Bioorganic & Medicinal Chemistry Letters 24 (2014) 177–180

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The identification of AF38469: An orally bioavailable inhibitor of the VPS10P family sorting receptor Sortilin

Tenna Juul Schrøder [a], Søren Christensen [a], Samsa Lindberg [a], Morten Langgård [a], Laurent David [a], Philip J. Maltas [a], Jørgen Eskildsen [a], Jan Jacobsen [a], Lena Tagmose [a], Klaus Bæk Simonsen [a], Lars Christian Biilmann Rønn [a], Inge E. M. de Jong [a], Ibrahim J. Malik [a], Jens-Jakob Karlsson [a], Christoffer Bundgaard [a], Jan Egebjerg [a], Jeffrey B. Stavenhagen [a], Dorthe Strandbygård [b], Søren Thirup [b], Jacob Lauwring Andersen [b], Srinivas Uppalanchi [c], Sridhar Pervaram [c], Shiva Prasad Kasturi [c], Pradheep Eradi [c], Durga Rao Sakumudi [c], Stephen P. Watson [a,*]

[a] Neuroscience Drug Discovery, H. Lundbeck A/S, Copenhagen, Denmark
[b] The Lundbeck Foundation Research Centre MIND, Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10C, Aarhus C 8000, Denmark
[c] Medicinal Chemistry, GVK BioScience, Hyderabad, India

ARTICLE INFO

Article history:
Received 16 October 2013
Revised 15 November 2013
Accepted 19 November 2013
Available online 27 November 2013

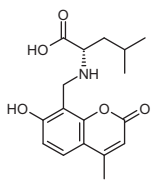
Keywords:
Sortilin
Neurotensin
AF38469
AF40431
VPS10P
Sorting receptor
Sortilin crystal structure
Protein-inhibitor complex

ABSTRACT

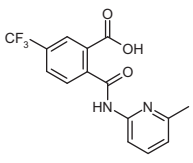
The identification of the novel, selective, orally bioavailable Sortilin inhibitor AF38469 is described. Structure–activity relationships and syntheses are reported, along with an X-ray crystal structure of the sortilin-AF38469 protein-inhibitor complex.

Sortilin is a type I membrane receptor belonging to the vacuolar protein sorting 10 protein (VPS10P) family of sorting receptors.[1] Sortilin is widely expressed in both the central nervous system and periphery. It mediates a number of important physiological functions via trafficking of, and signalling with, a variety of different protein partners. For example sortilin is involved in signalling via the neurotrophins, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Indeed, in complex with the protein p75, sortilin has been reported to form the receptor for pro-neurotrophin-mediated apoptotic effects leading to degeneration and cell death in cellular and animal models.[1] Sortilin has also been demonstrated to interact with apolipoprotein B100 in the Golgi and facilitate the export of apoB100-containing lipoproteins, thereby regulating plasma low-density lipoprotein (LDL) cholesterol levels, a key contributor to atherosclerosis and ischemic heart disease.[2] Recently, sortilin was also shown to function as a high affinity receptor for progranulin[3], and to mediate clearance of progranulin by binding followed by cellular uptake and distribution to lysosomes.

The 13 amino acid neuropeptide Neurotensin (NTS) is a sortilin ligand, indeed sortilin had been known as the neurotensin receptor 3 (NTR3[4]). Moreover the structure of the Neurotensin–Sortilin complex has been determined by X-ray crystallography.[5]



AF40431
Sortilin NTS IC_{50} 4μM



AF38469
Sortilin NTS IC_{50} = 330nM

* Corresponding author.
E-mail address: stwa@lundbeck.com (S.P. Watson).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.11.046

Thus due to its rich biology and association with pathological processes sortilin represents an interesting target. However there is currently a dearth of molecular tools available to understand and disease associate the biology of sortilin. We previously reported[6] the identification of the Sortilin inhibitor AF40431, the first small molecule ligand for sortilin. Unfortunately, the very low solubility and membrane permeability of AF40431 limited its use as a molecular probe.

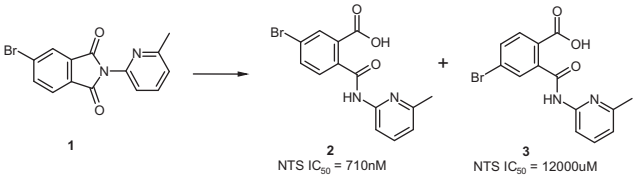
Herein we report the identification of a series of small molecule sortilin inhibitors, optimisation of which led to the orally bioavailable inhibitor AF38469. This molecule could prove a valuable molecular tool to further delineate and disease associate the biology of sortilin.

A high throughput screen (HTS) of the Lundbeck collection was performed using an NTS scintillation proximity assay (SPA) format binding assay.[7] A series of phthalimides, exemplified by compound 1 was one of the promising chemotypes that emerged from the HTS. Whilst these pthalimides were fully characterised, demonstrated good chemical stability in DMSO and DMSO/water solution they displayed a perplexing structure–activity relationship (SAR) which led to a more detailed investigation. LCMS analysis of assay wells revealed that phthalimide (**1**) degraded under assay conditions to afford the two corresponding phthalimide hydrolysis products, phthalamic acids **2** and **3**, respectively (scheme 1). Synthesis and testing of these two regioisomeric acids revealed **2** to be responsible for the observed NTS binding inhibition (with an IC_{50} of 710 nM), whilst compound **3** was only weakly active. Compound **2** thus represented an interesting Sortilin inhibitor hit which became the subject of an investigation/optimisation programme.

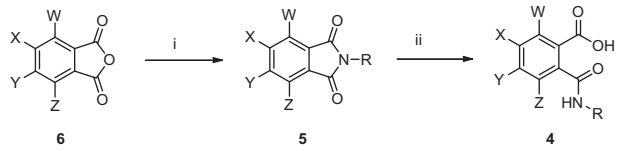
Analogues (**4**) of compound **2** were typically prepared via simple hydrolysis of the corresponding phthalimides (**5**) and separation of regioisomers via preparative HPLC. Phthalimides **5** were in turn prepared via condensation-*cyclo*-dehydration from the corresponding phthalic anhydrides **6** (scheme 2) (overall this route was more expedient than attempting a mono-coupling to the corresponding phthalic acids).

Alternatively compounds **7**, bearing just a 5-Bromo substituent on the phthalamic acid ring, were selectively prepared from corresponding phthalate mono-*t*-butyl ester **8** via coupling-deprotection (scheme 3). Intermediate **8** itself was prepared from the reaction of phthalic anhydride **9** with *tert*-butanol and separation of the regioisomeric mono esters by supercritical fluid chromatography (SFC). In addition, palladium-catalysed coupling could be used to replace the bromine of intermediate **8** (and also of compounds **7**) with alternative substituents.

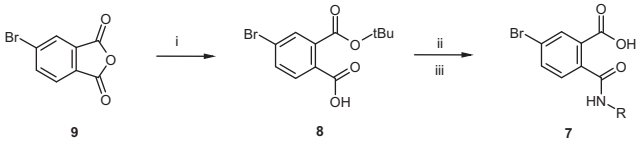
Table 1 shows the effect of modifications to the substituents of the phthalamic acid ring of compounds of type **10** on sortilin potency. Interestingly the compound with no such ring substituents (**10a**) is inactive. As seen with the initial hits **2** and **3** there is a clear preference for substituents in the 5-position, affording much higher potency compared to the 4-position, as is demonstrated by Br, Cl, Me and CF_3 substituents. Compounds **10b** and **10m**, bearing a methyl substituent at the 3- or 6-position respectively are inactive, as is the 5-phenyl analogue (**10g**). Interestingly the 4,5 di-chloro and 4,5-di-methyl analogues (**10k** and **10l**) show a slight increase in potency compared to their 5-chloro and



Scheme 1. Degradation of phthalamide **1** under assay[6] conditions.

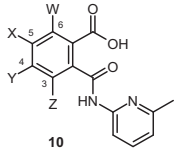


Scheme 2. Reagents and conditions: (i) $ArNH_2$ in AcOH, 5 h, reflux, (ii) LiOH, THF/Water, 1 h room temperature.



Scheme 3. Reagents and conditions: (i) tBuOH, DMPA, CH_2Cl_2, 2 h, then SFC, (ii) $ArNH_2$ HATU, CH_2Cl_2 18 h, room temperature, (iii) CH_2Cl_2/TFA 1:1, 4 h room temperature.

Table 1
Sortilin binding affinity of compounds **10**



Compound	W	X	Y	Z	NTS[6] IC_{50} (nM)
10a	H	H	H	H	15%*
10b	Me	H	H	H	5%*
2	H	Br	H	H	710
10c	H	Cl	H	H	1400
10d	H	Me	H	H	6400
10e	H	iPr	H	H	1100
AF38469	H	CF_3	H	H	330
10f	H	NO_2	H	H	1700
10g	H	Ph	H	H	2%*
3	H	H	Br	H	12000
10h	H	H	Cl	H	69%*
10i	H	H	Me	H	57%*
10j	H	H	CF_3	H	9100
10k	H	Cl	Cl	H	830
10l	H	Me	Me	H	3400
10m	H	H	H	Me	43%*

* % Inhibition at 50 μM for compounds too weak to fit a dose-response curve, IC_{50} > 50 μM.

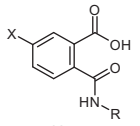
5-methyl mono-substituted counterparts (**10c** and **10d**) The trifluoromethyl group of AF38469 confers the highest potency of the substituents explored.

Table 2 shows the effect of modifications to the amide substituent of compounds of type **11** on sortilin potency.

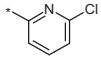
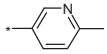
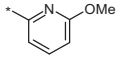
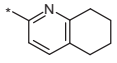
Simple alkyl amides were inactive e.g. **11a** and **11b**, as interestingly, is phthalamic acid **11c** with an unsubstituted phenyl group. However, relative to the unsubstituted phenyl (**11c**), introduction of a *m*-Me substituent or an *o*-pyridine nitrogen atom afforded compounds of modest potency (**11d** and **11e**) but a combination of both of these features shows a synergistic effect in the potency of the original hit compound **2**. Some other pyridine nitrogen and methyl substitutions afford active compounds but are all less potent than the arrangement in compound **2** and AF38469 (e.g. **11g** and **11j**). Pyridine 6,5-di-substitution in di-methyl compound **11n** affords a modest increase in potency, and in the tetrahydroquinoline **11o**, with an IC_{50} of 100 nM, the most potent compound prepared.

Methylation of the amide nitrogen of **2** in compound **12** completely ablates sortilin potency. Replacements of the carboxylic

Table 2
Sortilin binding affinity of compounds **11**

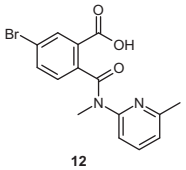


11

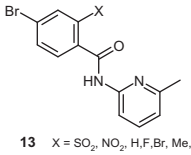
Compound	X	R	NTS[6] IC_{50} (nM)
11a	Br	sBu	30%*
11b	Br	iPr	35%*
11c	Br	Ph	69%*
11d	Br		5200
11e	Br		3600
2	Br		710
11f	Br		1600
11g	Br		2400
11h	Br		64%*
11i	Br		70%*
11j	Br		1800
11k	Br		490
AF38469	CF_3		330
11m	CF_3		1300
11n	CF_3		170
11o	CF_3		100

* % Inhibition at 50 μM for compounds too weak to fit a dose-response curve, IC_{50} > 50 μM.

acid functionality also ablated activity; all compounds **13** were inactive, indicative of an acidic interaction with the protein (as was subsequently confirmed by x-ray crystallography of the AF38469-Sortilin complex, Figures 1 and 2).



12



13 X = SO_2, NO_2, H,F,Br, Me, OMe

The more potent of compounds **11** typically demonstrated moderate lipophilicity with some of the more polar examples demonstrating promising Ligand Lipohilic Efficiency[8] (LLE) above **4** (Table 3).

AF38469 was progressed for further studies. It should be noted that Neurotensin itself exhibits a binding IC_{50} of 360 nM in the ^{3}HNTS assay[7], thus AF38469 is essentially equipotent with this sortilin substrate.

AF38469 showed no inhibition or stimulation of >50% at 10 μM in a standard selectivity panel of ca. 70 targets run at CEREP.[9] Importantly AF38469 showed no activity against the NTR1 receptor. In addition AF38469 showed no activity against a selected panel of targets known to bind acidic molecules (δ-Opioid, GPR40, PPARδ, EP1, Angiotensin AT1, Endothelin ETA & B, MMP-12). Thus overall the selectivity profiling of AF38469 demonstrated a highly specific interaction with sortilin.

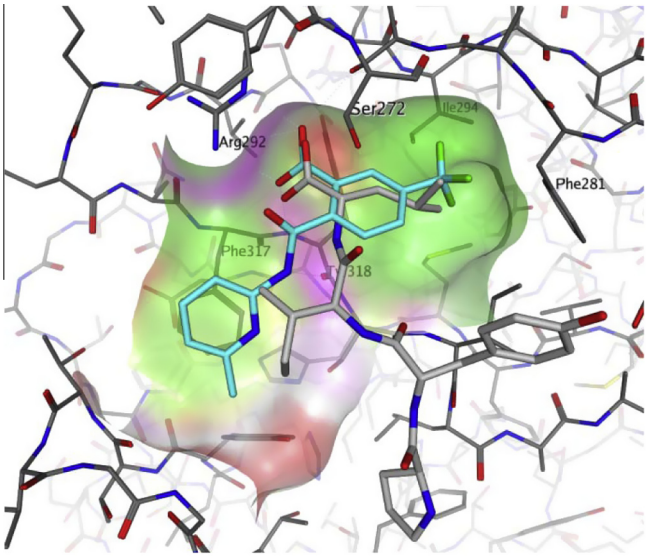


Figure 1. Overlay of X-ray crystal structures of sortilin bound AF38469 (PDB code 4N7E) and Neurotensin (C-terminus) (PDB code 3F6K).

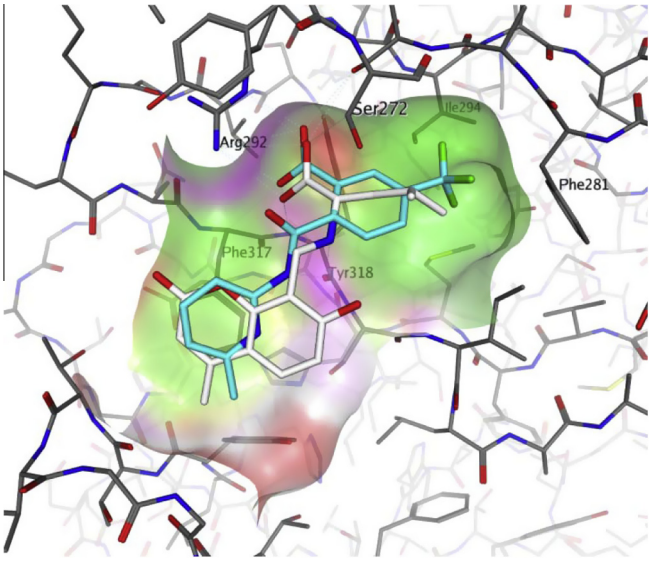


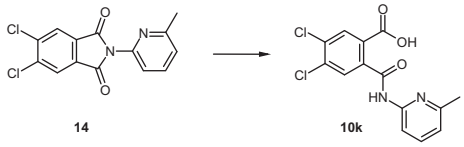
Figure 2. Overlay of X-ray crystal structures of sortilin bound AF38469 (PDB code 4N7E) and AF40431 (PDB code 4MSL)[5].

Table 3
Lipohilic ligand efficiency (LLE) of compounds **11**

Compound	clog*P*	LLE
2	2.2	3.9
11j	2.2	3.5
11k	1.6	4.7
11m	2.5	3.4
11n	2.7	4.1
11o	3.3	3.7
AF38469	2.2	4.3

Table 4
AF38469 pharmacokinetic parameters in rat

In Vitro	Solubility 134 μg/ml, plasma free fraction 2% intrinsic clearance 0.4 l/h/kg
Oral (2 mg/kg)	C_{max} 12850 ng/ml
i.v. (1 mg/kg)	Clb 0.03 L/h/Kg, $t_{1/2}$ 1.2 h, Vss 0.02 L/kg



Scheme 4. Symmetrical phthalamides as pro-drugs.

Table 4 describes key pharmacokinetic parameters for AF38469 in rat. The compound has a low volume of distribution (relative to blood volume of 0.7 l/kg) and low clearance (relative to liver blood flow of ca. 4.8 l/h/kg), and a half-life of 1.2 h. The initial exposure in plasma is high, due in part to the low volume of distribution. The oral bioavailability is 35%. Simple calculations based on C_{max}, free fraction and the sortilin potency suggest that a free plasma concentration of AF38469 ca. 2 fold higher than its IC_{50} is obtained at Cmax from an oral 2 mg/kg dose.

In principle a phthalimide could act as a pro-drug for an AF38469 type compound, with hydrolysis in vivo affording the corresponding phthalamic acid by an analogous process to that which lead to the liberation and identification of compound **2** itself (Scheme 1). Whilst an asymmetrically substituted phthalimide such as **1** would afford a mixture of regioisomers on hydrolysis (viz. **2** and **3**), a symmetrical phthalimide would afford a single phthalamic acid (e.g. Scheme 4). To this end phthalimide **14** was investigated as a potential oral prodrug for phthalamic acid **10k**. However, whilst oral administration of the phthalimide (**14**) did afford systemic exposure of phthalamic acid **10k**, the free exposure of the acid was not an improvement on the exposure of AF38469 obtained via oral administration.

A co-crystal of sortilin and AF38469 was successfully obtained and its structure determined[10] by X-Ray crystallography to a resolution of 2.78 Å. Figure 1 shows an overlay of the crystal structure of AF38469 with that of the structure of the C-terminal region of sortilin bound Neurotensin.[5] AF38469 makes similar interactions with sortilin as does the C-terminal Leu residue of Neurotensin. AF38469 makes a salt bridge to Arg292 via its carboxylic acid, the CF_3 group occupies the same hydrophobic binding pocket as the iPr of the Leu side-chain, and the amide N–H makes a hydrogen-bond donor interaction with Tyr318. The pyridyl methyl group makes a hydrophobic π-interaction with Phe317, similar to hydrophobic interaction of Ile12 of Neurotensin. The structure also serves to rationalise many of the structure–activity relationships outlined above, for example the key role of the pthalamic acid **5** substituent, the inactivity of the 5-Ph (**10f**) (due to size exclusion), and the pivotal role of the carboxylic acid functionality.

Figure 2 shows an overlay of the binding mode of AF38469 with the previously reported[6] small molecule inhibitor AF40431. AF38469 and AF40431 exhibit many common interactions, including the salt bridge and leucine pocket binding features of Neurotensin, but also interestingly the pyridine ring and methyl substituent of the former are virtually congruent with the pyran ring and methyl substituent of the latter.

It is anticipated that the structure of the sortilin-AF38469 complex will be used to further drive optimisation of the chemotype through classical structure based drug design.

In summary we have identified a potent, selective and orally bioavailable inhibitor for the VPS10P family sorting receptor Sortilin. We hope and anticipate that AF38469 will serve as an important tool to further delineate the biology of Sortilin, and to facilitate evaluation of the therapeutic potential of this protein.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.046.

References and notes

1. Nykjaer, A.; Willnow, T. E. *Trends Neurosci.* **2012**, *35*, 261.
2. Kjolby, M.; Andersen, O. M.; Breiderhoff, T.; Fjorback, A. W.; Pedersen, K. M.; Madsen, P.; Jansen, P.; Heeren, J.; Willnow, T. E.; Nykjaer, A. *Cell Metab.* **2010**, *12*, 213.
3. Hu, F.; Padukkavidana, T.; Vægter, C. B.; Zheng, Y.; Mackenzie, I. R.; Feldman, H. H.; Nykjaer, A.; Strittmatter, S. M. *Neuron* **2010**, *68*, 654.
4. The Neurotensin receptors, NTR1 and NTR2 are G-protein coupled receptors see Kitabgi, P. *Curr. Opin. Drug Discovery Dev.* **2002**, *5*, 764–768.
5. Quistgaard, E. M.; Madsen, P.; Grøftehauge, M. K.; Nissen, P.; Petersen, C. M.; Thirup, S. S. *Nature Struc. Mol. Biol.* **2009**, *9*, 46.
6. Andersen, J.L.; Schrøder, T.J.; Christensen, S.; Strandbygaard, D.; Pallesen, L.T.; García-Alai, M.M.; Lindberg, S.; Langgård,M.; Eskildsen, J.; David, L.; Tagmose, L.; Simonsen, K.B.; Maltas, P.J.; Biilmann Rønn, L.C.; de Jong, I.M.E.; Malik, I.J.; Egebjerg, J. Karlsson, J.J.; Uppalanchi, S.; Sakumudi, D.R., Eradi, P.; Watson, S.P.; Thirup, S. Acta Cryst. **2014**, *D70*, http://dx.doi.org/10.1107/S1399004713030149.
7. Compound affinity was determined by measuring the displacement of 3*H*-neurotensin binding to hSortilin in SPA format. Total volume of 40 μl in 50 mM HEPES pH 7.4 assay buffer containing 100 mM NaCl, 2.0 mM $CaCl_2$, 0.1% BSA and 0.1% Tween-20. Compound pre-incubation for 30 min at RT with 150 nM of 6his-Sortilin before 5 nM [3*H*]-Neurotensin and Ni chelate imaging beads (Perkin Elmer) were added, after 6 h plate was read on a ViewLux with 360 s exposure time. Dose-response evaluation of compounds was performed with 10 concentrations of drugs (covering 3 decades). IC_{50} values were calculated by nonlinear regression using the sigmoid concentration-response (variable slope) using Xlfit 4 (IDBS, UK). All values reported are average of at least 4 determination
8. Abad-Zapatero, C.; Metz, J. *Drug Discovery Today* **2005**, *10*, 464.
9. In earlier versions of the current CEREP ExpresSprofile, ADR CNS and blood panels—see http://www.cerep.fr/.
10. PDB Code 4N7E See Supplementary Data.