Discovery of 1-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-imidazolidinone (GSK163090), a Potent, Selective, and Orally Active 5-HT$_{1A/B/D}$ Receptor Antagonist

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In an effort to identify selective drug like pan-antagonists of the 5-HT$_1$ autoreceptors, studies were conducted to elaborate a previously reported dual acting 5-HT$_1$ antagonist/SSRI structure. A novel series of compounds was identified showing low intrinsic activities and potent affinities across the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors as well as high selectivity against the serotonin transporter. From among these compounds, 1-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-imidazolidinone (**36**) was found to combine potent in vivo activity with a strong preclinical developability profile, and on this basis it was selected as a drug candidate with the aim of assessing its potential as a fast-onset antidepressant/anxiolytic.

Introduction

Much preclinical and clinical evidence highlights the involvement of the serotonergic system in the regulation of mood.[1] Of particular clinical importance are the selective serotonin reuptake inhibitors (SSRIs[a]), which represent the first choice for the treatment of depression. The SSRIs are believed to owe their efficacy to increased serotonergic neurotransmission brought about through the elevation of synaptic 5-hydroxytyptamine (5-HT) levels. Despite their popularity, the SSRIs are associated with a number of undesirable characteristics including the latency to therapeutic onset, which typically requires several weeks of SSRI treatment. It has been hypothesized that this delay in therapeutic onset is attributable to the time required for the desensitization of the 5-HT$_{1A}$ receptors, allowing increased 5-HT levels to be maintained.[2] In support of this hypothesis, coadministration of a 5-HT$_{1A}$ antagonist with an SSRI has been reported to accelerate the onset of anxiolytic/

antidepressant activity of SSRIs both in preclinical[3] and clinical[4] settings and, accordingly, several researchers have endeavored to identify dual acting 5-HT$_{1A}$ antagonists/SSRIs that hold the promise of faster onset of therapeutic efficacy in the treatment of depression.[5]

5-HT$_1$ autoreceptors are located on both the cell bodies (5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptor subtypes) and nerve terminals (5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes) of 5-HT neurons.[6] They are widely distributed in the brain, and it has been documented that the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors play an important role in regulating the release of 5-HT on the 5-HT cell bodies[7] and on the nerve terminals.[8] Thus the concurrent blockade of all three autoreceptors, 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$, should lead to an elevation of extracellular 5-HT concentrations. Indeed, microdialysis studies in guinea pig have confirmed that the combination of 5-HT$_{1A}$ and 5-HT$_{1B}$ antagonism results in an acute synergistic enhancement of 5-HT levels,[9] comparable to that observed following a period of chronic SSRI treatment. It follows, therefore, that a pan-antagonist of the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors may be postulated to exhibit a similar therapeutic profile to a dual acting 5-HT$_{1A}$ antagonist/ SSRI through an acute and sustained increase of postsynaptic 5-HT neurotransmission. Consequently, a pan-antagonist of the 5-HT$_1$ autoreceptors may constitute a novel approach for the treatment of depression/anxiety with potential advantages in the latency to therapeutic onset.

This approach has been targeted within our laboratories, as well as by others,[10] and a series of recent publications have documented our progress.[11,12] A tactic that has proven successful in furnishing selective 5-HT$_1$ antagonist tools, such as **1**, is the modification of the benzoxazinone portion of the dual

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[a] Abbreviations: SSRI, selective serotonin reuptake inhibitor; 5-HT, 5-hydroxytyptamine; SerT, serotonin transporter; SAR, structure−activity relationship; GTPγS, guanosine 5′-O-[γ-thio]triphosphate; DMPK, drug metabolism and pharmacokinetics; CYP450, cytochrome P450; hERG, human ether-a-go-go-related-gene; CNS, central nervous system; PD, pharmacodynamic; 8-OH-DPAT, 8-hydroxy-N,N-dipropyl-2-aminotetralin; hLMA, hyperlocomotor activity; DDI, drug−drug interactions; P-gp, P-glycoprotein; MDCK, Madin−Darby canine kidney; hMDR1, human multidrug resistance 1 protein; BA, basolateral-to-apical; AB, apical-to-basolateral; IA, intrinsic activity; ND, value not determined; inv, inverse agonist; NMR, nuclear magnetic resonance; LCMS, liquid chromatography/mass sprectroscopy; Cl$_{int}$, intrinsic clearance; compd, compound; clogP, calculated octanol/water partition coefficient; Cl$_b$, blood clearance; $t_{1/2}$, half-life; F_{po}, oral bioavailability; C_{max}, maximum concentration.

acting 5-HT$_1$ antagonist/SSRI lead **2** (Figure 1) identified within our laboratories.[13] Exclusion of the benzoxazinone substructure, which is a key pharmacophoric requirement for potent inhibition of the serotonin transporter (SerT), readily removed SerT activity while judicious replacement with alternative groups allowed potent 5-HT$_1$ binding to be restored.[12] Recently, our laboratories have described a newer benzoxazinone dual acting 5-HT$_1$ antagonist/SSRI lead **3** (Figure 1),[14] endowed with increased 5-HT$_1$ antagonist activity and a superior pharmacokinetic profile. Here, we report our studies applying an analogous tactic, modifying the benzoxazinone moiety of **3**, that led to the discovery of a new potent and selective series of 5-HT$_1$ antagonists from which a preclinical candidate was selected.
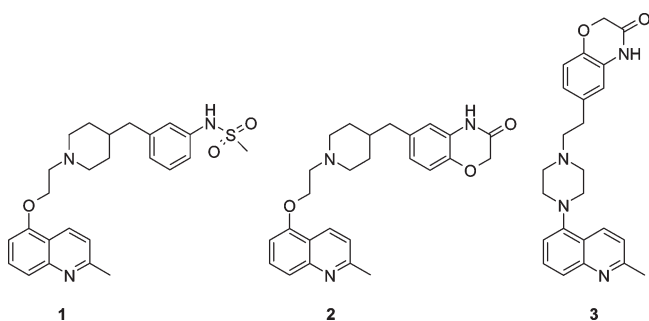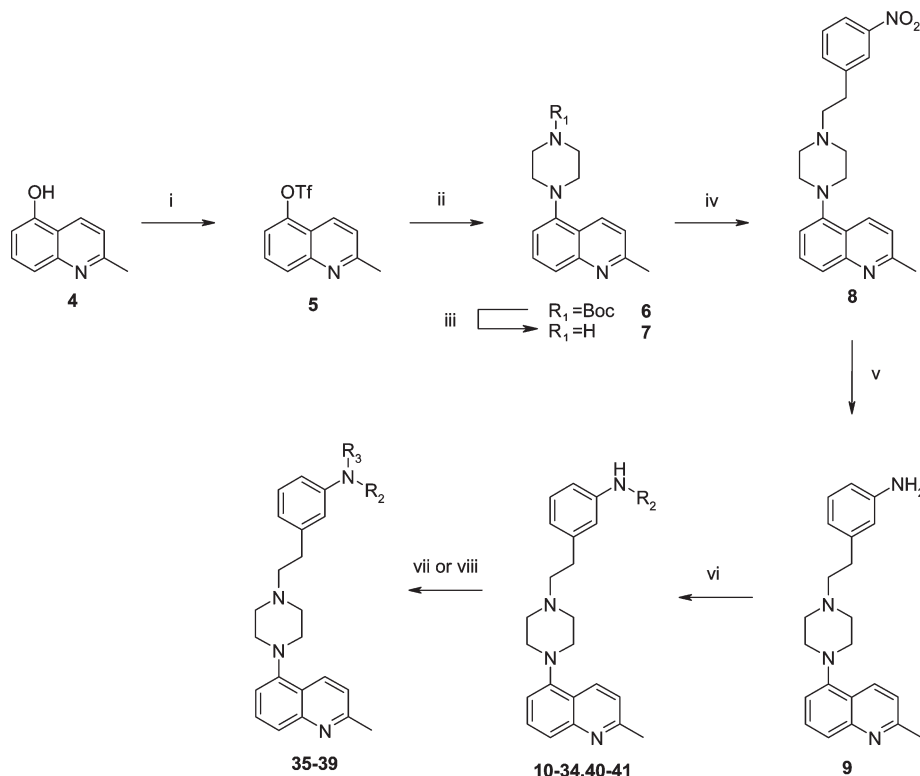
Results and Discussion

From the outset, the medicinal chemistry strategy was entirely focused on replacement of the benzoxazinone portion of molecule **3**. Experience gained working with related series of 5-HT$_{1A/B/D}$ antagonists and dual acting 5-HT$_{1A}$ antagonists/ SSRIs has taught that the 2-methylquinoline is fundamental for combining high 5-HT$_{1A/B/D}$ affinities with low intrinsic activities;[15] as such, the 2-methylquinoline group is considered optimal and hence this portion of the molecule was not targeted for modification. Likewise, modifications to the ethylpiperazine linker were not envisaged because our starting point, compound **3**, was itself the product of an extensive exploration of the linker group[14] and the ethylpiperazine linker was considered optimized.
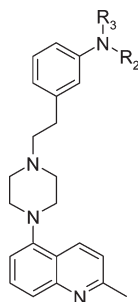
On the basis of the findings from the SAR exploration around lead **2** that had led to the identification of compound **1**,[12] we began by targeting the replacement of the benzoxazinone group in compound **3** with a 3-substituted phenyl group, in particular the methyl sulfonamide derivative. The target compounds were prepared following the synthetic sequence outlined in Scheme 1 that was designed to allow rapid parallel SAR expansion.

A slightly modified route to prepare 2-methyl-5-(1-piperazinyl)quinoline **7** than previously published was employed. A palladium catalyzed Buchwald–Hartwig coupling remains a key step in the synthetic sequence, but passing via the triflate ester of the quinoline group rather than the corresponding bromide afforded greatly improved yields and simplified purification procedures. Subsequent alkylation with (3-nitrophenyl)ethyl methanesulfonate and reduction of the nitro group with

Figure 1. Structures of **1**, a 5-HT$_1$ receptor antagonist, and **2** and **3**, dual acting 5-HT$_1$ receptor antagonist/serotonin reuptake inhibitors.

Scheme 1^a

^{a} Reagents and conditions: (i) Tf$_2$O, pyridine, dichloromethane, 0 °C, 92%; (ii) 1-Boc-piperazine, Cs$_2$CO$_4$, Pd(OAc)$_2$, BINAP, toluene, reflux, 62%; (iii) TFA, dichloromethane, room temp, 96%; (iv) 2-(3-nitrophenyl)ethyl methanesulfonate, DIPEA, DMF, 100 °C, 64%; (v) Fe powder, NH$_4$Cl, MeOH, reflux, 84%; (vi) for sulfonamides RSO$_2$Cl, pyridine, room temp, 44–62%; for amides RCO$_2$H, EDC, HOBt, dichloromethane/DMF, room temp or RCOCl, DIPEA, dichloromethane, room temp; for ureas RNCO, dichloromethane, room temp or triphosgene, TEA, RNH$_2$, 0 °C; for carbamates (RO)COCl, DIPEA, dichloromethane, 0 °C; (vii) MeI, NaH, THF, 0 °C; (viii) NaH, THF, 0 °C.

Table 1. Receptor Binding Affinity ($pK_i{}^a$) and Intrinsic Activities for 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ Receptors and Functional Inhibition of SerT for Novel Compounds

compd[b]	R$_2$	R$_3$	pK$_i$			
			5-HT$_{1A}$ (IA)	5-HT$_{1B}$ (IA)	5-HT$_{1D}$ (IA)	SerT
1			8.6 (inv)	8.7 (0.3)	9.3 (0.0)	6
3			9.6 (0.2)	9.3 (0.1)	9.8 (inv)	7.6
9	H	H	7.8 (ND)	8.1 (ND)	9.3 (ND)	ND
10	−SO$_2$Me	H	9.1 (0.0)	9.1 (0.1)	9.9 (inv)	5.5
11	−SO$_2$Pr	H	8.8 (ND)	8.7 (ND)	9.2 (ND)	ND
12	−COMe	H	9.0 (0.0)	9.3 (0.0)	9.6 (inv)	5.9
13	−COEt	H	8.7 (0.0)	8.9 (inv)	9.7 (inv)	5.9
14	−CO^{i}Pr	H	8.7 (inv)	8.8 (inv)	9.8 (inv)	5.9
15	−CO^{t}Bu	H	8.2 (inv)	8.7 (inv)	9.5 (inv)	5.8
16	−COPh	H	9.0 (inv)	8.6 (inv)	9.5 (inv)	5.7
17	−CO-2-F-Ph	H	9.0 (inv)	8.5 (0.0)	9.6 (inv)	6.2
18	−CO-3-F-Ph	H	9.2 (inv)	8.5 (inv)	9.5 (inv)	6.2
19	−CO-4-F-Ph	H	8.9 (inv)	8.3 (inv)	9.4 (0.0)	6.1
20	−CO-2-OMe-Ph	H	8.6 (ND)	8.4 (ND)	9.2 (ND)	6.1
21	−CO-3-OMe-Ph	H	8.7 (ND)	8.8 (ND)	9.8 (ND)	5.9
22	−CO-4-OMe-Ph	H	8.5 (inv)	8.4 (inv)	9.3 (ND)	5.9
23	−CO-pyrazin-2-yl	H	9.3 (ND)	8.9 (ND)	9.7 (ND)	6.4
24	−CO-2-Me-thiazol-4-yl	H	9.1 (inv)	8.5 (inv)	9.4 (inv)	6.3
25	−CO-4-Me-thiazol-5-yl	H	9.4 (inv)	8.7 (0.0)	9.6 (inv)	6.3
26	−CONHEt	H	8.7 (0.0)	8.9 (inv)	9.8 (inv)	6.3
27	−CONHPr	H	8.3 (ND)	8.7 (ND)	9.8 (ND)	6.1
28	−CONH^{t}Bu	H	8.1 (ND)	8.4 (ND)	9.6 (ND)	6.1
29	−CONHPh	H	8.4 (0.0)	8.5 (0.0)	9.5 (0.0)	5.7
30	−CONH-2-F-Ph	H	8.5 (ND)	9 (ND)	9.7 (ND)	5.7
31	−CONH-3-F-Ph	H	8.4 (0.0)	8.4 (inv)	9.2 (0.0)	5.6
32	−CONH-4-F-Ph	H	8.6 (ND)	8.5 (ND)	9.3 (ND)	5.9
33	−CO$_2$Me	H	9.4 (0.0)	9.3 (inv)	9.6 (0.0)	6.3
34	−CO$_2$Et	H	8.9 (ND)	8.9 (ND)	9.9 (ND)	6.1
35	−COMe	Me	8.7 (0.1)	7.9 (0.2)	9.5 (0.0)	ND
36	−CONHCH$_2$CH$_2$−		9.4 (0.0)	8.5 (0.0)	9.7 (0.0)	6.1
37	−CONHCH$_2$ CH$_2$CH$_2$−		8.5 (ND)	7.4 (ND)	9.5 (ND)	ND
38	−CO$_2$CH$_2$CH$_2$−		9.4 (0.0)	8.7 (0.0)	9.8 (inv)	6.3
39	−CO$_2$CH$_2$CH$_2$CH$_2$−		8.6 (ND)	7.5 (ND)	9.6 (ND)	ND
40	−COCH$_2$CH$_2$CO−		8.5 (ND)	7.4 (ND)	8.8 (ND)	4.7
41	−NCHCHCH−		9.3 (ND)	8.8 (ND)	9.8 (ND)	6.5

[a] Radioligand binding assay to determine affinity at human recombinant 5-HT receptors and functional [^{3}H]-5-HT uptake assays in rat cortical synaptosomes to determine potency for SerT. Each determination lies within 0.3 log units of the mean with a minimum of three replicates. Values in parentheses are intrinsic activity (IA) values relative to the maximum response elicited by the endogenous agonist 5-HT. inv = inverse agonist behavior was observed. ND = value not determined. [b] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

iron powder/ammonium chloride in methanol afforded the versatile intermediate **9**, ready for functionalization.

The in vitro target activities of the compounds described in this manuscript were assessed using a two-tier screening cascade. First, the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ affinities of all compounds prepared were evaluated in radioligand binding assays in stably transfected recombinant cell lines. Subsequently, the functional activities of selected compounds were assessed in [^{35}S]-GTPγS binding assays, again in cells stably transfected with the relevant receptor. Data are reported as pK$_i$ values from the binding assays and intrinsic activities (IA)

from the functional assays. Intrinsic activities are reported relative to the maximum response elicited by the endogenous agonist 5-HT (IA = 1.0); in some cases, test compounds were found to lower [^{35}S]-GTPγS binding below basal levels and these are indicated as inverse agonists (IA = inv). The desired profile for compound progression was set at pK$_i$ > 8.0 for all three targets and either silent antagonism (IA = 0.0) or inverse agonism at each receptor.

In confirmation of our working hypothesis, the initially prepared methyl sulfonamide derivative **10** was found to maintain subnanomolar affinity for the 5-HT$_{1A}$, 5-HT$_{1B}$, and

Table 2. Intrinsic Clearance (Cl$_{int}$) in Rat, Human, and Dog Liver Microsomes and in Vitro Cross-Screening (pK_i) in Radioligand Binding Assays[a]

compd[b]	Cl$_{int}$ (mL/min/g) rat/hum/dog	pK_i hERG	clogP[c]	| pK_i 5-HT | 2A	2B	2C	6	7	| pK_i dopamine | D2	D3
10	1.4/ < 0.5/14.4	6.3	3.7	6.1	6.4	5.5	< 5.6	7.0	6.4	6.7
12	< 0.5/ < 0.5/7.7	5.4	4.1	6.6	6.8	6.0	< 5.4	7.2	6.4	6.8
16	1.7/2.8/46.0	7.3	5.4	7.1	6.9	6.1	< 6.0	7.1	6.4	7.0
23	0.7/4.9/16.0	ND	4.3	6.8	6.1	< 5.8	6.1	6.7	6.7	7.1
24	1.8/3.1/9.9	6.5	5.4	ND	ND	ND	ND	ND	ND	ND
26	0.7/1.3/1.3	6.2	4.5	6.5	6.4	6.1	< 5.2	7.4	6.5	6.9
29	2.0/1.8/18.2	6.5	5.8	< 5.2	< 5.2	< 5.0	6.5	7.1	7.1	7.5
33	2.3/ < 0.5/-	ND	4.5	6.7	6.7	6.1	< 5.8	7.6	6.7	7.0
36	0.7/1.2/5.0	4.9	3.5	6.0	6.3	5.8	< 5.3	6.8	6.3	6.7
38	< 0.5/1.2/3.8	5.7	3.9	6.1	5.9	< 5.3	5.5	6.3	6.1	6.7
41	< 0.5/3.5/-	ND	5.0	6.4	7.1	5.7	< 5.0	6.9	6.4	7.3

[a] Radioligand binding assay to determine affinity at human recombinant 5-HT, dopamine receptors and hERG channel. Each determination lies within 0.3 log units of the mean with a minimum of two replicates. ND = value not determined. [b] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS. [c] Calculated octanol/water partition coefficient using Daylight 4.81 software.[16]

5-HT$_{1D}$ receptors while the SerT activity was reduced by more than 100-fold compared to the parent benzoxazinone structure **3** (Table 1). Furthermore, the intrinsic activities observed for **10** were even lower than those of **3**, eliminating any risk of inducing partial agonism in an in vivo setting;

Having verified that such a strategy was valid, we embarked on a broader exploration of the substituent at the 3-position of the phenyl ring. Aniline **9** reacted smoothly with a variety of electrophiles to generate a series of sulfonamide, amide, urea, and carbamate derivatives. Cyclic urea and carbamate derivatives were accessed by reaction with halogenated isocyanates or chloroformates and subsequent cyclization with sodium hydride. Gratifyingly, the majority of the analogues demonstrated high binding affinities (pK_i > 8) against all three target receptors, including the 5-HT$_{1B}$ receptor, which had proven the most elusive in previous pan 5-HT$_1$ antagonist series.[12] Moreover, SerT activities were uniformly low such that these analogues all exhibited > 100-fold selectivity over SerT as desired. In contrast to our previous experience,[12] the nature of the 3-substituent in this series was found to have little or no influence on the intrinsic activities, with all compounds exhibiting silent antagonism (IA = 0.0) or inverse agonism in GTPγS functional assays. Data for a selection of the compounds prepared is shown in Table 1.

It is noteworthy that such a large variety of functionalities are well tolerated, suggesting that the substituent at the 3-position does not represent a key pharmacophoric point, yet the intermediate **9** has modest affinities for 5-HT$_{1A}$ and 5-HT$_{1B}$ and *N*-methylation of the acetamide derivative leads to an appreciable drop in 5-HT$_{1B}$ affinity (**35** vs **12**), indicating that both hydrogen bond acceptor and hydrogen bond donor interactions may be contributing to the high affinities observed. Although there does not appear to be any particular restriction to the size of the substituent that is tolerated in this position, there is a tendency for the smallest substituents to have the highest affinities, especially at the 5-HT$_{1A}$ receptor. The marked preference for the 5- vs 6-membered cyclic urea/carbamate derivatives (**36** vs **37** and **38** vs **39**) is believed to be more a consequence of the spatial orientation of the hydrogen bond acceptor than a result of steric incompatibility.

With a large number of compounds satisfying progression criteria in our primary assays, the secondary screening cascade became the principal filter for triaging compounds. Typical in vitro DMPK screening consisting of determining metabolic stability toward rat/human liver microsomes and CYP450 inhibition also proved inefficient in prioritizing between

compounds, with essentially all compounds exhibiting good metabolic stability and low CYP450 inhibition (data not shown). However, monitoring metabolic stability in the higher species and screening for off-target selectivity against monoamine targets allowed differentiation of the best molecules for progression into in vivo studies. Data for some representative examples are shown in Table 2. As can be appreciated, selectivity against other 5-HT receptor subtypes and dopamine receptors was generally high, although some specific substitution patterns had modest 5-HT$_7$ or D3 affinities such that selectivity dropped below 50-fold. On the other hand, affinity toward the human ether-a-go-go-related-gene (hERG) potassium channel and high intrinsic clearance in dog liver microsomes represented an issue for a number of substrates.

Although low metabolic stability in the dog posed a problem, in that it could potentially preclude the possibility of reaching adequate systemic exposure in toxicological studies, the affinity of compounds toward the hERG channel was a much greater concern. Inhibition of the hERG channel is associated with prolongation of the cardiac QT interval, an effect which is in turn linked to life-threatening ventricular arrhythmias (Torsades de Pointes) in the clinic.[17] With the exception of a few outliers, such as the sulphonamide **10** which presumably has a specific interaction with the channel, the affinity for hERG grew steadily with increasing lipophilicity of the substrate. The substrates with lower hERG affinities, and hence lower lipophilicities, also tended to be those with lower intrinsic clearances, making them the obvious choice for progression into in vivo pharmacokinetic studies.[18]

In accord with the low intrinsic clearance values measured in vitro and their favorable physicochemical properties, the selection of compounds further profiled in rat pharmacokinetic studies showed low blood clearance, high oral bioavailability, and moderate half-lives (Table 3). Additionally, all substrates showed appreciable partitioning into the central nervous system (CNS), confirming their suitability as centrally acting 5-HT$_1$ antagonists.

To establish that the compounds were functionally exerting their pharmacological activity in vivo, they were investigated in a 5-HT$_{1A}$ pharmacodynamic (PD) model in rats. In this model, compounds are evaluated for their ability to reverse the hyperlocomotion induced by administration of the selective 5-HT$_{1A}$ agonist 8-OH-DPAT (0.2 mg/kg sc). Compounds **10**, **12**, and **36** all demonstrated clear dose-dependent inhibition of the 8-OH-DPAT-induced hyperlocomotor activity (hLMA), with ED$_{50}$ values ranging from 0.03 to 1 mg/kg

Table 3. Solubility,[a] Pharmacokinetic Properties in Rat[b] and in Vivo Activity in 5-HT$_{1A}$-Agonist Driven Pharmacodynamic (PD) Model in Rat[c]

compd[d]	solubility at pH1.2/pH5.5 (mg/mL)	Cl_b (mL/min/kg)	$t_{1/2}$ (h)	F_{po} %	C_{max} (ng/mL)	AUC_{0-inf} (ng·h/mL)	CNS Br/Bl	hLMA ED$_{50}$ (mg/kg)
10	>10/0.6	7	3.9	56	243	1446	0.5	1.0
12	>10/5.0	10	4.6	51	98	893	0.7	0.09
36	>10/0.5	11	3.2	58	122	889	0.5	0.03

[a] All compounds demonstrated pH dependent solubility profiles. Solubilities are shown for pH 1.2 (simulated gastric fluid, SGF) and pH 5.5 (fed state simulated intestinal fluid, FeSSIF) at 25 °C. The solubilities of the dihydrochloride salts of **10**, **12**, and **36** in water were > 10 mg/mL. [b] In vivo data were determined with dihydrochloride salts by 0.5 mg/kg iv study in rat; brain/blood ratio was measured after 1 h. Oral bioavailability (F_{po}) and area under the curve extrapolated to infinity (AUC_{0-inf}) were determined by an additional 1 mg/kg po rat PK study. [c] In vivo antagonist activity was determined by monitoring the inhibition of the hyperlocomotor activity (hLMA) elicited by sc administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT (0.2 mg/kg). [d] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

Table 4. Pharmacokinetic Properties of Compound **36** in Dog and Cynomolgous Monkey[a]

species	Cl_b (mL/min/kg)	$t_{1/2}$ (h)	F_{po} %	C_{max} (ng/mL)	AUC_{0-inf} (ng·h/mL)
dog	60	0.8	6	1305	2550
cynomolgus monkey	25	5.3	63	1390	4224

[a] In vivo data were determined with the dihydrochloride salt by 0.5 mg/kg iv study in dog and 1.0 mg/kg iv study in cynomolgus monkey. Oral bioavailability (F_{po}) in dog was determined by an additional 1 mg/kg po dog PK study. Oral bioavailability (F_{po}) in cynomolgus monkey as well as maximum blood exposure (C_{max}) and area under the curve extrapolated to infinity (AUC_{0-inf}) were determined by additional 10 mg/kg po dog and cynomolgus monkey PK studies.

(Table 3). In particular, attention was drawn to the exceptionally high in vivo potency observed for compound **36**, which was further complemented by the fact that **36** has the lowest hERG activity seen within the series, ensuring an ample safety margin toward cardiac QT effects.

Consequently, more in-depth profiling of compound **36** was conducted to ascertain its suitability for progression as a potential drug candidate.

A low potential for causing drug−drug interactions (DDI) was confirmed with low CYP450 inhibition being observed in the five major human isoforms expressed recombinantly (IC$_{50}$s for Gentest Supersomes, 1A2 = 96 μM, 2C9 = 60 μM, 2C19 = 76 μM, 2D6 = 86 μM, 3A4 = 67 μM) as well as in human liver microsomes (3A4 = 21−30 μM with midazolam, atorvastatin, and nifedipine as probes); furthermore, no evidence of time-dependent inhibition of 3A4 was observed in human liver microsomes with the same probes. Further selectivity screening performed within GlaxoSmithKline as well as a receptorgram screen run at CEREP[19] (Diversity profile) evidenced an excellent overall selectivity profile on > 200 targets with selectivity falling below 30-fold only against α adrenergic receptors (pIC$_{50}$ at rat native receptors, α$_{1A}$ = 7.6, α$_{1B}$ = 7.6); a follow-up study in rabbit thoracic aorta preparations determined that **36** was devoid of agonist activity at α$_1$ receptors, but rather it demonstrated a moderate functional antagonism of the phenylephrine-induced contraction of rabbit aorta (pIC$_{50}$ = 6.9). Taking into account the low predicted systemic concentrations at therapeutic doses, this degree of off-target activity was deemed tolerable for progression.

The susceptibility of compound **36** to the P-glycoprotein (P-gp) efflux transporter was evaluated in MDCK cells expressing hMDR1. At 1 μM concentration, the ratio of the apparent permeabilities measured from basolateral-to-apical (BA) to apical-to-basolateral (AB) indicated that **36** is a moderate P-gp substrate (BA/AB = 2.8; the BA/AB ratio drops to 1.6 in the presence of the specific P-gp inhibitor GF120918).

The extent of plasma protein binding was determined in rat, dog, and human plasma by equilibrium dialysis at a

concentration of 1 μg/mL showing values of 95.9, 95.5 and 95.9% bound, respectively.

Pharmacokinetic studies in higher preclinical species revealed very high blood clearance in dog (> 100% of liver blood flow) and consequently low oral bioavailability in this species (Table 4). It is likely that other elimination routes, besides hepatic extraction, are operating in this species. In spite of this, a supraproportional increase in systemic exposure was observed upon increasing the oral dose from 1 to 10 mg/kg, suggesting saturation of the hepatic first-pass metabolism, such that adequate exposures were achieved to be able to consider the dog as a possible toxicological test species. However, higher systemic exposures and a lower, albeit moderate, blood clearance (57% of liver blood flow) were seen in cynomolgus monkey (Table 4), and this species was elected as the second preclinical species for safety assessment studies.

When doses of compound **36** were escalated, some behavioral observations were noted in rat and cynomolgus monkey. Starting from 3 mg/kg in rat and 10 mg/kg in cynomolgus monkey, some of the animals treated with compound **36** were observed to have half-closed eyes and subdued behavior. These effects were temporary and reversible but increased in incidence and severity with increasing dose. It is not believed that these side effects are related to the 5-HT$_{1A/1B/1D}$ activity of **36**, but rather they are assumed to be the consequence of unspecified off-target activities. Although these behavioral side effects were not linked to any toxicological outcome in the animals, they may prudentially be considered a potentially limiting factor. As the systemic exposures associated with these effects (524 ng/mL in rat and 1390 ng/mL in cynomolgus monkey) were much higher than those required for efficacy in animal models of anxiety (ranging from 2 to 30 ng/mL in various rat and primate models), there was an ample therapeutic index to endorse the selection of compound **36** as a preclinical development candidate.

Conclusion

In conclusion, a novel series of selective 5-HT$_1$ antagonists were prepared via modification of the benzoxazinone moiety of a known dual acting 5-HT$_1$ antagonist/SSRI structure. The majority of compounds prepared showed potent affinity and low intrinsic activities at the autoreceptors 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ and high selectivity over SerT. From among these analogues, the cyclic urea derivative, 1-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-imidazolidinone **36** (GSK163090), emerged due to its low hERG affinity and excellent in vitro DMPK profile. The superior quality of **36** was further highlighted by its commendable in vivo pharmacokinetic profile in rat and its outstanding activity in the 5-HT$_{1A}$ PD model, where 50% efficacy was achieved at a blood concentration of 3 ng/mL. On the basis of these results

and its promising preclinical developability profile, compound **36** was selected as an appropriate development candidate for progression toward clinical proof-of-concept studies.

Evidence supporting fast-onset antidepressant/anxiolytic activity of compound **36** was obtained from both neurochemical studies and validated rodent and primate animal models of anxiety, which will be the subject of a separate publication.

Experimental Section

In Vitro Biological Test Methods. Receptor Affinities at Human Recombinant 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors. The affinities of the compounds toward the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors were determined by the following assay. CHO cells expressing 5-HT$_{1A}$ receptors (4×10^7 cells/mL) were homogenized in Tris buffer and stored in 1 mL aliquots. CHO cells expressing 5-HT$_{1B}$ receptors (4×10^7 cells/mL) were homogenized in Tris buffer and stored in 1.5 mL aliquots. CHO cells expressing 5-HT$_{1D}$ receptors (1×10^8/mL) were homogenized in Tris buffer and stored in 1 mL aliquots. Then 0.4 mL of a cell suspension is incubated with [^{3}H]-5-HT (4nM) for 5-HT$_{1B/1D}$ receptors and [^{3}H]-WAY100635 (1nM) for 5-HT$_{1A}$ receptors in Tris Mg HCl buffer (pH 7.7) and test drug at 37 °C for 45 min. Each test drug is tested at 10 concentrations (0.01−0.3 nM final concentration), with nonspecific binding defined using 0.01 mM 5-HT. The total assay volume is 0.5 mL. Incubation is stopped by rapid filtration using a Packard Filtermate and radioactivity measured by Topcount scintillation counting. pK_i values are calculated from the IC$_{50}$ generated by an iterative least-squares curve fitting program.

Functional Activities at Human Recombinant 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ Receptors. Intrinsic activities were determined according to the following assay. HEK293 cell membranes stably expressing human 5-HT$_{1A}$ receptors and CHO cell membranes stably expressing human 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors are homogenized in HEPES/EDTA buffer and stored in 1 mL aliquots, and [^{35}S]-GTPγS binding studies were carried out essentially as described by Lazareno et al.,[20] with some minor modifications. Membranes from 10^6 cells were preincubated at 30 °C for 30 min in 20 mM HEPES buffer (pH 7.4) in the presence of MgCl$_2$ (3 mM), NaCl (100 mM), GDP (10 μM), and ascorbate (0.2 mM), with or without test compounds. The reaction was started by the addition of 50 μL of [^{35}S]-GTPγS (100 pM, assay concentration), followed by a further 30 min incubation at 30 °C. Nonspecific binding was determined using nonradiolabeled GTPγS (20 μM) added prior to the membranes. The reaction was terminated by rapid filtration through Whatman GF/B grade filters. followed by 5 × 1 mL washes with ice cold HEPES (20 mM)/MgCl$_2$ (3 mM) buffer. Radioactivity was measured using liquid scintillation spectrometry.

Functional Activity for Inhibition of the Serotonin Reuptake Transporter (SerT). The efficacy to inhibit the reuptake of serotonin was measured in a 5-HT uptake assay by measurement of uptake of [^{3}H]-5-HT into LLCPK cells expressing human serotonin transporters. In brief, cells were harvested and plated onto 96-well plates (10000 cells per well). Twenty-four hours later, cells were washed twice with HBSSH (Hanks' balanced salt solution + 20 mM HEPES). Then 50 μL of test compound or vehicle was added to each well and incubated for 10 min. Subsequently, [^{3}H]-5-HT (final concentration 25 nM) was added, and the test mixture was incubated for a further 7 min. The reaction was terminated by aspiration of test mixture, and the cells were washed six times with HBSSH. Scintillation cocktail (50 μL, Microscint-20, Packard) was added to the cells, and the top and bottom of the plate was sealed. Plates were read, 30 min later, in a Packard TopCount instrument.

hERG [^{3}H]-Dofetilide Binding Assay. hERG activity was measured using [^{3}H]-dofetilide binding in a scintillation proximity assay (SPA) format. The activity was measured with a PerkinElmer Viewlux imager.

DMPK Test Methods. Intrinsic Clearance (Cl$_{int}$) Assay. Intrinsic clearance (Cl$_{int}$) values were determined in rat, dog, and human liver microsomes. Test compounds (0.5 μM) were incubated at 37 °C for 30 min in 50 mM potassium phosphate buffer (pH 7.4) containing 0.5 mg of microsomal protein/mL. The reaction was started by addition of cofactor (NADPH; 8 mg/mL). The final concentration of solvent was 1% of the final volume. At 0, 3, 6, 9, 15, and 30 min, an aliquot (50 μL) was taken, quenched with acetonitrile containing an appropriate internal standard, and analyzed by HPLC-MS/MS. The intrinsic clearance (Cl$_{int}$) was determined from the first-order elimination constant by nonlinear regression using Grafit v5 (Erithacus software, UK), corrected for the volume of the incubation and assuming 52.5 mg microsomal protein/g liver for all species. Values for Cl$_{int}$ were expressed as mL/min/g liver. The lower limit of quantification of clearance was determined to be when < 15% of the compound had been metabolized by 30 min, and this corresponded to a Cl$_{int}$ value of 0.5 mL/min/g liver. The upper limit was 50 mL/min/g liver.

CYP450 Inhibition Assay. Inhibition potential (IC$_{50}$) of test compound for inhibition of human CYP1A2, 2C9, 2C19, 2D6, and 3A4 was determined. A range of concentrations (0.1, 0.33, 1, 3.3, 10, 33, and 100 μM) of test compound prepared in methanol were preincubated at 37 °C for 10 min in 50 mM potassium phosphate buffer (pH 7.4) containing 0.1, 0.16, 0.2, or 0.4 mg recombinant human CYP450 microsomal protein mL^{-1} (Gentest Corporation, USA) and substrate. Following preincubation, 25 μL mL^{-1} of a NADPH regenerating system (7.8 mg glucose 6-phosphate, 1.7 mg NADP, and 6 U glucose 6-phosphate dehydrogenase mL^{-1} 2% w/v NaHCO$_3$) was added to each well to start the reaction. Production of fluorescent metabolite was then measured over a 10 min time-course using a Spectrafluor plus plate reader (Tecan). The rate of metabolite production (AFU/min) was determined for each concentration of compound and converted to a percentage of the mean control rate using Magellan v3.0 (Tecan software). The inhibitory potential (IC$_{50}$) was determined from the slope of the plot using Grafit v4.12 (Erithacus software, UK). Miconazole was added as a positive control to each plate.

CYP450 Inhibition Using Human Liver Microsomes. A range of concentrations (0.1, 0.33, 1, 3.3, 10, 33, and 100 μM) of test compound were prepared in methanol. These were preincubated at 37 °C for 20 min in 50 mM potassium phosphate buffer (pH 7.4) containing pooled human liver microsomal protein (0.1 mg/mL; Xenotech) and CYP3A4 substrate (midazolam, atorvastatin or nifedipine; 2.5, 10, or 50 μM, respectively). To start the reaction, NADPH regenerating system (7.8 mg glucose 6-phosphate, 1.7 mg NADP, and 6 units glucose-6-phosphate dehydrogenase/mL of 2% (w/v) NaHCO$_3$; 50 μL/mL) was added. Following a 5 min incubation, reactions were terminated with the addition of 250 μL of acetonitrile.

Quantification of 1′-hydroxy midazolam, o-hydroxy atorvastatin, and oxidized nifedipine metabolites was carried out using validated HPLC-MS analytical methods. Inhibition (IC$_{50}$) was determined using Grafit (Erithacus software, UK).

Metabolism-Dependent Inhibition of CYP3A4 in Human Liver Microsomes. A range of concentrations (0.1, 0.33, 1, 3.3, 10, 33, and 100 μM) of the test compound and troleandomycin (positive control) were prepared in methanol. These were pre-incubated at 37 °C for 20 min in 50 mM potassium phosphate buffer (pH 7.4) containing pooled human liver microsomal protein (0.1 mg/mL; Xenotech) and NADPH regenerating system (7.8 mg glucose 6-phosphate, 1.7 mg NADP, and 6 units glucose-6-phosphate dehydrogenase/mL of 2% (w/v) NaHCO$_3$; 50 μL/mL) [Cofactor preincubation samples] or CYP3A4 substrate (midazolam, atorvastatin, or nifedipine; 2.5, 10, or 50 μM, respectively) [substrate preincubation samples]. To start the reaction, CYP3A4 substrate (midazolam, atorvastatin, or nifedipine; 2.5, 10, or 50 μM, respectively) was added to the cofactor preincubation samples and NADPH regenerating system (50 μL/mL)

to the substrate preincubation samples. Following 5 min incubation for midazolam and 10 min incubation for atorvastatin or nifedipine, reactions were terminated with the addition of 250 μL of acetonitrile.

Quantification of 1'-hydroxy midazolam, o-hydroxy atorvastatin, and oxidized nifedipine metabolites was carried out using validated HPLC-MS analytical methods. Inhibition (IC_{50}) was determined using Grafit (Erithacus software, UK) and TDI assessed, comparing the difference in IC_{50} between the cofactor and substrate preincubation samples for each CYP3A4 substrate.

Rat Pharmacokinetics. Test compounds were administered to male Sprague−Dawley rats as cassette. The compounds were dosed intravenous (0.5 mg/kg, 2 mL/kg, 2.5% DMSO in glucosate solution; $n = 3$ for the iv pharmacokinetic study and $n = 3$ for the brain penetration study) and po (1 mg/kg, 10 mL/kg, 1% DMSO in aqueous methocel 0.5%, $n = 3$).

In the po and iv pharmacokinetic studies, blood samples were collected at intervals up to 24 h after each administration. In the brain penetration study, at 1 h after intravenous dosing, animals were anaesthetised using carbon dioxide. A blood sample was collected via a heart puncture and the brain removed.

Blood and brain samples were analyzed for test compounds using a method based on protein precipitation followed by LC-MS/MS analysis.

In Vitro P-Glycoprotein Assay. MDCK cells expressing human MDR1 were used to determine whether test compounds are substrates for human P-glycoprotein (P-gp). Compounds were tested in duplicate in two directions (apical-to-basolateral and basolateral-to-apical) and in the presence and absence of GF120918 (2 μM), a specific inhibitor of P-gp. Amprenavir (1 and 0.5 μM), a known P-gp substrate, was included as positive control. The apparent permeability value (P_{app}) was measured from the apical (which represents the blood lumen) to the basolateral (which simulates the brain side) compartment (P_{app} AB) and from basolateral to apical side (P_{app} BA) in order to determine the BA/AB ratio.

A compound is considered to be a substrate for P-gp when the BA/AB ratio in the absence of GF120918 is greater than 1.8 and is significantly reduced in the presence of GF120918.

In Vivo Biological Test Methods. All the work involving animals was carried out in accordance with European directive 86/609/EEC governing animal welfare and protection, which is acknowledged by Italian Legislative Decree no. 116, 27 January 1992, and according to internal review performed by the GlaxoSmithKline Committee on Animal Research & Ethics (CARE) and to the company Policy on the Care and Use of Laboratory Animals.

Adult male Sprague−Dawley rats (Charles River, Italy) were group-housed upon arrival and left to acclimatize for at least 5 days in a room controlled for temperature (23 $\pm$ 1 °C), humidity (60%), and lighting (12 h light−dark cycle; lights on 0600−1800 h) with ad libitum access to food pellets (Riepper, Italy) and tap water.

Rat Hyperlocomotion 5-HT$_{1A}$ Pharmacdynamic Model. Rats (250−350 g at test) were used. Rat locomotory activity was assessed as the total distance (cm) traveled by each rat in the test arena over a 30 min period using Digiscan analyzer system (Omnitech, model RXYZCM-8, Columbus, OH). Rats were placed individually into clear plexiglas boxes, measuring 40 cm × 40 cm × 30.5 cm and covered with a perforated plexiglas lid. Infrared monitoring sensors were located around the perimeter walls. Data were collected and analyzed by a Digiscan analyzer (Omnitech, model DCM-4, Columbus, OH), which in turn transferred information to a computer. Total distance was recorded during 30 min test period.

Rats were treated orally (5 mL/kg, body weight) with vehicle or compound **36** at 0.03, 0.1, or 0.3 mg/kg 60 min before a subcutaneous (sc, 2 mL/kg) injection of 0.2 mg/kg of 8-OH-DPAT, then immediately assessed for their locomotor activity. The standard 5-HT$_{1A}$ receptor antagonist WAY-100,635 was

also included in the experiment as a positive control (0.03 mg/kg sc 30 min before test). Eight to nine rats in each group of treatment were used.

Drugs: Compound **36** was suspended in Methocel 0.5% (w/v, HPCM Colorcon, Dow Chemical Company, Midland, MI). 8-OH-DPAT was purchased by Sigma (Italy) and dissolved in saline before use. WAY-100,635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride) was synthesized in-house and dissolved in sterile water before use. All compounds were prepared with correction for salt form such that doses cited refer to mg/kg of active principle.

Chemistry. General Methods. Proton magnetic resonance (NMR) spectra were recorded either on Varian instruments at 300, 400, or 500 MHz, or on a Bruker instrument at 300 MHz. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. The NMR spectra were recorded at a temperature ranging from 25 to 90 °C. When more than one conformer was detected, the chemical shifts for the most abundant one was reported. Mass spectra (MS) were typically taken on a 4 II triple quadrupole mass spectrometer (Micromass UK) or on a Agilent MSD 1100 mass spectrometer, operating in ES (+) and ES(−) ionization mode or on an Agilent LC/MSD 1100 mass spectrometer, operating in ES (+) and ES (−) ionization mode coupled with HPLC instrument Agilent 1100 series [LC/MS-ES (+): analysis performed on a Supelcosil ABZ +Plus (33 mm × 4.6 mm, 3 μm) (mobile phase, 100% [water + 0.1% HCO$_2$H] for 1 min, then from 100% [water + 0.1% HCO$_2$H] to 5% [water + 0.1% HCO$_2$H] and 95% [CH$_3$CN] in 5 min, finally under these conditions for 2 min; $T = 40$ °C; flux = 1 mL/min; LC/MS-ES: analysis performed on a Supelcosil ABZ +Plus (33 mm×4.6 mm, 3 μm) (mobile phase: 100% [water +0.05% NH$_3$] for 1 min, then from 100% [water +0.05% NH$_3$ to 5% [water +0.05% NH$_3$] and 95% [CH$_3$CN] in 5 min, finally under these conditions for 2 min; $T = 40$ °C; flux = 1 mL/min]]; in the mass spectra, only one peak in the molecular ion cluster is reported.

DAD chromatographic traces, mass chromatograms, and mass spectra were taken on a UPLC/MS Acquity system coupled with a Micromass ZQ mass spectrometer operating in ESI positive or negative. The phases used were: (A) H$_2$O/ACN 95/5 + 0.1% TFA; (B) H$_2$O/ACN 5/95 + 0.1% TFA. The gradient was: $t = 0$ min) 95%A 5%B, $t = 0.25$) 95%A 5%B, $t = 3.30$) 100%B, $t = 4.0$) 100%B, followed by 1 min of reconditioning. Flash silica gel chromatography was carried out on silica gel 230−400 mesh (supplied by Merck AG Darmstadt, Germany) or over Varian Mega Be−Si prepacked cartridges or over prepacked Biotage silica cartridges. SPE-SCX cartridges were ion exchange solid phase extraction columns supplied by Varian. The eluent used with SPE-SCX cartridges was methanol, followed by 2N ammonia solution in methanol. In a number of preparations, purification was performed using either Biotage manual flash chromatography (Flash+) or automatic flash chromatography (Horizon, SP1) systems. All these instruments work with Biotage Silica cartridges. SPE-Si cartridges were silica solid-phase extraction columns supplied by Varian. Microwave reactions were carried out in a Personal Chemistry Emrys Optimiser (300 W). All reactions were carried out under anhydrous nitrogen or argon atmosphere using standard Schlenk techniques. Most chemicals and solvents were analytical grade and used without further purification.

The purity of the compounds reported in the manuscript was established through HPLC methodology. All the compounds reported in the manuscript have a chemical purity ≥95%.

Chemistry. General Procedures. The general synthetic scheme used for the preparation of the reported compounds is shown in Scheme 1. Experimental procedures and analytical data for compounds **5−41** are described below.

2-Methyl-5-quinolinyl Trifluoromethanesulfonate (5). A solution of 2-methyl-quinolin-5-ol (2.5 g; 1 equiv) in dichloromethane

(25 mL) and pyridine (6.4 mL; 5 equiv) was cooled to 0 °C, and trifluoromethanesulfonic anhydride (4.2 mL; 1.6 equiv) was added dropwise over 10 min. The reaction mixture was stirred under an inert atmosphere at rt for 1 h, then poured into water (20 mL) and extracted into ethyl acetate (3 × 15 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by flash chromatography, eluting with ethyl acetate/cyclohexane (4/6), affording the title compound in 92% yield (4.2 g). MS (ES) m/z: 292.3 $[MH^+]$. $C_{11}H_8F_3NO_3S$ requires 291. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.05 (d, 1 H), 7.85 (d, 1 H), 7.64 (t, 1H), 7.48 (d, 1 H), 7.43 (d, 1 H), 2.48 (s, 3 H).

1,1-Dimethylethyl 4-(2-methyl-5-quinolinyl)-1-piperazinecar-boxylate (6). *tert*-Butyl 1-piperazine carboxylate (1.6 g; 1.2 equiv), cesium carbonate (1.7 g; 1.5 equiv), palladium acetate (0.33 g; 0.14 equiv), and 2,2′-bis(diphenylphosphino)-1,1′-bi-naphthyl (0.97 mg; 0.15 equiv) were added to a solution of intermediate 5 in toluene (20 mL) under an inert atmosphere. The reaction mixture was stirred at reflux under nitrogen for 8 h. The reaction was quenched at room temperature using a saturated aqueous solution of ammonium chloride (15 mL) and extracted into ethyl acetate (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by flash chromatography, eluting with ethyl acetate/cyclohexane (3/7), affording the title compound in 62% yield (1.4 g). MS (ES) m/z: 328.4 $[MH]^+$. $C_{19}H_{25}N_3O_2$ requires 327. ^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 8.40 (d, 1 H), 7.76 (d, 1 H), 7.61 (t, 1 H), 7.29 (d, 1 H), 7.06 (d, 1 H), 3.69 (bs, 4 H), 3.03 (bs, 4 H), 2.74 (s, 3 H), 1.51 (s, 9 H).

2-Methyl-5-(1-piperazinyl)quinoline (7). Intermediate 6 (1.1 g) in a 25% solution of trifluoroacetic acid in dichloromethane (10 mL) was stirred at rt under an inert atmosphere for 3 h. The reaction mixture was concentrated under reduced pressure and desalted by means of a 20 g SCX cartridge, affording the title compound in 96% yield (0.74 g). MS (ES) m/z: 228.4 $[MH]^+$. $C_{14}H_{17}N_3$ requires 227. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.34 (d, 1 H), 7.57 (m, 2 H), 7.35 (m, 1 H), 7.06 (m, 1 H), 2.93 (bm, 8 H), 2.62 (s, 3 H).

2-(3-Nitrophenyl)ethyl methanesulfonate used in the synthesis of 8 was prepared as follows: Methanesulfonyl chloride (0.28 mL) was added dropwise to a stirred solution of 2-(3-nitrophe-nyl)ethanol (0.5 g; 1 equiv) in dichloromethane (3 mL) and triethylamine (0.5 mL; 1.2 equiv) at 0 °C under an inert atmosphere. The solution was allowed to reach rt and stirred for 5 h. The reaction mixture was diluted with water (3 mL) and extracted into dichloromethane (3 × 3 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by flash chromatography, eluting with a gradient from dichloromethane to dichloromethane/MeOH (98/2), affording the title compound in 84% yield (0.62 g). ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.15 (m, 2 H), 7.53 (m, 2 H), 4.45 (t, 2 H), 3,15 (t, 2H), 2.92 (s, 3 H).

2-Methyl-5-{4-[2-(3-nitrophenyl)ethyl]-1-piperazinyl}quino-line (8). *N,N*-Diisopropylethylamine (0.8 mL; 5 equiv) was added to a solution of intermediate 7 (0.2 g; 1 equiv) and 2-(3-nitrophenyl)ethyl methanesulfonate (0.22; 1 equiv) in dimethyl-formamide (1.5 mL). The reaction mixture was heated to 100 °C for 10 h. The dark solution was concentrated under reduced pressure, diluted with water (3 mL) and brine (1 mL), and extracted into ethyl acetate (3 × 3 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by flash chromatography, eluting with a gradient from dichloromethane to dichloro-methane/MeOH (98/2), affording the title compound in 64% yield (0.21 g). MS (ES) m/z: 377.4 $[MH]^+$. $C_{22}H_{24}N_4O_2$ requires 376. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1 H), 8.11 (s, 1 H), 8.05 (d, 1 H), 7.70 (d, 1 H), 7.55 (m, 2 H), 7.45 (t, 1 H), 7.25 (m, 1 H), 7.05 (d, 1 H), 3.10 (mt, 4 H), 2.95 (bm, 2 H), 2.75 (bm, 6 H), 2.70 (s, 3 H).

3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}aniline (9). A solution of intermediate 8 (0.14 g; 1 equiv) in methanol (3 mL) was added dropwise to a suspension of iron powder (0.07 g; 3.2 equiv) and ammonium chloride (0.1 g; 5.3 equiv) in water (3 mL). The reactants were heated at reflux for 8 h, adding additional amounts of iron powder (total 0.07 g; 3.2 equiv) and ammonium chloride (total 0.1 g; 5.03 equiv) in 3 portions during the reaction. The reaction mixture was filtered using a Millipore filter. The filtrate was concentrated under reduced pressure, diluted with water (5 mL) and a saturated aqueous solution of sodium hydrogen carbonate (2 mL), extracted into ethyl acetate (3 × 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure, obtaining the title com-pound in 84% yield (0.11 g). MS (ES) m/z: 347.4 $[MH]^+$. $C_{22}H_{26}N_4$ requires 346. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1 H), 7.70 (d, 1 H), 7.55 (t, 1 H), 7.25 (d, 1 H), 7.08 (m, 2 H), 6.65 (md, 1 H), 6.55 (m, 2 H), 3.65 (bs, 2 H), 3.15 (t, 4 H), 2.80 (m, 4 H), 2.75 (s, 3 H), 2.70 (m, 4 H).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-methanesulfonamide (10). Methanesulfonyl chloride (8 μL; 1.2 equiv) was added dropwise to a solution of intermediate 9 (0.03 g; 1 equiv) in pyridine (0.5 mL). The reaction was stirred at rt overnight. The reaction mixture was concentrated under reduced pressure, diluted with water (1 mL) and a saturated aqueous solution of sodium hydrogen carbonate (1 mL), extracted into dichloromethane (3 × 2 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by flash chromatography, eluting with a gradient from dichloromethane to dichloromethane/MeOH (98/2), affording the title compound in 44% yield (0.016 g). MS (ES) m/z: 425.4 $[MH]^+$. $C_{23}H_{28}N_4O_2S$ requires 424. ^{1}H NMR (300 MHz, MeOD) δ (ppm): 8.40 (d, 1 H), 7.55 (m, 2 H), 7.30 (d, 1 H), 7.15 (t, 1 H), 7.10 (m, 2 H), 6.90 (bt, 2 H), 3.05 (bt, 4 H), 2.85 (s, 3 H), 2.83−2.63 (bm, 8 H), 2.60 (s, 3 H).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-1-propanesulfonamide (11). The title compound was prepared in 62% yield using a similar procedure to compound 10 starting from intermediate 9 and propanesulfonyl chloride. MS (ES) m/z: 453.4 $[MH]^+$. $C_{25}H_{32}N_4O_2S$ requires 452. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1 H), 7.70 (d, 1 H), 7.60 (t, 1 H), 7.30 (m, 2 H), 7.1 (m, 2H), 7.01 (d, 1 H), 3.30 (bm, 6 H), 2.80 (bm, 6 H), 2.60 (s, 3 H), 1.80 (m, 2 H), 1.0 (t, 3 H).

General Procedure for the Preparation of Amides and Their Corresponding Dihydrochloride Salts Starting from Intermediate 9. Method A. Triethylamine or diisopropylethylamine (1.7 equiv) and then an acyl chloride (1.5 equiv) were added dropwise to a stirred solution of intermediate 9 (1 equiv) in dichloromethane at room temperature under an inert atmosphere. The reaction was left under stirring for 16 h. The mixture was then washed with a saturated aqueous solution of NH$_4$Cl, a saturated aqueous solu-tion of NaHCO$_3$, brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude material was purified on SPE cartridge (Silica) using as eluent a gradient from dichloro-methane/MeOH 99/1 to dichloromethane/MeOH 98/2, affording the final compound (yields ranged from 30 to 80%).

Method B. EDC·HCl (1.5 equiv) and HOBt (1.5 equiv) were added sequentially to a stirred solution of a carboxylic acid (1.5 equiv) in dichloromethane/dimethylformamide (1/1) at room temperature. The reaction mixture was left under stirring for 30 min, and then intermediate 9 (1 equiv) dissolved in dichloro-methane/dimethylformamide (1/1) was added dropwise. The solution was stirred for 16 h and then diluted with dichloro-methane and washed with a saturated aqueous solution of NaHCO$_3$ and brine and then dried over Na_2SO_4. The solution was concentrated under reduced pressure, and the residual solvent was removed by means of an SCX cartridge. The crude material was purified on SPE cartridge (Silica) eluting from a gradient from dichloromethane/MeOH 99/1 to dichloromethane/MeOH 98/2, affording the final compound (yields ranged from 20 to 96%).

The free base could be converted into its dihydrochloride salt by dissolving the compound in dichloromethane and adding a

1 M ethereal solution of HCl (2.1 equiv) dropwise. A yellow solid precipitated and the suspension was stirred for 15 min. The solvent was removed under reduced pressure, affording a crude material which was triturated with Et_2O. The final compound was then recovered by filtration (yield quantitative).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-acetamide (12). The title compound was prepared in 52% yield according to the general procedure for the preparation of the amides (method A) starting from intermediate **9** and acetyl chloride. MS (ES) m/z: 389 [MH]$^+$. $C_{24}H_{28}N_4O$ requires 388. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 9.84 (s, 1 H), 8.33 (d, 1 H), 7.58 (m, 2 H), 7.46 (s, 1 H), 7.39 (m, 2 H), 7.19 (t, 1 H), 7.10 (dd, 1 H), 6.92 (d, 1 H), 3.03 (bm, 4 H), 2.73 (bm, 6 H), 2.62 (s + bm, 5 H), 2.02 (s, 3 H).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-propanamide (13). The title compound was prepared in 73% yield according to the general procedure for the preparation of the amides (method A) starting from intermediate **9** and propanoyl chloride. MS (ES/+) m/z: 403 [MH]$^+$. $C_{25}H_{30}N_4O$ requires 402. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1H), 7.70 (d, 1H), 7.55 (t, 1H), 7.50 (br s, 1H), 7.25 (m, 3H), 7.12 (br, 1H), 7.07 (d, 1H), 6.98 (br d, 1H), 3.20 (br m, 4H), 3.00−2.75 (br m, 8H), 2.73 (s, 3H), 2.37 (q, 2H), 1.23 (t, 3H).

2-Methyl-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-phenyl) propanamide (14). The title compound was prepared in 81% yield according to the general procedure for the preparation of the amides (method A) starting from intermediate **9** and 2-methylpropanoyl chloride. MS (ES/+) m/z: 417 [MH]$^+$. $C_{26}H_{32}N_4O$ requires 416. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1H), 7.70 (d, 1 H), 7.55 (m, 2H), 7.25 (m, 3H), 7.13 (br s, 1H), 7.08 (d, 1H), 6.98 (br d, 1H), 3.20 (br m, 4H), 3.00−2.75 (br m, 8 H), 2.73 (s, 3H), 2.48 (m, 1H), 1.25 (d, 6H).

2,2-Dimethyl-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]-ethyl}phenyl propanamide (15). The title compound was prepared in 66% yield according to the general procedure for the preparation of the amides (method A) starting from intermediate **9** and 2,2-dimethylpropanoyl chloride. MS (ES/+) m/z: 431 [MH]$^+$. $C_{27}H_{34}N_4O$ requires 430. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1H), 7.70 (d, 1H), 7.55 (t, 2H), 7.30−7.20 (m, 4H), 7.10 (d, 1H), 7.00 (m, 1H), 3.20 (br s, 4H), 2.85 (br s, 8H), 2.70 (s, 3H), 1.30 (s, 9H).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-benzamide (16). The title compound was prepared in 60% yield according to the general procedure for the preparation of the amides (method A) starting from intermediate **9** and benzoyl chloride. MS (ES/+) m/z: 451 [MH]$^+$. $C_{29}H_{30}N_4O$ requires 450. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1H), 7.87 (m, 2H), 7.80 (br s, 1H), 7.72 (d, 1H), 7.65 (br s, 1H), 7.6−7.4 (m, 5H), 7.30 (t, 1H), 7.27 (m, 1H), 7.08 (d, 1H), 7.05 (d, 1H), 3.18 (br s, 4H), 3.00−2.75 (br m, 8H), 2.72 (s, 3H).

2-Fluoro-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-phenyl) Benzamide Dihydrochloride Salt (17). The title compound was prepared in 96% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 2-fluorobenzoic acid. MS (ES/+) m/z: 469 [MH]$^+$. $C_{29}H_{29}FN_4O$ requires 468. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.00 (br s, 1H), 10.46 (s. 1H), 8.80 (br s, 1H), 8.00−7.72 (m, 4H), 7.65 (t, 1H), 7.58 (q, 1H), 7.52 (d, 1H), 7.73 (br s, 1H), 7.37−7.32 (m, 3H), 7.08 (d, 1H), 3.74 (d, 2H), 3.7−3.3 (m, 9H), 3.15 (m, 2H), 2.88 (s, 3H).

3-Fluoro-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-phenyl) Benzamide Dihydrochloride Salt (18). The title compound was prepared in 91% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 3-fluorobenzoic acid. HPLC/MS (ES/+): t_R = 6.45 min; assay >99% a/a; m/z: 469 [MH]$^+$. $C_{29}H_{29}FN_4O$ requires 468. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.18 (br s, 1H), 10.41 (s, 1H), 8.94 (br s, 1H), 7.97 (br s, 2H), 7.87 (br s, 2H), 7.80 (m, 2H), 7.62 (m, 2H), 7.48 (m, 2H), 7.10 (d, 1H), 3.80−3.30 (m, 10H), 3.18 (m, 2H), 2.92 (br s, 3H).

4-Fluoro-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-phenyl) Benzamide Dihydrochloride Salt (19). The title compound was prepared in 82% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 4-fluorobenzoic acid. HPLC/MS (ES/+): t_R = 6.45 min; assay 98.2% a/a; m/z: 469 [MH]$^+$. $C_{29}H_{29}FN_4O$ requires 468. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.29 (br s, 1H), 10.36 (s, 1H), 8.96 (br s, 1 H), 8.08 (m, 2H), 7.99 (br s, 1H), 7.86 (br s, 1H), 7.62 (d, 1H), 7.47 (br d, 1H), 7.40 (m, 3H), 7.09 (d, 1H), 3.70−3.30 (m, 10H), 3.18 (dd, 2H), 2.93 (br s, 3H).

2-(Methyloxy)-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]-ethyl}phenyl) Benzamide Dihydrochloride Salt (20). The title compound was prepared in 85% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 2-(methyloxy)benzoic acid. HPLC/MS (ES/+): t_R = 6.54 min; assay >99% a/a; m/z: 481 [MH+]. $C_{30}H_{30}N_4O_2$ requires 480. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.13 (br s, 1H), 10.17 (s, 1H), 8.93 (br s, 1H), 7.97 (s, 2H), 7.86−7.78 (br s, 1H), 7.64 (dd, 1H), 7.58−7.50(m, 2H), 7.47 (br s, 1H), 7.36 (t, 1H), 7.21 (d, 1H), 7.09 (dt, 1H), 7.07 (d, 1H), 3.92 (s, 3H), 3.76 (d, 2H), 3.70−3.30 (m, 8H), 3.17 (m, 2H), 2.91(br s, 3H).

3-(Methyloxy)-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]-ethyl}phenyl) Benzamide Dihydrochloride Salt (21). The title compound was prepared in 83% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 3-(methyloxy)benzoic acid. HPLC/MS (ES/+): t_R = 6.39 min; assay >99% a/a; m/z: 481[MH]$^+$. $C_{30}H_{30}N_4O_2$ requires 480. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.88 (br s, 1H), 10.30 (s, 1H), 8.86 (br s, 1H), 7.91 (br s, 2H), 7.87 (br s, 1H), 7.78 (br s, 2H), 7.63 (dd, 1H), 7.56 (d, 1H), 7.51 (m, 1H), 7.47 (t, 1H), 7.47 (br s, 1H), 7.38 (t, 1H), 7.19 (dm, 1H), 7.09 (d, 1H), 3.86 (s, 3H), 3.76 (d, 2H), 3.70−3.25 (m, 8H), 3.17 (m, 2H), 2.88 (br s, 3H).

4-(Methyloxy)-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]-ethyl}phenyl) Benzamide Dihydrochloride Salt (22). The title compound was prepared in 83% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 4-(methyloxy)benzoic acid. HPLC/MS (ES/+): t_R = 6.21 min; assay >99% a/a; m/z: 481 [MH]$^+$. $C_{30}H_{30}N_4O_2$ requires 480. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.09 (br s, 1H), 10.16 (s, 1H), 8.91 (br s, 1H), 7.97 (d, 2H), 7.94 (br s, 2H), 7.84 (s, 1H), 7.81 (br s, 1H), 7.60 (d, 1H), 7.44 (br s, 1H), 7.34 (t, 1H), 7.05 (m, 3H), 3.83 (s, 3H), 3.74 (br d, 2H), 3.60−3.40 (m, 6H), 3.33 (br t, 2H), 3.14 (dd, 2H), 2.89 (br s, 3H).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-pyrazine Carboxamide (23). The title compound was prepared in 89% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 2-pyrazinecarboxylic acid. MS (ES/+) m/z: 453 [MH]$^+$. $C_{27}H_{28}N_6O$ requires 452. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 9.65 (s, 1H), 9.50 (m, 1H), 8.80 (d, 1H), 8.60 (t, 1H) 8.38 (d, 1H), 7.75 (d, 1H), 7.70 (d, 1H), 7.58 (t, 1H), 7.55 (dd, 1H), 7.35 (t, 1H), 7.28 (d, 1H), 7.08 (m, 2H), 3.15 (br s, 4H) 2.95−2.70 (m, 8H), 2.70 (br s, 3H).

2-Methyl-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-phenyl)-1,3-thiazole-4-carboxamide Dihydrochloride Salt (24). The title compound was prepared in 52% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 2-methyl-1,3-thiazole-4-carboxylic acid. MS (ES/+) m/z: 472 [MH]$^+$. $C_{27}H_{29}N_5OS$ requires 471. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.70 (br s, 1H), 10.15 (s, 1H), 8.77 (br s, 1H), 8.26 (s, 1H), 7.87 (br s, 1H), 7.75−7.85 (m, 2H), 7.39 (br s, 1H), 7.34 (t, 1H), 7.06 (d, 1H), 3.80−3.20 (m, 10H), 3.12 (dd, 2H), 2.83 (br s, 3H), 2.76 (s, 3H).

4-Methyl-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-phenyl)-1,3-thiazole-5-carboxamide Dihydrochloride Salt (25). The title compound was prepared in 46% yield according to the general procedure for the preparation of the amides (method B) starting from intermediate **9** and 4-methyl-1,3-thiazole-5-carboxylic acid. MS (ES/+) m/z: 472 [MH]$^+$. $C_{27}H_{29}N_5OS$ requires 471. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.83 (br s, 1H), 10.28 (s, 1H), 9.13

(s, 1H), 8.86 (br s, 1H), 7.90 (br s, 2H), 7.80−7.74 (br s-s, 2H), 7.50 (d, 1H), 7.43 (br s, 1H), 7.35 (t, 1H), 7.08 (d, 1H), 3.9−3.2 (m, 10H), 3.13 (m, 2H), 2.87 (br s, 3H), 2.61 (s, 3H).

General Procedure for the Preparation of Ureas and Their Corresponding Dihydrochloride Salts Starting from Intermediate 9. Method C. An isocyanate was added to a stirred solution of intermediate **9** (1 equiv) in dichloromethane at room temperature under an inert atmosphere, and the reaction was left under stirring for 16 h. The solution was then poured into water and extracted with dichloromethane, the organic phase was dried over Na₂SO₄, and the solvent was removed under reduced pressure. The crude material was purified on SPE cartridge (Silica) using a gradient from dichloromethane to dichloromethane/MeOH 95/5 as eluent, affording the final compound (yields ranged from 30 to 80%).

The free base could be converted into its dihydrochloride salt by dissolving the compound in dichloromethane and adding a 1M ethereal solution of HCl (2.1 equiv) dropwise. A yellow solid precipitated and the suspension was stirred for 15 min. The solvent was removed under reduced pressure, affording a crude material which was triturated with Et₂O. The final compound was then recovered by filtration (yield quantitative).

N-Ethyl-N'-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-phenyl)urea Dihydrochloride (26). The title compound was prepared in 95% yield according to the general procedure for the preparation of ureas (method C) starting from intermediate **9** and isocyanatoethane. MS (ES/+) m/z: 418 [MH⁺] $C_{25}H_{31}N_5O$ requires 417. ¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.89 (bs, 1 H), 8.60 (bs, 1 H), 8.90 (s, 1 H), 7.92 (bs, 2 H), 7.80 (bs, 1 H), 7.44 (s, 2 H), 7.19 (m, 2 H), 6.81 (m, 1 H), 3.71 (d, 2 H), 3.6−3.2 (m, 10 H), 3.07 (m, 2 H), 2.87 (bs, 3 H), 1.02 (t, 3 H).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-N'-propylurea dihydrochloride (27). The title compound was prepared in 53% yield according to the general procedure for the preparation of ureas (method C) starting from intermediate **9** and 1-isocyanatopropane. MS (ES/+) m/z: 432 [MH⁺] $C_{26}H_{33}N_5O$ requires 431. ¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.5 (bs, 1 H), 8.75 (bs, 1 H), 8.54 (s, 1H), 7.83 (bs, 2 H), 7.7 (bs, 1 H), 7.45 (s, 1 H), 7.38 (bs, 1 H), 7.18 (m, 2 H), 6.81 (d, 1 H), 6.23 (bt, 1 H), 3.7−3.25 (bd, 4 H), 3.6−3.3 (m, 4 H), 3.4−3.02 (m, 6 H), 2.81 (bs, 3 H), 1.41 (m, 2 H), 0.85 (t, 3 H).

N-(1,1-Dimethylethyl)-N'-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)urea Dihydrochloride (28). The title compound was prepared in 79% yield according to the general procedure for the preparation of ureas (method C) starting from intermediate **9** and 2-isocyanato-2-methylpropane. MS (ES/+) m/z: 446 [MH⁺] $C_{27}H_{35}N_5O$ requires 445. ¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.48 (bs, 1 H), 8.75 (bs, 1 H), 8.37 (s, 1H), 7.83 (bs, 2 H), 7.7 (bs, 1 H), 7.51 (s, 1 H), 7.38 (bs, 1 H), 7.17 (t, 1 H), 7.04 (dd, 1 H), 6.79 (d, 1 H), 6.08 (s, 1 H), 3.71−3.24 (bd, bt, 4 H), 3.6−3.3 (m, 4 H), 3.40 (m, 2 H), 3.02 (m, 2 H), 2.81 (bs, 3 H), 1.27 (s, 9 H).

N-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-N'-phenylurea Dihydrochloride (29). The title compound was prepared in 73% yield according to the general procedure for the preparation of ureas (method C) starting from intermediate **9** and isocyanatobenzene. MS (ES/+) m/z: 466 [MH⁺] $C_{29}H_{31}N_5O$ requires 465. ¹H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.4 (bs, 1 H), 8.95 (bd, 2 H), 8.75 (bs, 1 H), 7.83 (bs, 2 H), 7.7 (bs, 1 H), 7.54 (s, 1 H), 7.45 (dd, 2 H), 7.38 (bs, 1 H), 7.27 (m, 4 H), 6.96 (m, 1 H), 6.91 (m, 1 H), 3.73 (bd, 2 H), 3.6−3.3 (m, 6 H), 3.24 (t, 2 H), 3.08 (dd, 2 H), 2.81 (bs, 3 H).

N-(2-Fluorophenyl)-N'-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)urea Dihydrochloride (30). The title compound was prepared in 75% yield according to the general procedure for the preparation of ureas (method C) starting from intermediate **9** and 1-fluoro-2-isocyanatobenzene. MS (ES/+) m/z: 484 [MH⁺] $C_{29}H_{30}FN_5O$ requires 483. ¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 10. 9 (bs, 1 H), 9.36 (s, 1 H), 8.8 (bs, 1H), 8.67 (s, 1 H), 8.11 (t, 1 H), 7.86 (bs, 2H), 7.71 (bs, 1 H), 7.49 (d, 1 H), 7.37 (bs,

1 H), 7.26 (m, 2 H), 7.20 (dd, 1 H), 7.07 (dd, 1 H), 6.98 (m, 1 H), 6.90 (m, 1 H), 3.69 (d, 2 H), 3.5−3.2(m, 8 H), 3.09 (m, 2 H), 2.82 (bs, 3 H).

N-(3-Fluorophenyl)-N'-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)urea Dihydrochloride (31). The title compound was prepared in 48% yield according to the general procedure for the preparation of ureas (method C) starting from intermediate **9** and 1-fluoro-3-isocyanatobenzene. MS (ES/+) m/z: 484 [MH⁺] $C_{29}H_{30}FN_5O$ requires 483. ¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.7 (bs, 1 H), 9.47 (s, 1 H), 9.24 (s, 1H), 8.75 (bs, 1 H), 7.81 (bs, 2 H), 7.68 (bs, 1H), 7.50 (d+bs, 2 H), 7.44 (bs, 1 H), 7.31 (t, 1 H), 7.3−7.24 (m, 2 H), 7.09 (d, 1 H), 6.89 (d, 1 H), 6.74 (td, 1 H), 3.7−3.2(m, 10 H), 3.07 (m, 2 H), 2.8 (bs, 3 H).

N-(4-Fluorophenyl)-N'-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)urea Dihydrochloride (32). The title compound was prepared in 64% yield according to the general procedure for the preparation of ureas (method C) starting from intermediate **9** and 1-fluoro-4-isocyanatobenzene. MS (ES/+) m/z: 484 [MH⁺]. $C_{29}H_{30}FN_5O$ requires 483. ¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.49 (bs, 1 H), 9.40 (s, 1H), 8.96 (s, 1H), 8.77 (bs, 1 H), 7.83 (bs, 2 H), 7.71 (bs, 1H), 7.51 (bs, 1 H), 7.45 (dd, 2 H), 7.37 (bs, 1 H), 7.25 (bd, 2 H), 7.10 (t, 2 H), 6.90 (bt, 1 H), 3.8−3.2(m, 10 H), 3.06 (dd, 2 H), 2.81 (bs, 3 H).

General Procedure for the Preparation of Carbamates and Their Corresponding Dihydrochloride Salts Starting from Intermediate 9. Method D. Diisopropylethylamine (1.5 equiv) and a chloroformate (1.2 equiv) were added sequentially to a stirred solution of intermediate **9** (1 equiv) in dichloromethane at 0 °C. The solution was stirred for 1 h at room temperature and then diluted with dichloromethane and washed with a saturated aqueous solution of NH₄Cl and brine and then dried over Na₂SO₄. The solution was concentrated under reduced pressure. The crude material was purified on SPE cartridge (Silica) eluting with a gradient from dichloromethane/MeOH 99/1 to dichloromethane/MeOH 98/2, affording the final compound (yields ranged from 43 to 78%).

The free base could be converted into its dihydrochloride salt by dissolving the compound in dichloromethane and adding a 1M ethereal solution of HCl (2.1 equiv) dropwise. A yellow solid precipitated and the suspension was stirred for 15 min. The solvent was removed under reduced pressure affording a crude material which was triturated with Et₂O. The final compound was then recovered by filtration (yield quantitative).

Methyl (3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)carbamate (33). The title compound was prepared in 41% yield according to the general procedure for the preparation of carbamates (method D) starting from intermediate **9** and methyl chloroformate. MS (ES) m/z: 405.4 [MH]⁺. $C_{24}H_{28}N_4O_2$ requires 404. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 8.33 (d, 1 H), 7.70 (d, 1 H), 7.6 (t, 1 H), 7.30 (bs, 1 H), 7.25 (t, 1 H), 7.22 (dd, 1 H), 7.20 (d, 1 H), 7.10 (d, 1 H), 6.95 (dd, 1 H), 6.55 (bs, 1 H), 3.8 (s, 3 H), 3.28 (bm, 4 H), 3.28 (t, 2 H), 2.85 (t, 2 H), 2.75 (bm, 4 H), 2.66 (s, 3 H).

Ethyl (3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)carbamate Fihydrochloride (34). The title compound was prepared in 79% yield according to the general procedure for the preparation of carbamates (method D) starting from intermediate **9** and ethyl chloroformate. MS (ES) m/z: 419 [MH⁺]. $C_{25}H_{30}N_4O_2$ requires 418. ¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.00 (bs, 1H), 9.74 (s, 1H), 8.95 (s, 1H), 8.00 (s, 2H), 7.87 (s, 1H), 7.57 (s, 1H), 7.51 (bs, 1H), 7.36 (m, 2H), 7.02 (d, 1H), 4.21 (q, 2H), 3.80 (d, 2H), 3.7−3.3 (m 8H), 3.17 (m, 2H), 2.96 (bs, 3H), 1.33 (t, 3H).

N-Methyl-N-(3-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl) Acetamide (35). A solution of intermediate **12** (50 mg) was dissolved in THF (2.5 mL), and sodium hydride (60% w/w oil dispersion, 0.005 g, 1 equiv) was added at 0 °C. After 5 min, iodomethane was added (5 µL, 1.0 equiv), and the mixture was warmed to room temperature. After 10 h, the solution was partitioned between water (3 mL) and ethyl acetate (3 × 5 mL).

The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude was purified by flash chromatography on silica gel, eluting with a gradient from dichloromethane to dichloromethane−methanol (98:2), affording the title compound in 61% yield (0.02 g). MS (ES) *m/z*: 403.4 [MH$^+$]. $C_{25}H_{31}ClNO_4$ requires 402. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 10.85 (bs, 1H), 8.79 (bs, 1H), 8−7 (bm, 16H), 3.8−3.0 (bm, 15H), 2.84 (bs, 3H), 1.79 (bs, 3H).

General Procedure for the Synthesis of Cyclic Ureas and Carbamates and Their Corresponding Dihydrochloride Salts Starting from Intermediate 9. Method E. Diisopropylethylamine (1.5 equiv) and a chloroformate or isocyanate (1.2eq) were added sequentially to a stirred solution of intermediate **9** (1 equiv) in dichloromethane at 0 °C. The solution was stirred for 1 h at room temperature and then diluted with dichloromethane and washed with a saturated aqueous solution of NH$_4$Cl and brine and then dried over Na$_2$SO$_4$. The solution was concentrated under reduced pressure. The crude material was dissolved in dimethylformamide, cooled to 0 °C, and NaH (1.1 equiv) was added portionwise under an inert atmosphere. The mixture was stirred for 2 h at room temperature, and then the solvent was removed by means of an SCX cartridge. The crude material was purified on SPE cartridge (Silica) eluting with a gradient from dichloromethane/MeOH 99/1 to dichloromethane/MeOH 98/2, affording the final compound (yields ranged from 22 to 87%).

The free base could be converted into its dihydrochloride salt by dissolving the compound in Et$_2$O and MeOH and adding an 1 M ethereal solution of HCl (2.1 equiv) dropwise. A yellow solid precipitated, and the suspension was stirred for 15 min. The solvent was removed under reduced pressure, affording a crude material which was triturated with Et$_2$O. The final compound was then recovered by filtration (yield quantitative).

1-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-imidazolidinone Dihydrochloride (36). The title compound was prepared in 22% yield according to the general procedure for the synthesis of cyclic ureas and carbamates (method E) starting from intermediate **9** and 1-chloro-2-isocyanatoethane. MS (ES) *m/z*: 416 [MH$^+$]. $C_{25}H_{29}N_5O$ requires 415. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.60 (bs, 1H), 8.77 (s, 1H), 7.85 (s, 2H), 7.71 (s, 1H), 7.59 (s, 1H), 7.38 (dd, 1H), 7.28 (t, 1H), 6.96 (bs, 1H), 6.92 (d, 1H), 3.83 (m, 2H), 3.71 (d, 2H), 3.7−3.2 (m, 10H), 3.08 (m, 2H), 2.82 (bs, 3H).

1-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-tetrahydro-2(1H)-pyrimidinone Dihydrochloride (37). The title compound was prepared in 87% yield according to the general procedure for the synthesis of cyclic ureas and carbamates (method E) starting from intermediate **9** and 1-chloro-3-isocyanatopropane. MS (ES) *m/z*: 430 [MH$^+$]. $C_{26}H_{31}N_5O$ requires 429. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.05 (bs, 1H), 8.94 (s, 1H), 7.97 (s, 2H), 7.84 (d, 1H), 7.46 (bs, 1H), 7.30 (m, 2H), 7.19 (dd, 1H), 7.08 (d, 1H), 6.58 (s, 1H), 3.70 (bm, 4H), 3.63 (t, 2H), 3.6−3.3 (bm, 6H), 3.24 (t, 2H), 3.12 (m, 2H), 2.91 (s, 3H), 1.95 (t, 2H).

3-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-1,3-oxazolidin-2-one Dihydrochloride (38). The title compound was prepared in 81% yield according to the general procedure for the synthesis of cyclic ureas and carbamates (method E) starting from intermediate **9** and (2-bromoethyl)carbamic chloride. MS (ES) *m/z*: 417 [MH$^+$]. $C_{25}H_{28}N_4O_2$ requires 416. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.99 (bs, 1H), 8.87 (bm, 1H), 7.91 (bm, 2H), 7.79 (bm, 1H), 7.57 (s, 1H), 7.40 (m, 3H), 7.08 (d, 1H), 4.43 (t, 2H), 4.07 (t, 2H), 3.72−3.3 (m, 10H), 3.15 (m, 2H), 2.87 (bs, 3H).

3-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-tetrahydro-2H-1,3-oxazin-2-one Dihydrochloride (39). The title compound was prepared in 75% yield according to the general procedure for the synthesis of cyclic ureas and carbamates (method E) starting from intermediate **9** and (3-chloropropyl)carbamic chloride. MS (ES) *m/z*: 431 [MH$^+$]. $C_{26}H_{30}N_4O_2$ requires 430. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.89 (bs, 1H), 8.84 (bm, 1H), 7.89 (bm, 2H), 7.76 (bm, 1H), 7.40 (m, 4H), 7.31 (s, 1H), 4.32 (t, 2H), 3.72−3.3 (m, 10H), 3.66 (t, 2H), 3.13 (m, 2H), 2.85 (bs, 3H), 2.10 (m, 2H).

1-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2,5-pyrrolidinedione (40). Dihydro-2,5-furandione (2 equiv) was added to a stirred solution of intermediate (**9**) (1 equiv) in toluene/pyridine (3:2) at room temperature under an inert atmosphere. The solution was stirred for 30 min at room temperature and then irradiated in a microwave reactor (PersonalChemistry Emrys Optimiser, 300W, 170 °C, 20 min, 4 cycles), diluted with dichloromethane, and washed with a saturated aqueous solution of NH$_4$Cl and brine and then dried over Na$_2$SO$_4$. The solution was concentrated under reduced pressure. The crude material was purified on SPE cartridge (Silica) eluting with a gradient from dichloromethane/MeOH 99/1 to dichloromethane/MeOH 98/2, affording the final compound in 76% yield.

The free base was converted into its dihydrochloride salt by dissolving the compound in Et$_2$O and MeOH and adding an 1 M ethereal solution of HCl (2.1 equiv) dropwise. A yellow solid precipitated, and the suspension was stirred for 15 min. The solvent was removed under reduced pressure, affording a crude material which was triturated with Et$_2$O to give the title compound. MS (ES) *m/z*: 429 [MH$^+$]. $C_{26}H_{28}N_4O_2$ requires 428. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.1 (bs, 1H), 8.91 (bs, 1H), 7.94 (bs, 2H), 7.81 (bs, 1H), 7.49 (t, 1H), 7.44 (bs, 1H), 7.37 (d, 1H), 7.22 (s, 1H), 7.18 (d, 1H), 3.73 (bm, 2H), 3.59 (bm, 2H), 3.48 (bm, 4H), 3.33 (m, 2H), 3.19 (m, 2H), 2.89 (bs, 3H), 2.80 (bs, 4H).

[3-(1H-Pyrazol-1-yl)phenyl]acetic acid used in the synthesis of 2-methyl-5-(4-{[3-(1H-pyrazol-1-yl)phenyl]acetyl}-1-piperazinyl)quinoline was prepared as follows: Pyrazole (1.2 equiv), Cs$_2$CO$_3$ (2.5 equiv), CuI (0.5 equiv), trans-1,2-cyclohexanediamine (0.6 equiv), and dodecane (1 equiv) were added to a stirred solution of 3-bromophenylacetic acid (1 equiv) in dioxane at room temperature under an inert atmosphere. The mixture was irradiated in a microwave reactor (PersonalChemistry Emrys Optimiser, 300 W, 160 °C, 20 min) and then added to a 1N aqueous solution of NaOH and extracted with Et$_2$O. The aqueous phase was acidified to pH = 3 with 2N HCl solution and then extracted with ethyl acetate; this phase was washed with brine and then dried over Na$_2$SO$_4$, and the solvent was removed under reduced pressure. The crude material was purified on SPE cartridge (Silica) eluting with a gradient from cyclohexane/ethyl acetate 8:2, to cyclohexane/ethyl acetate 1:1, affording the title compound in 65% yield. MS (ES) *m/z*: 203 [MH$^+$]. $C_{11}H_{10}N_2O_2$ requires 202. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 7.9 (m, 1H), 7.75 (m, 1H), 7.65 (m, 1H), 7.55 (d, 1H), 7.35 (t, 1H), 7.3−7.1 (m, 2H), 6.55 (m, 1H), 3.7 (s, 2H)

2-Methyl-5-(4-{[3-(1H-pyrazol-1-yl)phenyl]acetyl}-1-piperazinyl)quinoline used in the synthesis of intermediate **41** was prepared as follows: EDC·HCl (1.5 equiv), HOBt (2 equiv), and intermediate **7** (1 equiv) were added sequentially to a stirred solution of [3-(1H-pyrazol-1-yl)phenyl]acetic acid (1.1 equiv) in dimethylformamide at room temperature under an inert atmosphere. The solvent was removed by means of an SCX cartridge. The crude material was purified on SPE cartridge (Silica) eluting with a gradient from dichloromethane/MeOH 99/1 to dichloromethane/MeOH 98/2, affording the title compound in 74% yield. MS (ES) *m/z*: 412 [MH$^+$]. $C_{25}H_{25}N_5O$ requires 411. ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm): 8.36 (d, 1H), 7.93 (d, 1H), 7.75 (d, 1H), 7.71 (s, 1H), 7.69 (s, 1H), 7.58 (d, 1H), 7.55 (t, 1H), 7.42 (t, 1H), 7.25 (m, 2H), 7.00 (d, 1H), 6.46 (s, 1H), 3.87 (s, 2H), 4.0−3.7 (m, 4H), 3.1−2.9 (m, 4H), 2.72 (s, 3H).

2-Methyl-5-(4-{2-[3-(1H-pyrazol-1-yl)phenyl]ethyl}-1-piperazinyl)quinoline (41). A 1 M tetrahydrofuran solution of borane−tetrahydrofuran complex (3 equiv) was added to a stirred solution of 2-methyl-5-(4-{[3-(1H-pyrazol-1-yl)phenyl]acetyl}-1-piperazinyl)quinoline (1 equiv) in tetrahydrofuran at room temperature under an inert atmosphere. The solution was heated to 60 °C for 3 h. An aqueous 3N solution of HCl was added, and the solution was stirred at room temperature for 12 h. The solvent was

removed under reduced pressure. The crude material was purified by SCX cartridge, affording the title compound in 52% yield. MS (ES) m/z: 398 [MH$^+$]. $C_{25}H_{27}N_5$ requires 397. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.35 (d, 1H), 7.90 (d, 1H), 7.70 (d, 1H), 7.70 (s, 1H), 7.65 (t, 1H), 7.60 (t, 1H), 7.50 (dd, 1H), 7.35 (t, 1H), 7.25 (d, 1H), 7.15 (d, 1H), 7.05 (d, 1H), 6.45 (t, 1H), 3.20 (m, 4H), 3.0−2.7 (m, 8), 2.70 (s, 3H).

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