Bioorganic & Medicinal Chemistry Letters 23 (2013) 407–411

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

jour'nal homepage: www.elsevier.com/locate/bmcl

Design, optimization, and in vivo evaluation of a series of pyridine derivatives with dual NK_1 antagonism and SERT inhibition for the treatment of depression

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ARTICLE INFO

Article history:
Received 26 October 2012
Revised 16 November 2012
Accepted 20 November 2012
Available online 30 November 2012

Keywords:
Neurokinin 1 antagonists
Serotonin transporter inhibitors
Receptor occupancy
Depression

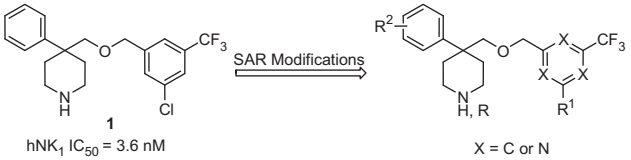
ABSTRACT

A series of substituted pyridines, ether linked to a phenylpiperidine core were optimized for dual NK_1/SERT affinity. Optimization based on NK_1/SERT binding affinities, and minimization of off-target ion channel activity lead to the discovery of compound **44**. In vivo evaluation of **44** in the gerbil forced swim test (a depression model), and ex-vivo NK_1/SERT receptor occupancy data support the potential of a dual acting compound for the treatment of depression.

The therapeutic potential of tachykinin receptor antagonists for the treatment of depression has been a subject of discussion for many years.[1] Although Phase II clinical trials with the NK_1 receptor antagonist Aprepitant[2] showed evidence of antidepressant efficacy, Phase III trials with Aprepitant as well as a follow-on NK_1 receptor antagonist Casopitant did not support these initial positive findings.[3] While these results have negatively impacted the development of NK_1 antagonists as single agents for the treatment of depression, there has been interest in the combination of NK_1 receptor antagonism with Serotonin Transporter (SERT) inhibitor activity as a potential means of potentiating the antidepressant effect of NK_1 antagonism.[4]

We set out to develop a series of molecules with dual acting NK_1 antagonism and SERT inhibition, with high affinity for both targets. Early efforts focused on identifying chemotypes that had good physicochemical properties and potential for brain uptake. A (4-phenylpiperidine-4-yl)-methyl ether chemotype, exemplified by **1**, was chosen as our starting point.[5] We designed a series of analogs directed toward improving NK_1/SERT affinity,[6] and decreasing off-target ion channel activity (Fig. 1). Initial SAR focus revolved around introducing a substituted pyridine on the ether side-chain as a means of increasing polarity in this series (Scheme 1).

Comparison of **1** with its direct pyridine analogue **6** showed that incorporation of a pyridine ring was tolerated, although potency was diminished three–four-fold for both NK_1 antagonism and SERT inhibition compared to **1** (Table 1). Modification around the left-hand phenyl ring significantly impacted both NK_1 and SERT binding affinities. In general, decreases in hERG flux activity paralleled the decrease in NK_1/SERT activity in this series. It should be noted that to date a 4-fluorophenyl substituent on the piperidine



1

hNK_1 IC_{50} = 3.6 nM
hSERT IC_{50} = 1.7 nM
Ca Flux IC_{50} = 5.2 μM
hERG Flux IC_{50} = 7.2 μM

X = C or N

Figure 1. SAR strategy around the 4-methyl-4-phenylpiperidine core.

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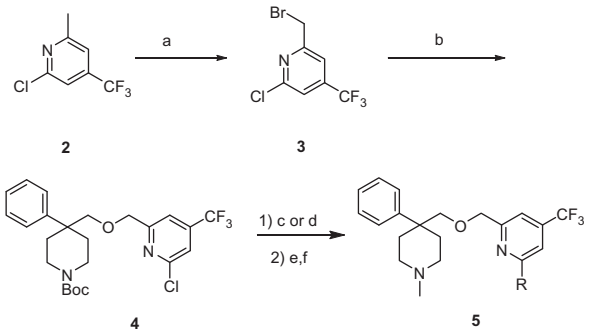
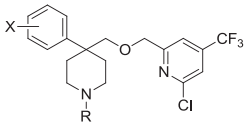
http://dx.doi.org/10.1016/j.bmcl.2012.11.094



Scheme 1. Reagents and conditions: (a) NBS/CCl_4; (b) KOtBu/THF, *tert*-butyl 4-(hydroxymethyl)-4-phenylpiperidine-1-carboxylate; (c) $Pd(Ph_3P)_4$/MeCN/KOH/120°C, R-B$(OH)_2$; (d) $Pd(OAc)_2$, NaOtBu, rac-BINAP, toluene, 120°C, R = 1° or 2° amine; (e) TFA, CH_2Cl_2; (f) formaldehyde, $NaCNBH_4$, MeCN.

Table 1
In vitro profiling data for the first generation pyridine analogues **6**–**11** produced via Scheme 1



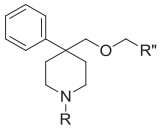
Compd.	R	X	hNK_1[a] IC_{50} (nM)	hSERT[a] IC_{50} (nM)	Ca flux IC_{50} (μM)	hERG flux IC_{50} (μM)
6	H	H	11	7.4	2	28
7	CH_3	H	12	6.3	7	25
8	CH_3	4-Cl	430	23	11	23
9	H	4-F	29	9.0	7	20
10	CH_3	4-F	45	4.3	5	6
11	CH_3	2-F	52	88	6.3	43

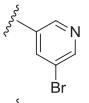
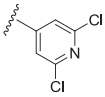
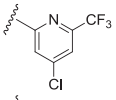
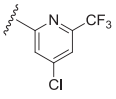
[a] Values are the mean of two experiments.

was the best tolerated modification on the 4-phenylpiperidine ring. Alkylation of the piperidine nitrogen with a methyl group improved SERT binding affinity slightly, while having little impact on NK_1 affinity. Additional alkyl groups were explored on the piperidine nitrogen, but a methyl substituent was determined to be optimal.[7]

Altering the position of the pyridine nitrogen impacted NK1 affinity significantly, demonstrating a clear preference for 2- and 4-substituted pyridines. Comparison of **6** and **15** (and **7**/**16**) shows a further preference for having the trifluoromethyl group in the 4-position relative to the pyridine nitrogen (Table 2). Removal of substitution on the 4-position significantly decreased NK_1 binding affinity as demonstrated by example **13**. In contrast to the above

Table 2
In vitro profiling data for 'right-hand' substituted pyridine analogues **12**–**16** produced via Scheme 2



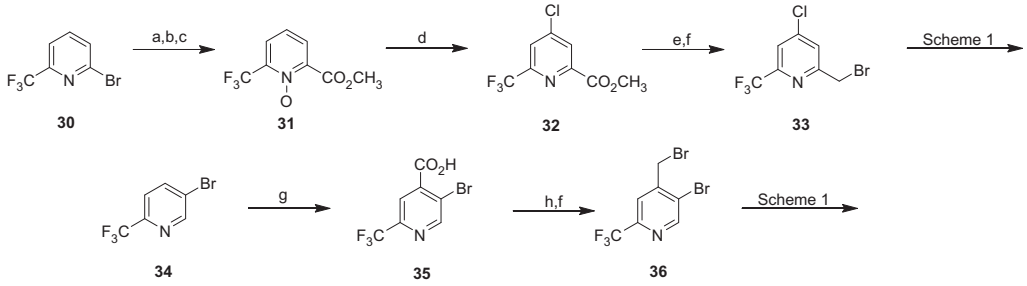
Compd.	R	R″	hNK_1[a] IC_{50} (nm)	hSERT[a] IC_{50} (nM)	Ca flux IC_{50}(μM)	hERG flux IC_{50} (μM)
12	H		600	10	21	15
13	CH_3		480	3.1	30	13
14	H		34	6.6	7	33
15	H		74	6.3	7	25
16	CH_3		66	14	7	12

[a] Values are the mean of two experiments.

findings the pyridine nitrogen position had minimal effects on SERT potency.

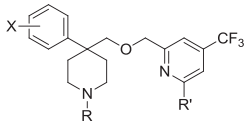
Additional modification of the pyridine ring was explored fixing the trifluoromethyl group in the 4-position while altering the 2-position with a variety of acyclic and cyclic amines. This exercise lead to analogues with significant improvements in NK_1 and SERT affinities, as well as in some examples, decreasing off-target ion channel activity in the flux assays (Table 3). Both acyclic and cyclic amines possessed good NK_1/SERT binding affinities. There was a clear preference for tertiary amines having better dual activity than secondary amines, although branching alpha to the nitrogen improved potency as in secondary amine **20**. Extended branching of the alkyl groups decreased both NK_1 and SERT affinities as in example **22**. Addition of a second basic amine as in the 4-methyl piperazine **28** also significantly decreased both NK_1 and SERT affinities.

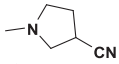
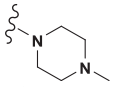
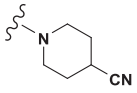
In vitro profiling of one of the better analogues in this series compound **25**, demonstrated that introduction of an amino functionality has a positive impact on protein free fraction (human,



Scheme 2. Reagents and conditions: (a) BuLi, THF, CO_2; (b) mCPBA, CH_2Cl_2; (c) MeOH, HCl; (d) $POCl_3$, 100°C; (e) $NaBH_4$, MeOH; (f) CBr_4, Ph_3, CH_2Cl_2.

Table 3
In vitro profiling data for 2-aminopyridine analogues **17–29** produced via Scheme 1



Compd.	X	R	R′	hNK$_1$[a] IC$_{50}$ (nM)	hSERT[a] IC$_{50}$ (nM)	Ca flux IC$_{50}$ (μM)	hERG flux IC$_{50}$ (μM)
17	4-F	H	–N(CH$_3$)$_2$	20	7.3	6.0	8.4
18	4-F	CH$_3$	–N(CH$_2$CH$_3$)$_2$	1.6	7.3	4.7	14
19	4-F	CH$_3$	–NHCH$_2$CH$_3$	760	4.8	56	80
20	4-F	CH$_3$	–NHCH(CH$_2$CH$_3$)$_2$	3.0	15	6.5	7.1
21	4-F	CH$_3$	–N(CH$_3$)CH$_2$CH$_3$	1.0	3.9	5.3	6.1
22	4-F	CH$_3$	–N(CH$_2$CH(CH$_3$)$_2$)$_2$	180	140	10	9.5
23	H	H		2.7	58	3.5	2.3
24	H	CH$_3$		1.2	6.6	2.9	7.4
25	4-F	CH$_3$		1.9	6.4	5.8	12
26	4-F	CH$_3$		6.3	4.0	5.5	8.7
27	4-F	CH$_3$		29	3.3	8.6	8.3
28	H	H		790	94	9.5	16
29	4-F	CH$_3$		13	2.4	3.4	7.7

[a] Values are the mean of two experiments.

6.9% free fraction, gerbil, 6.6% free fraction) and a modest decrease on hERG channel activity. Unfortunately, the microsomal stability data (human/rat/mouse: 100/43/51% remaining after 10 min @0.5 μM conc.) was modest for **25** and for the majority of compounds in this series. The major pathway for metabolism in the 2-aminopyridine series was oxidative dealkylation of the amine R groups.

As a means to eliminate this metabolic 'soft spot' we explored additional substituents at the 2-position, focusing on R groups that potentially were resistant to oxidation. Utilizing standard substitution chemistry on a 2-chloropyridine scaffold (Scheme 1) we explored a variety of substituents (Table 4). Reduction of the parent chloro compound **10** with hydrogen and Pd/C afforded analog **37** which suffered a significant loss in NK$_1$ receptor potency. Displacement of the chlorine with sodium cyanide or methoxide afforded compounds **38** and **39**. Compound **39** possessed good NK$_1$/SERT affinities, yet as in the amine series, this analogue had poor metabolic stability. Analogues **40–51** were synthesized via standard Suzuki chemistry from their corresponding boronic acids followed by either Pd/C reduction in the cases of the saturated analogues, or bycyclopropanation of the alkenylboronic esters using Diazomethane/Pd(OAc)$_2$, to afford the cyclopropyl analogues. From the in vitro screening data in Table 4, the 2-cyclopropyl analogue **44** possessed excellent dual NK$_1$/SERT affinity with a two-fold improvement in hERG flux activity over the lead 2-chloro-4-trifluoromethyl-phenyl analogue **1**.

Lastly, we turned our efforts to improving on compound **44** by fixing the 2-cyclopropyl position on the pyridine, modifying substitution at the 4-position of the pyridine, and the substituents on the 4-phenylpiperidine ring (Table 5).

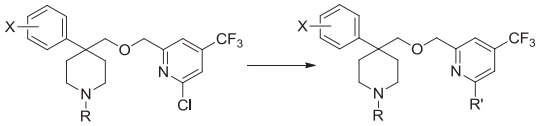
As in initial studies the 4-fluoro-substituent on the 4-phenyl piperidine ring afforded the best analogs (**44** vs **54–56**). Interestingly, replacing the 4-trifluoromethyl group with a cyclopropane as in **52**, afforded an equipotent analog to **44**. However, compound **44** had significantly better in vitro microsomal stability than **52**.[8] In addition, compound **44** had acceptable plasma protein free fraction across species (human% free = 2.0%, gerbil% free = 2.6%, rat% free = 3.9%, mouse% free = 2.9%). In contrast to cyclopropane **52**, significantly decreased NK$_1$ affinity was seen in **53** with the introduction of a nitrile in place of the trifluoromethyl group in the 4-position (**44** vs **53**). Based on these data, we chose **44** as a superior compound for in vivo evaluation.

Compound **44** was dosed po (0.25% methylcellulose suspension) 120 min prior to testing in our gerbil Forced Swim Test assay (gFST).[9] Test animals were compared against vehicle, with 10 mg/kg po fluoxetine included as a positive control. Total plasma and brain exposures were determined, as well as NK$_1$/SERT ex vivo receptor occupancy. In the gFST studies, compound **44** significantly decreased immobility (the measure of anti-depressant activity) at 1, 3, and 10 mg/kg po compared to vehicle, and demonstrated efficacy comparable to fluoxetine at the 3 mg/kg dose po (Fig. 2).

Exposure data from these studies indicated that compound **44** was efficacious at modest plasma levels. For example at 1 mg/kg, the total plasma concentration of **44** was 9.2 nM ± 8.1 nM. At 3 mg/kg and 10 mg/kg, plasma levels were 37 nM ± 19 nM, and 140 nM ± 40 nM respectively at t = 120 min. More importantly, compound **44** had total brain exposures that were on average 10-fold higher than total plasma levels over the dose range.

Ex vivo receptor occupancy was determined for both NK$_1$ and SERT target engagement. For NK$_1$ ex vivo studies we used ^{125}I-sub-

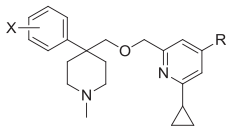
Table 4
In vitro profiling data for substituted pyridine analogues **17–28** produced via substitution of a 2-chloropyridine precursor



Compd	X	R	R'	hNK_1[a] IC_{50} (nM)	hSERT[a] IC_{50} (nM)	Ca flux IC_{50} (μM)	hERG flux IC_{50} (μM)
37	4-F	CH_3	H	700	2.2	11	29
38	H	CH_3	CN	380	4.3	16	33
39	4-F	CH_3	$-OCH_3$	22	2.8	7.3	9.9
40	4-F	CH_3	$-CH(CH_3)_2$	12	12	-	26
41	4-F	CH_3	$-CH_2CH(CH_3)_2$	16	8.9	4.4	12
42	4-F	CH_3	$CH{=}CHCH_3$	29	10	5.3	13
43	4-F	CH_3	$CH{=}C(CH_3)_2$	39	12	11	18
44	4-F	CH_3		1.9	2.1	5.0	13
45	H	CH_3		2.0	6.8	2.8	9.0
46	H	H		2.0	13	1.8	5.5
47	4-F	CH_3		9.1	28	6.5	34
48	4-F	CH_3		8.7	16	6.8	9
49	4-F	CH_3		5.3	5.6	3.5	4.3
50	H	CH_3		5.3	56	7.1	12
51	4-F	CH_3		6.1	6.8	8.2	12

[a] Values are the mean of two experiments.

Table 5
In vitro profiling data for 4-substituted-2-cyclopropyl pyridine analogues **52–56**



Compd.	X	R	hNK1[a] IC_{50} (nM)	hSERT[a] IC_{50} (nM)	Ca flux IC_{50} (μM)	hERG IC_{50} (μM)
52	4-F		1.8	1.5	7.4	17
53	4-F	–CN	290	1.6	8.9	17
54	4-Cl	$-CF_3$	11	32	3.4	3.1
55	3-Cl	$-CF_3$	6.4	9.1	4.5	9.2
56	3-F	$-CF_3$	3.7	8.3	3.6	9.0

[a] Values are the mean of two experiments.

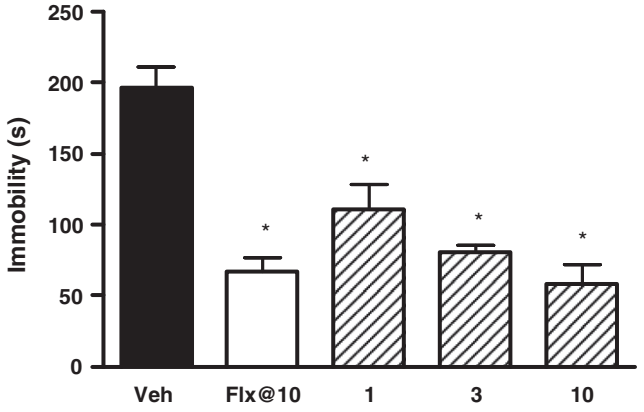


Figure 2. Gerbil forced swim test. Dose (mg/kg, po, $t = 120$ min). Fluoxetine at 10 mg/kg po as a positive control ($n = 4$). ANOVA $p < 0.001$ $^*p < 0.01$ Dunnett's t-test.

stance P.[10] SERT occupancy studies were performed with ^{3}H-Citalopram.[11] For all doses, we observed significant target engagement at both the NK_1 and SERT binding sites in the gerbil brain. Receptor occupancy increased proportionally with dose (Fig. 3).

Observations from the occupancy studies demonstrated that it is not necessary to saturate either target in order to see efficacy in the gFST. In fact, at the minimum efficacious dose of 1 mg/kg, only modest target engagement at both targets was required in order to obtain a significant behavioral effect in this assay.[12] These data support the synergistic relationship between modulation of the NK_1 and SERT pathways with respect to behavioral outcomes in models for depression.

In conclusion, we optimized a series of substituted pyridines with an ether-linkage to a 4-methyl-4-phenylpiperidine core. SAR studies focused on optimization of brain penetration, metabolic stability, and decreasing ion channel activity, while maintaining dual target affinities to afford compound **44**. Compound **44** (BMS-817693) demonstrated efficacy in our gerbil forced swim test at reasonable doses. Based on in vitro ion channel activity data, and the modest exposures necessary for efficacy, we have

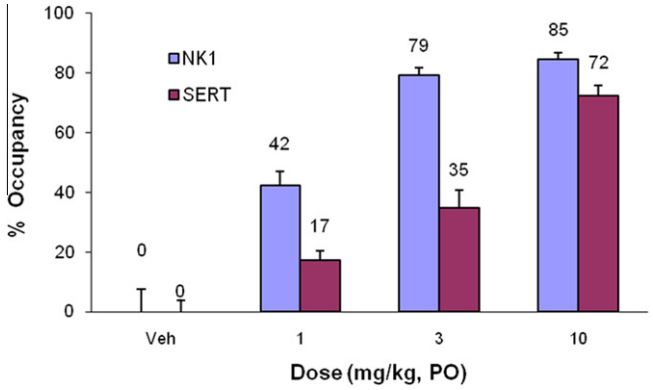


Figure 3. NK_1/SERT ex vivo receptor occupancy in the gerbil forced swim test ($n = 4$).

minimized the potential for significant off target cardiovascular toxicities at pharmaceutically relevant doses. Additional studies on the progress of compound **44** will be disclosed in due course.

Acknowledgments

The authors thank Drs. Kimberley A. Lentz and Joseph J. Raybon for helpful discussions on pharmacokinetic data supporting compound optimization.

References and notes

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6. (a) IC_{50} values were determined by filtration binding assays for both NK_1 and SERT. The NK_1 assay utilized ^{125}I-Substance P, and the SERT assay used ^{125}I-RTI-55 as the competitive inhibitors. Membranes were derived from stably expressing cultured cells; (b) for synthetic details on the preparation for compounds disclosed in this publication see: Parker, M. F.; Bronson, J. J.; Silva, M. V.; Gillman, K. W. U.S. Patent Application Publication 2009096941.
7. Data not shown. We explored a variety of alkyl substituents on the piperidine nitrogen and all of these significantly decreased SERT binding affinity compared to the methyl congener.
8. In vitro microsomal stability for 44 (human/rat/mouse: 99/43/87% remaining after 10 min @ 0.5 μM conc.); compound **52** (human/rat/mouse: 50/77/7% remaining after 10 min @ 0.5 μM conc.).
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12. Additional data relating to NK_1/SERT% occupancy and how these levels relate to efficacy in the gerbil forced swim test will be disclosed in the near future.