenylpiperazine (**13a**) on the inhibition of hERG tail currents was examined *in vitro*. Automated patch-clamp showed that no effect on hERG tail currents was observed at concentrations up to 1 μM (Figure 1f). At higher concentration a slight inhibition was observed (10 μM, 71.7 % current remaining), which was dose-dependently increased (50 μM, 21.5% remaining). However, the inhibitory potency (IC_{50}) of **13a** was determined to be 19.7 μM, a concentration that is 100-fold higher than the EC_{50} required for β-arrestin-2 recruitment at $D_{2S}R$ and even 24,000-fold higher than the binding affinity of **13a** at $D_{2S}R$ (K_i 0.81 nM).

CONCLUSIONS

Applied in combination with a peripheral decarboxylase inhibitor, L-DOPA remains the 'gold standard' in antiparkinsonian drug therapy. However, its use is limited by wearing-off and the occurrence of L-DOPA induced dyskinesia (LID) over the course of time.[3] Very recently, it has been demonstrated that dopamine receptor-mediated activation of β-arrestin-2 is required for the beneficial locomotor effects of L-DOPA and not involved in the pathogenesis of LIDs. Selective targeting of this signal transducer, e.g. by biased ligands, may therefore represent a valuable novel concept in the treatment of PD.[12] Aiming to develop novel dopaminergics that activate β-arrestin-2 in a functionally selective manner via the interaction with D_2R, we performed a formal structural hybridization of typical 1,4-DAPs and heterocyclic catecholamine surrogates leading to hydroxy-substituted heteroaryl-1,4-disubstituted piperazines and homopiperazines. In contrast to previously described β-arrestin-2 biased ligands for D_2R,[14, 27] these compounds are designed to mimic the interactions of the catechol substructure of the endogenous ligand dopamine with crucial serine residues in the orthosteric binding pocket. We found that two major factors determined the intrinsic efficacy of the ligands with this novel scaffold – the presence of an additional endocyclic H-bond donor rather than an H-bond acceptor, and the ring-size of the cyclic diamine. Notably, ligands **13a**,**c** and **17a** comprising an 8-hydroxyquinolin-

2(1*H*)-one or a 5-hydroxy-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one heterocycle and a homopiperazine substructure showed full agonist properties for the recruitment of β-arrestin-2, while they were substantially less efficacious for the induction of $D_{2S}R$-promoted guanine nucleotide exchange as a measure of canonical $G\alpha_o$ protein activation. Although the 8-hydroxycoumarine-substituted homopiperazine **13b** induced submaximal activation of both signaling pathways, this compound was identified as highly β-arrestin-biased using the operational model of agonism. A further evaluation of representative homopiperazines **13a** and **17a** comprising a 3,4-dihydroquinolin-2(1*H*)-one or pyrazolo[1,5-*a*]pyridine appendage revealed a low tendency to undergo metabolic transformation reactions in rat liver microsomes and identified a minor liability to inhibit hERG channels, an important off-target in the course of drug-development. The combination of typical agonist-type elements from known β_2-AR agonists such as procaterol[21, 22] and **4**[17, 24] with 1,4-DAP substructures known for their weak partial agonist characteristics[60] proved successful for the design of novel β-arrestin-biased D_2R agonists with unprecedented intrinsic efficacy. This novel scaffold might serve as a starting point for the development of functionally selective PD therapeutics with a lower propensity to induce LID. Nevertheless, the contribution of other dopamine receptor subtypes, especially the D_1R, to the efficacy and side effects associated with L-DOPA therapy should also be addressed in drug discovery.[6, 12] Medicinal chemistry specifically targeting D_1R-promoted β-arrestin-2 recruitment may lead to further promising innovations.

EXPERIMENTAL PART

Chemistry. Reagents and dry solvents were of commercial quality and used as purchased. If not stated otherwise, reactions were carried out under nitrogen atmosphere. MS was run on a BRUKER ESQUIRE 2000 or on a BRUKER amaZon SL mass spectrometer using ESI ionization. HRMS was run on a AB Sciex Triple TOF660 SCiex, source type ESI or at the Chair of Organic Chemistry,

Friedrich Alexander University Erlangen-Nuernberg on a Bruker Daltonik micrOTOF II focus or Bruker Daltonik maXis 4G, source type ESI or APPI. NMR spectra were recorded on a Bruker Avance 400, Bruker Avance 360 or a Bruker Avance 600 spectrometer at 300 K in the solvents indicated. Chemical shifts are given in ppm (δ) relative to TMS. IR spectra were obtained on a Jasco FT/IR 4100 spectrometer using a substance film on a NaCl crystal. Purification by column chromatography was performed with silica gel 60. TLC analyses were performed using Merck 60 F254 aluminum plates in combination with UV detection (254 nm) or ninhydrin staining. Analytical HPLC was conducted on an Agilent 1200 HPLC system employing a DAD detector and a ZORBAX ECLIPSE XDB-C8 (4.6 mm × 150 mm, 5 μm) column with the following binary solvent systems: System 1: eluent, methanol/0.1% aq trifluoroacetic acid, 10% methanol for 3 min, to 100% in 15 min, 100% for 6 min, to 10% in 3min, then 10% for 3min, flow rate 5 mL/min, λ = 210 or 254 nm; System 2: CH_3CN/0.1% aq trifluoroacetic acid, 10% CH_3CN for 3 min, to 100% in 15 min, 100% for 6 min, to 10% in 3min, then 10% for 3min, flow rate 5 mL/min, λ = 210 or 254 nm. Preparative HPLC was performed on an Agilent 1100 Preparative Series, using a MACHEREY-NAGEL Varioprep VP 250/32 Nucleodur C18 HTec (32 x 250 mm, 5 μm, flow rate 32 mL/min), a ZORBAX ECLIPSE XDB-C8 PrepHT (21.5 x 150 mm, 5 μm, flow rate 10 mL/min) or a MACHEREY-NAGEL Varioprep VP 250/10 Nucleodur C18 HTec (10 x 250 mm, 5 μm, flow rate 3 mL/min) column with the solvent systems indicated.

2,8-Bis(benzyloxy)-5-bromoquinoline (5d). To a solution of **5c** (863 mg, 2.6 mmol) and K_2CO_3 (1.08 g, 2.6 mmol) in DMF (26 mL) was added dropwise benzyl bromide (0.5 mL, 3.9 mmol). The mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was diluted with water. The product was extracted with EtOAc, the combined organic layers were washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (hexanes/EtOAc 30:1) yielded **5d** (990 mg, 91%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.31 (s, 2H), 5.58 (s, 2H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.05 (d, *J* = 9.1 Hz, 1H), 7.28 – 7.36 (m, 4H), 7.37

– 7.42 (m, 2H), 7.50 (d, J = 8.4 Hz, 1H), 7.52 – 7.58 (m, 4H), 8.33 (d, J = 9.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 68.16, 71.55, 112.9, 113.0, 114.7, 125.4, 127.4, 127.5, 128.0, 128.1, 128.5, 128.7, 128.8, 137.2, 137.2, 138.7, 139.5, 153.2, 161.6. IR (NaCl) ν 3030, 1610, 1597, 1502, 1404, 1272, 1253, 1095, 1006, 914, 826, 732, 695 cm^{-1}. HPLC (system 1) t_R = 23.1 min, purity >99 %, (system 2) t_R = 22.6 min, purity >99 %. ESI-MS *m/z* 420.0

***tert*-Butyl 4-[2,8-bis(benzyloxy)quinolin-5-yl]piperazine-1-carboxylate (5e).** To a solution of 1-boc-piperazine (1.3 g, 7.2 mmol) in toluene (51 mL) were added $Pd(OAc)_2$ (32 mg, 0.14 mmol) and *rac*-BINAP (286 mg, 0.43 mmol) under N_2-atmosphere. After stirring for 15 min at room temperature, **5d** (1.5 g, 3.6 mmol) and NaO^{t}Bu (0.5 g, 5.0 mmol) were added and the resulting mixture was stirred under reflux for 24 h. After cooling down to room temperature, EtOAc was added and the organic layer was washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (hexanes/EtOAC 20:1 to 2:1) yielded **5e** (1.2 g, 63%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.50 (s, 9H), 2.79 – 3.08 (m, 4H), 3.37 – 3.77 (m, 4H), 5.30 (s, 2H), 5.58 (s, 2H), 6.88 (d, J = 8.3 Hz, 1H), 6.98 (d, J = 9.0 Hz, 1H), 7.05 (d, J = 8.3 Hz, 1H), 7.28 – 7.33 (m, 2H), 7.33 – 7.40 (m, 4H), 7.47 – 7.67 (m, 4H), 8.39 (d, J = 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 28.62, 53.47, 67.85, 71.98, 79.99, 112.8, 113.0, 113.5, 122.2, 127.4, 127.8, 128.0, 128.5, 128.6, 128.7, 135.2, 137.6, 137.9, 139.7, 143.6, 150.3, 155.0, 161.3. IR (NaCl) ν 2924, 2854, 1695, 1610, 1411, 1261, 1246, 1169, 1003, 734, 696 cm^{-1}. HPLC (system 1) t_R = 23.1 min, purity >99 %, (system 2) t_R = 22.5 min, purity >99 %. ESI-MS *m/z* 526.7 [M+ H$^+$].

***tert*-Butyl 4-[2,8-bis(benzyloxy)quinolin-5-yl]-1,4-diazepane-1-carboxylate (5f).** To a solution of *N*-(*tert*-butyl-oxycarbonyl)-homopiperazine (380 mg, 1.9 mmol) in toluene (15 mL), $Pd(OAc)_2$ (8.5 mg, 0.04 mmol) and *rac*-BINAP (71 mg, 0.11 mmol) were added. After stirring for 15 min, **5d** (400 mg, 0.95 mmol) and NaO^{t}Bu (128 mg, 1.3 mmol) were added. The tube was sealed and the reaction mixture was stirred at 115°C for 1 d. After cooling down to room temperature, the

reaction mixture was diluted with EtOAc, the organic layer was washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (hexanes/EtOAc 40:1 to 10:1 to 2:1) yielded **1f** (350 mg, 68%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.50 (d, J = 9.8 Hz, 9H), 1.86 – 2.13 (m, 2H), 3.02 – 3.27 (m, 4H), 3.55 – 3.73 (m, 4H), 5.30 (s, 2H), 5.58 (s, 2H), 6.94 (dd, J = 8.2, 2.6 Hz, 1H), 6.98 (d, J = 9.0 Hz, 1H), 7.00 – 7.06 (m, J = 8.2, 2.0 Hz, 1H), 7.28 – 7.33 (m, 2H), 7.33 – 7.41 (m, 4H), 7.55 (dd, J = 11.8, 7.4 Hz, 4H), 8.44 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 28.69, (29.19), 29.35, (45.69), 46.48, (47.79), 48.14, (56.57), 56.97, (57.40), 57.46, 67.80, 71.98, (79.60), 79,64, (112.7), 113.1, (115.2), 115.2, 122.8, 127.4, 127.8, 128.0, 128.5, 128.6, 128.7, 135.6, (137.6), 137.6, 137.9, 139.5, (146.0), 146.1, (150.0), 150.0, (155.7), 155.8, 161.3 (signals in brackets represent resonances caused by rotamers). IR (NaCl) ν 3432, 2974, 1645, 1481, 1453, 1408, 1366, 1312, 1261, 1154, 736, 699 cm^{-1}. HPLC (system 1) t_R = 23.0 min, purity >98 %, (system 2) t_R = 22.7 min, purity >99 %. ESI-MS *m/z* 540.2 [M+ H$^+$].

4-[2,8-Bis(benzyloxy)quinolin-5-yl]piperazine (5g). To a solution of **5e** (550 mg, 0.95 mmol) in CH_2Cl_2 (13 mL) was added TFA (1.3 mL). The mixture was stirred at room temperature for 2 h. The reaction was stopped by adjusting the pH to 9 by addition of 1 M NaOH. The product was extracted with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4 and evaporated. Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **5g** (220 mg, 51 %) as a yellow solid. ^{1}H NMR ($CDCl_3$, 360 MHz) δ 3.11 (m, 4H), 3.26 (m, 4H), 3.45 (s, 1H), 5.31 (s, 2H), 5.58 (s, 2H), 6.94 (d, J = 8.3 Hz, 1H), 6.99 (d, J = 9.0 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.43 – 7.27 (m, 6H), 7.55 (t, J = 7.3 Hz, 4H), 8.36 (d, J = 9.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 45.54, 52.64, 67.89, 71.89, 112.8, 113.0, 114.0, 122.1, 127.4, 127.9, 128.0, 128.5, 128.6, 128.7, 135.0, 137.5, 137.8, 139.7, 143.0, 150.6, 161.3. IR (NaCl) ν 3439, 3031, 2946, 1610, 1411, 1264, 1005, 742, 693 cm^{-1}. HPLC (system 1) t_R = 19.9 min, purity 99 %, (system 2) t_R = 16.5 min, purity 93 %. ESI-MS *m/z* 426.1 [M+ H$^+$].

4-[2,8-Bis(benzyloxy)quinolin-5-yl]-1,4-diazepane (5h). Compound **5h** was prepared as described for **5g**, using a mixture of **5f** (200 mg, 0.37 mmol) and TFA (0.5 mL) in CH_2Cl_2 (5.0 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **5h** (129 mg, 79%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.95 – 2.12 (m, 2H), 3.09 – 3.40 (m, 8H), 5.30 (s, 2H), 5.58 (s, 2H), 6.94 – 7.01 (m, 2H), 7.04 (d, J = 8.4 Hz, 1H), 7.27 – 7.32 (m, 2H), 7.33 – 7.40 (m, 4H), 7.49 – 7.58 (m, 4H), 8.46 (d, J = 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 29.97, 46.92, 49.40, 56.66, 57.79, 67.82, 72.02, 112.8, 113.1, 115.4, 122.8, 127.4, 127.8, 127.9, 128.5, 128.6, 128.7, 135.6, 137.6, 137.9, 139.6, 145.9, 150.1, 161.3. IR (NaCl) ν 3432, 3034, 2937, 2848, 1650, 1559, 1506, 1411, 1260, 1093, 733, 697 cm^{-1}. HPLC (system 1) t_R = 20.0 min, purity 87 %, (system 2) t_R = 16.8 min, purity 91 %. ESI-MS *m/z* 440.2 [M+ H$^+$].

***tert*-Butyl 4-[8-(benzyloxy)-2-oxo-2*H*-chromen-5-yl]piperazine-1-carboxylate (6d).** $Pd_2(dba)_3$ (24 mg, 0.03 mmol) and *rac*-BINAP (49 mg, 0.08 mmol) were dissolved in toluene (9 mL) and stirred for 5 min under nitrogen atmosphere. Then Cs_2CO_3 (300 mg, 0.92 mmol), 1-boc-piperazine (307 mg, 1.6 mmol), and **6c** (220 mg, 0.66 mmol) were added and the reaction mixture was stirred in a sealed microwave reaction tube at 85°C for 1 d. After cooling down to room temperature, the reaction was diluted with EtOAc was added. The combined organic layer was washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (hexanes/EtOAc 20:1 to 5:1) yielded **6d** (172 mg, 59%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.49 (s, 9H), 2.75 – 3.08 (m, 4H), 3.45 – 3.88 (m, 4H), 5.20 (s, 2H), 6.43 (d, J = 9.8 Hz, 1H), 6.80 (d, J = 8.7 Hz, 1H), 7.03 (d, J = 8.7 Hz, 1H), 7.32 (t, J = 7.3 Hz, 1H), 7.38 (t, J = 7.5 Hz, 2H), 7.47 (d, J = 7.4 Hz, 2H), 8.07 (d, J = 9.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 28.58, 44.16, 53.55, 71.85, 80.20, 114.7, 115.4, 116.0, 116.9, 127.5, 128.2, 128.8, 136.6, 140.1, 143.0, 143.9, 145.5, 154.9, 160.3. IR (NaCl) ν 2975, 2917, 2857, 1727, 1690, 1487, 1454, 1424, 1267, 1250, 1170, 1127, 1069, 1011, 908, 810 cm^{-1}. HPLC

(system 1) t_R = 21.2 min, purity 94 %; (system 2) t_R = 19.8 min, purity 99 %. ESI-MS *m/z* 459.2 [M+Na$^+$].

***tert*-Butyl 4-[8-(benzyloxy)-2-oxo-2*H*-chromen-5-yl]-1,4-diazepane-1-carboxylate (6e).** Compound **6e** was prepared according to the protocol of **6d**, using a solution of $Pd_2(dba)_3$ (24 mg, 0.03 mmol), *rac*-BINAP (49 mg, 0.08 mmol), Cs_2CO_3 (300 mg, 0.92 mmol), *N*-(*tert*-butyl-oxycarbonyl)-homopiperazine (307 mg, 1.6 mmol), and **6c** (220 mg, 0.66 mmol) in toluene (9 mL). Purification by flash chromatography (hexanes/EtOAc 20:1 to 5:1) yielded **6e** (172 mg, 59%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.46 – 1.53 (m, 9H), 1.88 – 2.04 (m, 2H), 3.03 – 3.16 (m, 4H), 3.52 – 3.59 (m, 2H), 3.59 – 3.70 (m, 2H), 5.20 (s, 2H), 6.41 (d, *J* = 9.7 Hz, 1H), 6.85 (dd, *J* = 8.7, 4.0 Hz, 1H), 7.00 (dd, *J* = 8.6, 3.6 Hz, 1H), 7.31 – 7.33 (m, 1H), 7.36 – 7.40 (m, 2H), 7.45 – 7.48 (m, 2H), 8.07 – 8.14 (m, 1H). ^{13}C NMR (CDCl3, 101 MHz) δ 28.50, (28.85), 29.08, (45.46), 46.33, (47.25), 47.66, (56.34), 56.75, 57.19, 71.67, 115.6, 116.3, 116.8, 127.4, 128.0, 128.6, 129.5, 134.0, 136.5, 140.5 (signals in brackets represent resonances caused by rotamers) IR (NaCl) ν 3416, 2974, 2927, 1730, 1655, 1487, 1264, 1070 cm^{-1}. HPLC (system 1) t_R = 21.5 min, purity 96 %; (system 2) t_R = 20.1 min, purity 96 %. ESI-MS *m/z* 451.1 [M+H$^+$].

4-[8-(Benzyloxy)-2-oxo-2*H*-chromen-5-yl]piperazine (6f). Compound **6f** was prepared as described for **6g**, using a mixture of **6d** (170 mg, 0.39 mmol) and TFA (0.3 mL) in CH_2Cl_2 (5.3 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **6f** (85 mg, 65%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 360 MHz) δ 2.71 – 2.87 (m, 4H), 2.87 – 3.03 (m, 4H), 5.20 (s, 2H), 6.46 (d, *J* = 9.8 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 1H), 7.34 – 7.38 (m, 1H), 7.38 – 7.45 (m, 2H), 7.46 – 7.52 (m, 2H), 8.11 (d, *J* = 9.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 45.53, 54.00, 70.45, 114.2, 114.3, 115.1, 116.3, 127.8, 128.0, 128.5, 136.7, 140.7, 141.6, 144.2, 159.5. IR (NaCl) ν 3432, 1684, 1377, 1272, 1204, 1131, 1060 cm^{-1}. HPLC (system 1) t_R = 16.9 min, purity 99 %; (system 2) t_R = 14.0 min, purity 99 %. ESI-MS *m/z* 337.2 [M+H$^+$].

4-[8-(Benzyloxy)-2-oxo-2*H*-chromen-5-yl]-1,4-diazepane (6g). Compound **6g** was prepared as described for **5g**, using a mixture of **6e** (87 mg, 0.19 mmol) and TFA (0.25 mL) in CH_2Cl_2 (2.50 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **6g** (38 mg, 57%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.91 – 2.02 (m, 2H). 3.08 – 3.13 (m, 2H), 3.13 – 3.21 (m, 2H), 3.22 – 3.28 (m, 2H), 3.29 – 3.41 (m, 2H), 5.19 (d, *J* = 8.3 Hz, 1H), 6.48 (d, *J* = 9.8 Hz, 1H), 7.04 (d, *J* = 8.8 Hz, 1H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.34 – 7.38 (m, 1H), 7.39 – 7.45 (m, 2H), 7.46 – 7.52 (m, 2H), 8.24 (d, *J* = 9.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 27.33, 45.12, 47.02, 54.48, 55.86, 70.42, 115.0, 115.2, 116.3, 116.5, 127.8, 128.0, 128.5, 136.6, 141.2, 141.7, 143.9, 145.3, 159.5. IR (NaCl) ν 3421, 2959, 2922, 2850, 1722, 1651, 1488, 1454, 1270, 1192, 1134, 1067, 806, 699 cm^{-1}. HPLC (system 1) t_R = 17.1 min, purity 91 %; (system 2) t_R = 14.2 min, purity 88 %. ESI-MS *m/z* 351.1 [M+H$^+$].

4-Benzyl-5-(benzyloxy)-8-bromo-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (7d). To a solution of **7c** (3.7 g, 11.0 mmol) and K_2CO_3 (4.5 g, 33.0 mmol) in DMF (110 mL) was added dropwise benzyl bromide (1.9 mL, 16.5 mmol). The mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was diluted with water. The product was extracted with EtOAc, the combined organic layers were washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (hexanes/EtOAc 12:1 to 1:1) yielded **7d** (2.67 g, 57%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 4.65 (s, 2H), 4.95 (s, 2H), 5.37 (s, 2H), 6.50 (d, *J* = 9.0 Hz, 1H), 6.99 (d, *J* = 7.1 Hz, 2H), 7.13 (d, *J* = 9.0 Hz, 1H), 7.14 – 7.20 (m, 3H), 7.28 – 7.32 (m, 2H), 7.34 – 7.41 (m, 3H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 46.89, 69.24, 71.63, 102.8, 109.2, 120.2, 127.0, 127.1, 127.7, 128.2, 128.4, 128.6, 128.9, 135.8, 137.0, 146.7, 148.8, 166.2. IR (NaCl) ν 1692, 1485, 1456, 1386, 1279, 1103, 1016, 739, 702 cm^{-1}. HPLC (system 1) t_R = 21.7 min, purity 89 % (system 2) t_R = 20.2 min, purity 87 %. ESI-MS *m/z* 425.9 [M+H$^+$].

***tert*-Butyl 4-[4-benzyl-5-(benzyloxy)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl]piperazin-1-carboxylate (7e).** To a solution of 1-boc-piperazine (385 mg, 2.1 mmol) in toluene (13 mL) were added $Pd(OAc)_2$ (8.5 mg, 0.04 mmol) and *rac*-BINAP (70 mg, 0.13 mmol) under nitrogen atmosphere. After stirring for 15 min, **7d** (400 mg, 0.94 mmol) and Cs_2CO_3 (1.1 g, 3.3 mmol) were added. The resulting mixture heated to reflux for 72 h. After cooling down to room temperature, the reaction mixture was diluted with EtOAc, the organic layer was washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (H/EtOAC 12:1 to 2:1) yielded **7e** (313 mg, 66%) as a light brown solid. ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.48 (s, 9H), 2.80 – 2.95 (m, 4H), 3.49 – 3.65 (m, 4H), 4.57 (s, 2H), 4.92 (s, 2H), 5.39 (s, 2H), 6.52 (d, 1H, J = 9.0 Hz), 6.57 (d, 1H, J = 9.0 Hz), 6.95 – 7.05 (m, 2H), 7.08 – 7.21 (m, 3H), 7.28 – 7.51 (m, 5H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 28.60, 46.90, 51.25, 68.95, 71.61, 79.94, 107.8, 114.5, 120.2, 127.0, 127.0, 127.7, 128.3, 128.4, 128.8, 135.9, 136.4, 137.40, 142.9, 145.4, 154.9, 166.6. IR (NaCl) ν 2923, 2852, 1688, 1502, 1390, 1245, 1169, 1101, 1023, 911, 732 cm^{-1}. HPLC (system 1) t_R = 21.8 min, purity 89 % (system 2) t_R = 19.9 min, purity 91 %. ESI-MS *m/z* 530.1 [M+H$^+$].

***tert*-Butyl 4-[4-benzyl-5-(benzyloxy)-3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-8-yl]-1,4-diazepane-1-carboxylate (7f).** Compound **7f** was prepared as described for **7e**, using a solution of *N*-(*tert*-butyl-oxycarbonyl)-homopiperazine (467 mg, 2.3 mmol), $Pd(OAc)_2$ (9.5 mg, 0.04 mmol), *rac*-BINAP (74 mg, 0.12 mmol), **7d** (450 mg, 1.1 mmol) and Cs_2CO_3 (1.21 g, 3.7 mmol) in toluene (15 mL), allowing a reaction time of 48 h. Purification by flash chromatography (hexanes/EtOAc 12:1 to 2:1) yielded **7f** (218 mg, 38%) as a light brown solid. ^{1}H NMR ($CDCl_3$, 600 MHz, incl. rotamers) δ 1.45 (d, J = 10.8 Hz, 9H), 2.01 – 1.81 (m, 2H), 3.32 – 3.02 (m, 4H), 3.67 – 3.36 (m, 4H), 4.53 (s, 2H), 4.90 (d, J = 3.6 Hz, 2H), 5.38 (s, 2H), 6.48 (dd, J = 8.8, 4.9 Hz, 1H), 6.59 (d, J = 9.0 Hz, 1H), 7.07 – 6.99 (m, 2H), 7.19 – 7.10 (m, 3H), 7.32 – 7.29 (m, 2H), 7.39 – 7.32 (m, 3H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 21.17, 28.65, 45.92, 46.92, 48.06, 53.65, 54.17, 68.92, 71.71, 79.48, 108.0, 115.1, 120.3,

126.9, 127.1, 127.7, 128.3, 128.8, 136.6, 136.7, 137.5, 142.4, 144.4, 155.6, 166.8. IR (NaCl) ν 3026, 2946, 2830, 1686, 1503, 1454, 1385, 1267, 1023, 738, 695 cm^{-1}. HPLC (system 1) t_R = 21.8 min, purity 74 %. ESI-MS *m/z* 544.2 [M+H^+].

4-[4-Benzyl-5-(benzyloxy)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl]piperazine (7g). Compound **7g** was prepared as described for **5g**, using a mixture of **7e** (255 mg, 0.48 mmol) and TFA (0.6 mL) in CH_2Cl_2 (6.4 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **7g** (161 mg, 87%) as a light yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.00 – 2.95 (m, 4H), 3.13 – 3.03 (m, 4H), 4.57 (s, 2H), 4.92 (s, 2H), 5.39 (s, 2H), 6.53 (d, *J* = 9.0 Hz, 1H), 6.60 (d, *J* = 9.0 Hz, 1H), 7.03 – 6.99 (m, 2H), 7.18 – 7.10 (m, 3H), 7.32 – 7.29 (m, 2H), 7.39 – 7.33 (m, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 46.08, 46.85, 52.15, 68.89, 71.54, 107.8, 114.4, 120.1, 126.9, 127.0, 127.7, 128.3, 128.3, 128.8, 136.4, 136.4, 137.4, 142.8, 145.2, 166.7. IR (NaCl) ν 3432, 2851, 1655, 1504, 1457, 1389, 1258, 1101, 1262cm^{-1}. HPLC (system 1) t_R = 19.0 min, purity 99 % (system 2) t_R = 15.2 min, purity 96 %. ESI-MS *m/z* 430.0 [M+H^+].

4-[4-Benzyl-5-(benzyloxy)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl]-1,4-diazepane (7h). Compound **7h** was prepared according to the protocol of **5g**, using a mixture of **7f** (156 mg, 0.29 mmol) and TFA (0.4 mL) in CH_2Cl_2 (4.0 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **7h** (78 mg, 61%) as a light yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.82 – 1. 89 (m, 2H). 2.93 – 2.97 (m, 2H), 2.97 – 3.02 (m, 2H), 3.14 – 3. 21 (m, 4H), 4.58 (s, 2H), 5.00 (s, 2H), 5.29 (s, 2H), 6.63 (d, *J* = 9.1 Hz, 1H), 6.67 (d, *J* = 9.2 Hz, 1H), 6.99 – 7.04 (m, 2H), 7.11 – 7. 19 (m, 1H), 7.19 – 7. 26 (m, 2H), 7.30 – 7.45 (m, 5H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 28.74, 45.56, 46.07, 48.40, 51.31, 53.55, 68.23, 70.75, 108.0, 113.9, 119.6, 126.5, 126.8, 127.7, 127.9, 128.2, 128.5, 136.4, 136.6, 137.4, 141.2, 143.1, 166.3. IR (NaCl) ν 3026, 2946, 2830, 1686, 1503, 1454, 1385, 1267, 1023, 738, 695 cm^{-1}. HPLC (system 1) t_R = 19.3 min, purity 94 %. ESI-MS *m/z* 444.1 [M+H^+].

***tert*-Butyl 4-[8-(Benzyloxy)-2,3-dihydrobenzo[*b*][1,4]dioxin-5-yl]piperazine-1-carboxylate (8d).** $Pd_2(dba)_3$ (23 mg, 0.025 mmol) and *rac*-BINAP (46 mg, 0.07 mmol) were dissolved in toluene (9 mL) and stirred for 5 min under nitrogen atmosphere. Then NaO^tBu (83 mg, 0.87 mmol), 1-boc-piperazin (290 mg, 1.56 mmol) and **8c** (200 mg, 0.62 mmol) were added and the reaction mixture was stirred in a sealed microwave reaction tube at 85°C for 1 d. After cooling down to room temperature, the reaction was diluted with EtOAc was added. The combined organic layer was washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification by flash chromatography (hexanes/EtOAc 10:1 to 1:1) yielded **8d** (185 mg, 70%) as orange solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.48 (s, 9H), 2.89 – 2.94 (m, 4H), 3.53 – 3.63 (m, 4H), 4.29 – 4.33 (m, 2H), 4.33 – 4.40 (m, 2H), 5.09 (s, 2H), 6.38 (d, J = 8.9 Hz, 1H), 6.45 (d, J = 8.9 Hz, 1H), 7.27 – 7.32 (m, 1H), 7.33 – 7.38 (m, 2H), 7.42 – 7.45 (m, 2H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 28.61, 44.13, 51.29, 64.21, 64.39, 71.61, 79.83, 105.8, 109.2, 127.5, 128.0, 128.6, 134.9, 135.8, 137.4, 137.7, 144.5, 154.9. IR (NaCl) ν 2922, 2852, 1689, 1500, 1453, 1422, 1364, 1282, 1239, 1170, 1116, 998 cm^{-1}. HPLC (system 1) t_R = 20.2 min, purity 92 %; (system 2) t_R = 16.7 min, purity 91 %. ESI-MS *m/z* 427.1 [M+H$^+$].

1-[8-(Benzyloxy)-2,3-dihydrobenzo[*b*][1,4]dioxin-5-yl]piperazine (8e). Compound **8e** was prepared according to the protocol of **5g**, using a solution of **8d** (450 mg, 1.05 mmol) in CH_2Cl_2 (12 mL) and TFA (1.2 mL) Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **8e** (214 mg, 66%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.99 – 2.96 (m, 4H), 3.10 – 3.06 (m, 4H), 4.33 – 4.30 (m, 2H), 4.35 – 4.33 (m, 2H), 5.09 (s, 2H), 6.41 (d, J = 8.9 Hz, 1H), 6.45 (d, J = 8.9 Hz, 1H), 7.29 (t, J = 7.3 Hz, 1H), 7.36 (dd, J = 10.3, 4.7 Hz, 2H), 7.43 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 46.18, 52.19, 64.21, 64.36, 71.61, 105.8, 109.2, 127.6, 128.0, 128.6, 134.9, 136.2, 137.4, 137.7, 144.3. IR(NaCl) ν 3396, 2926, 2873, 1501, 1471, 1278, 1239, 1112, 996, 789 cm^{-1}. HPLC (system 1) t_R = 16.6 min, purity 93 %; (system 2) t_R = 13.5 min, purity 95 %. ESI-MS *m/z* 327.1 [M+H$^+$].

***tert*-Butyl 4-[8-(benzyloxy)quinolin-5-yl]piperazine-1-carboxylate (9d).** To a solution of 1-boc-piperazine (391 mg, 2.1 mmol) in toluene (15 mL) $Pd(OAc)_2$ (9 mg, 0.04 mmol) and *rac*-BINAP (0.13 mmol, 86 mg) were added. After stirring for 15 min, **9c** (330 mg, 1.0 mmol) and NaO^tBu (141 mg, 1.47 mmol) were added. The tube was sealed and the reaction mixture was stirred at 115°C for 1 d. After cooling down to room temperature, the reaction mixture was diluted with EtOAc, the organic layer was washed with 0.5 M HCl, water and brine, dried (Na_2SO_4) and evaporated. Purification of the crude product by flash chromatography (H/EtOAc 20:1 to 2:1) yielded **9d** (320 mg, 73%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.50 (s, 9H), 2.72 – 3.18 (m, 4H), 3.31 – 4.22 (m, 4H), 5.42 (s, 2H), 6.94 (d, *J* = 8.3 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 1H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.37 (dd, *J* = 10.4, 4.7 Hz, 2H), 7.46 (dd, *J* = 8.5, 4.2 Hz, 1H), 7.49 – 7.55 (m, 2H), 8.55 (dd, *J* = 8.5, 1.7 Hz, 1H), 8.99 (dd, *J* = 4.2, 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 28.59, 44.43, 53.37, 70.98, 80.01, 109.7, 115.8, 121.2, 125.5, 127.2, 127.9, 128.7, 132.0, 137.3, 141.3, 142.4, 149.4, 151.2, 155.0. IR (NaCl) ν 2974, 2928, 2858, 2815, 1691, 1608, 1454, 1365, 1247, 1170, 1103, 1008, 912, 734 cm^{-1}. HPLC (system 1) t_R = 19.1 min, purity 99 % (system 2) t_R = 16.3 min, purity 99 %. ESI-MS *m/z* 420.4 [$M+H^+$].

4-[8-(Benzyloxy)quinolin-5-yl]piperazine (9e). Compound **9e** was prepared following the protocol of **5g**, using a mixture of **9d** (600 mg, 1.43 mmol) and TFA (1.8 mL) in CH_2Cl_2 (18 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 15:1) yielded **9e** (443 mg, 97%) as a light yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.97 – 3.08 (m, 4H), 3.16 (t, *J* = 4.8 Hz, 4H), 5.42 (s, 2H), 6.94 (d, *J* = 8.3 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 7.33 – 7.38 (m, 2H), 7.45 (dd, *J* = 8.5, 4.1 Hz, 1H), 7.51 (d, *J* = 7.2 Hz, 2H), 8.53 (dd, *J* = 8.5, 1.7 Hz, 1H), 8.98 (dd, *J* = 4.1, 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 46.42, 54.23, 71.00, 109.7, 115.7, 121.1, 125.5, 127.2, 127.9, 128.7, 132.1, 137.4, 141.4, 142.9, 149.4, 151.1. IR (NaCl) ν 3416, 3062, 2943, 2821, 1607, 1472, 1364, 1304,

1253, 1099, 911, 816, 792, 734 cm^{-1}. HPLC (system 1) t_R = 14.2 min, purity 99 % (system 1) t_R = 11.5 min, purity 99 %. ESI-MS *m/z* 320.3 [M+H^+], 342.2 [M+Na^+].

7-(4-{4-[2,8-bis(benzyloxy)quinolin-5-yl]piperazin-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (10a). A solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (214 mg, 0.72 mmol) and NaI (162 mg, 1.08 mmol) in acetonitrile (3.6 mL) was heated to for 0.5 h before a solution **5g** (400 mg, 0.94 mmol) and triethylamine (150 µL, 1.08 mmol) in acetonitrile (1.8 mL) was added. The reaction mixture was stirred at 80°C for further 16 h. After cooling down to room temperature, saturated $NaHCO_3$ solution was added and the product was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated. Purification by flash chromatography (CH_2Cl_2/MeOH 40:1 to15:1) yielded **10a** (263 mg, 57%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.68 – 1.78 (m, 2H), 1.78 – 1. 90 (m, 2H), 2.49 – 2.56 (m, 2H), 2.61 (dd, *J* = 8.2, 6.8 Hz, 2H), 2.65 – 2.86 (m, 4H), 2.83 – 2.94 (m, 2H), 2.96 – 3.12 (m, 4H), 3.97 (t, *J* = 6.3 Hz, 2H), 5.30 (s, 2H), 5.58 (s, 2H), 6.33 (d, *J* = 2.4 Hz, 1H), 6.53 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.97 (d, *J* = 9.0 Hz, 1H), 7.02 – 7.08 (m, 2H), 7.27 – 7.33 (m, 2H), 7.33 – 7.43 (m, 4H), 7.50 – 7.60 (m, 4H), 8.07 (s, 1H), 8.38 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 23.58, 24.75, 27.43, 31.25, 53.48, 53.89, 58.45, 67.80, 68.06, 71.99, 102.3, 108.8, 112.5, 113.1, 113.2, 115.9, 122.1, 127.4, 127.8, 127.9, 128.5, 128.5, 128.7, 128.8, 135.5, 137.6, 138.0, 138.3, 139.7, 143.9, 150.0, 158.8, 161.2, 171.8. IR (NaCl) ν 3416, 2918, 2850, 2090, 1633, 1268 cm^{-1}. HPLC (system 1) t_R = 19.4 min, purity 96 %, (system 2) t_R = 16.3 min, purity 99 %. ESI-MS *m/z* 644.1 [M+ H^+].

7-(4-{4-[8-(Benzyloxy)-2-oxo-2*H*-chromen-5-yl]piperazin-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (10b). Compound **10b** was prepared as described for **10a,** using a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (27 mg, 0.092 mmol) and NaI (25 mg, 0.166 mmol) in acetonitrile (0.4 mL) and a solution of **6f** (34 mg, 0.101 mmol) and triethylamine (23 µL, 0.166 mmol) in acetonitrile (0.2 mL). Purification by flash chromatography (CH_2Cl_2/MeOH

40:1 to15:1) yielded **10b** (37 mg, 74%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.54 – 1.63 (m, 2H), 1.69 – 1.76 (m, 2H), 2.36 – 2.45 (m, 4H), 2.55 – 2.63 (m, 4H), 2.77 (t, J = 7.5 Hz, 2H), 2.88 (s, 4H), 3.92 (t, J = 6.4 Hz, 2H), 5.20 (s, 2H), 6.43 (d, J = 2.5 Hz, 1H), 6.47 (d, J = 9.8 Hz, 1H), 6.49 (dd, J = 8.3, 2.5 Hz, 1H), 6.95 (d, J = 8.8 Hz, 1H), 7.04 (d, J = 8.3 Hz, 1H), 7.30 (d, J = 8.8 Hz, 1H), 7.35 (t, J = 7.3 Hz, 1H), 7.42 (dd, J = 10.3, 4.7 Hz, 2H), 7.49 (d, J = 7.2 Hz, 2H), 8.08 (d, J = 9.8 Hz, 1H), 9.96 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 22.73, 23.98, 26.60, 30.74, 52.90, 53.14, 57.31, 67.28, 70.43, 101.7, 107.5, 114.2, 114.3, 115.2, 115.4, 116.3, 127.8, 128.0, 128.4, 128.5, 136.7, 139.2, 140.6, 141.6, 143.7, 144.1, 157.9, 159.5, 170.2. IR (NaCl) ν 3406, 1645, 1269, 1196, 1066 cm^{-1}. HPLC (system 1) t_R = 17.8 min, purity 99 %, (system 2) t_R = 15.3 min, purity 99 %. ESI-MS *m/z* 554.4 [M+ H$^+$].

7-(4-{4-[4-Benzyl-5-(benzyloxy)-2*H*-benzo[b][1,4]oxazin-3(4*H*)-one-8-yl]piperazin-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (10c). Compound **10c** was following the protocol for **10a** using a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (27 mg, 0.09 mmol) and NaI (24 mg, 0.16 mmol) in acetonitrile (0.9 mL) and a solution of **7g** (47 mg, 0.11 mmol) and triethylamine (20 µL, 0.16 mmol) in acetonitrile (0.4 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 40:1 to 15:1) yielded **10c** (40 mg, 68%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.73 – 1.77 (m, 2H), 1.78 – 1.98 (m, 2H), 2.53 – 2.58 (m, 2H), 2.57 – 2.68 (m, 2H), 2.72 – 2.82 (m, 4H), 2.85 – 2.96 (m, 2H), 3.02 – 3.11 (m, 4H), 3.96 (t, J = 6.1 Hz, 2H), 4.57 (s, 2H), 4.91 (s, 2H), 5.38 (s, 2H), 6.30 (d, J = 2.4 Hz, 1H), 6.50 – 6.53 (m, 1H), 6.53 (d, J = 9.0 Hz, 1H), 6.61 (d, J = 9.0 Hz, 1H), 6.97 – 7.03 (m, 2H), 7.04 (d, J = 8.3 Hz, 1H), 7.10 – 7.18 (m, 3H), 7.29 – 7.33 (m, 2H), 7.33 – 7.40 (m, 3H), 7.77 (s, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 22.96, 24.59, 27.13, 31.09, 46.73, 50.47, 53.08, 58.04, 67.76, 68.77, 71.40, 102.2, 107.7, 108.7, 114.2, 115.8, 119.9, 126.8, 126.9, 127.6, 128.2, 128.2, 128.6, 128.6, 135.5, 136.2, 137.2, 138.1, 142.6, 145.1, 158.6, 166.5, 171.7. IR (NaCl) ν 3437, 2948, 2823, 1650,

1387, 1272, 1187, 1097 cm^{-1}. HPLC (system 1) t_R = 19.3 min, purity 99 %, (system 2) t_R = 16.3 min, purity 99 %. ESI-MS *m/z* 647.5 [M+ H^+].

7-(4-{4-[8-(Benzyloxy)-2,3-dihydrobenzo[*b*][1,4]dioxin-5-yl]piperazin-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (10d). Compound **10d** was following the protocol for **10a** using a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (23 mg, 0.08 mmol) and NaI (20 mg, 0.14 mmol) in acetonitrile (0.5 mL) and a solution of **8e** (34 mg, 0.10 mmol) and triethylamine (19 µL, 0.14 mmol) in acetonitrile (0.3 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 40:1 to 15:1) yielded **10d** (27 mg, 64 %) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.68 – 1.77 (m, 2H), 1.78 – 1.87 (m, 2H), 2.41 – 2.53 (m, 2H), 2.54 – 2.65 (m, 2H), 2.67 – 2.74 (m, 4H), 2.86 – 2.93 (m, 2H), 2.95 – 3.16 (m, 4H), 3.96 (t, *J* = 6.3 Hz, 2H), 4.30 – 4.32 (m, 2H), 4.32 – 4.35 (m, 2H), 5.09 (s, 2H), 6.27 (d, *J* = 2.4 Hz, 1H), 6.41 (d, *J* = 8.9 Hz, 1H), 6.45 (d, *J* = 8.9 Hz, 1H), 6.52 (dd, *J* = 8.3, 2.4 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 7.32 – 7.28 (m, 1H), 7.33 – 7.39 (m, 2H), 7.42 – 7.44 (m, 2H), 7.45 (s, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 23.45, 24.78, 27.41, 31.27, 51.08, 53.51, 58.38, 64.20, 64.38, 68.09, 71.62, 102.3, 105.8, 108.8, 109.1, 115.9, 127.6, 128.0, 128.7, 128.9, 134.8, 137.5, 137.7, 138.2, 144.3, 158.8, 171.4. IR (NaCl) ν 3432, 2817, 1645, 1510, 1265, 1108 cm^{-1}. HPLC (system 1) t_R = 17.9 min, purity 98 %, (system 2) t_R = 15.2 min, purity 98 %. ESI-MS *m/z* 544.8 [M+ H^+].

7-(4-{4-[8-(Benzyloxy)quinolin-5-yl]piperazin-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (10e). Compound **10e** was prepared as described for **10a** using a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (80 mg, 0.267 mmol) and NaI (72 mg, 0.480 mmol) in acetonitrile (1.4 mL) and a solution of **9e** (102 mg, 0.320 mmol) and triethylamine (62 µL, 0.480 mmol) in acetonitrile (0.7 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 40:1 to 15:1) yielded **10e** (107 mg, 74%) as a light yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.59 – 1.68 (m, 2H), 1.71 – 1.81 (m, 2H), 2.37 – 2.46 (m, 4H), 2.52 – 2.73 (m, 4H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.87 – 3.05 (m, 4H), 3.94 (t, *J* = 6.4 Hz, 2H), 5.27 (s, 2H), 6.45 (d, *J* = 2.5 Hz, 1H), 6.50 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.04 (d, *J* = 8.3 Hz,

1H), 7.12 (d, *J* = 8.3 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.35 (t, *J* = 7.4 Hz, 1H), 7.40 – 7.44 (m, 2H), 7.52 – 7.58 (m, 3H), 8.47 (dd, *J* = 8.5, 1.7 Hz, 1H), 8.86 (dd, *J* = 4.1, 1.7 Hz, 1H), 9.97 (s, 1H). ^{13}C NMR (DMSO-d_6, 91 MHz) δ 23.34, 24.75, 27.37, 31.24, 53.12, 53.75, 58.33, 68.01, 71.04, 102.3, 108.8, 109.8, 115.7, 115.9, 121.1, 125.5, 127.3, 127.9, 128.7, 128.8, 132.1, 137.4, 138.3, 141.5, 142.5, 149.4, 151.1, 158.8, 171.7. IR (NaCl) ν 3432, 1640, 1453, 1369, 1266, 1191, 1097 cm^{-1}. HPLC (system 1) t_R = 16.1 min, purity 99 %, (system 2) t_R = 13.1 min, purity 99 %. ESI-MS *m/z* 537.6 [M+ H$^+$].

7-(4-{4-[2,8-Bis(benzyloxy)quinolin-5-yl]-1,4-diazepan-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (11a). Compound **11a** was prepared as described for **10a** using a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (60 mg, 0.20 mmol) and NaI (55 mg, 0.36 mmol) in acetonitrile (1.0 mL) and a solution of **5h** (98 mg, 0.22 mmol) and triethylamine (50 µL, 0.36 mmol) in acetonitrile (0.5 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 40:1 to 15:1) yielded **11a** (91 mg, 68%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.77 – 1.89 (m, 4H), 2.01 – 2.21 (m, 2H), 2.45 – 2.74 (m, 2H), 2.78 – 2.99 (m, 4H), 3.04 – 3.17 (m, 4H), 3.23 (t, *J* = 6.0 Hz, 2H), 3.30 – 3.46 (m, 2H), 3.89 – 4.15 (m, 2H), 5.30 (s, 2H), 5.58 (s, 2H), 6.33 (d, *J* = 2.4 Hz, 1H), 6.52 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.94 – 6.99 (m, 2H), 7.00 – 7.09 (m, 2H), 7.27 – 7.47 (m, 6H), 7.50 – 7.64 (m, 4H), 7.83 (s, 1H), 8.35 – 8.47 (m, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 23.21, 24.76, 27.09, 31.24, 54.18, 55.89, 56.50, 58.06, 67.79, 67.84, 71.97, 102.4, 108.8, 112.8, 113.1, 115.6, 116.1, 122.6, 127.4, 127.8, 128.0, 128.5, 128.6, 128.7, 128.9, 135.5, 137.6, 137.9, 138.3, 139.6, 150.2, 158.0, 161.3, 171.5. IR (NaCl) ν 3442, 3057, 2989, 1645, 1266, 736 cm^{-1}. HPLC (system 1) t_R = 20.2 min, purity 97 %, (system 2) t_R = 17.6 min, purity 97 %. ESI-MS *m/z* 657.4 [M+ H$^+$].

7-(4-{4-[8-(Benzyloxy)-2-oxo-2*H*-chromen-5-yl]-1,4-diazepan-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (11b). Compound **11b** was prepared as described for **10a** using a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (23 mg, 0.078 mmol) and NaI (17 mg, 0.117 mmol) in acetonitrile (0.8 mL) and a solution of **6g** (30 mg, 0.086 mmol) and triethylamine

(20 µL) in acetonitrile (0.4 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 40:1 to 15:1) yielded **11b** (20 mg, 45%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.74 – 1.95 (m, 4H), 2.15 – 2.04 (m, 2H), 2.58 (dd, *J* = 8.2, 6.8 Hz, 2H), 2.81 – 2.95 (m, 4H), 3.09 – 3.23 (m, 6H), 3.31 – 3.42 (m, 2H), 3.97 (t, *J* = 5.9 Hz, 2H), 5.17 (s, 2H), 6.39 (d, *J* = 9.8 Hz, 1H), 6.43 (d, *J* = 2.4 Hz, 1H), 6.50 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 7.01 (dd, *J* = 8.5, 5.0 Hz, 2H), 7.28 – 7.34 (m, 1H), 7.34 – 7.39 (m, 2H), 7.45 (dd, *J* = 7.9, 0.8 Hz, 2H), 8.12 (d, *J* = 9.8 Hz, 1H), 8.54 (s, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 23.02, 24.69, 26.88, 26.96, 31.21, 54.22, 55.99, 58.10, 67.74, 71.83, 102.5, 108.8, 115.7, 115.7, 116.0, 116.6, 117.1, 127.6, 128.2, 128.7, 136.6, 138.4, 140.7, 142.6, 145.2, 145.4, 158.5, 160.3, 171.9. IR (NaCl) ν 3406, 1645, 1270, 1191 cm^{-1}. HPLC (system 1) t_R = 18.0 min, purity 99 %, (system 2) t_R = 15.5 min, purity 99 %. ESI-MS *m/z* 568.2 [M+ H$^+$].

7-(4-{4-[4-Benzyl-5-(benzyloxy)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl]-1,4-diazepan-1-yl}butoxy)-3,4-dihydroquinolin-2(1*H*)-one (11c). Compound **11c** was prepared as described for **10a**, using a solution of 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(*1H*)-one (31 mg, 0.10 mmol) and NaI (23 mg, 0.15 mmol) in acetonitrile (1.0 mL) and a solution of **7h** (50 mg, 0.11 mmol) and triethylamine (20 µL, 0.15 mmol) in acetonitrile (0.5 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 50:1 to 15:1) yielded **11c** (46 mg, 68%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.80 – 1.91 (m, 2H), 1.94 – 2.06 (m, 2H), 2.24 – 2.32 (m, 2H), 2.53 – 2.63 (m, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 2.93 – 3.06 (m, 2H), 3.12 – 3.31 (m, 6H), 3.34 – 3.46 (m, 2H), 3.97 (t, *J* = 6.0 Hz, 2H), 4.52 (s, 2H), 4.92 (s, 2H), 5.38 (s, 2H), 6.35 (d, *J* = 2.3 Hz, 1H), 6.48 – 6.55 (m, 2H), 6.57 (d, *J* = 9.1 Hz, 1H), 7.00 – 7.05 (m, 3H), 7.10 – 7.20 (m, 3H), 7.28 – 7.32 (m, 2H), 7.32 – 7.40 (m, 3H), 8.00 (s, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 22.38, 24.77, 26.95, 29.84, 31.24, 39.31, 46.93, 50.61, 53.39, 56.67, 57.70, 67.57, 68.94, 71.74, 102.4, 108.0, 108.7, 114.5, 116.2, 120.3, 127.0, 127.1, 127.7, 128.3, 128.4, 128.8, 128.9, 136.4, 137.4, 138.4, 142.1, 144.6, 158.5, 166.5, 171.5. IR (NaCl) ν 3429, 1642, 1390, 1267, 1098 cm^{-1}.

HPLC (system 1) t_R = 19.7 min, purity 96 %, (system 2) t_R = 16.8 min, purity 97 %. ESI-MS *m/z* 661.3 [M+ H^+].

7-{4-[4-(8-Hydroxyquinolin-2(1*H*)-one-5-yl)piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one x TFA (12a). To a solution of **10a** (85 mg, 0.13 mmol) in acetic acid (1.8 mL) under nitrogen atmosphere a catalytic amount of 10% Pd/C was added. The flask was flushed with hydrogen and the reaction mixture was stirred at room temperature for 4 h. After filtering over celite and washing with MeOH, the solvent was evaporated. Purification of the crude product by preparative HPLC yielded the TFA-salt of **12a** (38 mg, 63%) as a brown solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.44 – 1.63 (m, 2H), 1.64 – 1.81 (m, 2H), 2.32 – 2.45 (m, 4H), 2.53 – 2.63 (m, 4H), 2.69 – 2.80 (m, 2H), 2.80 – 2.89 (m, 4H), 3.91 (t, *J* = 6.3 Hz, 2H), 6.40 – 6.44 (m, 1H), 6.44 – 6.52 (m, 2H), 6.70 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 9.8 Hz, 1H), 9.94 (s, 1H), 10.37 (s, 2H). ^{13}C NMR (DMSO-d_6, 91 MHz) δ 22.73, 23.98, 26.62, 30.73, 53.10, 53.16, 57.37, 67.30, 101.7, 107.6, 111.7, 114.5, 114.6, 115.4, 121.1, 128.3, 129.1, 136.2, 139.2, 139.7, 142.0, 157.7, 161.0, 170.2. IR (NaCl) ν 3427,2895, 2786, 1635, 1541, 1338, 1249, 1088, 889, 754, 680, 603 cm^{-1}. HPLC (system 1) t_R = 15.3 min, purity 97 %, (system 2) t_R = 12.3 min, purity 96 %. ESI-MS *m/z* 463.2 [M+ H^+]. HRMS-ESI calcd 463.2340; found 463.2339 [M+ H^+].

7-{4-[4-(8-Hydroxy-2-oxo-2*H*-chromen-5-yl)piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one (12b). To a solution of **10b** (32 mg, 0.058 mmol) in toluene (0.56 mL) was added TFA (0.24 mL). The reaction mixture was stirred under reflux for 8 h. After cooling down to room temperature, the pH was adjusted to 7 – 8 with $NaHCO_3$-solution and the product was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated. Purification of the crude product by preparative HPLC yielded the TFA-salt of **12b** (22 mg, 66%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.67 – 1.98 (m, 4H), 2.37 – 2.44 (m, 2H), 2.77 (t, *J* = 7.5 Hz, 2H), 2.94 – 3.09 (m, 2H), 3.12 – 3.36 (m, 6H), 3.52 – 3.61 (m, 2H), 3.94 (t, *J* = 5.7 Hz, 2H), 6.43 (d, *J* = 2.6 Hz, 1H),

6.45 (d, *J* = 9.9 Hz, 1H), 6.49 (dd, *J* = 8.2, 2.5 Hz, 1H), 6.94 (d, *J* = 8.6 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 1H), 8.16 (d, *J* = 9.8 Hz, 1H), 9.67 (s, 1H), 9.98 (s, 2H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 20.78, 24.45, 26.27, 31.21, 50.46, 51.82, 55.73, 67.11, 102.2, 107.9, 115.2, 115.7, 115.8, 116.2, 118.6, 128.9, 139.7, 140.7, 141.1, 142.0, 143.4, 158.1, 160.0, 170.7. IR (NaCl) ν 3432, 2958, 2848, 1675, 1478, 1376, 1275, 1200, 1139, 1025, 994, 832, 721 cm^{-1}. HPLC (system 1) t_R = 15.4 min, purity 97 %, (system 2) t_R = 12.8 min, purity 97 %. ESI-MS *m/z* 464.3 [M+ H$^+$]. HRMS-ESI calcd 464.2180; found 464.2174 [M+ H$^+$].

7-{4-[4-(5-Hydroxy-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl)piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one (12c). To a suspension of **10c** (9 mg, 0.01 mmol) in toluene (0.1 mL) was added MsOH (50 µL, 0.07 mmol). The reaction mixture was stirred under reflux for 3 h. After cooling down to room temperature, the solvent was removed. Purification of the crude product by flash chromatography yielded **12c** (5 mg, 77%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.69 – 1.79 (m, 2H), 1.79 – 1.89 (m, 2H), 2.38 – 2.47 (m, 2H), 2.76 – 2.85 (m, 2H), 2.89 – 3.00 (m, 2H), 3.11 – 3.18 (m, 2H), 3.18 – 3.24 (m, 2H), 3.30 – 3.35 (m, 2H), 3.48 – 3.61 (m, 2H), 3.94 (t, *J* = 6.0 Hz, 2H), 4.50 (s, 2H), 6.44 – 6.46 (m, 1H), 6.46 – 6.47 (m, 1H), 6.48 – 6.49 (m, 1H), 6.49 – 6.51 (m, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 9.66 (s, 1H), 9.80 (s, 1H), 9.87 (s, 1H), 10.01 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 20.59, 24.32, 26.16, 31.07, 48.00, 51.60, 55.57, 67.00, 67.11, 102.1, 107.8, 108.8, 112.7, 116.0, 117.1, 128.7, 132.0, 137.8, 139.5, 141.7, 158.0, 164.7, 170.6. IR (NaCl) ν 3341, 3030, 2921, 2851, 1611, 1598, 1478, 1280, 1250, 826, 725, 692 cm^{-1}. HPLC (system 1) t_R = 15.4 min, purity 96 %, (system 2) t_R = 12.4 min, purity 96 %. ESI-MS *m/z* 467.1 [M+ H$^+$].

7-(4-[4-(8-Hydroxy-2,3-dihydrobenzo[*b*][1,4]dioxin-5-yl)piperazin-1-yl]butoxy)-3,4-dihydroquinolin-2(1*H*)-one (12d). To a solution of **10d** (73 mg, 0.12 mmol) in acetic acid/methanol (0.17 mL, 1.7 mL) under nitrogen atmosphere a catalytic amount of 10% Pd/C was added. The flask was flushed with hydrogen and the reaction mixture was stirred at room temperature for 4 h. After

filtering over celite and washing with MeOH, the solvent was evaporated. Purification of the crude product by column chromatography (CH_2Cl_2/MeOH 20:1) yielded **12d** (32 mg, 61%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.50 – 1.64 (m, 2H), 1.66 – 1.79 (m, 2H), 2.36 – 2.46 (m, 4H), 2.73 – 2.82 (m, 2H), 2.83 – 2.92 (m, 4H), 3.17 (d, J = 4.7 Hz, 2H), 3.91 (t, J = 6.3 Hz, 2H), 4.17 – 4.24 (m, 4H), 6.27 (s, 2H), 6.43 (d, J = 2.5 Hz, 1H), 6.48 (dd, J = 8.2, 2.5 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 8.60 (s, 1H), 9.95 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 21.03, 23.98, 26.58, 30.74, 48.58, 50.65, 52.98, 57.34, 63.41, 63.65, 67.26, 101.7, 106.5, 107.6, 108.9, 115.4, 128.3, 132.9, 137.4, 139.2, 157.8, 170.2. IR (NaCl) ν 3419, 2944, 1674, 1503, 1475, 1376, 1271, 1190, 1099, 988, 798, 734 cm^{-1}. HPLC (system 1) t_R = 15.1 min, purity 96 %, (system 2) t_R = 12.5 min, purity 99 %. ESI-MS *m/z* 454.1 [M+ H$^+$]. HRMS-ESI calcd 454.2336; found 454.2338 [M+ H$^+$].

7-{4-[4-(8-Hydroxyquinolin-5-yl)piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one x TFA (12e). Compound **12e** was prepared as described for **12b**, using a solution **10e** (59 mg, 0.11 mmol) and TFA (0.8 mL) in toluene (0.8 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **12e** (38 mg, 62%) as an orange solid. ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.70 – 1.96 (m, 4H), 2.42 (dd, J = 8.3, 6.7 Hz, 2H), 2.79 (t, J = 7.5 Hz, 2H), 3.03 – 3.20 (m, 2H), 3.21 – 3.50 (m, 6H), 3.61 – 3.72 (m, 2H), 3.97 (t, J = 5.7 Hz, 2H), 6.45 (d, J = 2.4 Hz, 1H), 6.51 (dd, J = 8.2, 2.5 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 7.5 Hz, 1H), 7.63 – 7.77 (m, 1H), 8.70 (d, J = 7.4 Hz, 1H), 8.90 – 9.20 (m, 1H), 9.72 (bs, 1H), 10.00 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 20.24, 23.91, 25.76, 30.66, 49.78, 51.43, 55.24, 66.61, 101.7, 107.4, 111.5, 115.6, 117.7, 121.5, 124.3, 128.3, 134.1, 138.9, 139.1, 147.3, 149.1, 157.6, 170.2. IR (NaCl) ν 3411, 3245, 2953, 2854, 1679, 1593, 1522, 1374, 1272, 1201, 1130, 1029, 832, 801, 721 cm^{-1}. HPLC (system 1) t_R = 14.5 min, purity 99 %, (system 2) t_R = 11.7 min, purity 99 %. ESI-MS *m/z* 447.4 [M+ H$^+$]. HRMS-ESI calcd 447.2391; found 447.2386 [M+ H$^+$].

7-{4-[4-(8-Hydroxyquinolin-2(1*H*)-one-5-yl)-1,4-diazepan-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one x TFA (13a). Compound **13a** was prepared according to the protocol of **12c**, using a solution of **11a** (77 mg, 0.177 mmol) and MsOH (0.7 mL) in toluene (2 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **13a** (41 mg, 59%) as a brown solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.70 – 1.80 (m, 2H), 1.81 – 1.95 (m, 2H), 2.12 (m, 2H), 2.38 – 2.45 (m, 2H), 2.79 (t, J = 7.5 Hz, 2H), 3.06 (t, J = 5.9 Hz, 2H), 3.23 – 3.31 (m, 3H), 3.31 – 3.42 (m, 2H), 3.46 (dd, J = 14.5, 7.0 Hz, 1H), 3.54 – 3.63 (m, 2H), 3.95 (t, J = 6.1 Hz, 2H), 6.44 (d, J = 2.5 Hz, 1H), 6.48 – 6.53 (m, 2H), 6.83 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 8.3 Hz, 1H), 8.16 (d, J = 9.8 Hz, 1H), 9.72 (bs, 1H), 10.01 (bs, 1H), 10.11 (bs, 1H), 10.39 (bs, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 20.76, 23.97, 24.34, 25.83, 30.72, 50.72, 52.49, 54.68, 55.02, 55.39, 56.22, 66.69, 101.7, 107.4, 114.5, 114.5, 115.5, 115.7, 121.4, 128.4, 128.8, 136.6, 139.2, 140.2, 141.7, 142.8, 157.7, 161.1, 170.2. IR (NaCl) ν 3432, 1640, 1202, 1145, 1025, 986 cm^{-1}. HPLC (system 1) t_R = 15.4 min, purity 96 %, (system 2) t_R = 12.4 min, purity 96 %. HRMS-ESI calcd 447.2496; found 447.2491 [M+ H$^+$].

7-{4-[4-(8-Hydroxy-2-oxo-2*H*-chromen-5-yl)-1,4-diazepan-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one x TFA (13b). Compound **13b** was prepared as described for **12b**, using a solution of **11b** (12 mg, 0.02 mmol) and TFA (0.1 mL) in toluene (0.2 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **13b** (9.5 mg, 79%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.70 – 1.80 (m, 2H). 1.80 – 1.89 (m, 2H), 2.02 – 2.16 (m, 2H), 2.38 – 2.44 (m, 2H), 2.78 (t, J = 7.5 Hz, 2H), 3.05 – 3.12 (m, 2H), 3.16 – 3.26 (m, 2H), 3.42 – 3.54 (m, 4H), 3.94 (t, J = 6.0 Hz, 2H), 6.41 – 6.47 (m, 2H), 6.50 (dd, J = 8.2, 2.5 Hz, 1H), 6.99 (d, J = 8.6 Hz, 1H), 7.03 – 7.09 (m, 2H), 8.25 (d, J = 9.8 Hz, 1H), 10.01 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 20.77, 23.89, 24.35, 25.81, 30.64, 52.41, 54.46, 55.36, 56.14, 66.66, 101.6, 107.4, 114.9, 115.2, 115.6, 117.3, 118.2, 128.3, 139.1, 141.1, 141.1, 142.5, 142.9, 157.6, 159.6, 170.2. IR (NaCl) ν 3427, 1640, 1267, 1196,

1147 cm^{-1}. HPLC (system 1) t_R = 15.6 min, purity 99 %. ESI-MS *m/z* 478.1 [M+ H^+]. HRMS-ESI calcd 478.2336; found 478.2337 [M+ H^+].

7-{4-[4-(5-Hydroxy-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl)-1,4-diazepan-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one x TFA (13c). Compound **13c** was prepared as described for **12c**, using a solution of **7c** (25 mg, 0.038 mmol) and MsOH (70 µL) in toluene (0.54 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **13c** (12 mg, 53%) as a light brown solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.67 – 1.77 (m, 2H), 1.78 – 1.94 (m, 2H), 2.12 (s, 2H), 2.36 – 2.46 (m, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.97 – 3.14 (m, 1H), 3.16 – 3.26 (m, 3H), 3.44 – 3.58 (m, 2H), 3.94 (t, *J* = 6.0 Hz, 2H), 4.50 (d, *J* = 3.7 Hz, 2H), 6.42 (d, *J* = 8.7 Hz, 1H), 6.44 (d, *J* = 2.4 Hz, 1H), 6.46 (d, *J* = 8.8 Hz, 1H), 6.50 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 9.50 (s, 1H), 9.67 (s, 1H), 9.82 (s, 1H), 10.00 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 20.77, 23.99, 25.85, 30.74, 48.69, 49.97, 52.40, 55.15, 55.88, 66.71, 66.79, 101.7, 107.5, 108.3, 112.5, 115.7, 116.8, 128.4, 133.7, 136.9, 139.2, 140.1, 157.7, 164.5, 170.3. IR (NaCl) ν 3427, 2920, 2850, 1645, 1520, 1645, 1520, 1474, 1375, 1197, 1147, 1082 cm^{-1}. HPLC (system 1) t_R = 15.2 min, purity 99 %. ESI-MS *m/z* 481.1 [M+ H^+]. HRMS-ESI calcd 481.2445; found 481.2432 [M+ H^+].

5-(4-{4-[2,8-Bis(benzyloxy)quinolin-5-yl]piperazin-1-yl}butoxy)pyrazolo[1,5-*a*]pyridine (14a). A solution of 5-(4-bromobutoxy)pyrazolo[1,5-a]pyridine (253 mg, 0.94 mmol) and NaI (253 mg, 1.69 mmol) in acetonitrile (4.7 mL) was stirred under reflux for 0.5 h, before a solution of **5g** (439 mg, 1.03 mmol) and triethylamine (200 µL, 1.54 mmol) in acetonitrile (2.3 mL) was added. Stirring was continued at 80°C for 16 h. After cooling down to room temperature, saturated $NaHCO_3$ solution was added and the product was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated**.** Purification by flash chromatography (CH_2Cl_2/MeOH 50:1 to 30:1) afforded **14a** (382 mg, 66%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.59 – 1.69 (m, 2H), 1.75 – 1.87 (m, 2H), 2.40 – 2.47 (m, 2H), 2.57 – 2.76 (m, 4H), 2.87 – 3.01 (m, 4H), 4.07 (t, *J* = 6.3 Hz, 2H), 5.27

(s, 2H), 5.53 (s, 2H), 6.36 (dd, *J* = 2.1, 0.6 Hz, 1H), 6.54 (dd, *J* = 7.5, 2.6 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 2.6 Hz, 1H), 7.07 (d, *J* = 9.1 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.28 – 7.36 (m, 4H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.50 – 7.61 (m, 4H), 7.86 (d, *J* = 2.1 Hz, 1H), 8.37 (d, *J* = 9.1 Hz, 1H), 8.50 (d, *J* = 7.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 22.71, 26.24, 53.07, 53.13, 54.89, 66.78, 67.78, 70.50, 95.29, 95.63, 106.5, 112.1, 112.6, 113.1, 121.1, 127.2, 127.6, 127.7, 128.2, 128.3, 128.6, 129.6, 135.5, 137.2, 137.8, 138.6, 140.6, 142.4, 155.0, 160.2. IR (NaCl) ν 3442, 3050, 2951, 2822, 1648, 1610, 1445, 1409, 1264, 1191, 1009, 897 cm^{-1}. HPLC (system 1) t_R = 20.1 min, purity 99 %, (system 2) t_R = 17.6 min, purity 99 %. ESI-MS *m/z* 615.0 [M+ H$^+$].

5-(4-{4-[8-(Benzyloxy)-2-oxo-2*H*-chromen-5-yl]piperazin-1-yl}butoxy)pyrazolo[1,5-*a*]pyridine (14b). Compound **14b** was prepared as described for **14a,** using a solution 5-(4-bromobutoxy)-pyrazolo[1,5-*a*]pyridine (33 mg, 0.122 mmol) and NaI (33 mg, 0.220 mmol) in acetonitrile (0.6 mL) and a solution of **6f** (45 mg, 0.134 mmol) and triethylamine (30 µL, 0.220 mmol) in acetonitrile (0.2 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 40:1) yielded **14b** (42 mg, 65%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.55 – 1.70 (m, 2H), 1.70 – 1.92 (m, 2H), 2.39 – 2.47 (m, 2H), 2.55 – 2.74 (m, 4H), 2.80 – 2.97 (m, 4H), 4.06 (t, *J* = 6.5 Hz, 2H), 5.20 (s, 2H), 6.33 – 6.40 (m, 1H), 6.47 (d, *J* = 9.8 Hz, 1H), 6.54 (dd, *J* = 7.5, 2.7 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 7.02 (d, *J* = 2.6 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.42 (dd, *J* = 10.3, 4.7 Hz, 2H), 7.49 (d, *J* = 7.3 Hz, 2H), 7.86 (d, *J* = 2.1 Hz, 1H), 8.08 (d, *J* = 9.8 Hz, 1H), 8.50 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 22.57, 26.14, 52.80, 53.05, 57.10, 67.67, 70.34, 95.20, 95.53, 106.4, 114.1, 114.2, 115.1, 116.2, 127.7, 127.9, 128.7, 130.0, 136.6, 140.2, 140.5, 141.6, 142.3, 143.6, 144.0, 155.0, 159.4. IR (NaCl) ν 2255, 2135, 1655, 1025, 995, 822 cm^{-1}. HPLC (system 1) t_R = 17.8 min, purity 96 %, (system 2) t_R = 15.2 min, purity 92 %. ESI-MS *m/z* 525.3 [M+ H$^+$].

5-(4-{4-[4-Benzyl-5-(benzyloxy)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl]piperazin-1-yl}butoxy)pyrazolo[1,5-*a*]pyridine (14c). Compound **14c** was prepared as described for **14a**, using a

solution 5-(4-bromobutoxy)-pyrazolo[1,5-*a*]pyridine (100 mg, 0.37 mmol) and NaI (84 mg, 0.56 mmol) in acetonitrile (4.8 mL) and a solution of **7g** (176 mg, 0.41 mmol) and triethylamine (80 µL, 0.56 mmol) in acetonitrile (0.4 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 60:1 to 40:1) yielded **14c** (170 mg, 74%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.55 – 1.66 (m, 2H), 1.74 – 1.81 (m, 2H), 2.38 (t, *J* = 7.1 Hz, 2H), 2.49 – 2.50 (m, 4H), 2.84 – 2.91 (m, 4H), 4.04 (t, *J* = 6.5 Hz, 2H), 4.59 (s, 2H), 5.01 (s, 2H), 5.29 (s, 2H), 6.35 (dd, *J* = 2.1, 0.6 Hz, 1H), 6.54 (dd, *J* = 7.5, 2.7 Hz, 1H), 6.62 (d, *J* = 9.1 Hz, 1H), 6.70 (d, *J* = 9.1 Hz, 1H), 6.99 (d, *J* = 7.2 Hz, 2H), 7.01 (d, *J* = 2.6 Hz, 1H), 7.15 (dd, *J* = 8.3, 6.3 Hz, 1H), 7.19 – 7.24 (m, *J* = 7.4 Hz, 2H), 7.32 – 7.40 (m, 5H), 7.86 (d, *J* = 2.1 Hz, 1H), 8.50 (d, *J* = 7.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 22.64, 26.24, 45.62, 50.57, 52.82, 57.28, 67.77, 68.22, 70.63, 95.28, 95.61, 106.5, 107.8, 113.9, 119.4, 126.4, 126.8, 127.7, 128.0, 128.1, 128.2, 128.5, 129.6, 135.6, 136.5, 137.4, 140.6, 142.2, 142.4, 144.2, 155.0, 166.3. IR (NaCl) ν 3227, 291, 2812, 16861650, 1501, 1441, 1388, 1267, 1192, 1101, 1025, 792, 737, 698 cm^{-1}. HPLC (system 1) t_R = 19.3 min, purity 95 %, (system 2) t_R = 16.5 min, purity 92 %. ESI-MS *m/z* 618.2 [M+ H$^+$].

5-(4-{4-[8-(Benzyloxy)-2,3-dihydrobenzo[*b*][1,4]dioxin-5-yl]piperazin-1-yl}butoxy) pyrazolo[1,5-*a*]pyridine (14d). Compound **14d** was prepared as described for **14a**, using a solution 5-(4-bromobutoxy)-pyrazolo[1,5-*a*]pyridine (81 mg, 0.30 mmol) and NaI (81 mg, 0.54 mmol) in acetonitrile (4.3 mL) and a solution of **8e** (118 mg, 0.36 mmol) and triethylamine (70 µL) in acetonitrile (0.4 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 50:1) yielded **14d** (118 mg, 77%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.54 – 1.71 (m, 2H), 1.73 – 1.81 (m, 2H), 2.35 – 2.40 (m, 2H), 2.84 – 2.93 (m, 4H), 4.05 (t, *J* = 6.5 Hz, 2H), 4.20 – 4.22 (m, 2H), 4.22 – 4.24 (m, 2H), 4.99 (s, 2H), 6.38 – 6.31 (m, 2H), 6.51 (d, *J* = 8.9 Hz, 1H), 6.54 (dd, *J* = 7.5, 2.7 Hz, 1H), 7.02 (d, *J* = 2.6 Hz, 1H), 7.32 (ddd, *J* = 7.2, 3.9, 1.3 Hz, 1H), 7.36 – 7.40 (m, 2H), 7.41 – 7.44 (m, 2H), 7.86 (d, *J* = 2.1 Hz, 1H), 8.50 (d, *J* = 7.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 91 MHz) δ 22.64, 26.23,

50.53, 52.95, 57.30, 63.43, 63.61, 67.77, 70.31, 95.26, 95.61, 105.3, 106.5, 108.3, 127.6, 127.7, 128.3, 129.5, 134.3, 135.6, 137.2, 137.4, 140.6, 142.4, 143.3, 155.0. IR (NaCl) ν 2872, 2818, 1646, 1496, 1441, 1337, 1275, 1193, 1116, 999 cm^{-1}. HPLC (system 1) t_R = 17.9 min, purity 98 %, (system 2) t_R = 15.3 min, purity 95 %. ESI-MS *m/z* 515.4 [M+ H^+].

5-(4-{4-[8-(Benzyloxy)quinolin-5-yl]piperazin-1-yl}butoxy)pyrazolo[1,5-*a*]pyridine (14e). Compound **14e** was prepared according to the protocol of **14a,** using a solution 5-(4-bromobutoxy)-pyrazolo[1,5-*a*]pyridine (80 mg, 0.30 mmol) and NaI (80 mg, 0.53 mmol) in acetonitrile (1.5 mL) and a solution of **9e** (114 mg, 0.36 mmol) and triethylamine (75 µL, 0.53 mmol) in acetonitrile. Purification by flash chromatography (CH_2Cl_2/MeOH 60:1 to 40:1) afforded **14e** (103 mg, 68%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.61 – 1.80 (m, 2H), 1.82 – 1.95 (m, 2H), 2.47 – 2.61 (m, 2H), 2.66 – 2.80 (m, 4H), 2.99 – 3.17 (m, 4H), 4.03 (t, *J* = 6.3 Hz, 2H), 5.41 (s, 2H), 6.30 (d, *J* = 1.7 Hz, 1H), 6.44 (dd, *J* = 7.5, 2.6 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 6.99 (d, *J* = 8.3 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.43 (dd, *J* = 8.5, 4.2 Hz, 1H), 7.47 – 7.55 (m, 2H), 7.85 (d, *J* = 2.1 Hz, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.53 (dd, *J* = 8.5, 1.5 Hz, 1H), 8.97 (dd, *J* = 4.1, 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 23.57, 27.10, 53.36, 53.87, 58.33, 68.10, 71.04, 95.51, 95.55, 106.8, 109.8, 115.6, 121.0, 125.5, 127.2, 127.9, 128.7, 129.5, 132.1, 137.5, 141.2, 141.5, 142.9, 149.4, 151.1, 155.8. IR (NaCl) ν 2942, 2823, 1646, 1444, 1365, 1254, 1191, 1101 cm^{-1}. HPLC (system 1) t_R = 15.4 min, purity 95 %, (system 2) t_R = 13.1 min, purity 96 %. ESI-MS *m/z* 508.7 [M+ H^+].

7-(4-{4-[2,8-Bis(benzyloxy)quinolin-5-yl]-1,4-diazepan-1-yl}butoxy)pyrazolo[1,5-*a*]pyridine (15a). Compound **15a** was prepared as described for **14a** using a solution of 5-(4-bromobutoxy)-pyrazolo[1,5-*a*]pyridine (25 mg, 0.095 mmol) and NaI (21 mg, 0.142 mmol) in acetonitrile (0.95 mL) and a solution of **5h** (176 mg, 0.409 mmol) and triethylamine (80 µL, 0.558 mmol) in acetonitrile (0.4 mL). Purification by flash chromatography (CH_2Cl_2/MeOH 60:1 to 40:1) yielded **15a** (170 mg,74%) as a colorless solid. ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.85 – 1.98 (m,

4H), 2.07 – 2.19 (m, 2H), 2.77 – 2.94 (m, 2H), 3.06 – 3.19 (m, 4H), 3.24 (t, *J* = 6.0 Hz, 2H), 3.39 (m, 2H), 4.03 (t, *J* = 5.7 Hz, 2H), 5.30 (s, 2H), 5.58 (s, 2H), 6.30 (dd, *J* = 2.2, 0.7 Hz, 1H), 6.43 (dd, *J* = 7.6, 2.6 Hz, 1H), 6.73 (d, *J* = 2.5 Hz, 1H), 6.90 – 7.00 (m, 2H), 7.04 (d, *J* = 8.4 Hz, 1H), 7.28 – 7.43 (m, 6H), 7.50 – 7.62 (m, 4H), 7.85 (d, *J* = 2.2 Hz, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.40 (d, *J* = 9.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 91 MHz) δ 22.07, 26.64, 55.85, 56.55, 57.78, 67.38, 67.91, 71.80, 95.71, 95.81, 106.6, 112.8, 113.2, 116.3, 122.5, 127.4, 127.9, 128.0, 128.5, 128.6, 128.7, 129.6, 135.0, 137.4, 137.7, 139.5, 141.1, 143.0, 150.7, 155.4, 161.3. IR (NaCl) ν 3031, 2927, 2849, 2599, 1646, 1443, 1410, 1265, 1191, 1095, 1002 cm^{-1}. HPLC (system 1) t_R = 20.3 min, purity 83 %, (system 2) t_R = 17.9 min, purity 83 %. ESI-MS *m/z* 628.2 [M+ H$^+$].

5-{4-[4-(8-Hydroxyquinolin-2(1*H*)-one-5-yl)piperazin-1-yl]butoxy}pyrazolo[1,5-*a*]pyridine (16a). Compound **16a** was prepared as described for **12a**, using a solution of **14a** (378 mg, 0.62 mmol) and a catalytic amount of 10% Pd/C in AcOH/MeOH 1:1 (8.8 mL). Purification of the crude product flash chromatography (CH_2Cl_2/MeOH 25:1 + 1% NH_3) yielded **16a** (185 mg, 70%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.57 – 1.71 (m, 2H), 1.76 – 1.86 (m, 2H), 2.43 (t, *J* = 7.2 Hz, 2H), 2.55 – 2.64 (m, 4H), 2.82 – 2.90 (m, 4H), 4.06 (t, *J* = 6.5 Hz, 2H), 6.36 (d, *J* = 1.5 Hz, 1H), 6.48 (d, *J* = 9.8 Hz, 1H), 6.54 (dd, *J* = 7.5, 2.7 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 7.02 (d, *J* = 2.6 Hz, 1H), 7.86 (d, *J* = 2.1 Hz, 1H), 8.00 (d, *J* = 9.8 Hz, 1H) 8.50 (d, *J* = 7.5 Hz, 1H), 10.21 (s, 2H). ^{13}C NMR (DMSO-d_6, 101 MHz) δ 22.60, 26.16, 53.02, 53.09, 57.18, 67.68, 95.19, 95.50, 106.4, 111.6, 114.3, 114.5, 121.0, 129.0, 129.5, 136.1, 139.7, 140.5, 141.9, 142.3, 155.0, 161.0. IR (NaCl) ν 3421, 2949, 2819, 1643, 1485, 1442, 1369, 1345, 1442, 1276, 1190, 1145 cm^{-1}. HPLC (system 1) t_R = 15.1 min, purity 99 %, (system 2) t_R = 12.3 min, purity 97 %. HRMS-ESI calcd 434.2187; found 434.2183 [M+ H$^+$].

5-{4-[4-(8-Hydroxy-2-oxo-2*H*-chromen-5-yl)piperazin-1-yl]butoxy}pyrazolo[1,5-*a*]pyridine x TFA (16b). Compound **16b** was prepared as described for **12b**, using a solution of **14b**

(35 mg, 0.067 mmol) and TFA (0.29 mL) in toluene (0.67 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **16b** (21 mg, 57%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.79 – 1.91 (m, 4H), 3.0 – 3.12 (m, 2H), 3.12 – 3.39 (m, 6H), 3.53 – 3.69 (m, 2H), 4.09 (t, J = 5.5 Hz, 2H), 6.38 (d, J = 1.8 Hz, 1H), 6.47 (d, J = 9.8 Hz, 1H), 6.55 (dd, J = 7.5, 2.7 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 7.04 (d, J = 2.6 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H), 7.88 (d, J = 2.1 Hz, 1H), 8.18 (d, J = 9.8 Hz, 1H), 8.53 (d, J = 7.6 Hz, 1H), 9.71 (s, 1H), 10. 00 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 20.28, 25.47, 49.99, 51.37, 55.20, 67.16, 95.40, 95.74, 106.4, 114.8, 115.3, 115.3, 118.1, 129.7, 140.2, 140.5, 140.6, 141.6, 142.5, 142.9, 154.9, 159.5. IR (NaCl) ν 3406, 3077, 2941, 2845, 1715, 1648, 1439, 1198, 1128, 1047, 1024, 826, 718, 668 cm^{-1}. HPLC (system 1) t_R = 15.3 min, purity 99 %, (system 2) t_R = 12.9 min, purity 99 %. HRMS-ESI calcd 435.2027; found 435.2031 [M+ H$^+$].

5-{4-[4-(5-Hydroxy-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one-8-yl)piperazin-1-yl]butoxy}pyrazolo[1,5-*a*]pyridine x TFA (16c). Compound **16c** was prepared as described for **12c**, using a solution **14c** (99 mg, 0.16 mmol) and MsOH (0.31 mL) in toluene (2.3 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **16c** (24 mg, 28%) as colorless solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.73 – 1.94 (m, 4H), 2.91 (t, J = 11.8 Hz, 2H), 3.13 – 3.21 (m, 2H), 3.21 – 3.27 (m, 2H), 3.33 – 3.42 (m, 2H), 3.54 – 3.61 (m, 2H), 4.07 (t, J = 5.9 Hz, 2H), 4.51 (s, 2H), 6.38 (dd, J = 2.1, 0.7 Hz, 1H), 6.46 (d, J = 8.7 Hz, 1H), 6.50 (d, J = 8.8 Hz, 1H), 6.55 (dd, J = 7.5, 2.7 Hz, 1H), 7.03 (d, J = 2.6 Hz, 1H), 7.88 (d, J = 2.1 Hz, 1H), 8.53 (d, J = 7.6 Hz, 1H), 9.64 – 9.76 (m, 2H), 9.87 (s, 1H). ^{13}C NMR (DMSO-d_6, 91 MHz) δ 20.18, 25.38, 47.63, 51.23, 55.13, 66.69, 67.08, 95.29, 95.67, 106.3, 108.3, 112.4, 116.7, 129.6, 131.6, 137.4, 140.4, 141.3, 142.4, 154.8, 164.2. IR (NaCl) ν 3432, 2964, 2925, 2851, 1650, 1261, 1193, 1075 cm^{-1}. HPLC (system 1) t_R = 14.7 min, purity 95 %, (system 2) t_R = 12.1 min, purity 97 %. ESI-MS *m/z* 438.1 [M+ H$^+$]. HRMS-ESI calcd 438.2136; found 438.2135 [M+ H$^+$].

5-(4-[4-(8-Hydroxy-2,3-dihydrobenzo[*b*][1,4]dioxin-5-yl)piperazin-1-yl]butoxy) pyrazolo[1,5-*a*]pyridine (16d). Compound **16d** was prepared as described for **12a**, using a solution of **14d** (112 mg, 0.22 mmol) and a catalytic amount of 10% Pd/C in AcOH/MeOH 1:1 (2.2 mL). Purification of the crude product by flash chromatography (CH_2Cl_2/MeOH 60:1 to 30:1) yielded **16d** (74 mg, 79%) as a colorless solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.56 – 1.64 (m, 2H), 1.75 – 1.82 (m, 2H), 2.37 (t, J = 7.2 Hz, 2H), 2.46 – 2.49 (m, 4H), 2.77 – 2.94 (m, 4H), 4.04 (t, J = 6.5 Hz, 2H), 4.19 (dd, J = 3.4, 1.6 Hz, 2H), 4.21 (dd, J = 3.5, 1.5 Hz, 2H), 6.26 (s, 2H), 6.35 (dd, J = 2.2, 0.8 Hz, 1H), 6.54 (dd, J = 7.5, 2.6 Hz, 1H), 7.02 (d, J = 2.6 Hz, 1H), 7.86 (d, J = 2.1 Hz, 1H), 8.49 (d, J = 7.6 Hz, 1H), 8.62 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 22.69, 26.27, 50.77, 53.07, 57.38, 63.44, 63.67, 67.81, 95.31, 95.63, 106.5, 106.5, 108.9, 129.6, 132.9, 133.7, 137.4, 140.6, 141.8, 142.4, 155.1. IR (NaCl) ν 3406, 2952, 2879, 2825, 1645, 1445, 1343, 1267, 1189, 1093, 990 cm^{-1}. HPLC (system 1) t_R = 15.0 min, purity 93 %, (system 2) t_R = 12.5 min, purity 94 %. HRMS-ESI calcd 425.2184; found 425.2188 [M+ H$^+$].

5-{4-[4-(8-Hydroxyquinolin-5-yl)piperazin-1-yl]butoxy}pyrazolo[1,5-*a*]pyridine x TFA (16e). Compound **16e** was prepared according to the protocol of **12b**, using a solution of **14e** (70 mg, 0.14 mmol) and TFA (1.0 mL) in toluene (1.0 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **16e** (48 mg, 66%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.80 – 1.95 (m, 4H), 3.00 – 3.19 (m, 2H), 3.26 – 3.47 (m, 6H), 3.71 – 3.57 (m, 2H), 4.09 (t, J = 5.7 Hz, 2H), 6.37 (d, J = 1.7 Hz, 1H), 6.54 (dd, J = 7.5, 2.7 Hz, 1H), 7.03 (d, J = 2.6 Hz, 1H), 7.17 (d, J = 8.2 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.76 (dd, J = 8.0, 4.4 Hz, 1H), 7.86 (d, J = 2.1 Hz, 1H), 8.52 (d, J = 7.6 Hz, 1H), 8.80 (d, J = 8.4 Hz, 1H), 8.95 (d, J = 3.5 Hz, 1H), 9.91 (bs, 1H). ^{13}C NMR (DMSO-d_6, 91 MHz) δ 20.81, 26.02, 50.38, 52.04, 55.77, 67.70, 95.91, 96.29, 106.9, 112.8, 118.8, 122.2, 125.1, 130.2, 136.3, 141.1, 143.0, 147.3, 155.4, 158.9. IR (NaCl) ν 3401, 3096, 2921, 2851, 1680, 1445, 1385,

1198, 1132, 1027, 837, 799, 723 cm^{-1}. HPLC (system 1) t_R = 14.3 min, purity 98 %, (system 2) t_R = 11.6 min, purity 99 %. HRMS-ESI calcd 418.2238; found 418.2229 [M+ H^+].

5-{4-[4-(8-Hydroxyquinolin-2(1*H*)-one-5-yl)-1,4-diazepan-1-yl]butoxy}pyrazolo[1,5-*a*]pyridine x TFA (17a). Compound **17a** was prepared as described for **12c**, using a solution of **15a** (16 mg, 0.025 mmol) and MsOH (0.02 mL) in toluene (0.4 mL). Purification of the crude product by preparative HPLC yielded the TFA-salt of **17a** (6.7 mg, 48%) as a light brown solid. ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.75 – 1.96 (m, 4H), 2.11 – 2.18 (m, 2H), 3.07 (t, *J* = 5.7 Hz, 2H), 3.22 – 3.33 (m, 2H), 3.55 – 3.66 (m, 2H), 4.08 (t, *J* = 5.8 Hz, 2H), 6.38 (d, *J* = 1.3 Hz, 1H), 6.50 (d, *J* = 9.8 Hz, 1H), 6.54 (dd, *J* = 7.5, 2.6 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 7.87 (d, *J* = 1.8 Hz, 1H), 8.17 (d, *J* = 9.8 Hz, 1H), 8.52 (d, *J* = 7.6 Hz, 1H), 10.04 (s, 1H), 10.14 (s, 1H), 10.38 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 21.17, 24.75, 26.00, 51.15, 52.92, 55.12, 55.90, 56.60, 67.70, 95.85, 96.21, 106.9, 115.0, 115.0, 116.0, 116.7, 121.8, 129.2, 130.1, 137.1, 140.7, 141.0, 142.9, 143.3, 155.3, 158.3, 158.7, 161.6. IR (NaCl) ν 3427, 2927, 2854, 1653, 1560, 1445, 1193 cm^{-1}. HPLC (system 1) t_R = 15.4 min, purity 99 %, (system 2) t_R = 12.5 min, purity 96 %. ESI-MS *m/z* 448.2 [M+ H^+]. HRMS-ESI calcd 448.2330; found 448.2335 [M+ H^+].

***N*-(3-(4-(2,8-Bis(benzyloxy)quinolin-5-yl)-1,4-diazepan-1-yl)propyl)-4-(3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propoxy)-3-methoxybenzamide (18a).** Compound **5h** (25 mg, 0.06 mmol) and 4-(3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propoxy)-*N*-(3-oxopropyl)-3-methoxybenzamide (44 mg, 0.11 mmol) were dissolved in dichloroethane (2.8 mL) and $NaBH(OAc)_3$ (48 mg, 0.23 mmol) was added. The mixture was stirred at room temperature for 6 h. After addition of 1 M NaOH, the product was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated. After column chromatography (CH_2Cl_2/MeOH 20:1) **18a** was obtained as crude yellow solid. **18a** was used in further reactions without a final purification step. ESI-MS *m/z* 812.4 [M+ H^+]

***N*-(4-(4-(2,8-Bis(benzyloxy)quinolin-5-yl)-1,4-diazepan-1-yl)butyl)-4-(3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propoxy)-3-methoxybenzamide (18b).** Compound **18b** was prepared as described for **18a**, using a solution of **5h** (10 mg, 0.02 mmol) and 4-[3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propoxy]-*N*-(4-oxobutyl)-3-methoxybenzamide[38, 61] (18 mg, 0.04 mmol) and $NaBH(OAc)_3$ (19 mg, 0.09 mmol) in DCE (1.1 mL). Purification of the crude product by flash chromatography (CH_2Cl_2/MeOH 40:1) yielded **18b** (15 mg, 80%) as light yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.87 (t, J = 7.4 Hz, 3H), 1.22 (dq, J = 14.7, 7.3 Hz, 2H), 1.58 (dt, J = 14.1, 6.9 Hz, 2H), 1.69 – 1.80 (m, 4H), 1.98 – 2.11 (m, 2H), 2.15 (s, 2H), 2.77 (t, J = 7.5 Hz, 2H), 3.14 (t, J = 5.6 Hz, 2H), 3.24 (s, 2H), 3.29 – 3.33 (m, 2H), 3.41 – 3.52 (m, 4H), 3.59 (s, 2H), 3.81 (s, 3H), 4.05 (t, J = 6.3 Hz, 2H), 4.29 (t, J = 7.0 Hz, 2H), 5.29 (s, 2H), 5.53 (s, 2H), 6.99 (d, J = 8.9 Hz, 1H), 7.10 (t, J = 8.9 Hz, 2H), 7.21 (d, J = 8.5 Hz, 1H), 7.27 – 7.36 (m, 3H), 7.41 (dd, J = 17.7, 10.2 Hz, 2H), 7.44 – 7.51 (m, 2H), 7.53 – 7.59 (m, 3H), 7.90 (s, 1H), 8.45 (t, J = 5.6 Hz, 1H), 8.50 (d, J = 9.0 Hz, 1H), 10.06 (s, 1H). ^{13}C NMR (DMSO-d_6, 91 MHz) δ 13.23, 19.04, 21.30, 21.56, 24.32, 26.43, 28.44, 30.62, 31.66, 38.40, 48.83, 50.64, 52.50, 54.57, 55.17, 55.63, 56.22, 66.79, 67.48, 70.44, 110.9, 112.0, 112.4, 112.5, 115.8, 120.3, 121.8, 121.8, 126.8, 127.2, 127.6, 127.7, 128.2, 128.3, 128.5, 135.9, 137.2, 137.7, 138.4, 143.9, 146.0, 148.4, 149.5, 150.5, 157.8, 158.1, 160.3, 165.7. IR (NaCl) ν 3421, 2948, 2864, 1681, 1506, 1471, 1410, 1320, 1267, 1201, 1130, 1026 cm^{-1}. HPLC (system 1) t_R = 20.6 min, purity 94 %, (system 2) t_R = 18.0 min, purity 95 %. ESI-MS *m/z* 826.4 [M+ H$^+$]

***N*-(3-(4-(8-Hydroxyquinolin-2(1*H*)-one-5-yl)-1,4-diazepan-1-yl)propyl)-4-(3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propoxy)-3-methoxybenzamide (19a).** To a solution of **18a** (25 mg, 0.03 mmol) in toluene (0.44 mL) was added MsOH (20 µL) and the mixture was stirred at 80°C for 1 h. After cooling down to room temperature, the solvent was evaporated. The residue was purified by preparative HPLC to yield the TFA salt of **19a** (10 mg, 43%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.88 (t, J = 7.4 Hz, 3H), 1.18 – 1.28 (m, 2H), 1.69 – 1.83 (m, 2H), 1.92 – 2.02 (m, 2H), 2.0 – 2.09 (m, 2H),

2.09 – 2.16 (m, 2H), 2.78 (t, *J* = 7.6 Hz, 2H), 3.06 (t, *J* = 5.9 Hz, 2H), 3.20 – 3.31 (m, 3H), 3.32 – 3.43 (m, 4H), 3.43 – 3.52 (m, 2H), 3.54 – 3.65 (m, 3H), 3.82 (s, 3H), 4.06 (t, *J* = 6.4 Hz, 2H), 4.29 (t, *J* = 7.1 Hz, 2H), 6.49 (d, *J* = 9.8 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.9 Hz, 1H), 7.43 – 7.49 (m, 2H), 7.91 (s, 1H), 8.18 (d, *J* = 9.8 Hz, 1H), 8.56 (t, *J* = 5.7 Hz, 1H), 9.82 (s, 1H), 10.12 (s, 1H), 10.39 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 13.19, 18.98, 21.49, 24.34, 24.43, 28.36, 31.61, 36.34, 48.77, 50.68, 52.42, 54.53, 54.61, 55.30, 55.56, 67.40, 110.8, 111.8, 114.5, 115.4, 115.6, 117.6, 120.4, 121.3, 121.7, 126.4, 128.7, 136.5, 140.2, 142.7, 145.9, 148.3, 150.5, 157.8, 158.0, 161.0, 166.0. IR (NaCl) ν 3437, 2958, 2854, 1650, 1510, 1428, 1374, 1327, 1277, 1202, 1142, 1019, 843, 802, 720 cm^{-1}. HPLC (system 1) t_R = 16.7 min, purity >99 %. ESI-MS *m/z* 623.3 [M+ H$^+$]. HRMS-ESI calcd 316.6815; found 316.6815 [M+ H$^+$].

***N*-(4-(4-(8-Hydroxyquinolin-2(1*H*)-one-5-yl)-1,4-diazepan-1-yl)butyl)-4-(3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propoxy)-3-methoxybenzamide (19b).** To a solution of **18b** (19 mg, 0.02 mmol) in TFA/toluene (1:1, 0.4 mL) two drops of MsOH were added before the mixture was stirred at 80°C for 1 h. After cooling down to room temperature, the solvent was evaporated and the crude residue was purified by preparative HPLC to yield the TFA-salt **19b** (11 mg, 74%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.87 (t, *J* = 7.4 Hz, 3H), 1.14 – 1.35 (m, 2H), 1.66 – 1.51 (m, 2H), 1.84 – 1.69 (m, 4H), 2.10 – 2.02 (m, 2H), 2.21– 2.10 (m, 2H), 2.77 (t, *J* = 7.5 Hz, 2H), 3.09 – 2.99 (m, 2H), 3.24 – 3.19 (m, 2H), 3.32 – 3.24 (m, 4H), 3.60 – 3.51 (m, 2H), 3.82 (s, 3H), 4.05 (t, *J* = 6.3 Hz, 2H), 4.29 (t, *J* = 7.1 Hz, 2H), 6.49 (d, *J* = 9.8 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.99 (d, *J* = 9.0 Hz, 1H), 7.51 – 7.43 (m, 2H), 7.91 (s, 1H), 8.17 (d, *J* = 9.5 Hz, 1H), 8.48 (t, *J* = 5.6 Hz, 1H), 10.23 (s, 1H), 10.33 (s, 1H), 10.42 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz) δ 13.19, 18.98, 21.13, 21.50, 24.11, 26.33, 28.37, 31.60, 38.30, 48.48, 48.76, 50.53, 52.27, 54.45, 55.36, 55.56, 56.03, 67.38, 110.8, 111.8, 114.4, 114.5, 115.4, 116.2, 120.3, 121.2, 121.7, 126.7, 128.7, 136.6, 140.2, 142.7, 145.9, 148.3, 150.4, 157.8, 158.0, 161.1, 165.6. IR (NaCl) ν 3421, 1644, 1562, 1490, 1421, 1371, 1275, 1205, 1144,

1025, 982, 802, 719. cm^{-1}. HPLC (system 1) t_R = 16.7 min, purity >98 %. ESI-MS *m/z* 646.3 [M+ H^+]. HRMS-ESI calcd 323.6892; found 323.6890 [M/2+2H^+].

Membrane preparation. Membranes were obtained using a previously described protocol.[62] Briefly, HEK293T cells were grown to a confluence of 70 % and transiently transfected with the receptor of interest with or without co-transfection of a PTX insensitive $G\alpha_{o1}$ subunit using Mirus TransIT-293 transfection reagent (MoBiTec, Goettingen, Germany), $CaHPO_4$ precipitation or a solution of linear polyethyleneimine in PBS as described previously.[28, 29] After 24 h of incubation with the transfection complex, the medium was replaced. 48 h after transfection, cells were washed with ice cold phosphate buffered saline (PBS, pH 7.4) and detached with homogenate buffer (10 mM Tris-HCl, 0.5 mM EDTA, 5.4 mM KCl, 140 mM NaCl, pH 7.4). After centrifugation (8 min, 220 *g*) the pellet was resuspended in 10 mL ice cold homogenate buffer and cells were lysed with an ultraturrax. After ultracentrifugation (30 min 50,000 *g*), membranes were resuspended in binding buffer (50 mM Tris-HCl, 1 mM EDTA, 5 mM $MgCl_2$, 100 µg/mL bacitracin,5 µg/mL soybean trypsin inhibitor, pH 7.4) and homogenized with a glass-Teflon homogenizer at 4°C. Membrane preparations were shock-frozen in liquid nitrogen and stored at -80 °C. The protein concentration was determined with the method of Lowry[63] and bovine serum albumin as standard. Membranes from stably transfected CHO cells were obtained analogously.

Competition binding experiments. Affinities of the test compounds towards the human D_1R, $D_{2L}R$, $D_{2S}R$, D_3R, D_4R, D_5R, 5-$HT_{2A}R$ and porcine α_1-AR and 5-$HT_{1A}R$ were determined as described previously.[64] In brief, membranes containing human $D_{2L}R$, $D_{2S}R$, D_3R or D_4R obtained from CHO cells stably expressing the corresponding receptors were used together with the radioligand [^{3}H]spiperone (specific activity of 73 Ci/mmol, PerkinElmer, Rodgau, Germany) at a final concentration of 0.10–0.20 nM (K_D 0.035-0.10, B_{max} 610-1100 fmol/mg, protein concentration 4-6 µg/test tube), 0.15-0.20 nM (K_D 0.032-0.11, B_{max} 1500-8500 fmol/mg, protein concentration 1-4 µg/test tube), 0.20-0.50 nM (K_D 0.08-

0.55, B_{max} 3300-8500 fmol/mg, protein concentration 1-2 µg/test tube) and 0.20-0.50 nM (K_D 0.14-0.35, B_{max} 600-4500 fmol/mg, protein concentration 5-14 µg/test tube) for $D_{2L}R$, $D_{2S}R$, D_3R and D_4R, respectively. Competition binding experiments with the human D_1R, D_5R and 5-$HT_{2A}R$ were performed in an analogous manner with membranes from transiently transfected HEK293T cells together with [^{3}H]SCH23390 (80 Ci/mmol, Biotrend, Cologne, Germany, final concentration 0.20-0.50 nM, K_D 0.14-0.45 nM, B_{max} 2200-8900 fmol/mg, protein concentration 2-4 µg/test tube for D_1R and 0.40-0.60 nM, K_D 0.34-0.71 nM, B_{max} 450-2750 fmol/mg, 5-10 µg/test tube for D_5R) or [^{3}H]ketanserin (47 Ci/mmol, PerkinElmer, final concentration 0.30-0.50 nM, K_D 0.20-0.40 nM, B_{max} 1400-2400 fmol/mg, protein concentration 3-6 µg/test tube for 5-$HT_{2A}R$). Binding studies with α_1R or 5-$HT_{1A}R$ were carried out as described previously wit homogenates derived from porcine cerebral cortex,[64] in the presence of the radioligands [^{3}H]prazosin (84 Ci/mmol, PerkinElmer, 0.15-0.20 nM final concentration, K_D 0.060-0.170 nM, B_{max} 150-350 fmol/mg, protein concentration 20-40 µg/test tube for α_1R) or [^{3}H]WAY100635 (80 Ci/mmol, Biotrend, 0.20-0.35 nM final concentration, K_D 0.035-0.065 nM, B_{max} 40-120 fmol/mg, protein concentration 60-80 µg/test tube for 5-$HT_{1A}R$). Unspecific binding was determined in the presence of haloperidol (10 µM for D_1R-D_5R), WAY100635 (10 µM for 5-$HT_{1A}R$), ketanserin (10 µM for 5-$HT_{2A}R$) or prazosin (10 µM for α_1R).[64] Resulting competition curves were analyzed by nonlinear regression using the algorithms for one-site competition of PRISM 6.0 (GraphPad, San Diego, CA).

β-arrestin-2 recruitment. The PathHunter assay (DiscoverX, Birmingham, U.K.) was employed to determine β-arrestin-2 recruitment at $D_{2S}R$ as described previously.[28] In brief, (EA)-β-arrestin-2-HEK293 cells were transiently transfected with $D_{2S}R$-ARMS2-PK2 employing Mirus TransIT-293 (MoBiTec, Göttingen, Germany). Experiments in presence of GRK2 were performed analogously, using the cDNAs of $D_{2S}R$-ARMS2-PK2 and hGRK2 in a 2:1 ratio for transfection. 24 h after transfection, cells were detached with Versene (Life Technologies, Darmstadt, Germany), seeded

into white 384-well plates (Greiner, Frickenhausen, Germany) in assay medium at a density of 5000 cells/well and maintained for 24 h at 37 °C, 5 % CO_2. After incubation with the test compounds dissolved in PBS for 5 h at 37 °C, the detection mix was added and incubation was continued for 60 min at room temperature. Chemiluminescence was determined using the CLARIOstar plate reader (BMG LabTech, Ortenberg, Germany). Three to eight experiments per compound were performed, with each concentration in duplicate. All responses were normalized to the effect of 10 µM quinpirole (100%) and buffer conditions (0%) and analyzed using the algorithms for non-linear regression in PRISM 6.0.

[^{35}S]GTPγS binding experiments. [^{35}S]GTPγS incorporation assays were performed in analogy to previously described protocols.[28] Membranes from HEK293T cells transiently transfected with $D_{2S}R$ and a pertussis toxin insensitive variant of $G\alpha_{o1}$ (protein concentration 15 µg/mL, final volume 200 µL, B_{max} 9,900 ± 140 fmol/mg) were incubated with 10 µM GDP and the test compounds in assay buffer (20 mM HEPES, 10 mM $MgCl_2$ • 6 H_2O, 100 mM NaCl, 70 mg/L saponin) for 30 min at 37°C. After the addition of [^{35}S]GTPγS (0.1 nM final concentration, specific activity 1250 Ci/mmol, PerkinElmer), incubation was continued for 30 min at 37 °C before the reaction was terminated by filtration through Whatman GF/B filters. Bound radioactivity was determined as described previously.[62] Four to six experiments per compound were performed with each concentration (0.1 pM to 100 µM) in triplicate. Responses were normalized to the maximum effect of the full agonist quinpirole (100%) and buffer conditions (0%). Analysis of dose response curves was performed using the algorithms for non-linear regression in PRISM 6.0.

Operational model of agonism. Quantification of ligand bias was performed using the operational model of agonism[65] following recently published protocols.[66, 67] Using the algorithms implemented in PRISM6.0 (Graphpad), transduction ratios (τ/K_A) were obtained in their logarithmic form. Thus, normalized data from the functional experiments was analyzed using the following

equation:

$$Y = \text{basal} + \frac{(E_m - \text{basal})\left(\frac{\tau}{K_A}\right)^n [X]^n}{\left(\frac{\tau}{K_A}\right)^n [X]^n + \left(1 + \frac{[X]}{K_A}\right)^n}$$

with basal being the response of a the system in absence of an agonist, E_m the maximal response, [X] the concentration of the agonist and n the transducer slope. Parameter n was globally shared, while basal and E_m were constrained to 0 and 100 %, respectively. Transduction coefficients (τ/K_A) were then normalized to the response of endogenous agonist dopamine:

$$\Delta \log\left(\frac{\tau}{K_A}\right) = log\left(\frac{\tau}{K_A}\right)_{test\ ligand} - log\left(\frac{\tau}{K_A}\right)_{reference\ ligand}$$

Determination of bias was performed by calculation of the difference for two investigated pathways:

$$\Delta\Delta \log\left(\frac{\tau}{K_A}\right)_{1,2} = \Delta log\left(\frac{\tau}{K_A}\right)_{signal\ 1} - \Delta\ log\left(\frac{\tau}{K_A}\right)_{signal\ 2}$$

$$bias\ factor = 10^{\Delta\Delta log\left(\frac{\tau}{K_A}\right)}$$

Standard errors were estimated as described previously[66] using the following equation:

$$SEM_{1,2} = \sqrt{{SEM_1}^2 + {SEM_2}^2}$$

Statistical significance was calculated using one-way ANOVA followed by Dunnett's post hoc test at the level of $\Delta\Delta\log(\tau/K_A)$ and accepting $p < 0.05$ as level of significance.

Metabolic stability. Metabolic stability of the test compounds was assessed in rat liver microsomes in analogy to previously described procedures.[61, 68] Each incubation mixture (total volume 500 µL) contained the test compounds (concentration 20 µM from a 10 mM stock solution in DMSO) or a positive control (rotigotine, 20 µM from a 10 mM stock solution in DMSO), pooled male rat liver microsomes (Sprague Dawley, Sigma Aldrich, Germany) at a concentration of 0.5 mg microsomal protein/mL in Tris-$MgCl_2$ buffer (48 mM Tris, 4.8 mM $MgCl_2$, pH 7.4). Microsomal reactions were initiated by addition of 50 µL of a cofactor solution (NADPH, final concentration 1 mM, Carl Roth,

Germany) and carried out in polyethylene caps (Eppendorf, 1.5 mL) at 37°C. At 0, 15, 30 and 60 min, the enzymatic reactions were terminated by addition of 500 µL ice-cold acetonitrile (containing 8 µM internal standard), and precipitated protein was removed by centrifugation (15,000 *g*, 3 min). The supernatant was analyzed by HPLC (binary solvent system, eluent methanol in 0.1% aqueous formic acid, 10−90% methanol in 18 min, 90−95% methanol in 2 min, 95% methanol for 2 min, flow rate of 0.5 mL/min) using a Zorbax Eclipse XDB-C8 (4.6mm × 150 mm, 5 µm) column. For each time point, three independent experiments were performed and parallel control incubations in the absence of cofactor solution were used to determine unspecific binding to the matrix. Substrate remaining was calculated as a mean value ± S.E.M. by comparing the AUC substrate after predetermined incubation time to the AUC of substrate at time 0, corrected by a factor calculated from the AUC of internal standard at each time point. For each compound, the concentration of the substrate remaining was plotted in its logarithmic form as a function of time, to calculate an elimination rate constant (*k*), the half life ($T_{1/2}$) and the intrinsic clearance (CL_{int}) according to the following two equations as described previously:[54, 55]

$$T_{1/2}(\text{min}) = \frac{0.693}{k}$$

$$CL_{int}\left(\frac{\mu L}{min \times mg\ protein}\right) = \frac{volume\ of\ incubation\ (\mu L)}{protein\ in\ incubation\ (mg)} \times \frac{0.693}{T_{\frac{1}{2}}}$$

hERG inhibition. Inhibition of hERG (human-ether-à-go-go related gene) tail currents was examined using automated whole cell patch clamp and CHO cells stably transfected with the human *KCNH2* gene at B'SYS Analytics, Switzerland. All experiments were conducted at room temperature using a bath solution containing 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM D-glucose adjusted to pH 7.4 with NaOH. A solution of 130 mM KCl, 2 mM $CaCl_2$, 4 mM $MgCl_2$, 4 mM Na_2-ATP, 10 mM HEPES, 5 mM EGTA adjusted to pH 7.2 with KOH was used as intracellular solution. Outward tail currents were measured upon depolarization of the cell membrane

from a holding potential of -80 mV to +20 mV for 2 s (activation of channels) and upon subsequent repolarization to −40 mV for 3 s. After a waiting time of 5 s, the next pulse followed. The application protocol consisted of five periods, during which vehicle (DMSO 0.5%) and **13a** were applied in increasing concentrations. The voltage protocol was executed 20 times during each application period. As reference, the selective I_{Kr} inhibitor E-4031 (Sigma, Germany) was employed (final concentration 100 nM). The tail current amplitudes at the end of each period were used in further evaluation steps. The amount of current block was calculated as percentage of control and the data was analyzed with the following equation to generate a dose-response curve and to determine an IC_{50}, when n is the hill slope and $[X]$ the concentration of the test compound: $current_{peak,relative} = \frac{100}{1+\left(\frac{[X]}{IC_{50}}\right)^{n}}$

ASSOCIATED CONTENT

Supporting Information. Supplementary figures, data from functional assays, ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes. The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) grant GRK 1910. We thank Prof. Graeme Milligan (University of Glasgow, U.K.) and Prof. Michel Bouvier (IRIC, University of

Montreal) for providing the cDNAs for $G\alpha_{o1}$ and GRK2, respectively. We thank Dr. Stefan Löber (FAU Erlangen-Nuernberg) for helpful discussions.

ABBREVIATIONS USED

APPI, atmospheric pressure photoionization; β_x-AR, β adrenergic receptor, subtype x; CHO, chinese hamster ovary; CL_{int}, intrinsic clearance; 1,4-DAP, 1,4-disubstituted-aromatic piperazine; D_xR, dopamine D_x receptor; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid; $G\alpha_x$, G protein alpha subunit subtype x; GRK2, G protein-coupled receptor kinase subtype 2; GTPγS, guanosine 5'-*O*-(thiotriphosphate); hD_xR, human dopamine D_x receptor; $h5\text{-}HT_xR$, human serotonin 5-HT_x receptor; I_{Kr}, rectifying potassium current; L-DOPA, L-3,4-dihydroxyphenylalanine, LID, L-DOPA-induced dyskinesia; $p\alpha_xR$, porcine adrenergic α_x receptor; $p5\text{-}HT_xR$, porcine serotonin 5-HT_x receptor; PTX, pertussis toxin; QTc, corrected QT interval; S.D., standard deviation; S.E.M, standard error of mean; TdP, Torsade de Pointes arrhythmia.

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