Bioorganic & Medicinal Chemistry Letters 21 (2011) 271–275

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The identification, and optimisation of hERG selectivity, of a mixed NET/SERT re-uptake inhibitor for the treatment of pain

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ARTICLE INFO

Article history:
Received 17 September 2010
Revised 30 October 2010
Accepted 2 November 2010
Available online 6 November 2010

Keywords:
Noradrenaline re-uptake transporter inhibitor
Serotonin re-uptake transporter inhibitor
Monoamine re-uptake transporter inhibitor

ABSTRACT

Hit compound **1**, a selective noradrenaline re-uptake transporter (NET) inhibitor was optimised to build in potency at the serotonin re-uptake transporter (SERT) whilst maintaining selectivity against the dopamine re-uptake transporter (DAT). During the optimisation of **1** it became clear that selectivity against the Kv11.1 potassium ion channel (hERG) was also a parameter for optimisation within the series. Discrete structural changes to the molecule as well as a lowering of global *c*Log*P* successfully increased the hERG selectivity to afford compound **11m**, which was efficacious in a mouse model of inflammatory pain, complete Freund's adjuvant (CFA) induced thermal hyperalgesia and a rat model of neuropathic pain, spinal nerve ligation (SNL) induced mechanical allodynia.

A number of antidepressant medications have demonstrated efficacy in treating pain associated with nerve damage (neuropathic pain). In particular, the tricyclic antidepressants (TCAs) have proven clinical efficacy; one third of patients with neuropathic pain who took a TCA such as amitriptyline obtained moderate pain relief or better.[1] Although the TCAs are classical 'dirty' compounds with binding affinity at a range of receptor targets, there is a growing body of evidence which attributes the efficacy of the TCAs in neuropathic pain to the inhibition of serotonin and noradrenaline re-uptake at pre-synaptic transporters. This is further substantiated by evidence that the newer class of antidepressants venlafaxine and duloxetine (Fig. 1) have similar effectiveness to the TCAs with reduced side effects.[2] Both venlafaxine and duloxetine inhibit re-uptake of both of the key neurotransmitters, serotonin and noradrenaline, involved in the descending modulation of neuropathic pain.[3] Venlafaxine and duloxetine are serotonin–noradrenaline re-uptake inhibitors or SNRIs with an inhibitor profile of SERT > NET >> DAT, however of the two compounds only duloxetine is registered for the treatment of neuropathic pain by the FDA. The most frequently reported adverse events for duloxetine include nausea, somnolence, dizziness, and fatigue and it is likely these symptoms are caused by the elevation of serotonin rather than noradrenaline. Thus, we hypothesised that the optimal mixed re-uptake inhibitor profile for achieving the desired analgesic efficacy, without the side effects commonly attributed to serotonin re-uptake, would be an inhibitor with a potency profile of NET > SERT >> DAT; an 'NSRI' re-uptake inhibitor with reduced potency at SERT.

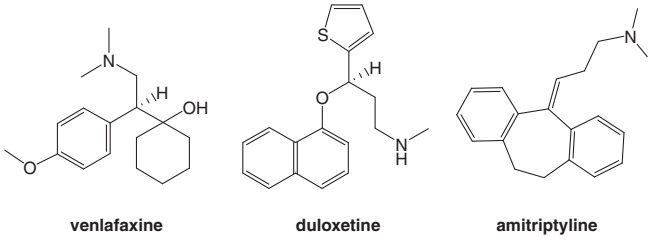

Figure 1.

An in-house transporter project looking at triple re-uptake inhibitors had identified a number of interesting leads including compound **1** which was selected as a start point for our NSRI programme (Fig. 2). Compound **1** was a selective NET inhibitor and thus the challenge was to dial in SERT affinity without compromising the DAT selectivity and NET potency. During the optimisation of **1** it became clear that hERG selectivity was also a

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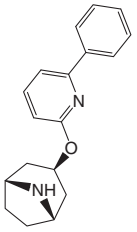
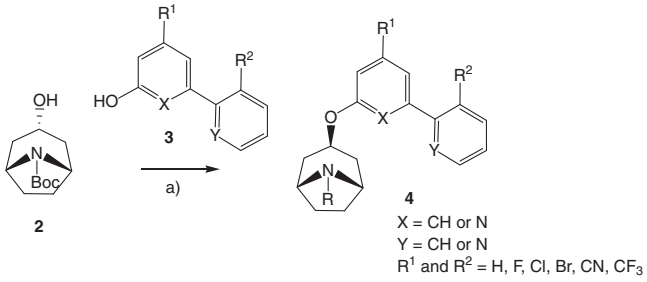
doi:10.1016/j.bmcl.2010.11.021



1

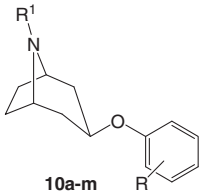
SERT IC_{50} 7.6μM
NET IC_{50} 26nM
DAT IC_{50} >10μM
hERG K_i 1.3 μM

Figure 2.



Scheme 1. Reagents and conditions: (a) PPh_3, DEAD, THF, rt, 18 h.

Table 1
NET, DAT and SERT re-uptake assay results for compounds **10a**–**10m**



10a-m

	R_1	R	NET[a] (IC_{50}/nM)	SERT[a] (IC_{50}/nM)	DAT[a] (IC_{50}/nM)
10a	H	H	54 ± 32	5655 ± 2235	737 ± 198
10b	Me	H	86 ± 5	>10,000	437 ± 200
10c	H	*p*-Chloro	347 ± 203	43 ± 6	2261 ± 94
10d	H	*m*-Chloro	19 ± 7	135 ± 46	380 ± 52
10e	H	*o*-Chloro	188 ± 142	881 ± 166	1318 ± 127
10f	H	*m*-Bromo	13 ± 4	136 ± 62	201 ± 33
10g	H	*m*-Iodo	12 ± 3	106 ± 37	804 ± 141
10h	H	*m*-Fluoro	67 ± 16	1245 ± 136	750 ± 141
10i	H	*m*-Methyl	92 ± 28	624 ± 190	2427 ± 28
10j	H	*m*-Nitro	91 ± 15	86 ± 30	2010 ± 584
10k	H	*m*-CF3	128 ± 51	99 ± 17	3861 ± 426
10l	H	*m*-*OMe*	222 ± 111	369 ± 49	2775 ± 771
10m	H	*m*-*CN*	126 ± 56	25 ± 4	1404 ± 378

[a] Values are mean ± SEM of at least three independent experiments carried out in triplicate.

parameter for optimisation within the series. We report herein the results of those optimisation studies, including the observation that discrete structure–activity relationships (SAR) at hERG could be used to dial out hERG affinity without significant changes to global *c*Log*P*.

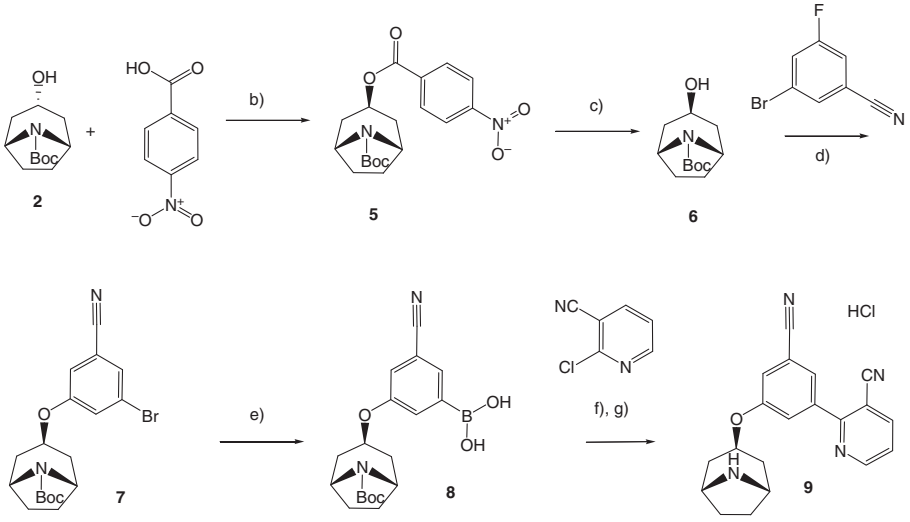
Scheme 1 illustrates a typical synthetic route which was used to prepare analogues **4**.[4] Where phenol or pyridinone derivatives **3** were available, or readily accessible from Suzuki-Miyaura coupling of commercially available 3-bromophenols or 6-bromopyridones, the final compounds **4** were prepared via mitsunobu reaction of compounds **3** with Boc protected *endo*-8-azabicyclo[3.2.1]octan-3-ol **2**.

In cases where the requisite phenol or pyridone **3** was not available then the final compounds were prepared from Boc protected *exo*-8-azabicyclo[3.2.1]octan-3-ol **6**, using an alkylation, borylation sequence to obtain the intermediate boronic acid as illustrated for compound **8**. The final compound **9** was synthesized via a Suzuki-Miyaura coupling of **8** with 3-cyano, 2-chloropyridine and subsequent Boc deprotection (Scheme 2).

The compounds thus prepared were assayed using whole-cell re-uptake assays as described by Haunso and Buchanan[5] using MDCK cells overexpressing NET, and HEK293 cells overexpressing DAT and SERT. Inhibition of re-uptake of ^{3}H-noradrenaline, ^{3}H-dopamine or ^{3}H-serotonin, respectively, was then measured.[6] The human Kv11.1 potassium ion channel (hERG) dofetilide binding assay was carried out as previously described.[7]

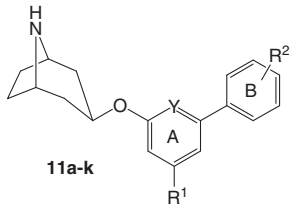
Studies in our laboratories with substituted monoaryl tropane ethers such as **10**, had indicated that the transporter profile could be modified by appropriate selection of substitution pattern on the aromatic ring (Table 1).

In particular we had observed that potency at SERT could be increased by substitution at the *meta*- or *para*-position of the aryl ring. In the case of *para*-substitution as in example **10c** this was



Scheme 2. Reagents and conditions: (b) PPh_3, DEAD, THF, rt, 18 h, 75%; (c) 4 N NaOH (aq), THF, 72 h, 89%; (d) NaH, DMF, rt then μw 180 °C, 900 s; (e) (i) *n*-BuLi −78 °C, B(O*i*-Pr)$_3$ −78 °C to rt (ii) HCl 2 M aq 90%; (f) Pd_2dba_3, K_3PO_4, PCy_3, dioxane/water 2.5:1 100 °C, 30 min, 59%; (g) (i) TFA, DCM (ii) SCX (iii) HCl/diethyl ether 2 M 87%.

Table 2
NET, DAT and SERT re-uptake assay results for compounds



	Y	R_1	R_2	NET[a] (IC_{50}/nM)	SERT[a] (IC_{50}/nM)	DAT[a] (IC_{50}/nM)
1	N	H	H	26 ± 10	7644 ± 1166	>10,000
11a	CH	CF3	H	891 ± 166	4624 ± 593	>10,000
11b	CH	Br	H	132 ± 63	2610 ± 1359	>10,000
11c	CH	Cl	H	30 ± 10	452 ± 129	5329 ± 1723
11d	CH	F	H	85 ± 8	905 ± 360	8710 ± 1290
11e	CH	CN	H	148 ± 73	100 ± 14	9550 ± 282
11f	N	CN	H	90 ± 32	208 ± 34	>10,000
11g	CH	H	*p*-CN	60 ± 9	105 ± 29	173 ± 100
11h	CH	H	*m*-CN	31 ± 19	<10,000	481 ± 49
11i	CH	H	*o*-CN	5 ± 2	2550 ± 216	8066 ± 1934
11j	N	H	*o*-CN	9 ± 1	8981 ± 1019	>10,000
11k	N	CN	*o*-CN	13 ± 2	48 ± 6	>10,000

[a] Values are mean ± SEM of at least three independent experiments carried out in triplicate.

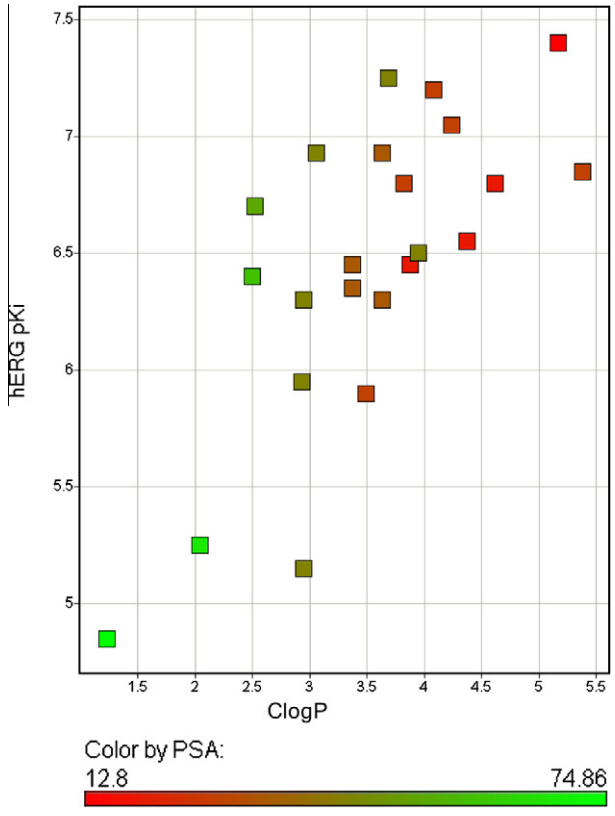


Figure 3. Graph to show the correlation between *cLogP* and hERG dofetilide binding, and the inverse relationship between polar surface area and hERG dofetilide binding. Data points are shaded to reflect polar surface area increasing from red to green.

usually accompanied by a reduction in NET affinity which ran counter to our desired NSRI-like profile. However, in the case of *m*-substitution as in **10d** the SERT potency could be raised without a loss of affinity at the NET transporter. Electron withdrawing groups in the *meta*-position were preferred by the SERT with the nitrile **10m** as the optimal substituent. Our strategy for the optimisation of hit **1** was thus to see if this SAR could be translated to the biaryl series via the synthesis of compounds such as **11a–k**. For synthetic expediency the initial R_1 SAR screen was carried out on the bi-phenyl series (Table 2). We were delighted to observe that the SAR did appear to translate; again the most pronounced boost in SERT potency was seen for the chloro and nitrile analogues **11c** and **11e,f**, with the nitrile affording a ~37-fold increase in SERT affinity (**11f** vs **1**).

We also carried out a SAR screen in the second phenyl ring B, illustrative examples **11g–i** are given in Table 2. In this case the potency at SERT was most enhanced by *para*-substitution **11g**, whereas the NET potency could be significantly boosted by addition of an *ortho*-substituent **11i**. Importantly this SAR was tolerant of re-introduction of the A-ring pyridyl nitrogen from hit **1** as in compound **11j** and was additive with the SAR for the A-ring, since re-introduction of the nitrile R_1 again boosted the SERT potency and afforded a potent NSRI **11k**.

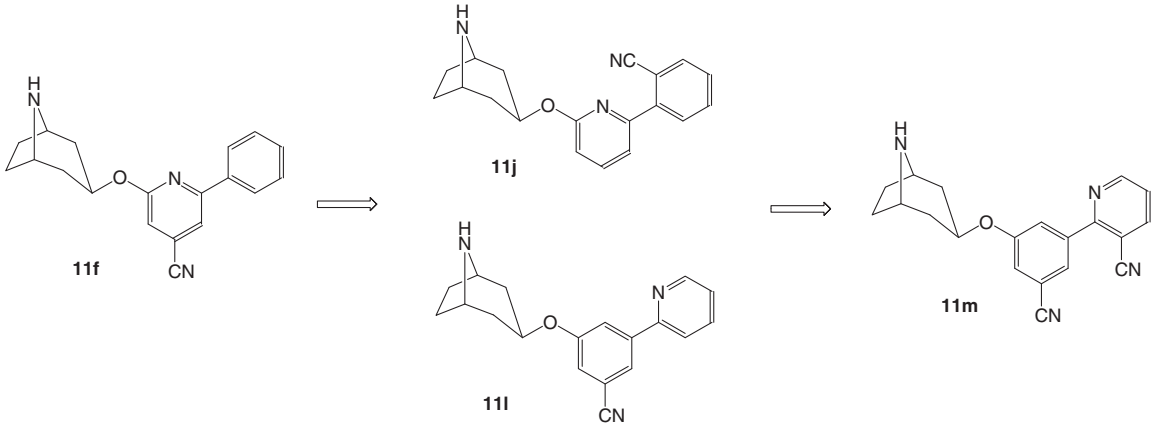
A ubiquitous issue in the development of ligands for monoamine receptors is hERG selectivity. Mindful of this liability, we introduced the hERG dofetilide binding assay[7] early in our screening cascade. It quickly became evident that the biaryl series based around hit **1** has inherent hERG affinity and our optimisation goals were thus focussed on four assays SERT, NET and the selectivity assays, DAT and hERG. As is frequently observed,[8] we identified a positive correlation between *cLogP* and hERG dofetilide binding within the series and a negative correlation with polar surface area (Fig. 3).

Over and above this correlation we also identified some discrete hERG SAR which could be used to modify the hERG selectivity (Table 3). In particular we observed that moving the nitrile to the second aryl ring as in **11j** reduced the hERG affinity by 60-fold, without a significant effect on the *cLogP*. Indeed compound **11j** is an interesting compound in its own right as a very potent NRI. We also observed that moving the nitrogen from the A-ring to the B-ring as in compound **11l** lead to a reduction in hERG potency, but this time retaining the SERT potency. The effect can perhaps be attributed to a change in the pK_a of the pyridine conjugate acid. Combining the above changes into a single compound **11m**, afforded a potent NSRI with reduced hERG affinity relative to **11f**.

Compound **11m** had the best balance of NET/SERT potency and selectivity in the hERG dofetilide assay and was selected for further profiling. The compound was screened against our in-house selectivity panel and was selective against a range of receptors (including <60% inhibition at 10 μM for M1, M2, M3, M4, $5HT_{1a}$, $5HT_{2b}$, $5HT_{2c}$, α_{1A} α_{2a} D2 and H1 and 64% at 10 μM for $5HT_{2a}$). The compound had moderate Caco2 permeability (A–B 178 nm/s and B–A 402 nm/s ER 2.2) and a brain/plasma ratio of 1.5 (1 mpk iv, ICR male mice) predictive of reasonable brain exposure. In vitro microsome and hepatocyte assays predicted low intrinsic clearance for the majority of analogues within the series and **11m** was no exception (rat Clint <12 μL/min/mg protein, and human Clint <12 μL/min/mg protein in microsomes) however, this did not translate into the in vivo clearance which was measured as 103 mL/min/Kg for compound **11m**. Compound **11m** was shown to have a blood/plasma ratio of 3:1 and a high V_{ss} which may in part explain this discrepancy.

Compound **11m** was selected as a tool compound to evaluate the NSRI profile in our in-house in vivo pain models. The compound was tested in a mouse model of inflammatory pain, complete Freund's adjuvant (CFA) induced thermal hyperalgesia and a rat model of neuropathic pain, spinal nerve ligation (SNL) induced mechanical allodynia. In the mouse CFA model **11m** completely reversed the thermal hyperalgesia induced by CFA after oral administration of 100 μmol/kg. Similarly **11m** dose dependently

Table 3
hERG dofetilide binding of analogues **11f**, **j**, **l**, **k** and **m**



Compound	hERG dofetilide (K_i/nM)	*cLogP*	NET[a] (IC_{50}/nM)	SERT[a]	DAT[a] (IC_{50}/nM)
11f	119 ± 14	3.06	90 ± 33	208 ± 34	>10,000
11j	7080 ± 864	2.95	9 ± 1	8981 ± 1019	>10,000
11l	1621 ± 834	2.39	111 ± 88	169 ± 45	>10,000
11k	251 ± 147	2.50	13 ± 4	94 ± 7	>10,000
11m	3802 ± 1130	2.01	23 ± 8	101 ± 11	>10,000

[a] Values are mean ± SEM of at least three independent experiments carried out in triplicate.

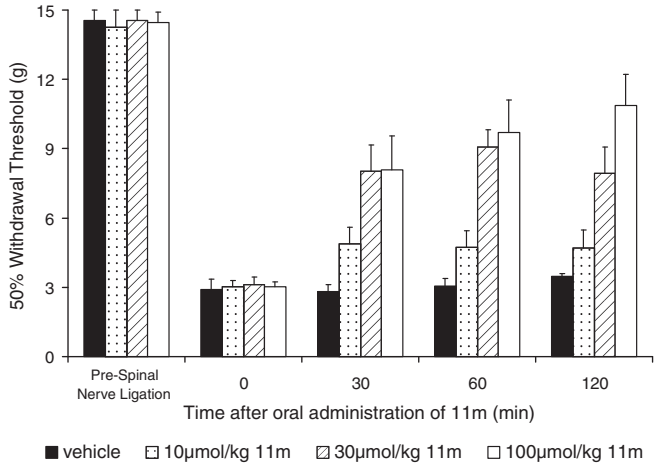


Figure 4. Effect of compound **11m** in a rat model of neuropathic pain, spinal nerve ligation (SNL) induced mechanical allodynia.

attenuated the mechanical allodynia induced by SNL. This effect was significant compared to vehicle treated animals after a dose of 30 or 100 μmol/kg PO (Fig. 4). Both NRIs and to a more modest extent SNRIs (e.g., duloxetine) have proven efficacy in the SNL model,[9] however, in our hands, SRIs (e.g., fluoxetine) are not active, indicating that noradrenaline re-uptake inhibition is key for analgesic efficacy in this model. Addition of the component of serotonin re-uptake inhibition as in compound **11m** may confer additional benefits compared with NRIs hence **11m** provides a tool compound for wider pharmacological profiling with comparator NRI/SNRI/SSRI compounds in neurochemistry studies and neuropathic pain models (Table 4).

In summary we have optimised the NET selective hit **1** to afford an NSRI **11m** with reduced hERG liability compared with the starting compound. The key discovery which allowed us to build in the SERT inhibition, without introducing DAT inhibition was the incorporation of a nitrile in the *meta*-position of the A-ring. The chemical series was optimised further to dial out hERG binding affinity, via a dual strategy of global *cLogP* reduction and discrete structural changes, to afford compound **11m** which had demonstrated efficacy in animal models of inflammatory and neuropathic pain. Further characterisation of compound **11m** identified that the window between the estimated peak plasma exposure at the MED (minimum effective dose) and adverse effects in the Langendorf model,[10] a model of cardiovascular conductance, was not sufficient and development of this series was stopped in favour of more promising chemotypes. Compound **11m** is however a useful tool for evaluating the NET > SERT >> DAT profile in neuropathic pain models.

Table 4
In vivo DMPK data for **11m**

	1 mg/Kg iv[a]			10 mg/Kg p.o[a]		
	V_{ss} (L/Kg)	Cl (mL/min/kg)	AUC (ng/mL h)	*F* (%)	T_{max} (h)	$t_{1/2}$ (h)
11m	6.9	103	563	34	1.7	3.2

[a] Male wistar rat.

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6. Cells were seeded the day before the experiment at 35,000 cells/well in 96 well plates. On the day of the experiment, cells were washed and pre-incubated with assay buffer (HBSS supplemented with pargyline and ascorbic acid) containing monoamine transport inhibitors for 5 min. Subsequent incubation with ^{3}H-dopamine, ^{3}H-serotonin or ^{3}H-noradrenaline (final concentration of 20 nM) for 3, 5 and 10 min for DAT, SERT and NET, respectively, termination of the assay by cold HBSS wash and addition of MicroScint-20 scintillation mixture to determine ^{3}H-monoamine uptake was carried out. All incubations were performed at room temperature except for NET cells which were incubated at 37 °C. IC_{50} values were determined form 6-point concentration–response curves from three independent experiments carried out in triplicates.
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