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Synthesis and SAR of 3- and 4-Substituted Quinolin-2-ones: Discovery of Mixed 5-HT_{1B}/5-HT_{2A} Receptor Antagonists

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Abstract—Quinolin-2-ones bearing a heteroaryl-piperazine linked by a two carbon chain at the 3- or 4-position were synthesised and evaluated as mixed 5-HT_{1B}/5-HT_{2A} receptor antagonists. Potent mixed antagonists were obtained with thieno[3,2-c]pyridine derivatives. In this series, compound **2.1** (SL 65.0472) proved to be functional antagonist at both the 5-HT_{2A} receptor (rat in vivo 5-HT-induced hypertension model) and the 5-HT_{1B} receptor (dog in vitro saphenous vein assay).

Serotonin (5-HT), a well-known neurotransmitter and vasoactive agent is stored in platelets in the cardiovascular system. Upon platelet activation by endothelial damage or dysfunction, 5-HT is liberated in the blood where it exerts deleterious effects such as vasoconstriction and amplification of platelet aggregation. Platelet aggregation in response to serotonin is mediated by the activation of 5-HT_{2A} receptors and 5-HT_{2A} antagonists, such as ketanserin, inhibit this response.[1,2] 5-HT-induced vasoconstriction is mediated through two distinct receptor subtypes, the 5-HT_{2A} receptor and a subtype previously known as 5-$HT_{1\text{-like}}$[3] but now considered likely to be the 5-HT_{1B} receptor (formerly 5-HT_{1D} beta receptor).[4] Hence, a number of blood vessels have been identified in which sumatriptan, a 5-$HT_{1B/D}$ receptor agonist, causes vasoconstriction that is resistant to antagonism by ketanserin which has low affinity for 5-HT_{1B} receptors.[5] Molecular biological and pharmacological analyses demonstrate that sumatriptan-induced constriction of dog saphenous vein,[6,7] human coronary artery,[8–12] human cerebral arteries[11,13] and human pulmonary artery[14] is probably due to 5-HT_{1B} receptor activation. In haemodynamic studies in man, sumatriptan causes coronary, pulmonary and systemic vasoconstriction[15,16] and has been reported to provoke cardiac ischaemia.[17] Isolated tissue studies and clinical investigations indicate that contractions of human coronary arteries produced by 5-HT are only partially and inconsistently blocked by ketanserin because they result from stimulation of a mixed population of 5-HT_{2A} and 5-HT_{1B} receptors.[8,9,18–21]

These findings led us to initiate a program directed toward the discovery of mixed 5-HT_{2A}/5-HT_{1B} antagonists permitting a blockade of the two receptors subtypes responsible for the adverse cardiovascular effects of the 5-HT. Our objective was to identify compounds showing high affinity ($\leq$10 nM) for both 5-HT_{2A} and 5-HT_{1B} receptors. These compounds should also be orally active, with good haemodynamic tolerance and poor cerebral penetration. Back screening of compounds from a former 5-HT antagonist program, yielded the lead compound **1**, which was a good 5-HT_{2A} antagonist and had moderate 5-HT_{1B} antagonistic activity. In this paper, we describe the synthesis, affinity and in vivo functional antagonism at the 5-HT_{2A} receptor site an in

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vitro functional antagonism at the 5-HT_{1B} receptor site of ligands with the basic structure of compounds **2** and **3** (Chart 1). These compounds were obtained during the optimization process of 4-[2-(4-naphtalen-1-yl)-ethyl]-1*H*-quinolin-2-one (**1**) which ultimately led to the discovery of compound **2.1** (SL 65.0472), currently evaluated in clinical trials.

Chemistry

Early structure–activity relationships (SARs) around the lead naphtalene (**1**) showed that most potent 5-HT_{1B} activity was obtained with 4-substituted quinolinones (**2**) and 3-substituted 3,4-dihydroquinolinones (**3**). Analogues of **2** (Table 1) were readily prepared by *N*-alkylation of heteroaryl piperazines (**10**) with different 4-(2-chloroethyl)-quinolinones (**9**) (Scheme 1).[22–25] The heteroaryl piperazines (**10**) were prepared by published procedures or modifications thereof.[25–29]

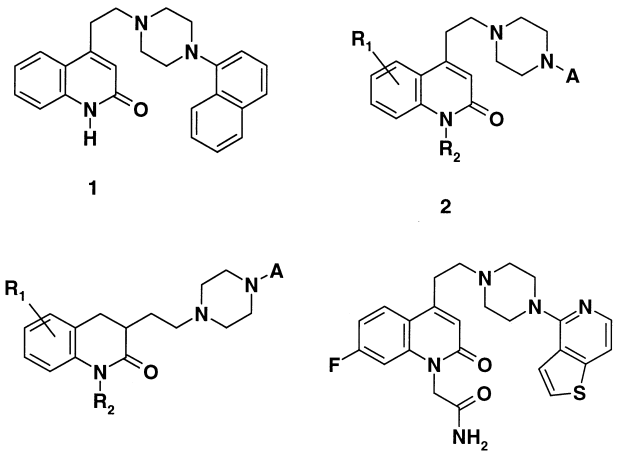


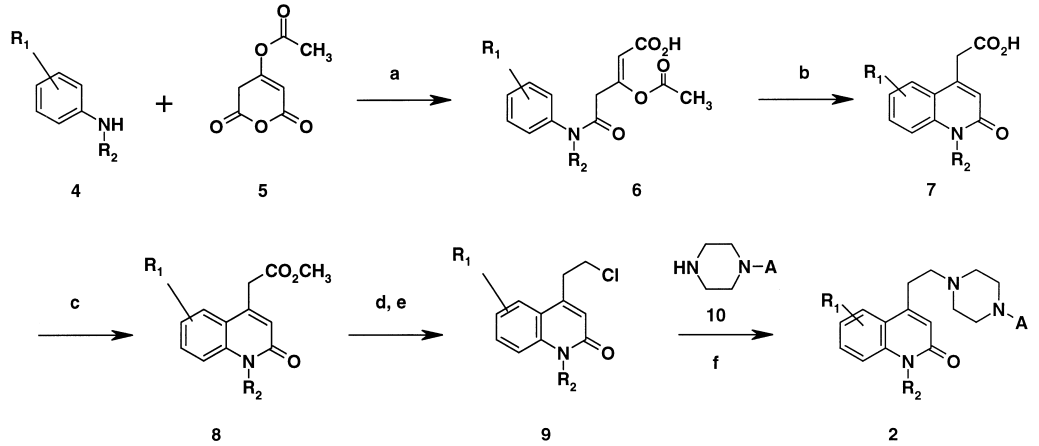
Chart 1.

The substituent R_2 at the 1-position of the quinolinone can either be introduced on the starting aniline (**4**) or at the end of the synthesis (**2′** or **3′**, $R_2 \neq H$), by phase transfer alkylation (Scheme 2).

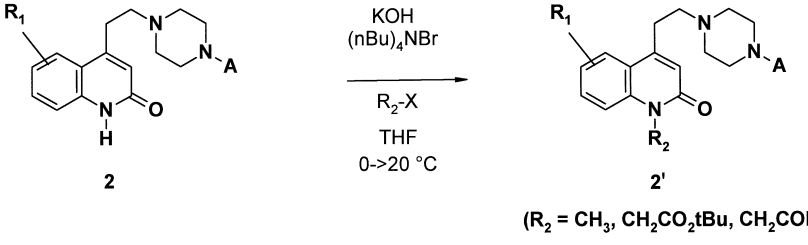
Synthesis of 3-substituted 3,4-dihydroquinolinone analogues (**3**) was accomplished by *N*-alkylation of heteroaryl-piperazines (**10**), with different 3-(2-chloroethyl)-3,4-dihydroquinolinone derivatives (**15**) or alternatively by reductive amination of the 3-acetaldehyde compounds (**13**). The 3-(2-hydroxyethyl)-3,4-dihydroquinolinones (**12**) were synthesized by reductive cyclization of (*ortho*-nitrophenyl)methylenebutyrolactones (**11**).[26] Their subsequent transformation to compound **3** is shown in Scheme 3 (Table 1).

Pharmacology

The in vitro affinity for 5-HT_{2A} receptors of the compounds reported in Table 2 (**1**–**2.l**) was determined by displacement of [^{3}H]spiroperidol from rat cerebral cortex membranes.[30] Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) were determined through three or four independent experiments



Scheme 1. Reagents and conditions: (a) CH_3CO_2H, room temperature; (b) H_2SO_4 or CH_3SO_3H, 80–100 °C; (c) $SOCl_2$, CH_3OH, reflux; (d) $NaBH_4$, THF, reflux; (e) $SOCl_2$, $CHCl_3$, reflux (f); **10**, K_2CO_3, KI, CH_3CN/DMF, 70 °C.



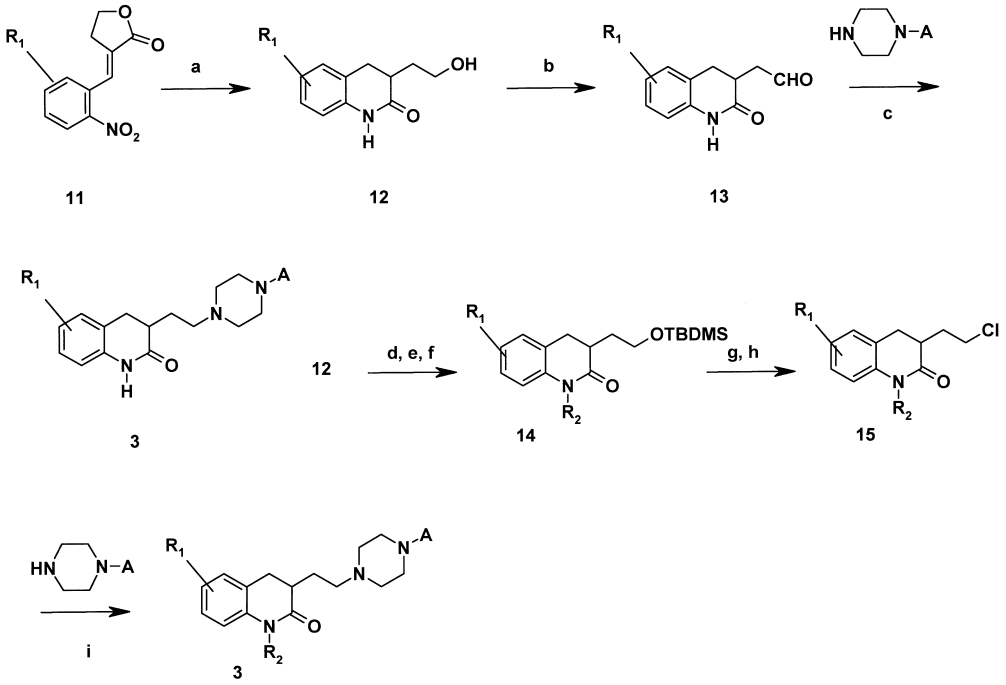
Scheme 2. *N*-alkylation of quinolines **2**.

using different concentrations of the test compound. Specific binding defined in the Experimental represented more than 75% of total binding. The obtained IC_{50} nM values are listed in Table 2.

In addition, the in vivo 5-HT_{2A} functional antagonistic activity was measured as the ability to block serotonin-induced hypertension in anesthetized pithed rats (RS2A).The ID_{50} is defined as the antagonist dose required to reduce induced pressor response by a half. 5-HT_{1B} functional antagonistic activity was evaluated as the ability of the test compounds to block sumatriptan-induced constriction of dog saphenous veins in vitro.[7,31] The pharmacological profile of lead compound **2.l** was further characterized by various functional assays demonstrating the usefulness of a double 5-HT_{2A}/5-HT_{1B} blockade.

Results and Discussion

Table 2 shows that in the 4-substituted quinolinone series **2**, 5-HT_{1B} activity can be enhanced by introducing a nitrogen atom (**2.a**) into the napthylpiperazine moiety of compound **1**. Substitution on the isoquinoline part of **2.a** gave no advantages. Isosteric replacement for a thienopyridine derivative (**2.b**) provided an equipotent compound. The introduction of a fluorine atom at the 7 position of quinolinone **2.c** increased both 5-HT_{2A} and 5-HT_{1B} potency. *N*-Methylation of **2.c** shows that the



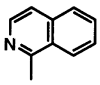
Scheme 3. Reagents and conditions: (a) H_2, Pd/C, THF, AcOH, room temperature; (b) Dess–Martin periodinane, CH_2Cl_2, room temperature; (c) **10**, $Ti(OiPr)_4$, $NaBH_3CN$, $EtOH/CH_2Cl_2$, room temperature; (d) TBDMSi-Cl, imidazole, DMF, room temperature; (e) NaH, THF, room temperature; (f) R_2-Hal, THF, room temperature; (g) $(nBu)_4NF$, THF, room temperature; (h) $SOCl_2$, $CHCl_3$, reflux; (i) **10**, K_2CO_3, CH_3CN, reflux.

Table 1. Physicochemical properties of 3- and 4-substituted quinolin-2-one derivatives **2** and **3**

Compound	Formula	Mp (°C)	Salt	M + H	% Yield (last step)
2.a	$C_{24}H_{24}N_4O$	226–227	None	385	63
2.b	$C_{22}H_{22}N_4OS$	215–217	2 HCl	391	59
2.c	$C_{22}H_{21}FN_4OS$	252	2 HCl	409	42
2.d	$C_{23}H_{23}FN_4OS$	280	2 HCl	423	68
2.e	$C_{22}H_{22}N_4O_2$	220–221	2 HCl	375	55
2.f	$C_{23}H_{22}FN_5O$	246–248	None	404	46
2.g	$C_{23}H_{22}FN_5O$	224	None	404	56
2.h	$C_{22}H_{21}FN_6O$	266	None	405	47
2.i	$C_{21}H_{20}FN_5OS$	194–196	2 HCl	410	54
2.j	$C_{23}H_{24}FN_5O$	213	2 HCl	406	42
3.a	$C_{22}H_{24}N_4OS$	260	2 HCl	393	49
3.b	$C_{23}H_{26}N_4OS$	178	2 HCl	407	64
3.c[a]	$C_{23}H_{26}N_4OS$	52–53	None	407	—
3.d[b]	$C_{23}H_{26}N_4OS$	52–53	None	407	—
2.k	$C_{24}H_{23}FN_4O_3S$	218–220	2 HCl	467	95
2.l	$C_{24}H_{24}FN_5O_2S$	269–270	2 HCl	466	74

[a]$[\alpha]_D$ +30.0 (*c* 0.75; $CHCl_3$).
[b]$[\alpha]_D$ −32.3 (*c* 0.75; $CHCl_3$).

Table 2. 5-HT_{2A} affinity (IC_{50} in nM), 5-HT_{2A} antagonistic activity (RS2A; ID_{50} in mg/kg) and 5-HT_{1B} antagonistic activity (DSV; pA_2)[a]

Compound[b]	R_1	R_2	A	5-HT_{2A} binding IC_{50} (nM)	RS2A (mg/kg)	DSV (pA_2)
1	H	H		12	ID_{50} iv = 0.075	7.06
2.a	H	H		3.8	ID_{50} iv = 0.015	7.66
2.b	H	H		21	ID_{50} iv = 0.002	7.69
2.c	7-F	H		0.2	ID_{50} po = 0.021	8.21
2.d	7-F	CH_3		0.5	ID_{50} po = 0.08	8.22
2.e	7-F	H		1.7	N.D.	7.28
2.f	7-F	H		> 100	N.D.	< 6
2.g	7-F	H		95	N.D.	< 6
2.h	7-F	H		> 100	N.D.	< 6
2.i	7-F	H		16	N.D.	< 6
2.j	7-F	H		3.2	N.D.	< 6
3.a	H	H		2.8	ID_{50} po = 0.085	9.08
(±)-**3.b**	H	CH_3		9.1	42% inh @ 0.1 po ID_{50} iv = 0 .011	8.66
(+)-**3c**[c]	H	CH_3		2.5	4% inh @ 0.1 (po) ID_{50} iv = 0 .0036	8.88
(−)-**3d**[c]	H	CH_3		7	ID50 po = 0.1 ID_{50} iv = 0 .021	9.23
2.k	7-F	CH_2CO_2H		> 100	N.D.	6.64
2.l	7-F	CH_2CONH_2		6	ID_{50} po = 0.031 ID_{50} iv = 0 .0014	8.17

[a]Data are the mean of three or four independent determinations.
[b]All compounds were characterized by ^1H NMR, mass spectroscopy and gave satisfactory CHN analyses.
[c]Separated by preparative chiral HPLC.

H-donating property of the amide is not necessary for activity at either of the 5-HT receptor subtypes.

Among the phenyl bioisosteres tried, it is of interest to note that besides the thienopyridine moiety, only the furan derivative **2.e** showed high affinity for the 5-HT_{2A} receptor and reasonable antagonistic activity in the 5-HT_{1B} DSV assay. The two azaquinoline analogues **2.f** and **2.g** and the pyrimidine analogue **2.h** were inactive, and this was a general rule we observed for six-membered rings fused to the pyridine. Among all the five-membered heterocycles investigated, only the isothiazole **2.i** and the pyrrole **2.j** maintained 5-HT_{2A} activity, but none of them showed any 5-HT_{1B} activity.

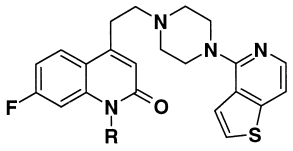
Potent mixed antagonists were also obtained in the 3,4-dihydroquinolinone series (**3**) with piperazinylthieno[3,2-c]pyridine; however, here, substitution on the dihydroquinolinone moiety presented no distinct advantages. Since the chiral compounds **3.c** and **3.d** did not show superior activity over the non-chiral derivatives (**2**) and due to the fact that the chiral center was very difficult to control synthetically, the non-chiral 4-substituted quinolinones were pursued. In order to circumvent any potential central 5-HT receptor-mediated effects, we sought to decrease the possible crossing of the blood brain barrier by the introduction of an acetic acid substituent at position number 1 (quinolinone **2.c**). However, the carboxylic acid induces a sharp decrease in activity for both 5-HT receptor subtypes. Introduction of the non-charged acetamide group (**2.l**), on the other hand, resulted in a potent, balanced, mixed antagonist with limited brain penetration as estimated by the 5-HT-induced head twitch test (HTA) in rats[32] (Table 3).

Compound **2.l**, SL 65.0472 inhibited serotonin-induced hypertension in rats in a dose-dependent manner, with an ID_{50} value of 1.4 μg/kg iv and 31 μg/kg po, indicating it to be a potent antagonist of vascular 5-HT_{2A} receptors.[33] It remains active after oral administration for 24 h. **2.l** also inhibited serotonin-induced vasconstriction in rabbit aorta.[34] Under adrenergic blockade, aortic rings serotonin caused concentration-dependent contractions with an EC_{50} of 1.1 μM ($n=10$). Compound **2.l** (0.01–1 μM) was able to antagonize this 5-HT_{2A} receptor-mediated response with an apparent pK_B of 8.58. In comparison, ketanserin had a pA_2 value of 7.90 in this tissue. Compound **2.l** is also a potent inhibitor of human platelet aggregation induced by 5-HT + ADP (IC_{50} 49 nM) and by 5-HT + collagen (IC_{50} 48 nM), equivalent to that of ketanserin.[35] Compound **2.l** demonstrates potent anti-thrombotic activity in the Folts model of dog coronary artery thrombosis; 10–30 μg/kg iv provides complete protection.[33]

The affinity of **2.l** for human 5-HT_{1B} receptors was assessed on membranes of Hela cells transfected by the recombinant receptor.[36] Compound **2.l** inhibited the specific [^{3}H]-GR125743 binding to the recombinant 5-HT_{1B} receptor in a competitive and concentration-dependent manner ($K_i=19$ nM). Functional 5-HT_{1B} receptor antagonistic activity was investigated on sumatriptan-elicited contraction of isolated dog saphenous vein.[7] We have previously demonstrated using molecular biological techniques that dog saphenous vein contains the 5-HT_{1B} receptor subtype, which shows a very high degree of homology with its human counterpart.[6] However, no evidence of 5-HT_{1D} receptor was detected. Sumatriptan caused a concentration-related contraction which was significantly shifted to the right by **2.l** (0.01–0.1 μM) without reducing its maximal effect. The slope of the regression line derived from Schild plot analysis was not significantly different from unity. The pA_2 value was 8.17.[34] In comparison, similar results were obtained with methiothepin, a non-selective 5-HT antagonist, its pA_2 value being 8.43. **2.l** also blocked 5-HT-induced constriction of atherosclerotic human coronary arteries consistently and potently (pA_2 8.8).[34]

Finally, the in vivo interaction with the 5-HT_{1B} receptor was studied on the sumatriptan-induced venoconstriction in anesthetized dogs. Intravenous bolus injection of sumatriptan produced a significant constriction of the saphenous vein as measured by a reduction in saphenous vein diameter of ~30%. Compound **2.l** antagonized this venoconstriction by 90% at 30 μg/kg iv.[33] It should be noted that, at this dose, no adverse effects on hemodynamics and ECG parameters including mean arterial pressure, heart rate and PR and QT intervals were observed. The high potency of **2.l** in this in vivo functional model is consistent with its affinity for the 5-HT_{1B} receptor.

Table 3. Effect of N-1 substitution on CNS penetration of piperazinylthieno[3,2-*c*]pyridine-quinolinones



Compound	R	HTA ID_{50} (mg/kg, po)	RS2A ID_{50} (mg/kg, po)	RS2A/HTA
2.c	H	0.13	0.021	6.19
2.d	CH_3	0.03	0.08	0.38
2.l	CH_2CONH_2	1.0	0.031	32.26

Conclusion

The work presented here has allowed us to combine and optimize 5-HT_{1B} and 5-HT_{2A} antagonistic activity in the same molecule, leading to the development of SL 65.0472 (7-fluoro-2-oxo-4-[2-[4-(thieno[3,2-*c*]pyridin-4-yl)piperazin-1-yl]-1,2-dihydroquinoline-1-acetamide), an orally active mixed antagonist that is currently under clinical study.[37]

Experimental

Chemistry

Melting points were determined using open capillary tubes on a Buchi 530 apparatus and are uncorrected. Merck Kieselgel 60 (230–400 μm) was used for flash chromatography and Kieselgel 60 F_{254} silica plates (0.2 mm) were used for TLC. The structures of the compounds were confirmed spectroscopically by proton and carbon NMR ($CDCl_3$ or DMSO-d_6) with TMS as the internal standard using a Brucker AC-250 instrument, by IR (KBr) on a Perkin-Elmer 297 spectrophotomer and by their mass spectra (MS-ES) which were recorded on a VG Autospec (Fisons Instruments) spectrometer. The purity of the compounds was analyzed by TLC, HPLC and elementary analysis. Results obtained were within $\pm 0.4\%$ of the theoretical values. Reagent grade chemicals were purchased from Sigma-Aldrich-Fluka. All solvents were analytical grade and anhydrous reactions were performed in oven-dried glassware under atmosphere of argon.

General procedure for the synthesis of 3-(acetoxy)-5-phenylamino-5-oxo-2-enoic acids (6). To a stirred solution of the appropriate aniline derivative **4** (53.1 mmol) in glacial acetic acid (25 mL), 4-acetoxy-2H, 3H-pyrane-2,6-dione (9.93 g, 58.4 mmol) was added by small portions. Following addition, the reaction mixture was warmed to 35 °C for 2 h. After cooling at room temperature, the mixture was diluted in ice/water (500 mL). The resulting precipitate was isolated by filtration, rinsed with water and vacuum dried to yield the desired acid **6** (76–93%).

General procedure for the synthesis of 2-oxo-1,2-dihydroquinolin-4-acetic acids (7). A suspension of the 3-(acetoxy)-5-phenylamino-5-oxo-2-enoic acid **6** (107 mmol) in concentrated (95–97%) sulfuric acid (130 mL) was heated at 90 °C for 90 min After cooling to room temperature, the solution was poured into a mixture of ice (500 g) and water (500 mL). The precipitate formed was isolated by filtration, rinsed with water and vacuum-dried. The product was triturated in ether yielding **7** (45–85%).

General procedure for the synthesis of methyl-2-oxo-1,2-dihydroquinolin-4-acetates (8). To a stirred suspension of the 2-oxo-1,2-dihydroquinolin-4-acetic acid **7** (68 mmol) in methanol (250 mL) at room temperature, thionyl chloride (16 mL, 219 mmol) was added dropwise. After the addition, the reaction mixture was heated to reflux for 16 h. The solvent was removed at reduced pressure and the residue was redissolved in dichloromethane and washed with saturated sodium hydrogenocarbonate solution. The organic layer was then washed with water, dried (Na_2SO_4), filtered and concentrated. The crude product obtained was chromatographed on silica gel (dichloromethane/methanol, 97:3) to furnish the ester **8** (85–98%).

General procedure for the synthesis of 4-(2-chloroethyl)-quinolin-2-(1H)-one (9). To a suspension of the methyl 2-oxo-1,2-dihydroquinolin-4-acetate **8** (12.5 mmol) in dry tetrahydrofuran (100 mL) and methanol (1 mL) at room temperature, sodium borohydride (19.0 g, 50 mmol) was added in small portions. Following addition, the mixture was heated to reflux for 24 h. After cooling to 5 °C, methanol (3 mL) was added dropwise. The solvents were removed under reduced pressure and the residue was taken up by a mixture of dichloromethane (200 mL) and aqueous HCl 1 N (100 mL). The organic layer was separated, washed with water, dried (Na_2SO_4), filtered and concentrated, affording 74–87% of the 4-(2-hydroxethyl)quinolin-2-(1H)-one.

To a suspension of the above alcohol (9,95 mmol) in chloroform (100 mL), pyridine (3 drops), dimethylformamide (3 drops) and thionyl chloride (3 mL, 41.0 mmol) were added. The mixture was then heated to a gentle reflux for 4 1/2 h. After cooling to room temperature, water (50 mL) was added dropwise and the solution was stirred for 30 min The organic phase was decanted, washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure yielding **9** (89–97%).

General procedure for the synthesis of 4-[2-[4-heteroaryl-piperazinyl-1-yl]ethyl] quinolin-2(1H)-ones (2). A mixture of the 4-(2-chloroethyl)quinolin-2(1H)-one **9** (16.4 mmol), the *N*-substituted piperazine **10** (15.1 mmol), sodium hydrogenocarbonate (1.6 g, 19.05 mmol) and a catalytic quantity (100 mg) of potassium iodide in acetonitrile (75 mL) and DMF (5 mL) was heated at 70 °C for 14–18 h. The solvent was then removed under reduced pressure and the residue was redissolved in dichloromethane. The solution was washed with saturated sodium hydrogenocarbonate and then with water. The organic layer was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (dichloromethane/methanol/NH_4OH 95:4.5:0.5) to obtain the desired **2** (41–74%).

General procedure for the *N*-alkylation of 4-[2-piperazinyl-1-yl]ethyl]quinolin-2(1H)-ones (2.d, 2.k, 2.l). A 0.5 M solution of the appropriate electrophile (*tert*-butyl bromoacetate, methyl iodide or bromoacetamide; 1.45 mmol) in dry tetrahydrofuran (2.9 mL) was added dropwise at 0 °C to a suspension of the 4-[2-piperazinyl-1-yl]ethyl]quinolin-2(1H)-one **2** (1.3 mmol), freshly ground potassium hydroxide (105 mg, 1.8 mmol) and *tetra*butylammonium bromide (125 mg, 0.4 mmol) in dry tetrahydrofuran (25 mL). After stirring at 0 °C for 30 min, the temperature was allowed to rise to 20 °C and stirring was continued for another 6–10 h. The solvent was then removed under reduced pressure and the

residue is redissolved in dichloromethane. The organic phase was washed with water, dried (Na_2SO_4), filtered and condensed. The crude product was purified by flash chromatography (AcOEt/MeOH/NH_4OH), providing the *N*-alkyl quinolines **2** (45–70%).

General procedure for chlorhydrate salts. To a stirred solution of piperazinylqunolinone **2** (0.7 mmol) in methanol (5–7 mL) at room temperature was added an excess of anhydrous HCl/diethyl ether solution 3 N (1 mL). The mixture was stirred at room temperature for 30 min and the precipitate was isolated by filtration, rinced several times with diethyl ether and vacuum-dried.

General procedure for the synthesis of 3-[2-nitrophenyl)-methylene]dihydrofuran-2(3H)-ones (11). α-[γ-Butyrolactonylidene]triphenylphophine (8.3 g, 24 mmol) was added to a solution of the appropriately-substituted *ortho*-nitrobenzaldehyde (27 mmol) in toluene (200 mL) at room temperature. The solution was heated to reflux for 6 h then cooled to room temperature overnight. The precipitate was collected by vacuum filtration, rinsed with toluene and oven-dried at 50 °C, yielding the expected olefin **11** (62–88%).

General procedure for the synthesis of 3-(2-hydroxyethyl)-3,4-dihydroquinolin-2(1H)-ones (12). A mixture of the 3-[2-nitrophenyl)methylene]dihydrofuran-2(3H)-one **11** (20 mmol) and 5% Pd/C (0.7 g) in dry tetrahydrofuran (100 mL) and acetic acid (0.6 mL) was hydrogenated with H_2 (40 psi) at room temperature for 9 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was crystallized from a minimum of dichloromethane, yielding **12** (58–76%).

General procedure for the synthesis of 3-[2-tert butyldimethylsilyloxyethyl]-1-methyl-3,4-dihydroquinolin-2(1H)-ones (14). To a solution of the 3-(2-hydroxyethyl)-3,4-dihydroquinolin-2(1H)-one **12** (10.84 mmol) and imidazole (1.36 g, 20 mmol) in dry DMF (20 mL) at room temperature was added *tert*-butyldimethylsilyl chloride (4.0 g, 26.5 mmol). After the addition, the mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and the residue was redissolved in diethyl ether. The organic solution was washed with water, dried ($MgSO_4$), filtered and concentrated. The compounds thus obtained in a quantitative yield were *N*-alkylated as follows: sodium hydride (60% mineral oil dispersion; 0.6 g, 15 mmol) was added to the above *O*-protected dihydroquinolin-2(1H)-one (11.23 mmol) in solution in tetrahydrofuran (100 mL) at room temperature stirring was continued for 1 h then methyl iodide (1.6 mL, 25.6 mmol) was added. The mixture was stirred at room temperature for 20 h and the solvent was removed under reduced pressure. The compound was extracted with diethyl ether and the organic extracts were washed with water, dried ($MgSO_4$), filtered and concentrated. The crude product was filtered through a pad of silica gel (CH_2Cl_2/cylcohexane) providing the *N*-methyl dihydroquinolinones **14** in near quantitative yields.

General procedure for the synthesis of 3-(2-chloroethyl)-1-methyl-3,4 dihydroquinolin-2(1H)-ones (15). To a solution of **14** (13.7 mmol) in dry tetrahydrofuran (80 mL) to which was added 15 mL (15 mmol) of a 1 M solution of *tetra*butylammonium fluoride in THF. The mixture was stirred at room temperature for 3 h and the solvent was evaporated. The residue was dissolved in diethyl ether and the organic solution was washed several times with water, dried ($MgSO_4$), filtered and concentrated. The alcohol was purified by chromatoflash (dichloromethane/methanol, 98:2) and isolated in 64–82% yields.

Thionyl chloride (0.7 mL, 9.6 mmol) was added dropwise to a solution of the 3-(2-hydroxyethyl)-1-methyl-3,4-dihydroquinolin-2(1H)-one (6,37 mmol) in chloroform (30 mL) at room temperature. After the addition, the solution was heated at reflux for 1 h. The solvent was removed under reduced pressure and the crude product was filtered on a pad of silical gel (CH_2Cl_2) yielding the chloro derivative **15** in a 92–97% yield.

General procedure for the synthesis of 3-[2-[4-heteroaryl-piperazin-1-yl)ethyl]-1-methyl-3,4-dihydroquinolin-2(1H)-ones (3). A mixture of 3-(2-chloroethyl)-1-methyl-3,4 dihydroquinolin-2(1H)-one **15** (3.9 mmol), the appropriate heteraoarylpiperazine (3.9 mmol) and sodium bicarbonate (0.65 g, 7.8 mmol) in acetonitrile (20 mL) was heated at reflux for 7 h. The solvent was then removed under reduced pressure and the crude product was purified by flash chromatography (dichloromethane/MeOH/NH_4OH, 96.5:3:0.5). The free base obtained was transformed into its chlorhydrate salt with 41–74% combined yields for the two steps.

General procedure for the synthesis of 2-oxo-1,2,3,4-tetrahydroquinolin-3-acetaldehydes (13). A solution of 1,1,1-triacetoxy-1,1-dihydro-1,2-benzodioxol-3(1H)-one (Dess–Martin reagent; 6.4 g, 14 mmol) in dichloromethane (100 mL) was added drop wise at room temperature to a stirred solution of the 3-(2-hydroxyethyl)-3,4-dihydroquinolin-2(1H)-one **12** (12 mmol) in dichloromethane (25 mL). After the addition, the mixture was stirred at room temperature for 20 h. Dichloromethane and a saturated sodium hydrogenocarbonate solution were added. The organic layer was separated and the aqueous phase was re-extracted twice with dichloromethane. The combined organic extracts were washed with water, dried ($MgSO_4$), filtered and concentrated. The crude aldehydes **13** are obtained in 90–95% yields and are used immediately in the next step.

General procedure for the synthesis of 3-[2-[4-(heteroaryl)-piperazin-1-yl]ethyl]-3,4-dihydroquinolin-2(1H)-ones (3, R_1 = H). To a stirred mixture of the 2-oxo-1,2,3,4-tetrahydroquinolin-3-acetaldehyde **13** (4.5 mmol) and the appropriate *N*-heteroarylpiperazine (4.8 mmol) in 5 mL of absolute ethanol at room temperature was added titanium *tetra*isopropoxide (2.6 mL ; 9 mmol). After stirring for 4 h, sodium cyanoborohydride (1.0 g, 18 mmol) was added and the mixture was stirred at room temperature an additional 20 h. The milky solution was then diluted with dichloromethane (100 mL) and saturated

ammonium chloride solution (100 mL). After stirring for 5 min, the mixture was filtered through Celite and the organic layer of the filtrate was separated. The aqueous layer was then re-extracted twice with dichloromethane and the combined organic solutions were dried ($MgSO_4$), filtered and concentrated. The compounds **3** where $R_2 = H$ were purified by flash chromatography (dichloromethane/methanol, 95:5), and obtained in 69–94% yields.

Pharmacology

5-HT$_{2A}$ binding assay. Radioligand binding assay was performed following a published procedure.[30] Frontal cortical regions of male Sprague–Dawley rats (Charles River) were dissected on ice and homogenized (1:20 w/v) in ice-cold buffer solution (50 nM Tris–HCl, 120 nM NaCl, 5 mM KCl) and centrifuged at 40,000 *g* for 10 min The pellet was resuspended in buffer, rehomogenized and recentrifuged. The final pellet was resuspended in buffer (500 mg/10 mL) containing 10^{-5} M pargyline.

Assays were performed in triplicate in a 1.0 mL volume containing 5 mg wet weight of tissue and 0.3 nM [^{3}H] spiroperidol (19 Ci/mmol; New England Nuclear). Tubes were incubated for 20 min at 37 °C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters. The filters were washed twice with 5 mL of ice-cold Tris–HCl buffer, and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [^{3}H] spiroperidol binding was defined as the difference between binding in the absence or the presence of 100 μM 5-HT.

The following studies were conducted in accordance with ethical guidelines edited by the European Community (EC directive 86/609), the council of Europe (Convention ETS 123) and the French Government (decree of 19.10.87).

5-HT2A antagonism in the pithed rat. Rats (Sprague–Dawley Charles River France were anesthetised with pentobarbital sodium (60 mg/kg IP), intubated and pithed. They were mechanically ventilated on a small rodent ventilator (Harvard™ Apparatus). Polyethylene cannulas were inserted into the right carotid artery for blood pressure measurement, and in the femoral vein for drug infusions. The arterial cannula was connected to a pressure transducer, and systemic blood pressure was continuously recorded with the use of data acquisition software (IOX®, EMKA technologies).

Three to four cumulative doses of the drug were given intravenously (0.1 mL/min/rat for 5 min) during the same experiment, so that the rats were their own controls. Serotonin (30 μg/kg/iv bolus) was administered 5 min before the beginning of the infusion of the first dose of the drug tested, and repeated 5 min after the end of the infusion of each of the doses of compound.

The hypertensive effect of serotonin was calculated for each injection as the difference between the peak increase in mean blood pressure (MBP) and the MBP recorded just before the serotonin injection. The antagonistic effect of drugs was calculated for each dose of the drug by the percentage of inhibition of the serotonin-induced hypertension:

$$\%\ \text{inhibition} = \left[1 - (\Delta)\text{MBP}_{(\text{dose x})}/\Delta\text{MBP}_{(\text{control})}\right] \times 100$$

where $\Delta MBP_{(dose\ x)}$ was absolute variation in MBP following serotonin injection after the end of the infusion of the dose 'x' of the drug; $\Delta MBP_{(control)}$ was the absolute variation in MBP following serotonin injection before the administration of the first dose of the drug. The ID_{50} was defined as the antagonist dose reducing the serotonin-induced pressor response by a half.

Canine isolated saphenous vein model of 5-HT$_{1B}$ antagonism[7,31]

Saphenous veins were removed from Beagle or Anglopoitevin dogs (ECDL, either sex, 25–34 kg) anesthetised with pentobarbital (35 mg/kg iv). Helical strips approximately 0.4 cm in width and 0.5 cm in length were prepared by mounting the vein on metal cannula and then suspended in 20-mL tissue baths containing Krebs' solution of the following millimolar composition: NaCl 118.0; KCl 4.7; $MgCl_2$ 1.2; $CaCl_2$ 2.6; $NaHCO_3$·25.0; glucose 11.1; ascorbic acid 0.11; (pH 7.4; 37 °C) and aerated continuously with 95% O_2–5% CO_2. The solution also contained atropine 1 μM (to inhibit muscarinic receptors); mepyramine 1 μM (to inhibit histamine H_1 receptors).

Contractile responses were recorded isometrically using Hugo Sachs model 351 force–displacement transducers (March Hugstentten, Germany), connected to a Gould 2400S polygraph (Courtaboeuf, France). Optimal responses were obtained when the tissues were subjected to an initial tension of 2 g. Data acquisition was performed automatically using the JAD.2 Notocord software (Croissy, France) on a PC Compaq. Strips were allowed to equilibrate for 30 min with several washes before exposure to a 3 μM noradrenaline challenge to test the viability of the preparation. Following a 30 min resting period, the first concentration–response curve to sumatriptan was obtained by exposing the preparations to cumulative additions of the compounds until the maximal response was obtained. Another resting period with several washes allowed the strips to return to the basal tension. The antagonist compound was then added to the bath and left in contact with the tissue for 15 min prior to the second concentration-response curve to sumatriptan. Responses were expressed as a percentage of the maximum contraction obtained with the first sumatriptan curve. pA_2 values were calculated using the Schild plot method.

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