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Novel selective and potent 5-HT reuptake inhibitors with 5-HT_{1D} antagonist activity: chemistry and pharmacological evaluation of a series of thienopyran derivatives

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Abstract—A series of compounds combining the naphthylpiperazine and thienopyran scaffolds has been prepared and evaluated for 5-HT reuptake inhibition with 5-HT_{1D} antagonist activity. The design of these compounds has been based on the 'overlapping type' strategy where two pharmacophores are linked in a single molecule. The resultant dual pharmacological profile has the potential to deliver a more efficient treatment for depression.

1. Introduction

Serotonin (5-HT), a biogenic amine neurotransmitter with diverse physiological actions in both the central and peripheral nervous systems, operates through various distinct membrane receptors. Disturbances in the central serotonin system have been associated with the pathogenesis of depression, and the antidepressant effect of the selective 5-HT reuptake inhibitors (SSRIs) is due to an enhancement of postsynaptic 5-HT levels by blockade of the reuptake of synaptic 5-HT.[1] Although the SSRIs offer a significant advance in the treatment of major depression, there are limitations to their effectiveness. Some reports suggest that up to 30–50% of patients fail to show an adequate response,[2] and in those that do respond, therapeutic improvement is not immediate, but requires treatment for 2–4 weeks.[3] This delayed onset of therapeutic action is thought to be due to the time required to desensitize terminal 5-$HT_{1B/1D}$ and somatodendritic 5-HT_{1A} autoreceptors.[4] Therefore, one approach to more efficient and fast acting antidepressant drugs would be to mimic pharmacologically 5-HT autoreceptor desensitization by the concomitant blockade of terminal autoreceptors with selective antagonists.[5] This combination would prevent feedback inhibition of terminal 5-HT release leading to an increased effectiveness of the selective serotonin reuptake inhibitor on sypnatic levels of 5-HT, and potentially shorten the onset of the antidepressant effect in depressed patients.[6]

Receptor mapping studies have shown that the 5-HT_{1B} receptor is widely distributed in the central nervous system (CNS) in neural and vascular tissues, whereas the 5-HT_{1D} receptor is believed to be restricted to neural tissues. In addition, recent studies on the localization of 5-HT_{1D} and 5-HT_{1B} receptors have indicated that only 5-HT_{1D} receptors are located on human trigeminal sensory neurons whereas only 5-HT_{1B} receptors were detected on dural arteries.[7] There is a potential for coronary artery constriction, possibly through activation of 5-HT_{1B} receptors, which may preclude use in patients with known heart disease.[8] In the belief that the efficacy of antidepressants can be attributed to either one or other of the 5-$HT_{1B/1D}$ receptors, we sought to

Keywords: 5-HT_{1D} Antagonist; 5-HT Reuptake inhibitor; Thienopyran scaffold.
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identify compounds that discriminate between these two receptors, searching for a 5-HT_{1D} ligand with a potentially lower side-effect profile.[9]

In summary, we believe that a single compound combining 5-HT reuptake inhibition (SRI) with 5-HT_{1D} receptor antagonism would elevate synaptic levels of 5-HT above those evoked by reuptake inhibition alone, and to levels that are only obtained following chronic SSRI treatment. In consequence, such a drug would be expected to produce a more rapid onset in clinical antidepressant activity, and be a more effective antidepressant.

Instead of combining the administration of a SSRI with a 5-HT_{1D} antagonist to potentiate postsynaptic 5-HT levels,[6b] we report here the synthesis of compounds with a dual pharmacological profile exhibiting both 5-HT reuptake inhibition and 5-HT_{1D} antagonism within a single molecule.

2. SAR strategy

Our chemical strategy for constructing hybrid molecules from 5-HT_{1D} antagonists and SSRIs has utilized the approach previously described as the 'overlapping type' (Fig. 1).[10]

Hybrid molecules have been designed by taking advantage of the simultaneous presence in both types of compounds (5-HT_{1D} antagonists and SSRIs) of a basic nitrogen, creating a shared central structural unit in the form of a piperazine or its piperidine bioisostere. These novel heterodimers have been evaluated for potency at 5-HT_{1D} and selectivity versus 5-HT_{1B} receptors and as SRIs (our initial lead requirements were set as: binding affinity on 5-HT_{1D} $K_i < 50$ nM, 5-HT_{1B} $K_i > 150$ nM; functional antagonist potency on 5-HT_{1D} $K_B < 10$ nM, 5-HT_{1B} $K_B > 50$ nM; and 5-$HT_{transporter}$ binding $K_i < 10$ nM). As serotonergic receptor ligands are frequently observed to show undesirable cross-reactivities at adrenergic and dopaminergic receptors, the pharmacological testing cascade included radioligand binding assays to assess α_1 adrenergic and dopamine D_2 affinities. From previous experience on related serotoninergic projects at Lilly, we have established that binding affinity requirements of $\alpha_1 > 100$ nM and $D_2 > 200$ nM (10-fold and 20-fold with respect to SRI binding) were sufficient to give satisfactory margins of safety when comparing no adverse effect levels in models of adrenergic (spontaneously hypertensive rat) and dopaminergic (inhibition of apomorphine-induced climbing in rats) activity with minimum effective doses in in vivo measures of serotonin elevation (microdialysis in the hypothalamus of freely-moving guinea pigs).

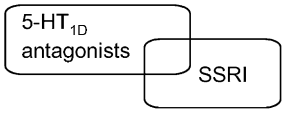


Figure 1.

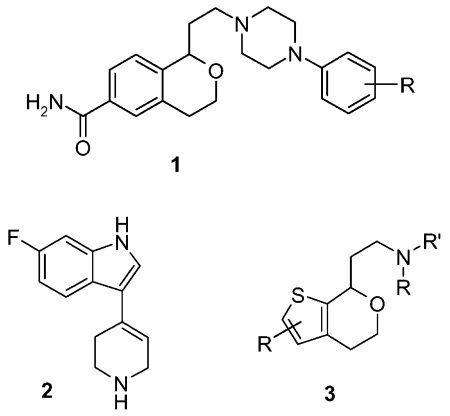
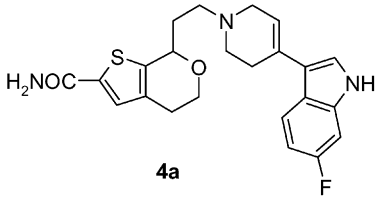


Figure 2.

Examining the reported platforms that have shown good selectivity for the 5-HT_{1D} receptor, the benzopyran **1**[11] was recognized as having the potential for replacement of the phenyl piperazine by 5-HT reuptake inhibitor pharmacophores of the 4-(indol-3-yl)piperidine type, such as **2**[12] (Fig. 2). Initial studies with the benzopyran platform[13a,b] demonstrated that the 5-HT_{1D} binding site could not only tolerate the presence of the more bulky biaryl tetrahydropyridine, but also, importantly, that this additional steric interaction influenced the functional activity of the resultant hybrid, imparting a shift from the receptor agonism seen with the benzopyran **1** to our desired 5-HT_{1D} antagonist profile.[13a] These early studies also demonstrated that this dual pharmacology leads to an elevation of extracellular 5-HT levels in the guinea pig hypothalamus significantly above that obtained after a maximally effective acute dose of the SSRI fluoxetine. Continuing the development of the SAR around this 'overlapping' approach, we now report the evaluation of the isosteric thienopyran platform **3** as a modified scaffold for this series of 5-HT_{1D} antagonist/5-HT reuptake inhibitor hybrids.

Synthesis of the parent 4-(6-fluoroindol-3-yl)piperidine in this series (**4a**, Fig. 3) showed that in comparison with the corresponding benzopyran (**4b**, Fig. 3) it exhibited improved potency and selectivity for the human 5-HT_{1D} receptor (with respect to the 5-HT_{1B} receptor) while maintaining excellent SRI activity against the rat 5-HT reuptake transporter. In addition, it demonstrated potent antagonism in a 5-HT_{1D} GTP-γS binding assay. Although significant cross-reactivity was observed at α_1 adrenergic and dopamine D_2 receptors, this compound demonstrated that the thienopyran platform could offer a different pharmacological profile and was considered as a lead meriting further SAR optimization.

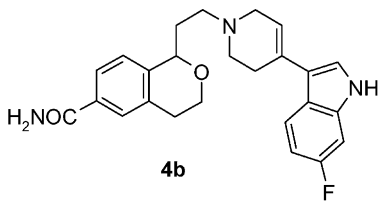
Our initial SAR strategy, aimed at ameliorating the unwanted cross-reactivity versus α_1 and D_2 receptors, was focused on replacement of the indolylpiperidine by the naphthylpiperazine scaffold, a modification, which had proved successful with the benzopyran platform[13a] and also found in recent related literature.[13c] In particular, the 1-naphthylpiperazines, shown in Figure 4, were evaluated. Following the previous SAR developed at Lilly[13a,14] around the known SRI indol-



4a

Binding affinities	K_i (nM)
h-5-HT_{1D}	8.3±1.8
h-5-HT_{1B}	203±31
rat-5-$HT_{transporter}$	0.11±0.01
α_1	0.68 (n = 2)
D_2	42 (n = 2)

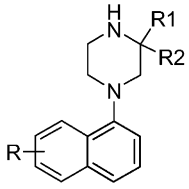
h-5-HT_{1D} GTP-γS K_B = 0.95nM



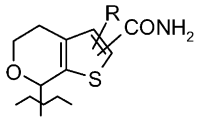
4b

Binding affinities	K_i (nM)
h-5-HT_{1D}	56
h-5-HT_{1B}	281
rat-5-$HT_{transporter}$	0.11
α_1	16
D_2	4.7

Figure 3.



R= F, OMe
R_1, R_2= H, Me



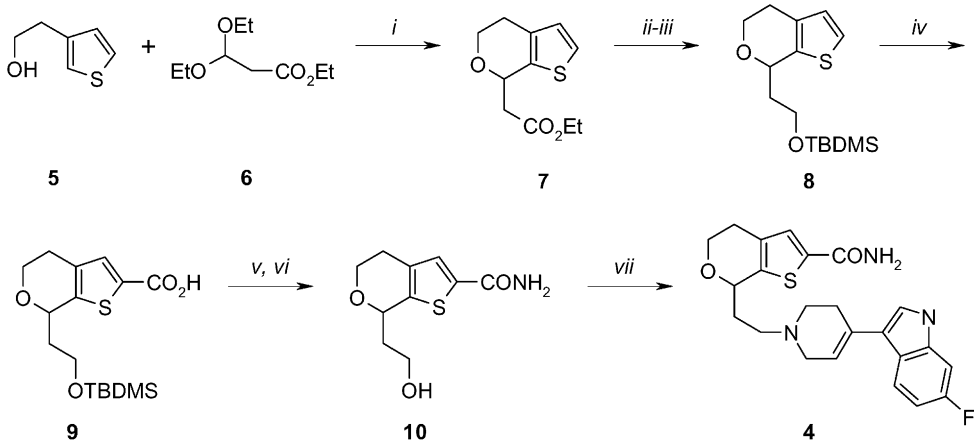
R= F, Cl, Me

Figure 4.

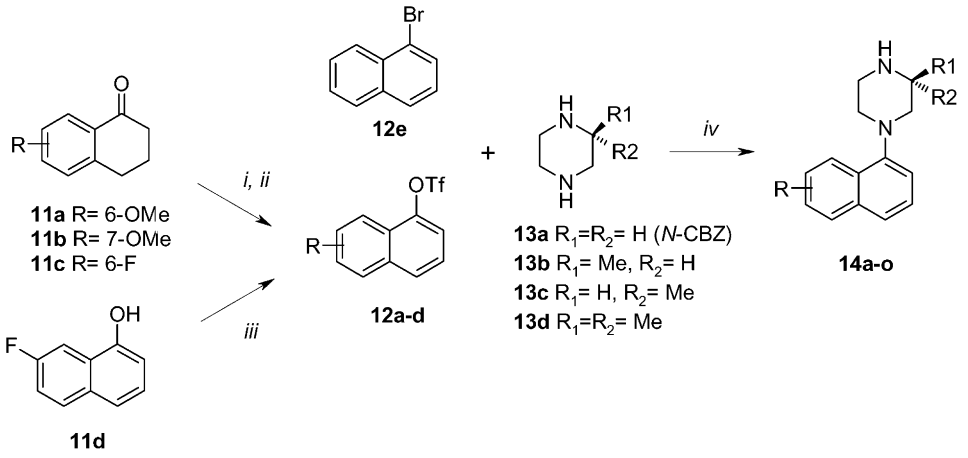
ylpiperidine featured in **4**, we wished to translate promising substitutions and substituents from the indolyl to the naphthyl ring (F and MeO at positions 6 and 7). At the same time and with the same purpose, we wished to evaluate the influence of substitution α to the critical basic nitrogen of the piperazine, as we had also previously observed that α-methylation of the piperidine nucleus could increase SRI activity and ameliorate D_2 cross-reactivity.[13a,15] Finally, the SAR was expanded to investigate the effect of modifications to the thienopyran core, including replacement by the isomeric [3,2-*c*] nucleus, substitution adjacent to the carboxamide and one amide isostere.

3. Results and discussion

The synthesis of compound **4** (Scheme 1) was conducted by initial preparation of the thienopyran core, followed by alkylation of the reuptake pharmacophore, as shown in Scheme 1. The [2,3-*c*]-thienopyran **7** was synthesized from 2-(3-thienyl)ethanol (**5**) by treatment with ethyl 3,3-diethoxypropionate (**6**) in the presence of a Lewis acid. Optimal yields were obtained by the use of boron trifluoride etherate.[16] The ester functionality was reduced with DIBAL-*H*, followed by protection of the resulting alcohol with TBDMS chloride. The carboxylic acid functionality was then selectively introduced at the C-2 position of the thienopyranyl ring via metallation with *n*-BuLi and subsequent carboxylation with carbon dioxide. The acid **9** was then transformed into the carboxamide **10** in two steps: initial treatment with CDI to obtain the activated ester, followed by the addition



Scheme 1. Reagents and conditions: (i) $Et_2O{\cdot}BF_3$, −78 °C to rt, CH_2Cl_2, 82%; (ii) DIBAL-*H*, THF, 98%; (iii) TBDMSCl, imidazole, THF, 90% over two steps; (iv) (1) *n*-BuLi, (2) CO_2 (g), 90%; (v) (1) CDI, THF, (2) NH_3 in dioxane, 78%; (vi) *n*-Bu_4NF, THF, 75%; (vii) (1) MsCl, Et_3N, DMF, (2) K_2CO_3, **2**, CH_3CN, 75%.



Scheme 2. Reagents and conditions: (i) (1) LHMDS, THF, −78 °C, 1 h, (2) $PhNTf_2$, −78 °C to rt, 69%; (ii) DDQ, dioxane, 100 °C, 64%; (iii) *t*-BuOK, $PhNTf_2$, THF, 99%; (iv) $Pd(OAc)_2$, (*R*)-BINAP, Cs_2CO_3, toluene, 110 °C, 15–94%.

of ammonia in dioxane, and deprotection of the silyl ether with tetrabutylammonium fluoride. The desired compound **4** was obtained by displacement of the corresponding mesylate derived from **10** with the SRI pharmacophore **2**.

The required substituted 1-naphthylpiperazines **14a–o** were synthesized as shown in Scheme 2. Thus the commercially available 6-methoxy and 7-methoxy tetralones **11a–b**, and 6-fluorotetralone (**11c**) (obtained from commercially available 3-fluoro-1-bromobenzene according to the synthetic route described by Plumet and co-workers[17]) were treated with lithium hexamethyldisilazide, followed by *N*-phenyl bis-(trifluoromethanesulfonimide), to afford the desired enol triflates, which were then aromatized with DDQ in hot dioxane to give **12a–c**. The 7-fluoro derivative **12d** was obtained from 7-fluoronaphthol (**11d**) (prepared by condensation of furan with the benzyne derived from commercially available 2,4-difluoro-1-bromobenzene;[18a] or by catalytic dehydrogenation of the 7-fluorotetralone[18b]). The intermediates **12** were then coupled under Buchwald conditions[19] with the appropriate piperazines **13a–d** (**13a–c** are commercially available; **13d** was prepared according to the method of Bøgesø et al.[20]) to yield the desired SRI pharmacophores **14a–o** (Table 1).

Finally, the piperazines **14a–o** were coupled with the thienopyran **10**, under the conditions shown in Scheme 1, to yield the final analogous **15a–o**. The biological data are shown in Table 2. Note that initial compounds in this dataset were tested for SRI activity against the rat 5-HT reuptake transporter, but as the human recombinant cell-line became available, later members of the series were tested on the corresponding human transporter; where affinities were measured on both preparations, comparable potencies were observed.

Introduction of the naphthylpiperazines, as featured in **15a–b**, maintained the required 5-HT_{1D} receptor affinity and selectivity over 5-HT_{1B}, but reduced the 5-HT reuptake inhibition activity. Significantly, however, the undesired α_1 and D_2 cross-reactivities were appreciably reduced, although they still did not meet our desired profile. Thus, we considered the new naphthylpiperazine moiety as a promising replacement for the known indolylpiperidine pharmacophore.

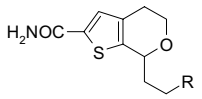
Table 1. Compounds **14a–o** synthesized

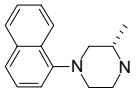
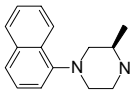
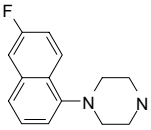
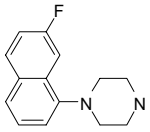
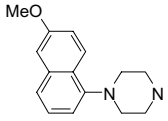
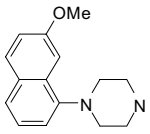
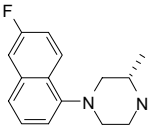
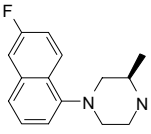
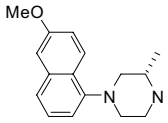
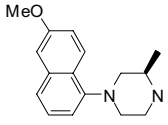
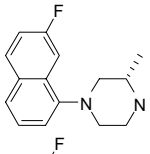
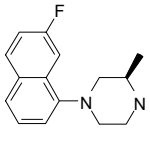
Compd	R	R1	R2
14a	H	Me	H
14b	H	H	Me
14c	6-F	H	H
14d	7-F	H	H
14e	6-OMe	H	H
14f	7-OMe	H	H
14g	6-F	Me	H
14h	6-F	H	Me
14i	6-OMe	Me	H
14j	6-OMe	H	Me
14k	7-F	Me	H
14l	7-F	H	Me
14m	7-OMe	Me	H
14n	7-OMe	H	Me
14o	6-F	Me	Me

Next, we investigated the influence of substitution in the naphthyl ring and of α-substitution in the piperazine on improving the 5-HT reuptake activity, whilst maintaining the good 5-HT_{1D} binding affinity and selectivity observed with **15a–b**. In particular we focused on 6- and 7-fluoro and methoxy substitutions, as being isomeric with the 5- and 6-substituted indoles previously recognized as potent SRI pharmacophores.[14]

Initially we examined the compounds without piperazine α-methyl substitution (**15c–f**) and observed a significant decrease in SRI activity, with a concurrent increase in D_2 cross-reactivity, excepting when the naphthyl was substituted with a 6-methoxy group (**15e**). We therefore looked at further examples possessing either the (*R*)- or (*S*)-methyl piperazine moieties, as similar α-substitution on the corresponding 4-aryl piperidines[13a,15] had previously proved beneficial for SRI activity. Looking at the subfamily of 6-fluoronaphthyl derivatives **15g–h**

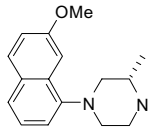
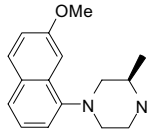
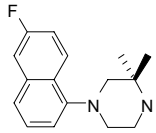
Table 2. Receptor binding affinities of **15a–o** at 5-HT_{1D}, 5-HT_{1B}, 5-$HT_{transporter}$, α_1 and D_2 receptors



Compd	R	K_i (nM)[a]					
		5-HT_{1D}[b]	5-HT_{1B}[c]	α_1[d]	D_2[e]	Rat 5-HT_{trans}[f]	Hum 5-HT_{trans}[g]
15a		22 ± 7	>1000	54	136	14 ± 3	
15b		37 ± 20	137 ± 53	64	150	6.8 ± 0.7	
15c		126 ± 55	119 ± 14	46	17	15 ± 2	
15d		68 ± 30	92 ± 15	57	12.3	37 ± 15	
15e		38 ± 19	264 ± 44	116	324	46 ± 0.3	
15f		170 ± 52	12.5 ± 7.7	72	32	20 ± 2	
15g		21 ± 3	673 ± 108	93	101	3.0 ± 0.2	
15h		9.4 ± 2.4	343 ± 15	89	133	3.8 ± 0.2	
15i		104 ± 24	>1000	164	>1000	41 ± 8	
15j		29 ± 7	>1000	172	>1000	25 ± 8	21 ± 1
15k		15.3 ± 2.6	428 ± 20	68	81	24 ± 3	28 ± 4
15l		23 ± 6	>1000	61	101	9.1 ± 0.4	24 ± 1

(*continued on next page*)

Table 2 (*continued*)

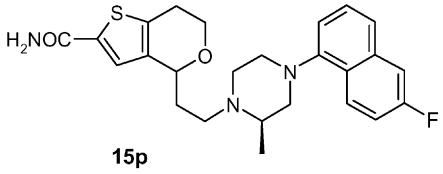
Compd	R	K_i (nM)[a]					
		5-HT_{1D}[b]	5-HT_{1B}[c]	α_1[d]	D_2[e]	Rat 5-HT_{trans}[f]	Hum 5-HT_{trans}[g]
15m		12.4 ± 2.7	61 ± 9	211	122		>100
15n		13.8 ± 2.8	41 ± 6	113	112		39 ± 12
15o		164 ± 41	>1000	182	604	0.20 ± 0.05	1.5 ± 0.2

[a] Mean K_i values and SEM determined from at least three experiments, only otherwise stated.
[b] Affinities for 5-HT_{1D} receptors were measured on human 5-HT_{1D} expressed in LM(tk-) cells using [^{3}H]-GR125743 as radioligand.
[c] Affinities for 5-HT_{1B} receptors were measured on human 5-HT_{1B} expressed in LM(tk-) cells using [^{3}H]-GR125743 as radioligand.
[d] Affinities for α_1 receptors were measured on rat cortex membranes using [^{3}H]-prazosin as radioligand (mean of two experiments).
[e] Affinities for D_2 receptors were measured on rat caudate membranes cells using [^{3}H]-raclopride as radioligand (mean of two experiments).
[f] Affinities for 5-$HT_{transporter}$ were measured on rat cortex membranes using [^{3}H]-citalopram as radioligand.
[g] Affinities for 5-$HT_{transporter}$ were measured on human 5-$HT_{transporter}$ expressed in HEK293 cells using [^{3}H]-citalopram as radioligand.

and **15o**, we observed the expected improvement in SRI activity on introduction of methyl groups to the piperazine, in particular with dimethyl substitution; we also saw an improvement in the cross-reactivity with respect to α_1 and D_2, again significantly in the disubstituted analogue **15o**. However the 5-HT_{1D} receptor binding, while tolerating a single 2-methyl substituent, decreased sharply when the *gem*-dimethyl group was introduced (**15o**), demonstrating that steric space is limited in this region of the serotonin receptor. With respect to substitution of the naphthyl nucleus, it was observed that, in general, the methoxy group afforded compounds with worse 5-HT reuptake activity than did fluorine substitution; and that 6-fluoro substitution afforded better 5-HT reuptake activity than the 7-fluoro isomer.

The regiosisomeric [3,2-*c*]-thienopyran bicyclic system, which its modified steric relationship between the key 2-carboxamido functionality and the ethylamine side chain, was also investigated. These analogous were prepared from 2-(2-thienyl)ethanol, following an analogous synthetic route to that shown in Scheme 1. Compound **15p** (Fig. 5) emerged as the most attractive member of this series and will be the only example described here as. Although the cross-reactivity against α_1 and D_2 was slightly better, the SRI and 5-HT_{1D} results with this isostere were found to be worse than observed with the regioisomer **15h**. We therefore chose to continue the SAR study with the [2,3-*c*]-thienopyran system.

Compounds **15g** and **15h** demonstrated the best profile of the compounds evaluated up to this point. We chose to progress the SAR by further utilizing the reuptake pharmacophore featured in **15h** as it tended to impart better 5-HT_{1D} binding activity.



15p

	Ki (nM)
5-HT_{1D}	5.6±0.1
5-HT_{1B}	499±86
h-5-$HT_{transporter}$	34±1
α_1	102 (n = 2)
D_2	234 (n = 2)

Figure 5.

The next phase of the SAR study was to introduce variation into the thienopyran moiety including substitution at C-3; transposing the carboxamide from C-2 to C-3; or replacing the carboxamide by bioisosteres. Furthermore, it was decided to assay the chirally separated diastereomers and not the mixtures as initially performed. Although the final compounds were diastereomeric mixtures, due to the presence of the chirally resolved 2-methylpiperazine, the chromatographic separations were always performed under chiral HPLC conditions.

The preparation of **16** (Fig. 6) was conducted by application of the method used for the preparation of **15** but using ethyl acetoacetate instead of the acetal **6** (see Scheme 1).

For the preparation of analogous **19a–c**, the carboxylic acid **9** was used as a common starting material (Scheme 3).

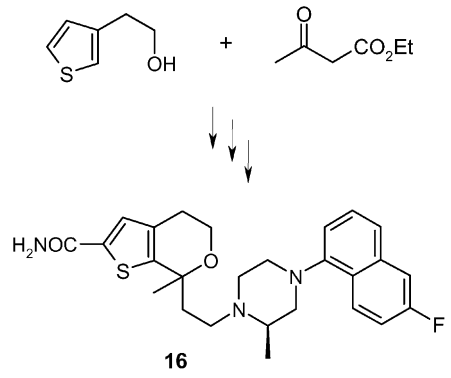
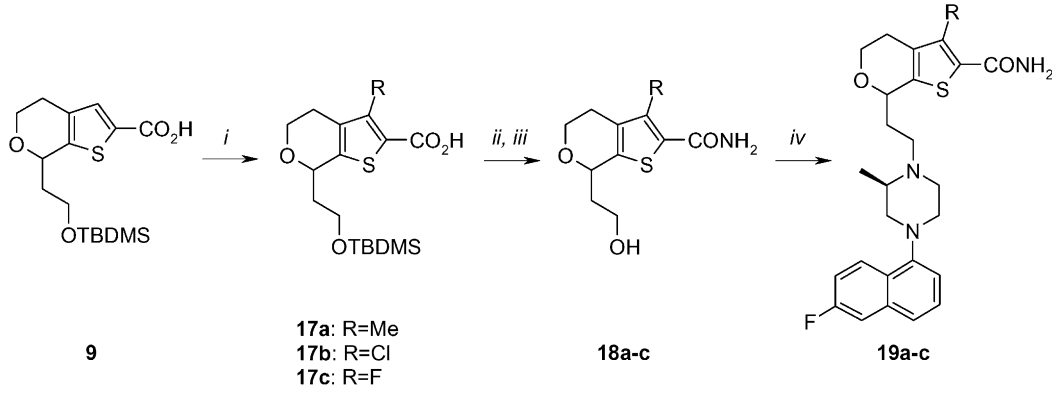


Figure 6.

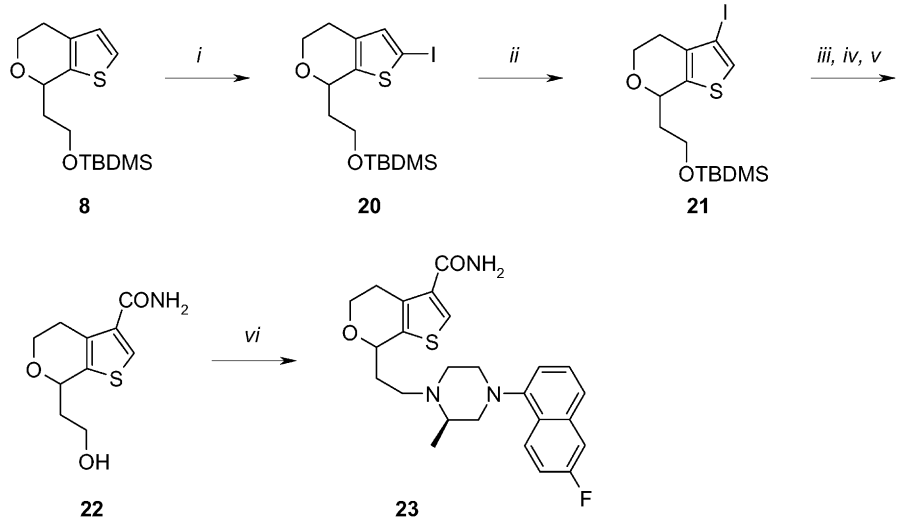
The key step was the synthesis of **17**, via *ortho*-metallation at C-3 assisted by the carboxylic acid.[21] The transformation of **17** into **18** required the use of methanesulfonyl chloride to give increased activation of the carboxylic acid, as a more reactive acylating species was required to form the amide, presumably due to the increased steric hindrance imparted by the C-3 substituent. Subsequent steps followed the conditions shown in Scheme 1.

The synthesis of the derivative **23** started with the intermediate **8** (Scheme 4). The key step involved iodine rearrangement from C-2 (**20**) to C-3 (**21**), known to occur on lithiation of the thiophene heterocycle.[22] Introduction of the cyano functionality under palladium catalysis,[23] subsequent hydrolysis of this group and desilylation afforded the 3-carboxamido intermediate **22**. This compound was finally transformed into **23** using the conditions described in Scheme 1.

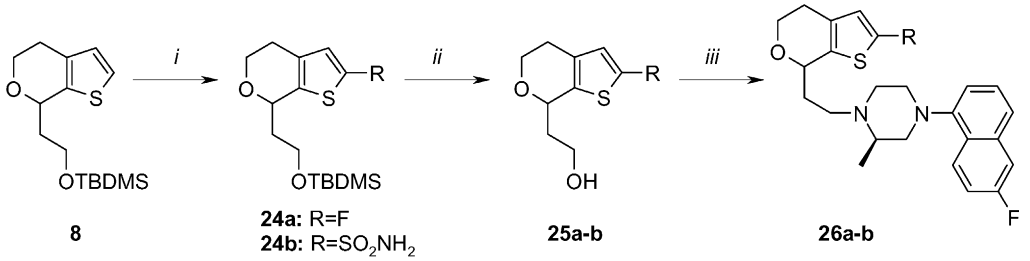
Finally, the preparation of analogous **26** also started from the intermediate **8** (Scheme 5). The introduction of the sulfonamide group in **24b** was conducted following



Scheme 3. Reagents and conditions: (i) For R = Cl and Me: (1) *sec*-BuLi/TMEDA, THF, −78 °C, 1.5 h, (2) MeI or NCS, −78 °C to rt, 65% and 50%. For R = F: (1) *t*-BuLi, THF, −78 °C, 1 h, (2) NFSI, −78 °C to rt, 40%; (ii) (1) MsCl, Et_3N, THF, (2) NH_3 in dioxane, 25–55%; (iii) and (iv) see steps (vi) to (vii) in Scheme 1, but using **14h** in the final step, 45–65% both steps.



Scheme 4. Reagents and conditions: (i) (1) *n*-BuLi, THF, −78 °C, 1 h, (2) 1,2-diiodoethane, −78 °C to rt, 80%; (ii) LDA, THF, −45–35 °C, 20 min, 50%; (iii) KCN, CuI, $Pd(PPh_3)_4$, THF, reflux, 65%; (iv) H_2O_2 (33%), 2 N NaOH, Bu_4NHSO_4, CH_2Cl_2, ultrasound, 1 h, quant. (v) and (vi) see steps (vi) to (vii) in Scheme 1, but using **14h** in the final step, 67% two steps.

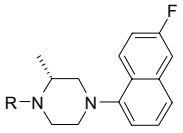


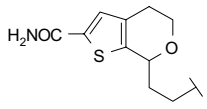
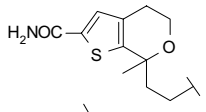
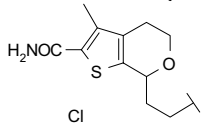
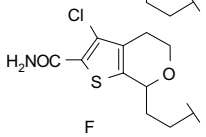
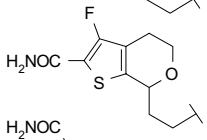
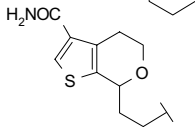
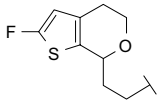
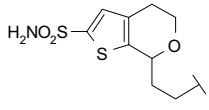
Scheme 5. Reagents and conditions: (i) For R = F (1) *n*-BuLi, THF, −78 °C, 1 h, (2) NFSI, −78 °C to rt, 30%. For R = SO_2NH_2 (1) *n*-BuLi, THF, −78 °C, 1 h, (2) SO_2, 20 min at −78 °C, 2 h at rt, (3) NCS, CH_2Cl_2, rt, 1 h, (4) NH_3, dioxane, rt, 16 h. Overall yield 65% (ii) and (iii) see steps (vi) to (vii) in Scheme 1, but using **14h** in the final step, 30–35% two steps.

a literature procedure.[24] The final transformations of **24** into **26** were conducted as described in Scheme 1.

The biological data for compounds **15**, **16**, **19**, **23** and **26** are described in Table 3.

Table 3. Receptor binding affinities of the pure isomers of **15**, **16**, **19**, **23** and **26** at 5-HT_{1D}, 5-HT_{1B}, 5-$HT_{transporter}$, α_1 and D_2 receptors; and GTP-γ[^{35}S] binding at 5-HT_{1D} and 5-HT_{1B}



Compd	R	Isomer[a]	K_i (nM)				
			5-HT_{1D}[b]	5-HT_{1B}[c]	α_1	D_2	5-$HT_{transporter}$[d]
15h		Isomer A	69 ± 82 (0.02 ± 0.02)	102 ± 24 (20 ± 14)	65	72	12 ± 5
		Isomer B	78 ± 12 (11 ± 6)	344 ± 61 (>1000)	65	187	11 ± 2
16		Isomer A	68 ± 3 (nt)	>1000	169	537	>100
		Isomer B	23 ± 7 (nt)	>1000	124	21	17 ± 8
19a		Isomer A	22 ± 8 (0.66 ± 0.16)	113 ± 15 (232 ± 126)	134	196	2.3 ± 0.4
		Isomer B	49 ± 6 (2.4 ± 0.3)	410 ± 65 (>300)	108	421	>100
19b		Isomer A	45 ± 7 (0.12 ± 0.02)	>1000 (>300)	140	291	2.1 ± 0.6
		Isomer B	42 ± 5 (3.5 ± 1.0)	264 (>300)	100	489	2.4 ± 0.8
19c		Isomer A	32 ± 1 (137%@30 nM)	338 (>300)	129	160	7.4 ± 0.2
		Isomer B	79 ± 1 (73%@30 nM)	>1000 (>300)	89	271	1.5 ± 0.2
23		Isomer A	25 ± 4 (1.2 ± 0.1)	421 ± 142 (215 ± 25)	98	27	6.6 ± 0.2
		Isomer B	11.4 ± 0.6 (4.2 ± 1.1)	259 ± 57 (58%@300 nM)	94	73	23 ± 2
26a		Isomer A	70 ± 3 (1.0 ± 0.1)	>1000	>1000	644	37 ± 7
		Isomer B	80 ± 1 (29 ± 9)	>1000	>1000	313	30 ± 2
26b		Isomer A	34 ± 1 (115%@30 nM)	>1000 (53%@300 nM)	111	337	11.9 ± 1.5
		Isomer B	>1000 (>30 nM)	>1000 (91%@300 nM)	93	205	17.5 ± 0.3

nt = Not tested.

[a] Diastereomers separated by chiral hplc—'Isomer A' signifies the isomer with the shorter hplc retention time.

[b] Figures in brackets are 5-HT_{1D} GTP-γS binding affinities expressed as K_B (nM) or % displacements at 30 nM.

[c] Figures in brackets are 5-HT_{1B} GTP-γS binding affinities expressed as K_B (nM) or % displacements at 300 nM.

[d] Affinities for 5-$HT_{transporter}$ were measured on human 5-$HT_{transporter}$ expressed in HEK293 cells using [^{3}H]-citalopram as radioligand.

The results obtained from this part of the SAR, where the diastereomers of each compound were separated, showed that the stereochemistry at C-1 of the thienopyran had little effect on 5-HT_{1D} binding; but the isomers with shorter HPLC retention times (Isomer A) displayed better 5-HT_{1D} antagonist potency on the functional GTP-γS binding assay. With respect to 5-HT reuptake inhibition, there was mostly no difference seen in potency between the two diastereomers, with the exception of **16** and **19a**.

Importantly the cross-reactivity was improved with many of the compounds compared to the parent **15h**, satisfying our preset binding requirements of $\alpha_1 > 100$ nM and $D_2 > 200$ nM (vs SRI < 10 nM). Only analogue **23**, where the carboxamide has been shifted to C-3, gave worse cross-reactivity than the parent **15h**. Replacement of the carboxamide by fluorine (**26a**) was detrimental to the reuptake activity; while the incorporation of a sulfonamide (**26b**) maintained both 5-HT_{1D} and SRI activities. Finally, the introduction of substituents at C-3 (**19a–c**) provided the best overall profile, improving 5-HT reuptake inhibition whilst maintaining good 5-HT_{1D} affinity, functional antagonism and selectivity with respect to 5-HT_{1B}.

4. Conclusion

The SAR study conducted under the 'overlapping type' strategy described in this manuscript has led to the discovery of a new series of compounds with dual pharmacological activity as inhibitors of 5-HT reuptake and as antagonists of the 5-HT_{1D} receptor. This novel series, combining the thienopyran and naphthylpiperazine scaffolds, in addition to displaying the desired potency with respect to 5-HT_{1D} and 5-HT reuptake inhibition, also demonstrates selectivity versus 5-HT_{1B}. Positive approaches to influencing cross-reactivity against α_1 and D_2 receptors have also been identified. The results indicate that the 5-HT_{1D} activity is not only due to the thienopyran portion of the molecule but also is modulated by the naphthylpiperazine moiety. At the same time, the 5-HT reuptake activity, though residing mainly in the naphthylpiperazine structure, is similarly influenced by the appended thienopyran scaffold.

Among the new compounds prepared to date within the SAR, the analogue **19b**, featuring a chloro substituent at C-3 position of the thienopyran, has shown the most promising profile fulfiling all of our preset lead requirements, including those related to cross-reactivity. These results encourage us to further evaluate these compounds in vivo in order to test the augmentation hypothesis.

5. Biology

5.1. Receptor binding studies

Standard receptor binding assay methods were used to evaluate the ability of compounds to interact with human 5-HT_{1B}, 5-HT_{1D}, α_1 and D_2 receptors, as well as with the rat and human 5-HT transporters. The 5-HT_1 binding assays were performed on LM(tk-) cells stably transfected with human 5-HT_{1B} or 5-HT_{1D} receptors, using [^{3}H]-GR125743 as radioligand, under the assay conditions described previously.[25] The α_1 binding affinity was measured on rat cerebral cortex membranes, using [^{3}H]-prazosin as radioligand.[26] The D_2 binding affinity was measured on rat caudate membranes, using [^{3}H]-raclopride as radioligand (see method below). Initially inhibition of the serotonin transporter was measured on rat cerebral cortex membranes, as previously described,[25] using [^{3}H]-citalopram as radioligand; latterly the assay was performed in HEK293 cells stably transfected with the human 5-$HT_{transporter}$ (see method below). The K_i values were calculated from the corresponding IC_{50} values using the method of Cheng and Prusoff.[27]

5.2. Binding to rat D_2 receptors

Caudate tissue from male Listar Hooded rats was homogenized in ice-cold assay buffer (50 mM Tris·HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) (30 vol) and, after centrifugation at 40,000*g* for 10 min at 4 °C, the pellet was resuspended as before, incubated for 10 min at 37 °C and again spun at 40,000*g*. The resulting pellet was resuspended in assay buffer (100 vol wet weight) and used in the assay.

Competition studies were performed in 600 μL assay buffer (50 mM Tris·HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) containing 200 μL membrane protein, 100 μL [^{3}H]-raclopride (0.8 nM, specific activity 79.3 Ci/mmol) and 100 μL of appropriate concentrations of the competing ligand (prepared in 20% aqueous DMSO). Nonspecific binding was defined using 100 μL spiperone (1 μM). Samples were incubated at room temperature in the dark for 1 h, followed by filtration through GF/B filters presoaked with 0.9% saline containing 0.1% (w/v) polyethylenimine, using a 96-well Brandel cell harvester. Filters were washed three times with ice-cold 0.9% saline, dried for 2 min in a microwave, prior to Meltilex® treatment (solid scintillation fluid) and counting using a Wallac β plate counter.

5.3. Binding to rat 5-$HT_{transporter}$

Competition studies were performed on rat cerebral cortex membranes with [^{3}H]-citalopram as radioligand, using the method previously described.[25] The results were analyzed using an automatic spline fitting program (Multicalc, Packard, Milton Keynes, UK) to provide K_i values for each of the test compounds.

5.4. Binding to human 5-$HT_{transporter}$

Competition studies were performed on 96-well microtitre plates in 75 μL assay buffer (50 mM Tris·HCl containing 300 mM NaCl, 5 mM KCl, pH 7.4) containing 50 μL membrane protein (0.2 mg/mL), 50 μL WGA PVT SPA beads (10 mg/mL), 50 μL [^{3}H]-citalopram (2 nM, specific

activity 70–87 Ci/mmol) and 25 μL of appropriate concentrations of the competing ligand (prepared in 20% aqueous DMSO). Nonspecific binding was defined using 25 μL desipramine (10 μM). Plates were incubated at room temperature for 10 h, prior to reading in a Trilux scintillation counter. The results were analyzed using an automatic spline fitting program (Multicalc, Packard, Milton Keynes, UK) to provide K_i values for each of the test compounds.

5.5. GTP-γ[^{35}S] binding studies

The method used was as described previously.[28] The dextral shift of the 5-HT dose–response curves for the binding of GTP-γ[^{35}S] to human 5-HT_{1B} or 5-HT_{1D} receptors stably expressed into LM(tk-) cells was measured and the results expressed as K_B values.

6. Experimental section

6.1. General information

All solvents and reagents were purchased from commercial sources, unless otherwise indicated. Solvents were used dry, either distilled before use (THF from Na/benzophenone ketyl) or purchased dry (DMF). All reactions were performed under positive pressure of nitrogen or argon. ^{1}H NMR and ^{13}C NMR data were recorded on a Bruker AC-200P or Bruker AC-300. Chemical shifts are reported in δ units (ppm) relative to tetramethylsilane or the solvent. Analytical thin-layer chromatography (TLC) was performed on 60 F_{254} silica gel (Merck). Chromatographic separations were performed using 230–400 mesh silica gel (Merck). Mass spectra were obtained on an Agilent 1100 series instrument. High resolution mass spectra (HRMS) were acquired on a Thermo Electron LTQ-FT Fourier Transform Ion Cyclotron Resonance mass spectrometer with an instrument resolution of 200,000, using external calibration. Sample ionization was achieved using Electrospray and measurement of pseudo-molecular $[M+H]^+$ ions was carried out. Infrared spectra (IR) were recorded on a Perkin–Elmer 1310 spectrophotometer. Melting points were measured with a capillary melting point apparatus and are uncorrected.

6.1.1. Ethyl (4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)acetate (7). Boron trifluoride etherate (0.148 mL, 1.17 mmol) was added dropwise at −78 °C to a solution of 2-(3-thienyl)ethanol (**5**) (0.150 g, 1.17 mmol) and ethyl 3,3-diethoxypropionate (**6**) (0.266 g, 1.40 mmol) in dichloromethane (6 mL). The resulting solution was allowed to warm to room temperature overnight. A brine/HCl mixture (1:1) was added and the aqueous phase was extracted with dichloromethane (3×). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. The crude product was purified by flash column chromatography on silica gel (hexane/EtOAc, 9:1) to afford pure **7** as a pale yellow oil (0.216 g, 82%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.15 (d, J = 5.0 Hz, 1H), 6.81 (d, J = 5.0 Hz, 1H), 5.27 (t, J = 7.0 Hz, 1H), 4.22 (q, J = 7.0 Hz, 2H), 4.2–4.0 (m, 1H), 3.8 (dt, J = 10.7, 4.0 Hz, 1H), 3.0–2.7 (m, 1H), 2.8 (d, J = 6.6 Hz, 2H), 2.7–2.5 (m, 1H), 1.29 (t, J = 7.0 Hz, 3H) ppm.

6.1.2. *tert*-Butyl-[2-(4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)ethoxy]dimethylsilane (8). To a solution of **7** (1.09 g, 4.86 mmol) in THF was slowly added DIBAL-*H* (11.67 mL, 1.0 M solution in THF, 11.67 mmol) at 0 °C and the mixture stirred for 1 h at room temperature before being quenched by careful addition of 3 N HCl at 0 °C. The resulting mixture was filtered through Celite, the phases separated and the aqueous phase extracted with dichloromethane (3×). The combined organic phases were dried ($MgSO_4$), filtered and evaporated to give crude 2-(4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)-ethanol as a yellowish oil, which was used without further purification. Imidazole (0.364 g, 5.30 mmol) and *tert*-butyldimethylsilyl chloride (0.800 g, 5.30 mmol) were added sequentially to a solution of 2-(4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)-ethanol (0.89 g, 4.86 mmol) dissolved in dry DMF (6 mL). After stirring at room temperature for 2 h, water was added and the aqueous phase extracted with diethyl ether (3×). The combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) to afford **8** as a yellow oil (1.30 g, 90% over two steps). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.12 (d, J = 5.0 Hz, 1H), 6.79 (d, J = 5.0 Hz, 1H), 5.0–4.8 (m, 1H), 4.16 (ddd, J = 11.3, 5.6, 2.2 Hz, 1H), 4.0–3.6 (m, 3H), 3.0–2.7 (m, 1H), 2.7–2.4 (m, 1H), 2.2–1.8 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H) ppm.

6.1.3. 7-(2-*tert*-Butyldimethylsilanyloxyethyl)-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxylic acid (9). *n*-Butyllithium (3.0 mL, 1.6 M solution in THF, 4.80 mmol) was added dropwise to a suspension of **8** (1.30 g, 4.36 mmol) in THF at −78 °C and the resulting solution stirred for 1 h. Gaseous carbon dioxide was bubbled through the solution at −78 °C for 10 min before allowing the reaction to warm to room temperature. Quenching with satd ammonium chloride (caution: exothermic reaction!), addition of a mixture of H_2O/brine/Et_2O (1:1:1) and extraction with diethyl ether (3×) afforded a yellowish solid, which was triturated with hexane and filtered to give pure **9** as a white solid (1.34 g, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.58 (s, 1H), 5.0–4.8 (m, 1H), 4.2 (ddd, J = 11.3, 5.6, 2.2 Hz, 1H), 4.0–3.6 (m, 3H), 3.0–2.7 (m, 1H), 2.7–2.5 (m, 1H), 2.2–1.8 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H) ppm.

6.1.4. 7-(2-Hydroxyethyl)-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (10). Carbonyl diimidazole (1.30 g, 8.05 mmol) was added to a suspension of **9** (1.10 g, 3.22 mmol) in THF (50 mL) and the resulting solution stirred at room temperature for 24 h. The solution was concentrated and the residue treated with a dioxane solution of ammonia (16.0 mmol). The resulting solution was stirred at room temperature for 24 h. The solvent was removed in vacuo and the residue partitioned between dichloromethane and water. The aqueous phase was extracted with dichloromethane (3×) and the combined organic layers were dried ($MgSO_4$),

filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (ethyl acetate 100%) to afford pure 7-[2-(*tert*-butyldimethylsilanyloxy)ethyl]-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide as a pale yellow oil (0.86 g, 78%). To a solution of the *tert*-butyldimethylsilyloxy intermediate (0.80 g, 2.35 mmol) in THF (16 mL) was added TBAF (2.58 mL, 1.0 M solution in THF, 2.58 mmol) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. The solvent was concentrated and the crude yellow oil purified by flash column chromatography on silica gel (methanol/dichloromethane, 5:95) to yield pure **10** as a white solid (0.40 g, 75%). The crude product could also be purified by trituration with a mixture of diethyl ether in hexanes (1:1). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.26 (s, 1H), 4.89 (dq, J = 7.9, 1.9 Hz, 1H), 4.14 (ddd, J = 11.5, 5.7, 2.2 Hz, 1H), 3.8–3.6 (m, 3H), 2.9–2.7 (m, 1H), 2.51 (dq, J = 16.0, 2.0 Hz, 1H), 2.1–1.8 (m, 2H) ppm.

6.1.5. 7-{2-[4-(6-Fluoro-1*H*-indol-3-yl)-3,6-dihydro-2*H*-pyridin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (4). To a solution of alcohol **10** (0.150 g, 0.66 mmol) in dry DMF (1 mL) were added sequentially, at 0 °C, triethylamine (0.18 mL, 1.32 mmol) and methanesulfonyl chloride (0.055 mL, 0.69 mmol). The resulting solution was stirred at room temperature for 1 h. The solvent was removed in vacuo and the crude mesylate intermediate was redissolved in dry acetonitrile (1.5 mL). Potassium carbonate (0.228 g, 1.65 mmol) and indole **2** (0.143 g, 0.66 mmol) were added to the above solution under inert atmosphere and the resulting mixture was heated at 80 °C for 20 h. Water was added and the aqueous phase was extracted with dichloromethane (3×). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to obtain pure **4** as a pale yellow solid (0.210 g, 75%). Mp: 184–185 °C. IR (cm^{-1}, KBr): 3478 and 3416 (NH), 1618 (CO). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 11.15 (broad s, 1H), 7.87 (broad s, 1H), 7.77 (dd, J = 8.8, 5.4 Hz, 1H), 7.48 (s, 1H), 7.36 (d, J = 2.3 Hz, 1H), 7.33 (broad s, 1H), 7.12 (dd, J = 10.0, 2.3 Hz, 1H), 6.86 (td, J = 9.3, 2.2 Hz, 1H), 6.10 (broad s, 1H), 4.9–4.7 (m, 1H), 4.2–4.1 (m, 1H), 3.8–3.6 (m, 1H), 3.12 (broad s, 2H), 2.8–2.5 (m, 8H), 2.1–1.8 (m, 2H) ppm.

6.2. General procedure for the synthesis of naphthalen-1-yl-trifluoromethanesulfonates 12a–c

To a solution of tetralone (1 equiv) in THF (1.2 mL/mmol tetralone **11**) cooled to −78 °C was added LiHMDS (1.2 equiv) in THF (6 mL/mmol tetralone) and the resulting solution was stirred for 1 h. N-Ph-triflimide (1.2 equiv) was added in one portion to the above solution and the reaction allowed to warm to room temperature. Stirring was continued for 2 h and after solvent removal, the residue was dissolved in ethyl acetate and washed with 2 N NaOH. The organic phase was dried ($MgSO_4$), filtered and evaporated. The crude product was purified on a silica gel column (hexanes 100%) to afford pure product. DDQ (1.5 equiv) was then added to a solution of the 3,4-dihydronaphthalen-1-yl trifluoromethanesulfonate (1 equiv) in dioxane (7 mL/mmol triflate), and the resulting suspension heated at reflux for 2 days. Most of the dioxane was removed by evaporation in vacuo and the residue was passed through a silica gel chromatography column (hexanes 100%) to afford the desired product.

6.2.1. 6-Methoxynaphthalen-1-yl trifluoromethanesulfonate (12a). Colourless oil, 82%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.90 (d, J = 9.0 Hz, 1H), 7.34 (t, J = 8.0 Hz, 1H), 7.3–7.1 (m, 2H), 7.09 (d, J = 2.8 Hz, 1H), 3.82 (s, 3H) ppm.

6.2.2. 7-Methoxynaphthalen-1-yl trifluoromethanesulfonate (12b). Colourless oil, 84%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.8–7.7 (m, 2H), 7.5–7.2 (m, 4H), 3.96 (s, 3H) ppm.

6.2.3. 6-Fluoronaphthalen-1-yl trifluoromethanesulfonate (12c). Yellow oil, 43%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.10 (dd, J = 9.2, 5.3 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.6–7.3 (m, 4H) ppm.

6.2.4. 7-Fluoronaphthalen-1-yl trifluoromethanesulfonate (12d). *t*-BuOK (1.31 g, 11.75 mmol) was added in one portion to a solution of 7-fluoronaphthol (**11d**) (1.73 g, 10.68 mmol) in THF (60 mL) at 0 °C. The cooling bath was removed and the yellow solution stirred for 15 min at room temperature. N-Ph-triflimide (4.20 g, 11.75 mmol) was added in one portion to the above solution and the resulting orange mixture was stirred for 1 h at room temperature. The reaction was quenched by addition of satd aqueous potassium carbonate and the resulting suspension was stirred for 30 min before adding hexane. The organic phase was washed with satd potassium carbonate (5×), dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (hexanes 100%) to give **12d** as a yellow oil (3.11g, 99%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.93 (dd, J = 9.0, 5.4 Hz, 1H), 7.89 (dd, J = 6.0, 1.7 Hz, 1H), 7.68 (dd, J = 9.8, 2.5 Hz, 1H), 7.39 (td, J = 8.6, 2.5 Hz, 1H), 7.6–7.4 (m, 2H) ppm.

6.3. General procedure for the synthesis of naphthylpiperazines 14a–o

Piperazine **13** (1.2 equiv), (*R*)-BINAP (7.5%), $Pd(OAc)_2$ (5%) and Cs_2CO_3 (1.4 equiv) were added to a solution of triflate (or bromide) **12** (1 equiv) in toluene (0.5 M). The resulting suspension was heated at 110 °C for 16 h. Upon cooling, the mixture was filtered through a short Celite pad (EtOAc), the filtrate concentrated and the crude product purified by flash column chromatography on silica gel. Cbz-protected naphthylpiperazines were deprotected by catalytic hydrogenation in the presence of 10% Pd/C (0.05 equiv) under a hydrogen atmosphere (1 atm) to give the desired unprotected naphthylpiperazines. The final products were purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 7:3) to afford the pure desired products.

6.3.1. 1-(Naphthalen-1-yl)-3-(*S*)-methylpiperazine (14a). Yellow solid, 74%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.3–8.1 (m, 1H), 7.9–7.7 (m, 1H), 7.6–7.3 (m, 4H), 7.07 (d, J = 7.3 Hz, 1H), 3.3–3.2 (m, 5H), 3.0–2.7 (m, 1H), 2.6–2.4 (m, 1H), 1.18 (d, J = 6.1 Hz, 3H) ppm.

6.3.2. 1-(Naphthalen-1-yl)-3-(*R*)-methylpiperazine (14b). Yellow solid, 70%. ^{1}H NMR consistent with data for **14a**.

6.3.3. 1-(6-Fluoronaphthalen-1-yl)piperazine (14c). Pale yellow solid, 79%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.22 (dd, J = 9.3, 5.8 Hz, 1H), 7.5–7.4 (m, 3H), 7.3–7.2 (m, 1H), 7.04 (dd, J = 6.9, 1.6 Hz, 1H), 3.2–3.0 (m, 8H) ppm.

6.3.4. 1-(7-Fluoronaphthalen-1-yl)piperazine (14d). Yellow solid, 56%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.9–7.7 (m, 2H), 7.55 (d, J = 8.2 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.3–7.2 (m, 1H), 7.13 (d, J = 7.4 Hz, 1H), 3.2–3.0 (m, 8H) ppm.

6.3.5. 1-(6-Methoxynaphthalen-1-yl)piperazine (14e). Pale yellow solid, 72%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.13 (d, J = 10.3 Hz, 1H), 7.5–7.3 (m, 2H), 7.2–7.1 (m, 2H), 6.96 (dd, J = 7.1, 1.4 Hz, 1H), 3.91 (s, 3H), 3.2–3.0 (m, 8H) ppm.

6.3.6. 1-(7-Methoxynaphthalen-1-yl)piperazine (14f). Pale yellow solid, 80%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.73 (d, J = 8.9 Hz, 1H), 7.56 (d, J = 2.6 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.3–7.2 (m, 1H), 7.2–7.1 (m, 2H), 3.94 (s, 3H), 3.2–3.1 (m, 8H) ppm.

6.3.7. 1-(6-Fluoronaphthalen-1-yl)-3-(*S*)-methylpiperazine (14g). Dark red oil, 68%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.20 (dd, J = 9.3, 5.8 Hz, 1H), 7.5–7.3 (m, 3H), 7.22 (ddd, J = 9.2, 8.4, 2.6 Hz, 1H), 6.99 (dd, J = 6.8, 1.6 Hz, 1H), 3.2–3.0 (m, 5H), 2.77 (td, J = 11.6, 2.8 Hz, 1H), 2.44 (t, J = 10.8 Hz, 1H), 1.96 (broad s, 1H), 1.10 (d, J = 6.3 Hz, 3H) ppm.

6.3.8. 1-(6-Fluoronaphthalen-1-yl)-3-(*R*)-methylpiperazine (14h). Dark red oil, 30%. ^{1}H NMR consistent with data for **14g**.

6.3.9. 1-(6-Methoxynaphthalen-1-yl)-3-(*S*)-methylpiperazine (14i). Yellow solid, 62%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.2–8.1 (m, 1H), 7.5–7.3 (m, 2H), 7.3–7.1 (m, 2H), 6.94 (dd, J = 7.1, 1.4 Hz, 1H), 3.91 (s, 3H), 3.3–3.1 (m, 5H), 2.9–2.7 (m, 1H), 2.5–2.4 (m, 1H), 1.92 (broad s, 1H), 1.12 (d, J = 6.3 Hz, 3H) ppm.

6.3.10. 1-(6-Methoxynaphthalen-1-yl)-3-(*R*)-methylpiperazine (14j). Yellow solid, 73%. ^{1}H NMR consistent with data for **14i**.

6.3.11. 1-(7-Fluoronaphthalen-1-yl)-3-(*S*)-methylpiperazine (14k). Orange oil, 48%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.9–7.7 (m, 2H), 7.56 (d, J = 8.2 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.24 (td, J = 8.6, 2.7 Hz, 1H), 7.14 (d, J = 7.4 Hz, 1H), 3.5–3.2 (m, 7H), 3.0–2.8 (m, 1H), 2.6–2.5 (m, 1H), 1.21 (d, J = 6.3 Hz, 3H) ppm.

6.3.12. 1-(7-Fluoronaphthalen-1-yl)-3-(*R*)-methylpiperazine (14l). Dark red oil, 47%. ^{1}H NMR consistent with data for **14k**.

6.3.13. 1-(7-Methoxynaphthalen-1-yl)-3-(*S*)-methylpiperazine (14m). Yellow solid, 72%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.73 (d, J = 8.9 Hz, 1H), 7.6–7.4 (m, 2H), 7.3–7.2 (m, 2H), 7.2–7.0 (m, 2H), 3.94 (s, 3H), 3.3–3.1 (m, 5H), 3.0–2.8 (m, 1H), 2.6–2.4 (m, 1H), 1.96 (broad s, 1H), 1.18 (d, J = 6.2 Hz, 3H) ppm.

6.3.14. 1-(7-Methoxynaphthalen-1-yl)-3-(*R*)-methylpiperazine (14n). Yellow solid, 70%. ^{1}H NMR consistent with data for **14m**.

6.3.15. 1-(6-Fluoronaphthalen-1-yl)-3,3-dimethylpiperazine (14o). Dark red oil, 14%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.15 (dd, J = 9.3, 5.7 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.5–7.4 (m, 2H), 7.27 (ddd, J = 9.2, 8.3, 2.6 Hz, 1H), 7.08 (d, J = 6.7 Hz, 1H), 3.47 (broad s, 2H), 3.25 (broad s, 2H), 3.05 (s, 2H), 1.61 (broad s, 6H) ppm.

6.4. General procedure for the synthesis of final analogous 15a–o

To a solution of the amide-alcohol **10** (1 equiv) in DMF (0.3 M) was added methanesulfonyl chloride (1.05 equiv) at 0 °C and the resulting yellowish solution was stirred at room temperature for 1 h. The DMF was removed in vacuo and the residue redissolved in CH_3CN (0.2 M). Potassium carbonate (2 equiv) and the appropriately substituted naphthylpiperazine (1.1 equiv) were added to the above solution and the resulting suspension heated at 80 °C for 24 h. Water was added and the aqueous phase was extracted with CH_2Cl_2 (3×). The combined organic phases were dried ($MgSO_4$), filtered and concentrated. The crude was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 95:5) to afford the pure coupled product.

6.4.1. 7-{2-[2-(*S*)-Methyl-4-(naphthalen-1-yl)-piperazin-1-yl]ethyl}4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15a). White solid, 62%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.3–8.1 (m, 1H), 7.9–7.7 (m, 1H), 7.6–7.3 (m, 4H), 7.25 (s, 1H), 7.07 (dd, J = 7.3, 0.9 Hz, 1H), 5.74 (broad s, 2H), 4.9–4.8 (m, 1H), 4.21 (dd, J = 9.3, 5.5 Hz, 1H), 3.76 (td, J = 10.8, 3.9 Hz, 1H), 3.2–2.5 (m, 11H), 2.1–2.0 (m, 2H), 1.18 (dd, J = 6.0, 1.8 Hz, 3H) ppm. HRMS (M+1): calcd for $C_{25}H_{29}N_3O_2S$ 436.20532. Found 436.20530.

6.4.2. 7-{2-[2-(*R*)-Methyl-4-(naphthalen-1-yl)piperazin-1-yl]ethyl}4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15b). White solid, 59%. IR (cm^{-1}, KBr): 3341 and 3186 (NH), 1608 (CO). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.3–8.1 (m, 1H), 7.9–7.7 (m, 1H), 7.6–7.3 (m, 4H), 7.26 (s, 1H), 7.07 (dd, J = 7.3, 0.8 Hz, 1H), 5.96 (broad s, 2H), 4.9–4.8 (m, 1H), 4.3–4.1 (m, 1H), 3.76 (td, J = 10.5, 3.9 Hz, 1H), 3.2–2.5 (m, 11H), 2.1–2.0 (m, 2H), 1.19 (dd, J = 6.0, 1.9 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 163.9, 149.5, 144.0 (isomer 1), 143.8 (isomer 2), 134.9, 134.7, 134.5, 129.4, 128.9, 128.4, 125.9, 125.8,

125.3, 123.5, 123.4, 114.7, 73.6, 64.2, 60.3 (isomer 1), 60.2 (isomer 2), 55.3, 53.2, 51.7, 49.4 (isomer 1), 49.2 (isomer 2), 33.6, 26.1, 16.4 ppm. HRMS (M+1): calcd for $C_{25}H_{29}N_3O_2S$ 436.20533. Found 436.20526.

6.4.3. 7-{2-[4-(6-Fluoronaphthalen-1-yl)piperazin-1-yl]-ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15c). Pale yellow solid, 50%. Mp: 192–193 °C. IR (cm^{-1}, KBr): 3342 and 3169 (NH), 1611 (CO). ^1H NMR ($CDCl_3$, 300 MHz): δ 8.18 (dd, $J = 9.1$, 5.8 Hz, 1H), 7.5–7.3 (m, 3H), 7.3–7.2 (m, 2H), 7.04 (d, $J = 6.8$ Hz, 1H), 5.84 (broad s, 2H), 4.88 (broad s, 1H), 4.21 (dd, $J = 10.9$, 3.6 Hz, 1H), 3.76 (td, $J = 10.9$, 3.6 Hz, 1H), 3.2–2.9 (m, 3H), 2.9–2.5 (m, 11H), 2.2–2.0 (m, 2H) ppm. HRMS (M+1): calcd for $C_{24}H_{26}FN_3O_2S$ 440.18025. Found 440.18020.

6.4.4. 7-{2-[4-(7-Fluoronaphthalen-1-yl)piperazin-1-yl]-ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15d). White solid, 46%. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.8–7.7 (m, 2H), 7.55 (d, $J = 8.0$ Hz, 1H), 7.36 (t, $J = 7.5$ Hz, 1H), 7.27 (s, 1H), 7.23 (td, $J = 8.6$, 2.9 Hz, 1H), 7.14 (d, $J = 7.4$ Hz, 1H), 5.62 (broad s, 2H), 5.0–4.8 (m, 1H), 4.22 (ddd, $J = 11.5$, 8.1, 2.5 Hz, 1H), 3.78 (td, $J = 10.8$, 4.0 Hz, 1H), 3.2–3.0 (m, 4H), 2.9–2.5 (m, 8H), 2.2–2.0 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 164.2, 160.6 (d, $J = 240$ Hz), 149.2 (isomer 1), 149.1 (isomer 2), 143.6, 135.1, 134.5, 130.7 (d, $J = 10$ Hz), 129.9, 129.4, 125.0, 123.4, 116.2, 116.0 (d, $J = 25$ Hz), 115.8, 107.1 (d, $J = 25$ Hz), 73.4, 64.1, 54.2, 53.7, 52.7 (2C), 50.4, 34.2, 26.0 ppm. HRMS (M+1): calcd for $C_{24}H_{26}FN_3O_2S$ 440.18025. Found 440.18029.

6.4.5. 7-{2-[4-(6-Methoxynaphthalen-1-yl)-piperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15e). Pale yellow solid, 55%. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.09 (d, $J = 9.9$ Hz, 1H), 7.4–7.3 (m, 2H), 7.24 (s, 1H), 7.2–7.1 (m, 2H), 6.94 (d, $J = 6.4$ Hz, 1H), 6.12 (broad s, 2H), 4.9–4.8 (m, 1H), 4.2–4.1 (m, 1H), 3.89 (s, 3H), 3.74 (td, $J = 10.7$, 3.7 Hz, 1H), 3.1–3.0 (m, 4H), 2.9–2.6 (m, 8H), 2.1–2.0 (m, 2H) ppm. HRMS (M+1): calcd for $C_{25}H_{29}N_3O_3S$ 452.20024. Found 452.20018.

6.4.6. 7-{2-[4-(7-Methoxynaphthalen-1-yl)piperazin-1-yl]-ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15f). Pale yellow solid, 60%. IR (cm^{-1}, KBr): 3327 and 3191 (NH), 1652 (CO). ^1H NMR ($CDCl_3$, 200 MHz): δ 7.73 (d, $J = 8.8$ Hz, 1H), 7.51 (s, 1H), 7.3–7.2 (m, 3H), 7.1–7.0 (m, 2H), 5.7 (broad s, 2H), 5.0–4.8 (m, 1H), 4.22 (dd, $J = 10.6$, 5.4 Hz, 1H), 3.94 (s, 3H), 3.77 (td, $J = 10.8$, 3.4 Hz, 1H), 3.3–3.0 (m, 4H), 2.9–2.6 (m, 6H), 2.2–2.0 (m, 2H) ppm.

6.4.7. 7-{2-[4-(6-Fluoronaphthalen-1-yl)-2-(*S*)-methyl-piperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15g). Pale brown solid, 40%. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, $J = 9.2$, 5.8 Hz, 1H), 7.5–7.3 (m, 3H), 7.26 (s, 1H), 7.22 (td, $J = 9.0$, 2.5 Hz, 1H), 7.01 (dd, $J = 6.9$, 1.4 Hz, 1H), 6.19 (broad s, 2H), 4.9–4.8 (m, 1H), 4.3–4.2 (m, 1H), 3.75 (td, $J = 10.6$, 3.8 Hz, 1H), 3.2–2.5 (m, 11H), 2.1–2.0 (m, 2H), 1.17 (dd, $J = 5.9$, 1.7 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 164.1, 160.6 (d, $J = 245$ Hz), 149.8, 144.0 (isomer 1), 143.8 (isomer 2), 135.6 (d, $J = 10$ Hz), 135.0, 134.4, 129.3, 127.1, 126.3 (d, $J = 10$ Hz), 125.9, 122.6 (d, $J = 5$ Hz), 115.2 (d, $J = 25$ Hz), 114.0, 111.2 (d, $J = 20$ Hz), 73.5, 64.1, 60.4 (isomer 1), 60.3 (isomer 2), 55.2, 53.2, 51.6, 49.3 (isomer 1), 49.1 (isomer 2), 33.6, 26.0, 16.4 ppm. HRMS (M+1): calcd for $C_{25}H_{28}FN_3O_2S$ 454.19590. Found 454.19590.

6.4.8. 7-{2-[4-(6-Fluoronaphthalen-1-yl)-2-(*R*)-methyl-piperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15h). Pale brown solid, 23%. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, $J = 9.2$, 5.8 Hz, 1H), 7.5–7.3 (m, 3H), 7.26 (s, 1H), 7.22 (td, $J = 8.9$, 2.5 Hz, 1H), 7.02 (dd, $J = 6.8$, 1.3 Hz, 1H), 6.14 (broad s, 2H), 4.9–4.8 (m, 1H), 4.3–4.1 (m, 1H), 3.75 (td, $J = 10.6$, 3.9 Hz, 1H), 3.2–2.5 (m, 11H), 2.1–1.9 (m, 2H), 1.17 (dd, $J = 5.9$, 1.6 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 164.0, 160.6 (d, $J = 245$ Hz), 149.8, 144.0 (isomer 1), 143.8 (isomer 2), 135.6 (d, $J = 10$ Hz), 135.0, 134.4, 129.3, 127.1, 126.3 (d, $J = 10$ Hz), 125.9, 122.6 (d, $J = 5$ Hz), 115.2 (d, $J = 25$ Hz), 114.0, 111.2 (d, $J = 20$ Hz), 73.5, 64.2, 60.4 (isomer 1), 60.3 (isomer 2), 55.2, 53.3, 51.6, 49.3 (isomer 1), 49.1 (isomer 2), 33.6, 26.0, 16.2 ppm. HRMS (M+1): calcd for $C_{25}H_{28}FN_3O_2S$ 454.19590. Found 454.19582.

6.4.9. 7-{2-[4-(6-Methoxynaphthalen-1-yl)-2-(*S*)-methyl-piperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15i). Pale brown solid, 52%. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.12 (d, $J = 9.4$ Hz, 1H), 7.5–7.3 (m, 2H), 7.25 (s, 1H), 7.2–7.1 (m, 1H), 7.11 (s, 1H), 6.93 (dd, $J = 7.2$, 1.1 Hz, 1H), 6.25 (s, 2H), 4.9–4.8 (m, 1H), 4.19 (dd, $J = 11.3$, 4.1 Hz, 1H), 3.90 (s, 3H), 3.73 (td, $J = 10.8$, 3.8 Hz, 1H), 3.2–2.5 (m, 11H), 2.1–1.9 (m, 2H), 1.16 (dd, $J = 5.9$, 1.7 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 164.1, 157.5, 149.7, 143.9 (isomer 1), 143.7 (isomer 2), 136.0, 135.0, 134.4, 129.4, 126.5, 125.2, 124.1, 122.3, 117.7, 112.6, 106.4, 73.5, 64.1, 60.4 (isomer 1), 60.2 (isomer 2), 55.2, 53.2, 51.5, 49.4 (isomer 1), 49.1 (isomer 2), 33.5, 26.0, 16.2 ppm. HRMS (M+1): calcd for $C_{26}H_{31}N_3O_3S$ 466.21589. Found 466.21586.

6.4.10. 7-{2-[4-(6-Methoxynaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15j). Pale brown solid, 53%. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.12 (d, $J = 8.9$ Hz, 1H), 7.5–7.3 (m, 2H), 7.25 (s, 1H), 7.2–7.1 (m, 1H), 7.11 (s, 1H), 6.93 (dd, $J = 7.1$, 1.2 Hz, 1H), 6.28 (broad s, 2H), 4.9–4.8 (m, 1H), 4.19 (dd, $J = 11.4$, 4.0 Hz, 1H), 3.89 (s, 3H), 3.73 (td, $J = 10.8$, 3.8 Hz, 1H), 3.2–2.5 (m, 11H), 2.1–1.9 (m, 2H), 1.16 (d, $J = 5.1$ Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 164.2, 157.5, 149.7, 143.9 (isomer 1), 143.7 (isomer 2), 136.0, 135.1, 134.4, 129.3, 126.5, 125.2, 124.1, 122.3, 117.7, 112.6, 106.4, 73.5, 64.1, 60.4 (isomer 1), 60.2 (isomer 2), 55.2, 53.2, 51.6, 49.4 (isomer 1), 49.1 (isomer 2), 33.5, 26.0, 16.2 ppm. HRMS (M+1): calcd for $C_{26}H_{31}N_3O_3S$ 466.21589. Found 466.21584.

6.4.11. 7-{2-[4-(7-Fluoronaphthalen-1-yl)-2-(*S*)-methyl-piperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15k). Pale yellow solid, 42%. ^1H NMR

($CDCl_3$, 200 MHz): δ 7.9–7.7 (m, 2H), 7.53 (d, J = 8.2 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.27 (s, 1H), 7.21 (td, J = 8.8, 2.6 Hz, 1H), 7.11 (d, J = 7.4 Hz, 1H), 5.96 (broad s, 2H), 4.9–4.8 (m, 1H), 4.22 (dd, J = 11.5, 4.3 Hz, 1H), 3.76 (td, J = 10.5, 4.0 Hz, 1H), 3.2–2.5 (m, 11H), 2.1–2.0 (m, 2H), 1.18 (d, J = 4.5 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 163.9, 160.6 (d, J = 245 Hz), 149.2, 144.0 (isomer 1), 143.8 (isomer 2), 134.9, 134.4 (d, J = 5 Hz), 131.6, 130.7 (d, J = 10 Hz), 130.1 (d, J = 10 Hz), 129.5, 125.1, 123.4, 115.8 (d, J = 30 Hz), 115.8, 107.2 (d, J = 20 Hz), 73.5, 64.2, 60.2 (isomer 1), 60.1 (isomer 2), 55.2, 53.0, 51.6, 49.4 (isomer 1), 49.1 (isomer 2), 33.6, 26.1, 16.2 ppm. HRMS (M+1): calcd for $C_{25}H_{28}FN_3O_2S$ 454.19590. Found 454.19584.

6.4.12. 7-{2-[4-(7-Fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15l). Pale yellow solid, 40%. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.9–7.7 (m, 2H), 7.53 (d, J = 8.2 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.27 (s, 1H), 7.22 (td, J = 8.7, 2.5 Hz, 1H), 7.11 (d, J = 7.3 Hz, 1H), 5.98 (broad s, 2H), 4.9–4.8 (m, 1H), 4.21 (dd, J = 11.1, 3.6 Hz, 1H), 3.76 (td, J = 10.8, 3.9 Hz, 1H), 3.2–2.5 (m, 11H), 2.2–2.0 (m, 2H), 1.18 (d, J = 5.3 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 163.9, 160.6 (d, J = 245 Hz), 149.2, 144.0 (isomer 1), 143.8 (isomer 2), 134.9, 134.5, 131.6, 130.7 (d, J = 10 Hz), 130.1 (d, J = 10 Hz), 129.5, 125.1, 123.4, 116.0 (d, J = 25 Hz), 115.8, 107.2 (d, J = 20 Hz), 73.5, 64.2, 60.2 (isomer 1), 60.1 (isomer 2), 55.2, 53.1, 51.6, 49.4 (isomer 1), 49.1 (isomer 2), 33.6, 26.1, 16.2 ppm. HRMS (M+1): calcd for $C_{25}H_{28}FN_3O_2S$ 454.19590. Found 454.19591.

6.4.13. 7-{2-[4-(7-Methoxynaphthalen-1-yl)-2-(*S*)-methylpiperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15m). Pale brown solid, 55%. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.76 (d, J = 9.0 Hz, 1H), 7.6–7.51 (m, 2H), 7.35–7.26 (m, 2H), 7.21–7.11 (m, 2H), 5.82 (broad s, 2H), 4.94–4.91 (m, 1H), 4.29–4.22 (m, 1H), 3.98 (s, 3H), 3.81 (td, J = 10.5, 3.8 Hz, 1H), 3.27–2.60 (m, 11H), 2.2–2.0 (m, 2H), 1.27 (broad s, 3H).

6.4.14. 7-{2-[4-(7-Methoxynaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15n). Pale brown solid, 53%. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.76 (d, J = 9.0 Hz, 1H), 7.6–7.51 (m, 2H), 7.35–7.26 (m, 2H), 7.21–7.11 (m, 2H), 5.75 (broad s, 2H), 4.94–4.91 (m, 1H), 4.25–4.20 (m, 1H), 3.93 (s, 3H), 3.77 (td, J = 10.5, 3.8 Hz, 1H), 3.27–2.60 (m, 11H), 2.2–2.0 (m, 2H), 1.27 (broad s, 3H).

6.4.15. 7-{2-[4-(6-Fluoronaphthalen-1-yl)-2,2-dimethylpiperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (15o). ^1H NMR ($CDCl_3$, 200 MHz): Pale brown solid, 14%. Mp: 89–90 °C. IR (cm^{-1}, NaCl): 3337 and 3188 (NH), 1653 (CO), 1605, 1579, 1466, 1431, 1373, 787, 761. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.8.29 (dd, J = 9.3, 5.9 Hz, 1H), 7.49–7.36 (m, 3H), 7.28–7.18 (m, 2H), 7.03 (dd, J = 6.9, 1.5 Hz, 1H), 5.70 (broad s, 2H), 4.93–4.88 (m, 1H), 4.22 (ddd, J = 11.6, 5.9, 2.2 Hz, 1H), 3.77 (ddd, J = 11.4, 10.5, 4.0 Hz, 1H), 3.13–2.76 (m, 8H), 2.70–2.50 (m, 2H), 2.04–1.91 (m, 2H), 1.25 (s, 3H), 1.21 (s, 3H). IR (NaCl, cm^{-1}): 3337, 3188, 1653, 1605, 1579, 1466, 1431, 1373, 787, 761.

6.4.16. 4-{2-[4-(6-Fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-6,7-dihydro-4*H*-thieno[3,2-*c*]pyran-2-carboxamide (15p). Compound **15p** was prepared from [3,2-*c*]-thienopyran as a pale pink solid in 34% yield by the same procedure as described for **4**. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, J = 9.2, 5.8 Hz, 1H), 7.5–7.1 (m, 5H), 7.03 (d, J = 6.9 Hz, 1H), 4.8–4.6 (m, 1H), 4.24 (ddd, J = 11.0, 5.5, 2.6 Hz, 1H), 3.77 (ddd, J = 11.4, 9.6, 3.9 Hz, 1H), 3.2–2.5 (m, 11H), 2.2–1.9 (m, 2H), 1.16 (d, J = 5.0 Hz, 3H). MS: 454 (M^++1). HRMS (M+1): calcd for $C_{25}H_{28}FN_3O_2S$ 454.19590. Found 454.19580.

6.4.17. 7-{2-[4-(6-Fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-7-methyl-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxylic acid amide (16). Compound **16** was prepared, starting from 2-(3-thienyl)ethanol and ethyl acetoacetate, in an overall yield of 41% yield (white solid, mixture of both isomers) by the same procedure as described for **4**. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.2–8.1 (m, 1H), 7.5–7.3 (m, 3H), 7.3–7.1 (m, 2H), 7.01 (dd, J = 7.0 and 1.4 Hz, 1H), 5.87 (broad s, 2H), 4.0–3.8 (m, 2H), 3.2–2.6 (m, 11H), 2.1–2.0 (m, 2H), 1.58 (s, 3H), 1.10 (d, J = 5.8 Hz, 3H).

The diastereomers were separated using a Chiralcel OJ column (10 μm, 20 × 250 mm) under isocratic conditions (solvent system: hexane/0.2% DMEA in ethanol, 80:20; flow rate of 0.5 mL/min). Isomer A: de > 99%, purity > 98%, retention time 16.7 min; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.16 (dd, J = 9.4, 6.0 Hz, 1H), 7.86 (br s, 1H), 7.65 (dd, J = 10.5, 2.6 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.5–7.4 (m, 2H), 7.37 (dd, J = 9.0, 2.6 Hz, 1H), 7.30 (br s, 1H), 7.05 (d, J = 7.5 Hz, 1H), 4.0–3.75 (m, 2H), 3.15–3.05 (m, 2H), 2.95–2.77 (m, 2H), 2.76–2.45 (m, 6H), 2.1–1.85 (m, 2H), 1.49 (s, 3H), 1.01 (d, J = 6.0 Hz, 3H). MS: 468 (M^++1). HRMS (M+1): calcd for $C_{26}H_{30}FN_3O_2S$ 468.21155. Found 468.21144.

Isomer B: retention time 18.3 min (contaminated with 15% of Isomer A); ^1H NMR (DMSO-d_6, 300 MHz): δ 8.16 (dd, J = 9.4, 6.0 Hz, 1H), 7.86 (br s, 1H), 7.67 (dd, J = 10.2, 2.6 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.5–7.4 (m, 2H), 7.36 (dd, J = 9.0, 2.6 Hz, 1H), 7.31 (br s, 1H), 7.06 (d, J = 7.5 Hz, 1H), 4.0–3.75 (m, 2H), 3.15–3.0 (m, 2H), 2.95–2.7 (m, 2H), 2.75–2.5 (m, 5H), 2.3–2.1 (m, 1H), 2.1–1.85 (m, 2H), 1.48 (s, 3H), 1.01 (d, J = 6.0 Hz, 3H). MS: 468 (M^++1). HRMS (M+1): calcd for $C_{26}H_{30}FN_3O_2S$ 468.21155. Found 468.21149.

6.4.18. 7-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-3-methyl-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxylic acid (17a). To a solution of TMEDA (0.6 mL, 3.99 mmol) in dry THF (2 mL), under an inert atmosphere, was added *sec*-BuLi (2.8 mL, 1.3 M in cyclohexane/hexane, 3.65 mmol) at −78 °C. After 10 min, a solution of **9** (0.390 g, 1.14 mmol) in THF (10 mL) was added to the above solution and the resulting mixture stirred at

−78 °C for 90 min. Neat iodomethane (0.21 mL, 3.42 mmol) was then added and the reaction mixture allowed to warm gradually to room temperature (approx. 4 h). The reaction was quenched by addition of satd NH_4Cl and the aqueous phase was extracted with CH_2Cl_2 (3×). The combined organic phases were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) to obtain 0.250 g (62% yield) of pure **17a** as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.1–5.0 (m, 1H), 4.32 (ddd, J = 9.5, 6.5 and 2.7 Hz, 1H), 4.0–3.7 (m, 3H), 2.7–2.3 (m, 2H), 2.26 (s, 3H), 2.0–1.6 (m, 2H), 0.92 (s, 9H), 0.10 (s, 6H).

6.4.19. 7-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-3-chloro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxylic acid (17b). Compound **17b** was prepared following the same procedure as described for the synthesis of **17a**, starting with 9 (1.00 g, 2.92 mmol) and using *N*-chlorosuccinimide (1.36 g, 10.23 mmol) as the electrophile. The initial product was contaminated with succinimide so this mixture was diluted in hexanes and filtered. The filtrate was evaporated to dryness to yield 0.530 g (48% yield) of pure **17b** as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.0–4.7 (m, 1H), 4.3–4.1 (m, 1H), 4.0–3.6 (m, 3H), 2.9–2.4 (m, 2H), 2.1–1.7 (m, 2H), 0.89 (s, 9H), 0.07 (s, 6H).

6.4.20. 7-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-3-fluoro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxylic acid (17c). A solution of **9** (1.00 g, 2.92 mmol) in THF (30 mL), under an inert atmosphere, was treated with *t*-BuLi (6.23 mL, 1.5 M in hexanes, 9.34 mmol) at −78 °C for 1 h. Solid *N*-fluorobenzenesulfonimide (NFSI, 2.95 g, 9.34 mmol) was added to the above solution and the resulting reaction mixture was allowed to warm to room temperature over 2 h. The reaction was quenched by addition of aq 1 M HCl until approx. pH 3. The aqueous layer was extracted with CH_2Cl_2 (2×) and the organic extracts dried (Na_2SO_4), filtered and evaporated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) affording 0.585 g of **17c** contaminated with some starting material **9** (ratio 4/1 by ^{1}H NMR), which was used in the next step without purification. The yield was estimated at 35%.

6.4.21. 7-(2-Hydroxyethyl)-3-methyl-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (18a). Compound **18a** was prepared from **17a** (0.50 g, 1.41 mmol) in 86% yield (0.29 g, white solid) following the same procedure as described for the synthesis of **10**. ^{1}H NMR (CD_3OD, 200 MHz): δ 4.8–4.7 (m, 1H), 4.3–4.1 (m, 1H), 3.9–3.6 (m, 3H), 2.8–2.6 (m, 1H), 2.5–2.4 (m, 1H), 2.33 (s, 3H), 2.1–1.8 (m, 2H).

6.4.22. 7-(2-Hydroxyethyl)-3-chloro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (18b). A solution of **17b** (0.685 g, 1.82 mmol) and triethylamine (0.5 mL, 3.63 mmol) in THF (6 mL), under an inert atmosphere, was treated with methanesulfonyl chloride (0.17 mL, 2.18 mmol) at 0 °C. The resulting solution was stirred for 30 min before 0.5 M solution of ammonia in dioxane (18.2 mL, 9.1 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 24 h. Aq 1 M HCl was then added until approx. pH 2 and the aqueous phase was extracted with CH_2Cl_2 (3×). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated. The crude mixture was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 9.5:0.5) to afford the silyloxycarboxamide intermediate as a white solid. This intermediate, following the same procedure as described for the synthesis of **10**, was transformed into **18b** (0.251 g, 53% yield, white solid). ^{1}H NMR (CD_3OD, 200 MHz): δ 5.0–4.8 (m, 1H), 4.3–4.1 (m, 1H), 3.9–3.6 (m, 3H), 2.8–2.4 (m, 2H), 2.2–1.8 (m, 2H).

6.4.23. 7-(2-Hydroxyethyl)-3-fluoro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (18c). Compound **18c** was prepared from **17c** (0.58 g, 1.77 mmol) in 47% yield (0.15 g, white solid) following the same procedure as described for the synthesis of **18b**. ^{1}H NMR (CD_3OD, 200 MHz): δ 4.9–4.8 (m, 1H), 4.3–4.1 (m, 1H), 3.9–3.6 (m, 3H), 2.8–2.4 (m, 2H), 2.1–1.7 (m, 2H).

6.5. Synthesis of analogous 19

Analogs **19** were prepared as a mixture of diastereomers from the intermediate alcohol **18** and the piperazine **14j**, following the same procedure as described for the synthesis of **4** (pale brown solids; yield: **19a**, 70%; **19b**, 65%; **19c**, 51%). The diastereomers were separated using a Chiralcel OD column (10 μm, 4.6 × 250 mm) under isocratic conditions (solvent system: hexane/0.2% DMEA in 2-propanol, 1:4; flow rate of 1.3 mL/min for **19a** and 0.75 mL/min for **19b** and **19c**); all de > 99% and purity > 98%.

6.5.1. 7-{2-[4-(7-Fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-3-methyl-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (19a). Isomer A: retention time 5.71 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.23 (dd, J = 9.0, 5.7 Hz, 1H), 7.6–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.06 (d, J = 7.2 Hz, 1H), 5.57 (broad s, 2H), 4.9–4.8 (m, 1H), 4.4–4.2 (m, 1H), 3.80 (td, J = 10.8, 3.9 Hz, 1H), 3.3–2.9 (m, 5H), 2.9–2.5 (m, 6H), 2.43 (s, 3H), 2.2–1.9 (m, 2H), 1.23 (d, J = 6.3 Hz, 3H). MS: 468 (M^++1). HRMS (M+1): calcd for $C_{26}H_{30}FN_3O_2S$ 468.21155. Found 468.21149.

Isomer B: retention time 13.34 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, J = 9.0, 5.7 Hz, 1H), 7.6–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.07 (d, J = 7.2 Hz, 1H), 5.63 (broad s, 2H), 4.9–4.8 (m, 1H), 4.4–4.2 (m, 1H), 3.80 (td, J = 10.5, 3.9 Hz, 1H), 3.4–2.6 (m, 11H), 2.43 (s, 3H), 2.3–2.0 (m, 2H), 1.23 (d, J = 6.3 Hz, 3H). MS: 468 (M^++1). HRMS (M+1): calcd for $C_{26}H_{30}FN_3O_2S$ 468.21155. Found 468.21144.

6.5.2. 7-{2-[4-(7-Fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-3-chloro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (19b). Isomer A: retention time 15.55 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, J = 9.3, 5.8 Hz, 1H), 7.5–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.03 (dd, J = 6.9, 1.5 Hz, 1H), 4.9–4.8 (m, 1H), 4.3–4.2

(m, 1H), 3.79 (td, $J = 10.8$, 4.1 Hz, 1H), 3.3–2.9 (m, 5H), 2.9–2.4 (m, 6H), 2.2–1.9 (m, 2H), 1.17 (d, $J = 5.8$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 162.0, 160.7 (d, $J = 245$ Hz), 149.7, 143.8, 135.6 (d, $J = 9.3$ Hz), 133.4, 129.7, 127.2, 126.3 (d, $J = 8.9$ Hz), 126.1, 123.5, 122.8 (d, $J = 4.8$ Hz), 115.3 (d, $J = 25.0$ Hz), 114.0, 111.2 (d, $J = 20.3$ Hz), 73.3, 64.1, 60.5, 55.2, 53.3, 51.7 (broad), 49.0, 33.1, 25.0, 16.3 (broad). MS: 488 (M^++1). HRMS (M+1): calcd for $C_{25}H_{27}ClFN_3O_2S$ 488.15693. Found 488.15685.

Isomer B: retention time 22.31 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, $J = 9.2$, 5.8 Hz, 1H), 7.0–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.03 (dd, $J = 7.0$, 1.3 Hz, 1H), 4.9–4.7 (m, 1H), 4.3–4.2 (m, 1H), 3.78 (td, $J = 10.8$, 4.3 Hz, 1H), 3.2–2.5 (m, 11H), 2.2–1.9 (m, 2H), 1.19 (d, $J = 6.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 162.0, 160.7 (d, $J = 244$ Hz), 149.9, 143.5, 135.7 (d, $J = 9.1$ Hz), 133.4, 129.7, 127.2, 126.3 (d, $J = 8.9$ Hz), 126.0, 123.5, 122.8 (d, $J = 4.6$ Hz), 115.3 (d, $J = 24.8$ Hz), 114.1, 111.2 (d, $J = 20.0$ Hz), 73.4, 64.0, 60.2, 55.3, 53.2, 51.7 (broad), 49.3, 33.0, 24.9, 16.3 (broad). MS: 488 (M^++1). HRMS (M+1): calcd for $C_{25}H_{27}ClFN_3O_2S$ 488.15693. Found 488.15688.

6.5.3. 7-{2-[4-(7-Fluoronaphthalen-1-yl)-2-(*R*)-methyl-piperazin-1-yl]ethyl}-3-fluoro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-carboxamide (19c). Isomer A: retention time 10.30 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, $J = 9.3$, 5.8 Hz, 1H), 7.5–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.03 (dd, $J = 6.9$, 1.5 Hz, 1H), 6.3–5.6 (very broad d, 2H), 4.9–4.7 (m, 1H), 4.3–4.2 (m, 1H), 3.76 (td, $J = 11.0$, 4.1 Hz, 1H), 3.3–2.9 (m, 5H), 2.9–2.4 (m, 6H), 2.1–1.9 (m, 2H), 1.17 (d, $J = 5.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 161.3 (d, $J = 3.1$ Hz), 160.7 (d, $J = 244$ Hz), 153.7 (d, $J = 264$ Hz), 149.9, 142.9 (d, $J = 8.3$ Hz), 135.7 (d, $J = 9.3$ Hz), 127.2, 126.3 (d, $J = 8.9$ Hz), 126.0, 123.8 (d, $J = 26.1$ Hz), 122.8 (d, $J = 4.8$ Hz), 115.4 (d, $J = 24.7$ Hz), 115.2 (d, $J = 28.6$ Hz), 114.1 (d, $J = 1.4$ Hz), 111.3 (d, $J = 20.3$ Hz), 73.4, 63.8, 60.4, 55.2, 53.2, 51.7, 48.9, 32.7, 22.9, 16.4 (broad). MS: 472 (M^++1).

Isomer B: retention time 16.83 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, $J = 9.3$, 5.7 Hz, 1H), 7.5–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.03 (d, $J = 7.0$ Hz, 1H), 6.3–5.6 (very broad d, 2H), 4.8–4.7 (m, 1H), 4.3–4.2 (m, 1H), 3.76 (td, $J = 10.8$ and 4.1 Hz, 1H), 3.3–2.9 (m, 5H), 2.9–2.4 (m, 6H), 2.2–1.9 (m, 2H), 1.18 (d, $J = 5.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 161.4 (d, $J = 2.9$ Hz), 160.7 (d, $J = 244$ Hz), 153.7 (d, $J = 237$ Hz), 149.9, 142.8 (d, $J = 8.6$ Hz), 135.6 (d, $J = 9.1$ Hz), 127.2, 126.3 (d, $J = 8.9$ Hz), 125.9, 123.8 (d, $J = 26.3$ Hz), 122.8 (d, $J = 4.9$ Hz), 115.3 (d, $J = 24.5$ Hz), 115.2 (d, $J = 28.4$ Hz), 114.1 (d, $J = 1.7$ Hz), 111.2 (d, $J = 20.0$ Hz), 73.5, 63.8, 60.3, 55.3, 53.2, 49.2, 32.7, 22.9. MS: 472 (M^++1). HRMS (M+1): calcd for $C_{25}H_{27}F_2N_3O_2S$ 472.18648. Found 472.18647.

6.5.4. *tert*-Butyl-[2-(2-iodo-4,5-dihydro-7*H*-thieno[2,3-*c*]-pyran-7-yl)ethoxy]dimethylsilane (20). To a solution of **8** (1.20 g, 4.02 mmol) in THF (20 mL) was added *n*-BuLi (2.9 mL, 1.6 M solution in hexanes, 4.63 mmol) at −78 °C and the resulting solution stirred for 45 min. Solid 1,2-diiodoethane (1.50 g, 5.23 mmol) was added in one portion and the resulting reaction mixture was allowed to warm to room temperature and stirred for 5 h. 10% $Na_2S_2O_3$ (10 mL) was then added and the two phases separated. The aqueous phase was extracted with CH_2Cl_2 (2×) and the combined organic extracts were dried (Na_2SO_4), filtered and evaporated to afford 1.67 g of **20** as a yellow oil. The ^{1}H NMR of this oil showed a 10:1 mixture of desired compound **20** and starting material **8**. This mixture was used without further purification in the next step. The yield was estimated at 80%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.95 (s, 1H), 4.9–4.8 (m, 1H), 4.2–4.0 (m, 1H), 3.9–3.6 (m, 3H), 2.9–2.7 (m, 1H), 2.6–2.4 (m, 1H), 2.0–1.8 (m, 2H), 0.91 (s, 9H), 0.08 (s, 6H).

6.5.5. *tert*-Butyl-[2-(3-iodo-4,5-dihydro-7*H*-thieno[2,3-*c*]-pyran-7-yl)ethoxy]dimethylsilane (21). A solution of 8.49 mmol of LDA (prepared by addition of *n*-BuLi (5.30 mL, 1.6 M solution in hexanes, 8.49 mmol) to a solution of diisopropylamine (1.19 mL, 8.49 mmol) in THF (20 mL) at 0 °C and stirring for 30 min at this temperature) was cooled to −40 °C. *tert*-Butyl-[2-(2-iodo-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)-ethoxy]-dimethylsilane (**20**) (1.20 g, 2.83 mmol) dissolved in THF (12 mL) was added to the above solution and the resulting reaction mixture stirred for 20 min. The reaction was quenched with water and the mixture allowed to warm to room temperature. The two phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2×). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 98:2) to yield 0.60 g (50% yield) of pure **21** as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.31 (d, $J = 0.8$ Hz, 1H), 4.9–4.8 (m, 1H), 4.3–4.2 (m, 1H), 4.0–3.6 (m, 3H), 2.8–2.6 (m, 1H), 2.5–2.4 (m, 1H), 2.3–1.8 (m, 2H), 0.91 (s, 9H), 0.08 (s, 6H).

6.5.6. 7-(2-Hydroxyethyl)-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-3-carboxamide (22). A mixture of **21** (0.90 g, 2.12 mmol), potassium cyanide (0.157 g, 2.34 mmol), copper(I)iodide (0.041 g, 0.21 mmol) and $(PPh_3)_4Pd$ (0.123 g, 0.11 mmol) in THF (15 mL) was stirred at 95 °C, under an inert atmosphere of nitrogen, for 6 h. The solvent was removed and the residue purified by column chromatography on silica gel (hexane/EtOAc, 9:1) to obtain 0.450 g of the desired 3-cyano derivative as a yellow oil. To a solution of this 3-cyano derivative (0.390 g, 1.20 mmol) in CH_2Cl_2 (4 mL) was added Bu_4NHSO_4 (0.105 g, 0.30 mmol), followed by 33% H_2O_2 (0.62 mL, 6.03 mmol) and 2 N NaOH (1.2 mL, 2.41 mmol). This mixture was sonicated for 1 h. The reaction was quenched by addition of satd $KHSO_4$ (4 mL) and diluted with CH_2Cl_2. The two phases were separated and the organic layer was washed with satd $Na_2S_2O_4$, dried (Na_2SO_4), filtered and concentrated to afford 0.415 g of pure 3-carboxamide intermediate as a pale yellow solid, which was then desilylated following the procedure described for the synthesis of **9**, to obtain

0.260 g (61% combined yield) of **22** as a white solid. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.84 (d, J = 0.6 Hz, 1H), 5.0–4.8 (m, 1H), 4.2–4.1 (m, 1H), 3.9–3.6 (m, 3H), 3.1–2.8 (m, 2H), 2.2–1.8 (m, 2H).

6.5.7. 7-{2-[4-(6-Fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-3-carboxamide (23). Compound **23** was prepared as a mixture of diastereomers by coupling of **22** (0.315 g, 1.39 mmol) and **14j** (0.476 g, 1.46 mmol) in a 76% yield (0.476 g) following the same procedure as described for the synthesis of **4**. The separation of isomers was conducted in a Chiralcel OD column (10 μm, 4.6 × 250 mm) under isocratic conditions (solvent system hexane/0.2% DMEA in 2-propanol, 1:4; flow rate of 1.3 mL/min).

Isomer A: Purity > 98%, de > 99%. Retention time: 4.19 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, J = 9.3, 5.9 Hz, 1H), 7.62 (d, J = 0.6 Hz, 1H), 7.6–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.03 (dd, J = 6.9, 1.5 Hz, 1H), 5.68 (broad s, 2H), 4.9–4.8 (m, 1H), 4.3–4.2 (m, 1H), 3.9–3.7 (m, 1H), 3.4–2.5 (m, 11H), 2.2–2.0 (m, 2H), 1.19 (d, J = 5.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 165.5, 160.7 (d, J = 245 Hz), 149.8, 139.9, 135.7 (d, J = 8.6 Hz), 134.8, 133.5, 127.2, 126.3 (d, J = 8.6 Hz), 125.9 (2C), 122.8 (d, J = 4.8 Hz), 115.4 (d, J = 24.7 Hz), 114.1 (d, J = 1.4 Hz), 111.3 (d, J = 19.9 Hz), 73.5, 64.4, 60.4, 55.3, 53.2, 51.7 (broad), 49.3, 33.6, 26.7, 16.3 (broad). MS: 454 (M^++1). HRMS (M+1): calcd for $C_{25}H_{28}FN_3O_2S$ 454.19590. Found 454.19592.

Isomer B: Purity > 98%, de > 99%. Retention time: 6.51 min. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, J = 9.3, 5.9 Hz, 1H), 7.63 (s, 1H), 7.6–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.04 (dd, J = 7.0, 1.2 Hz, 1H), 5.65 (broad s, 2H), 4.9–4.8 (m, 1H), 4.3–4.2 (m, 1H), 3.9–3.7 (m, 1H), 3.3–2.6 (m, 11H), 2.3–1.9 (m, 2H), 1.20 (d, J = 5.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.4, 160.7 (d, J = 238 Hz), 149.7, 139.6, 135.8 (d, J = 8.4 Hz), 134.8, 133.6, 127.2, 126.2 (d, J = 9.5 Hz), 125.9 (2C), 122.9 (d, J = 5.0 Hz), 115.4 (d, J = 23.9 Hz), 114.2 (d, J = 1.7 Hz), 111.3 (d, J = 19.1 Hz), 73.4, 64.3, 60.0, 55.4, 53.1, 49.4, 33.5, 26.7. MS: 454 (M^++1). HRMS (M+1): calcd for $C_{25}H_{28}FN_3O_2S$ 454.19590. Found 454.19591.

6.5.8. *tert*-Butyl-[2-(2-fluoro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)ethoxy]dimethyl-silane (24a). To a solution of **8** (0.100 g, 0.34 mmol) in THF (1.5 mL) was added *n*-BuLi (0.23 mL, 1.6 M solution in hexanes, 0.37 mmol) at −78 °C and the mixture stirred for 1 h. Solid *N*-fluorobenzenesulfonimide (0.300 g, 0.95 mmol) was added in one portion and the resulting reaction mixture allowed to warm to room temperature (approx. 4 h). The reaction was quenched with satd NH_4Cl and extracted with CH_2Cl_2. The organic phase was washed with brine, dried ($MgSO_4$), filtered and evaporated. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 3:1) to obtain 0.062 g (30% yield) of **24a** as a colourless oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.09 (s, 1H), 4.8–4.7 (m, 1H), 4.1–4.0 (m, 1H), 3.9–3.7 (m, 3H), 2.8–2.7 (m, 1H); 2.5–2.4 (m, 1H), 2.0–1.9 (m, 1H), 0.83 (s, 9H), 0.08 (s, 6H). MS: 317 (M^++1).

6.5.9. 7-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-2-sulfonamide (24b). To a solution of **8** (1.00 g, 3.36 mmol) in THF (20 mL) was added *n*-BuLi (2.2 mL, 1.6 M solution in hexanes, 3.52 mmol) at −78 °C and the mixture stirred for 1 h. Gaseous sulfur dioxide was bubbled through for 20 min at −78 °C and the reaction mixture allowed to warm to room temperature while the stirring was continued for 2 h. The solvent was removed and the crude mixture redissolved in dry CH_2Cl_2 (20 mL). To this solution was added *N*-chlorosuccinimide (480 mg, 3.53 mmol) in one portion. After stirring for 1 h, the reaction mixture was filtered through a Celite pad and the filtrate evaporated to dryness. The crude product was redissolved in dioxane (10 mL) and gaseous ammonia bubbled through for 10 min. The resulting reaction mixture was stirred at room temperature for 16 h. The solvent was removed and the residue purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to afford pure **24b** as a white solid (65%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.36 (s, 1H), 5.28 (broad s, 2H), 4.90–4.84 (m, 1H), 4.22–4.12 (m, 1H), 3.94–3.65 (m, 2H), 2.91–2.75 (m, 1H), 2.62–2.52 (m, 1H), 2.01–1.90 (m, 1H), 0.88 (s, 9H), 0.06 (s, 6H). MS: 378 (M^++1).

6.6. Synthesis of analogous 25

Analogs **25** were prepared by deprotection of silanyloxy derivatives **24** following the same procedure as described for the synthesis of **10** (white solids; yield: **25a**, 75%; **25b**, 73%).

6.6.1. 2-(2-Fluoro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)ethanol (25a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.18 (s, 1H), 4.9–4.6 (m, 1H), 4.2–4.1 (m, 1H), 3.8–3.7 (m, 3H), 2.8–2.7 (m, 1H), 2.4–2.3 (m, 1H), 2.0–1.8 (m, 2H). MS: 203 (M^++1).

6.6.2. 7-(2-Hydroxyethyl)-4,5-dihydro-7*H*-thieno[2,3-*c*]-pyran-2-sulfonamide (25b). ^{1}H NMR (CD_3OD, 200 MHz): δ 7.36 (s, 1H), 5.0–4.9 (m, 1H); 4.3–4.2 (m, 1H), 3.9–3.7 (m, 3H), 2.9–2.7 (m, 1H); 2.7–2.5 (m, 1H), 2.1–1.9 (m, 2H). MS: 294 (M^++1).

6.7. Synthesis of analogous 26

Analogs **26** were prepared as a mixture of diastereomers by coupling of the alcohol intermediates **25** and piperazine **14j** following the same procedure as described for the synthesis of **4** (pale brown solids; yield: **26a**, 30%; **26b**, 37%). The diastereomers were separated using a Chiralcel OD column (5 μm, 4.6 × 250 mm) under isocratic conditions (solvent system: hexane/0.2% DMEA 0.2% in 2-propanol, 7:3 for **26a** and 3:7 for **26b**; flow rate of 1 mL/min for **26a** and 0.75 mL/min for **26b**); all de > 99% and purity > 98%.

6.7.1. 1-[2-(2-Fluoro-4,5-dihydro-7*H*-thieno[2,3-*c*]pyran-7-yl)ethyl]-4-(6-fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazine (26a). Isomer A: retention time 4.25 min. ^{1}H NMR

($CDCl_3$, 200 MHz): δ 8.1–8.0 (m, 1H), 7.4–7.3 (m, 3H), 7.2–7.1 (m, 1H), 6.95 (d, $J = 7.1$ Hz, 1H), 6.09 (s, 1H), 4.64 (broad s, 1H), 4.1–4.0 (m, 1H), 3.7–3.6 (m, 1H), 3.2–3.0 (m, 3H), 2.9–2.5 (m, 3H), 2.4–2.2 (m, 1H), 1.9–1.8 (m, 2H), 1.2–1.1 (m, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 164.3 (d, $J = 288$ Hz), 160.7 (d, $J = 244$ Hz), 149.4, 135.6 (d, $J = 9.1$ Hz), 129.5 (d, $J = 4.3$ Hz), 127.2, 126.1 (d, $J = 8.9$ Hz), 125.8, 125.1, 123.0 (d, $J = 4.6$ Hz), 115.5 (d, $J = 24.6$ Hz), 114.2, 111.3 (d, $J = 20.3$ Hz), 107.1 (d, $J = 10.3$ Hz), 72.3, 64.4, 59.8, 55.4, 52.7, 49.2, 32.9, 25.9. MS: 429 (M^++1). HRMS (M+1): calcd for $C_{24}H_{26}F_2N_2OS$ 429.18067. Found 429.18063.

The oil was treated with 1.0 M HCl in ethyl acetate and the precipitate formed was filtered and washed with diethyl ether to obtain the hydrochloride salt of **26a** (isomer A) as a pale brown solid. MS: 429 (M^++1).

Isomer B: retention time 9.30 min. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.09 (dd, $J = 9.2$, 5.8 Hz, 1H), 7.5–7.3 (m, 3H), 7.2–7.1 (m, 1H), 6.99 (d, $J = 7.3$ Hz, 1H), 6.12 (d, $J = 1.2$ Hz, 1H), 4.67 (broad s, 1H), 4.2–4.1 (m, 1H), 3.69 (dt, $J = 10.9$, 3.8 Hz, 1H), 3.2–3.0 (m, 3H), 3.0–2.7 (m, 2H), 2.7–2.6 (m, 1H), 2.4–2.3 (m, 1H), 2.1–1.9 (m, 2H), 1.3–1.2 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.4 (d, $J = 288$ Hz), 160.7 (d, $J = 244$ Hz), 148.9, 135.6 (d, $J = 9.1$ Hz), 129.9 (d, $J = 4.3$ Hz), 125.8, 125.8 (d, $J = 8.3$ Hz), 124.2, 123.4 (d, $J = 3.7$ Hz), 115.6 (d, $J = 24.6$ Hz), 114.6 (br s), 111.4 (d, $J = 20.0$ Hz), 107.2 (d, $J = 10.3$ Hz), 71.9, 64.4, 58.4, 55.9, 52.0, 49.1, 31.5, 25.9, 22.6, 14.1. MS: 429 (M^++1). HRMS (M+1): calcd for $C_{24}H_{26}F_2N_2OS$ 429.18067. Found 429.18064.

The oil was treated with 1.0 M HCl in ethyl acetate and the precipitate formed was filtered and washed with diethyl ether to obtain the hydrochloride salt of **26a** (isomer 2) as a pale brown solid. MS: 429 (M^++1).

6.7.2. 7-{2-[4-(6-Fluoronaphthalen-1-yl)-2-(*R*)-methylpiperazin-1-yl]ethyl}-4,5-dihydro-7*H*-thieno[2,3-*c*]-pyran-2-sulfonamide (26b). Isomer A: retention time 9.39 min. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.19 (dd, $J = 9.4$, 5.9 Hz, 1H), 7.5–7.4 (m, 3H), 7.3–7.2 (m, 1H), 7.1–7.0 (m, 1H), 4.9–4.8 (m, 2H), 4.3–4.2 (m, 1H), 3.8–3.7 (m, 1H), 3.3–2.5 (m, 11H), 2.1–2.0 (m, 2H), 1.18 (d, $J = 5.9$ Hz, 3H). MS: 490 (M^++1).

Isomer B: retention time 21.26 min. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.21 (dd, $J = 9.1$, 5.9 Hz, 1H), 7.5–7.3 (m, 3H), 7.3–7.2 (m, 1H), 7.03 (dd, $J = 7.0$, 1.6 Hz, 1H), 5.0–4.8 (m, 2H), 4.3–4.2 (m, 1H), 3.8–3.7 (m, 1H), 3.2–2.5 (m, 11H), 2.1–2.0 (m, 2H), 1.18 (d, $J = 6.2$ Hz, 3H). MS: 490 (M^++1).

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References and notes

1. (a) Leonard, B. E. *CNS Drugs* **1995**, *4*(Suppl 1), 1–12; (b) Leonard, B. E. *J. Clin. Psychiatry* **1996**, *57*(Suppl 4), 26–33; (c) Kilts, C. D. *Am. J. Med.* **1994**, *97*(Suppl 6A), 6A-11; (d) Goodwin, G. M.; Phil, D.; Edin, F. R. C. P.; Psych, F. R. C. *J. Clin. Psychiatry* **1996**, *57*(Suppl 4), 9–13.
2. (a) Thase, M. E.; Rush, A. J. In *Psychopharmacology, The Fourth Generation of Progress*; Bloom, F. E., Kupfer, D. J., Eds.; Raven: New York, 1995; pp 1081–1098; (b) Fawcett, J.; Barkin, R. L. *J. Clin. Psychiatry* **1997**, *58*, 32–39; (c) Fava, M. *J. Clin. Psychiatry* **2000**, *61*, 26–32.
3. Montgomery, S. A. In *Psychopharmacology, The Fourth Generation of Progress*; Bloom, F. E., Kupfer, D. J., Eds.; Raven: New York, 1995; pp 451–461.
4. (a) Goodwin, G. M. *J. Clin. Psychiatry* **1996**, *57*, 9–13; (b) Leonard, B. E. *J. Clin. Psychiatry* **1996**, *57*, 26–33.
5. (a) Glennon, R. A.; Westkaemper, R. B. *Drugs New Perspect.* **1993**, *6*, 390–405; (b) Briley, M.; Moret, C. *Clin. Neuropharmacol.* **1993**, *16*, 387–400; (c) Moret, C.; Briley, M. *Eur. J. Pharmacol.* **2000**, *404*, 1–12.
6. (a) Davidson, C.; Stamford, J. A. *Br. J. Pharmacol.* **1995**, *114*, 1107–1109; (b) Rollema, H.; Clarke, T.; Sprouse, J. S., D. W.; Schulz, D. W. *J. Neurochem.* **1996**, *67*, 2204–2207.
7. Longmore, J.; Shaw, D.; Smith, D.; Hopkins, R.; McAllister, G.; Pickard, J. D.; Sirinathsinghji, D. J. S.; Butler, A. J.; Hill, R. G. *Cephalalgia* **1997**, *17*, 833–842.
8. Bouchelet, I.; Cohen, Z.; Case, B.; Seguela, P.; Hamel, E. *Mol. Pharmacol.* **1996**, *50*, 219–223.
9. (a) Sternfeld, F.; Guiblin, A. R; Jelley, R. A.; Matassa, V. G.; Reeve, A. J.; Hunt, P. A.; Beer, M. S.; Heald, A.; Stanton, J. A.; Sohal, B.; Watt, A. P.; Street, L. J. *J. Med. Chem.* **1999**, *42*, 677–690; (b) Chambers, M. S.; Street, L. J.; Goodacre, S.; Hobbs, S. C.; Hunt, P.; Jelley, R. A.; Matassa, V. G.; Reeve, A. J.; Sternfeld, F.; Beer, M. S.; Stanton, J. A.; Rathbone, D.; Watt, A. P.; MacLeod, A. M. *J. Med. Chem.* **1999**, *42*, 691–705; (c) van Niel, M. B.; Collins, I.; Beer, M. S.; Broughton, H. B.; Cheng, S. K. F.; Goodacre, S. C.; Heald, A.; Locker, K. L.; MacLeod, A. M.; Morrison, D.; Moyes, C. R.; O'Connor, D.; Pike, A.; Rowley, M.; Russell, M. G. N.; Sohal, B.; Stanton, J. A.; Thomas, S.; Verrier, H.; Watt, A. P.; Castro, J. L. *J. Med. Chem.* **1999**, *42*, 2087–2104; (d) Russell, M. G. N.; Matassa, V. G.; Pengilley, R. R.; van Niel, M. B.; Sohal, B.; Watt, A. P.; Hitzel, L.; Beer, M. S.; Stanton, J. A.; Broughton, H. B.; Castro, J. L. *J. Med. Chem.* **1999**, *42*, 4981–5001.
10. Kawanishi, Y.; Ishihara, S.; Tsushima, T.; Seno, K.; Miyagashi, M.; Hagashita, S.; Ishikawa, M.; Shima, M.;

Shimamura, M.; Ishihara, Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1421–1426.
11. Ennis, M. D.; Tenbrink, R. E. WO 97/02259, 1997.
12. (a) Gueremy, C.; Audiau, F.; Champseix, A. *J. Med. Chem.* **1980**, *23*, 1306–1310; (b) Matzen, L.; van Amsterdam, C.; Rautenberg, W.; Greiner, H. E.; Harting, J.; Seyfried, C. A.; Bottcher, H. *J. Med. Chem.* **2000**, *43*, 1149–1157.
13. (a) Timms, G. H.; Boot, J. R.; Broadmore, R. J.; Carney, S. L.; Cooper, J.; Findlay, J. D.; Gilmore, J.; Mitchell, S.; Moore, N. A.; Pullar, I.; Sanger, G. J.; Tomlinson, R.; Tree, B.; Wedley, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2469–2472; (b) Agejas-Chicharro, J.; Bueno Melendo, A. B.; Camp, N. P.; Gilmore, J.; Jimenez-Aguado, A. M.; Lamas-Peteira, C.; Marcos-Llorente, A.; Mazanetz, M. P.; Montero Salgado, C.; Timms, G. H.; Williams, A. C. WO 02/50067, 2002; (c) Orús, L.; Pérez-Silanes, S.; Oficialdegui, A.-M.; Martínez-Esparza, J.; Del Castillo, J.-C.; Mourelle, M.; Langer, T.; Guccione, S.; Doncella, G.; Krovat, E. M.; Poptodorov, K.; Laceras, B.; Ballaz, S.; Hervias, I.; Tordera, R.; Del Río, J.; Monge, A. *J. Med. Chem.* **2002**, *45*, 4128–4139.
14. Kohlman, D. T.; Liang, S. X.; Xu, Y.-C. WO 01/23381, 2001.
15. Fairhurst, J.; Gallagher, P. WO 01/87881, 2001.
16. Unterhalt, B.; Kissenbeck, N. *Sci. Pharm.* **1999**, *67*, 97–102.
17. Esteban, G.; López-Sánchez, M. A.; Martínez, M. E.; Plumet, J. *Tetrahedron* **1998**, *54*, 197–212.
18. (a) Brun, P.; Guglielmetti, R.; Anguille, S. *Appl. Organomet. Chem.* **2002**, *16*, 271–276; (b) Adcock, W.; Dewar, M. J. S. *J. Am. Chem. Soc.* **1967**, *89*, 386–390.
19. Ahman, J.; Buchwald, S. L. *Tetrahedron Lett.* **1997**, *38*(36), 6363–6366.
20. Bøgesø, K. P.; Arnt, J.; Frederiksen, K.; Hansen, H. O.; Hyttel, J.; Pedersen, H. *J. Med. Chem.* **1995**, *38*, 4380–4392.
21. Carpenter, A. J.; Chadwick, D. J. *Tetrahedron Lett.* **1985**, *26*, 1777–1780.
22. Reinecke, M.; Wayne Adickes, H.; Pyun, C. *J. Org. Chem.* **1971**, *36*, 2690–2692.
23. Anderson, B.; Bell, E.; Ginah, F.; Harn, N.; Pagh, L.; Wepsiec, J. *J. Org. Chem.* **1998**, *63*, 8224–8228.
24. Prugh, J.; Hartman, G.; Mallorga, P.; McKeever, B.; Michelson, S.; Murcko, M.; Schwam, H.; Smith, R.; Sondey, J.; Springer, P.; Sugrue, M. *J. Med. Chem.* **1991**, *34*, 1805–1818.
25. Pullar, I. A.; Carney, S. L.; Colvin, E. M.; Lucaites, V. L.; Nelson, D. L.; Wedley, S. *Eur. J. Pharmacol.* **2000**, *407*, 39–46.
26. Greengrass, P.; Bremner, R. *Eur. J. Pharmacol.* **1979**, *55*, 323–326.
27. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
28. Pullar, I. A.; Boot, J. R.; Carney, S. L.; Cohen, M. L.; Colvin, E. M.; Conway, R. G.; Hardy, C. H. L.; Lucaites, V. L.; Nelson, D. L.; Schenck, K. W.; Tomlinson, R.; Wedley, S. *Eur. J. Pharmacol.* **2001**, *432*, 9–17.