Bioorganic & Medicinal Chemistry Letters 18 (2008) 4308–4311

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

N-Benzyl-*N*-(pyrrolidin-3-yl)carboxamides as a new class of selective dual serotonin/noradrenaline reuptake inhibitors

Florian Wakenhut [a,*], Paul V. Fish [a], M. Jonathan Fray [a], Ian Gurrell [b], James E. Mills [a], Alan Stobie [a], Gavin A. Whitlock [a]

[a] Department of Genitourinary Chemistry, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK
[b] Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

ARTICLE INFO

Article history:
Received 23 May 2008
Revised 24 June 2008
Accepted 25 June 2008
Available online 28 June 2008

Keywords:
N-benzyl-*N*-(pyrrolidin-3-yl)carboxamides
Serotonin reuptake
Noradrenaline reuptake
Metabolic profile
Metabolic stability
CYP2D6 inhibition
Lipophilicity
SNRI

ABSTRACT

The structure–activity relationship and the synthesis of novel *N*-benzyl-*N*-(pyrrolidin-3-yl)carboxamides as dual serotonin (5-HT) and noradrenaline (NA) monoamine reuptake inhibitors are described. Compounds such as **18** exhibited dual 5-HT and NA reuptake inhibition, good selectivity over dopamine (DA) reuptake inhibition and drug-like physicochemical properties consistent with CNS target space. Compound **18** was selected for further preclinical evaluation.

Selective inhibition of serotonin (5-HT) and noradrenaline (NA) reuptake constitutes an attractive dual pharmacology approach to the treatment of a number of diseases. For example, venlafaxine **1** and duloxetine **2** have become established drugs for the treatment of depression.[1,2] Duloxetine has also shown clinical efficacy for the treatment of diabetic neuropathic pain[3] and stress urinary incontinence (SUI).[4]

As part of our research efforts to identify new dual serotonin and noradrenaline reuptake inhibitors (SNRI) as potential drug candidates, we recently reported several new templates[5–8] that delivered potent selective dual serotonin/noradrenaline reuptake inhibitors (SNRI), and notably a 3-amino-pyrrolidine template that furnished compound **3** (Fig. 1). Unfortunately, when the metabolic profile of compound **3** was investigated, we found that it was mainly metabolized by the cytochrome P450 enzyme CYP2D6 through oxidation of the benzylic ring (Fig. 2).

As a consequence of the potential for drug–drug interactions and the risk of overexposure in poor metabolizers,[9] the progression of compound **3** was halted. In order to block the CYP2D6 mediated oxidation pathway, our initial strategy was to replace the 2,3-di-Me benzylic substituents of compound **3** with 2,3-di-Cl (Fig. 3) leading to compound **4**. This strategy was successful and N-dealkylation was identified to be the major route of metabolism for compound **4**. However, the metabolizing enzyme had also swapped in the process and this metabolic pathway was now mainly mediated by CYP2D6 and not CYP3A4, as for compound **3**, resulting in no mitigation of the drug–drug interaction risk.

We therefore decided to try blocking the N-dealkylation pathway and to do so we elected to replace the 4-tetrahydropyranyl group with carboxamides (Fig. 4). The structure–activity relationship of these amides **8**–**29** is reported in this letter.

Test compounds were conveniently prepared from commercially available homochiral *N*-BOC 3-amino pyrrolidine **5** by the general method described in Scheme 1. Standard reductive amination with the appropriate benzaldehydes followed by acylation of the resulting amines **6** afforded amides **7**. Finally HCl or TFA deprotection of the *N*-BOC amines provided required targets **8**–**29**. It is worth noting that this synthetic route was highly amenable to parallel synthesis and this greatly facilitated SAR exploration. Initial investigations focused on the variation of the carboxamide group (R^1) with the benzylic substitution (R) maintained as 2,3-di-Cl and 2,4-di-Cl (Table 1). A series of analogues **8**–**21** were prepared with R^1 alkyls of increasing size. Interestingly, both benzylic substitution patterns afforded good dual serotonin/noradrenaline reuptake inhibition although 2,4-di-Cl analogues generally exhib-

* Corresponding author. Tel.: +44 1304 640950; fax +44 1304 651987.
E-mail address: florian.wakenhut@pfizer.com (F. Wakenhut).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.078

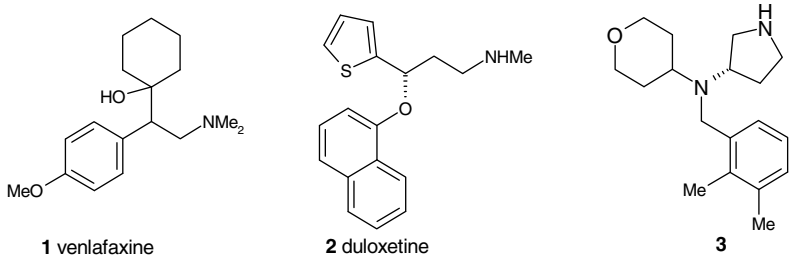


Figure 1. Structures of duloxetine, venlafaxine and recently disclosed SNRI **3**.

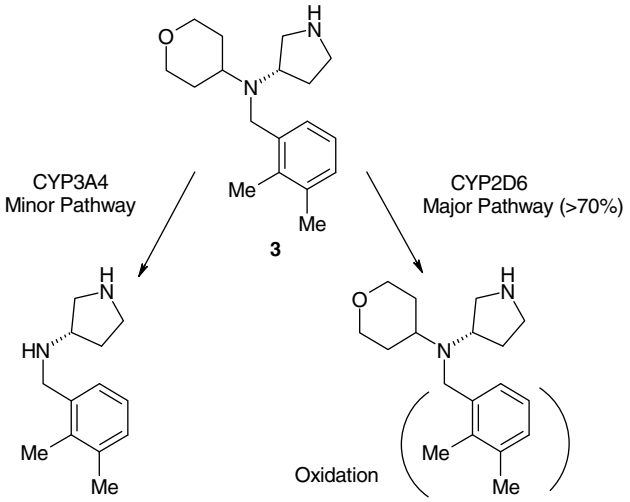


Figure 2. Metabolic profile of compound **3**.

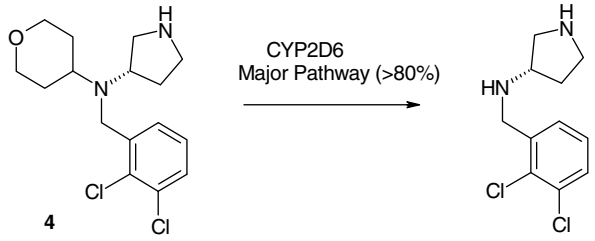


Figure 3. Metabolic profile of compound **4**.

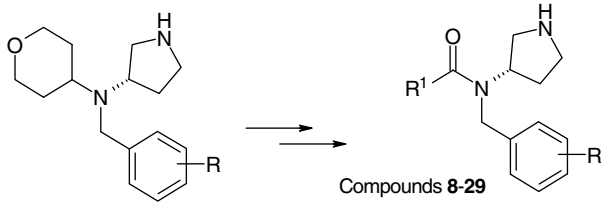


Figure 4. Structure of new amino-pyrrolidine carboxamides **8–29**.

ited slightly weaker activity for the 5-HT transporter, as can be seen when comparing compounds **19** and **12**. From this analysis, the isopropyl group emerged as our preferred R^1 substituent and compounds **11** and **18** gave potent SNRI activity, and excellent selectivity against dopamine reuptake activity (DRI). Replacing the alkyl groups with either a phenyl group (compound **17**) or a more polar branched 4-tetrahydropyranyl group (compound **21**) resulted in a drop in both SRI and NRI activities.

Having identified the isopropyl group as our preferred R^1 substituent a broader set of R substituents with different substitution patterns was investigated (compounds **22**–**28**). This analysis afforded additional compounds with excellent SNRI activity and good selectivity against dopamine reuptake activity (compound **24**). Interestingly, it also demonstrated that, depending on the choice of R substituents, the series could deliver selective inhibitors of the noradrenaline transporter (NRI) as illustrated by compound **22** with a single 2-Ph substituent, or selective inhibitors of the serotonin transporter (SRI), for example, compound **26** with a 2-F, 4-CF_3 substitution pattern. Finally, it is worth noting that both aminopyrrolidine enantiomers possessed SNRI activity but that the (*S*) enantiomer provided more balanced SNRI activities (compounds **18** and **29**).

Based on that observation, assuming that the remaining substructures overlap, there are two distinct overlaps possible for the pyrrolidine portion of the two enantiomers. In an overlap driven by H-bonding complementarity (Fig. 5), in this case, the projection of the N–H moieties to a common acceptor atom position, the rings do not sterically overlap perfectly, but certainly should be able to occupy the same pocket.

In an overlap driven by steric complementarity (Fig. 6), the two nitrogen atoms do not superimpose, and it is assumed that the presumably acidic moiety of the transporter can flex to accommodate these alternative positions.

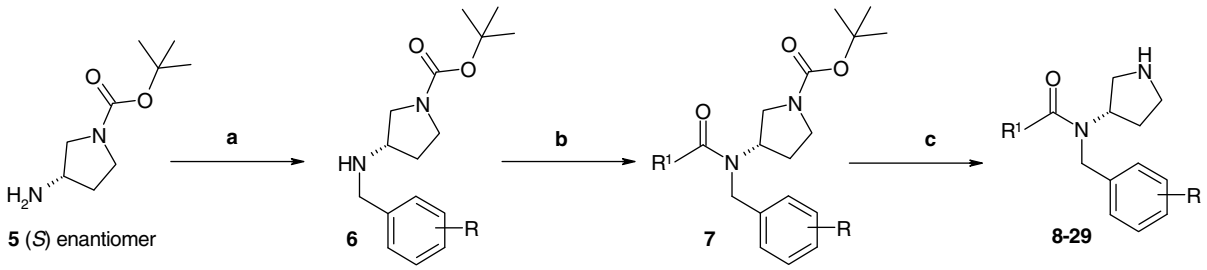
From these experiments, compounds **11**, **18** and **24** emerged as preferred compounds, exhibiting a superior combination of dual SNRI activity and good selectivity against dopamine reuptake activity. The three compounds also exhibited attractive drug-like physicochemical properties consistent with CNS target space[11] as illustrated in Table 2.

Additional screening in in vitro ADME assays (Table 3) demonstrated that compounds **11**, **18** and **24** all exhibited good metabolic stability in human liver microsomes, therefore predicting low in vivo clearance. To further rank them, the three analogues were tested in a human hepatocyte assay and compound **18** was found to be the more metabolically robust, therefore, predicting lowest clearance. All three compounds were weak CYP2D6 inhibitors with compound **24** being the most potent. This may be attributed to its higher lipophilicity as it is well established that lipophilic compounds tend to have an increased risk of off-target promiscuity.[12]

Compounds **11**, **18** and **24** all exhibited good membrane permeability in the CaCO-2 cell line,[13] therefore, predicting good oral absorption.

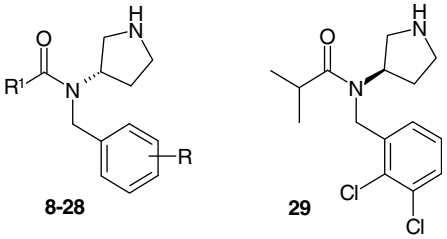
Compounds **11**, **18** and **24** were tested for their ability to block the hERG channel, as it is well established that compounds that block this ion channel have the potential to cause QT prolongation and cardiac arrhythmia in man.[14] The three analogues were found to be selective with compound **24** being the least selective probably also as a result of its higher lipophilicity.

Based on its promising profile, including notably exquisite metabolic stability and weak CYP2D6 inhibition, compound **18** was selected to further evaluate the series.



Scheme 1. Reagents and conditions: (a) Benzaldehyde, toluene, Dean–Stark apparatus, reflux, 18 h then $NaBH_4$, MeOH, RT, 2 h; (b) R^1COCl, dioxane, Et_3N, 70 °C, 2 h; (c) TFA, CH_2Cl_2, RT, 2–18 h or 4 N HCl in dioxane, rt, 2–18 h.

Table 1
In vitro inhibition of human monoamine reuptake,[a,b] and *c*log*P* for compounds **8**–**29**



Compound	R	R^1	Stereochemistry	5-HT IC_{50} (nM)	NA IC_{50} (nM)	DA IC_{50} (nM)	*c*log*P*
3				9	7	727	
8	2,4-di-Cl	Ethyl	*S*	25	60	NT[c]	3.1
9	2,4-di-Cl	Propyl	*S*	72	31	NT	3.6
10	2,4-di-Cl	Butyl	*S*	33	70	NT	4.1
11	2,4-di-Cl	Isopropyl	*S*	13	28	>40,000	3.4
12	2,4-di-Cl	Isobutyl	*S*	93	31	NT	4
13	2,4-di-Cl	Tertbutyl	*S*	41	92	NT	3.7
14	2,4-di-Cl	Neopentyl	*S*	27	39	10,200	4.4
15	2,4-di-Cl	Cyclopropyl	*S*	38	25	NT	3.1
16	2,4-di-Cl	Cyclobutyl	*S*	41	64	NT	3.4
17	2,4-di-Cl	Phenyl	*S*	195	94	NT	4.2
18	2,3-di-Cl	Isopropyl	*S*	12	23	1270	3.3
19	2,3-di-Cl	Isobutyl	*S*	17	62	NT	3.9
20	2,3-di-Cl	Tertbutyl	*S*	26	>322	NT	3.8
21	2,3-di-Cl	4-Tetrahydropyranyl	*S*	123	300	NT	2
22	2-Phenyl	Isopropyl	*S*	>400	14	NT	3.5
23	2-Cl	Isopropyl	*S*	91	114	NT	2.7
24	2,3,4-tri-Cl	Isopropyl	*S*	5	16	5870	3.8
25	2,3,5-tri-Cl	Isopropyl	*S*	44	29	21,600	4
26	2-F, 4-CF_3	Isopropyl	*S*	24	>400	NT	2.9
27	2-Cl, 4-F	Isopropyl	*S*	161	98	NT	2.8
28	2-F,4-F	Isopropyl	*S*	>400	>400	NT	2.2
29	2,3-di-Cl	Isopropyl	*R*	5	37	1120	3.3

[a] See Ref. 10 for complete details of assay conditions.
[b] Monoamine reuptake IC_{50} are geometric means of a minimum of two experiments. Differences of <2-fold should not be considered significant.
[c] NT denotes not tested.

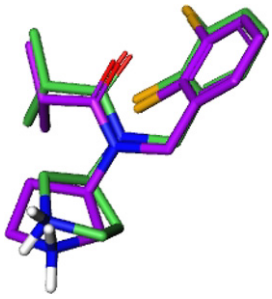


Figure 5. Overlap of compounds **18** and **29** driven by H-bonding complementarity.

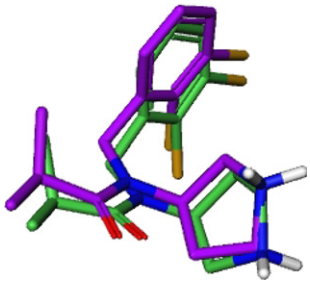


Figure 6. Overlap of compounds **18** and **29** driven by steric complementarity.

Table 2
Physicochemical properties of compounds **11**, **18** and **24**[a]

	11	18	24
MW	315	315	348
*c*log*P*	3.4	3.3	3.8
Log$D_{7.4}$	0.8	0.8	1.3
HBD/HBA count	1/3	1/3	1/3
pK_a	ND	9.4	ND
TPSA	32	32	32

[a] ND denotes not determined.

Table 3
ADME profiles and K^+ channel affinities of compounds **11**, **18** and **24**[a]

	11	18	24
HLM, $t_{1/2}$ (min)	>120	>120	>120
h.heps, Clint (μl/min/million cells)	4.8	2.7	8.8
CYP2D6 inhibition, IC_{50} (μM)	21.8	27.1	17.6
CaCO-2, AB/BA at 10 μM	16/24	19/28	15/18
K^+, hERG binding IC_{50} (nM)	40,200	24,700	>6870[b]

[a] Maximum measurable half life ($t_{1/2}$) was 120 min in human liver microsomes.
[b] Geometric mean of nine experiments, two of which >10,000 nM.

Table 4
Pharmacokinetics of compound **18** in dog[a]

Blood parameter	Dog (n = 2)
IV	
Dose (mg/kg)	0.01
$t_{1/2}$ (h)	5.7
Cl_s (ml/min/kg)	1.1
Vd (L/kg)	0.5
Oral	
Dose (mg/kg)	0.013
$t_{1/2}$ (h)	5.7
F (%)	91

[a] Dog liver microsomes $t_{1/2}$ = 35 min.

Compound **18** exhibited no significant inhibition of other CYP450 enzymes (1A2, 2C9, 2C19, IC_{50}s >30 μM; 3A4 IC_{50} 17.9 μM). We were also pleased to find that it demonstrated >200-fold selectivity for serotonin and noradrenaline-reuptake inhibition when assessed against the CEREP/bioprint™ panel of receptors, enzymes and ion channels.

When we investigated the metabolic profile of compound **18**, we were pleased to find that our strategy of blocking the route of metabolism had been successful. Indeed, the compound was so metabolically stable in in vitro microsomal preparations that no measurable turnover was observed. Although this result was very encouraging, it does not guarantee that compound **18** will not be a CYP2D6 substrate in human and differences in exposures between poor and extensive metabolizers need to be monitored in clinical studies.

Finally, pharmacokinetic data for compound **18** were generated in the dog following intravenous and oral administration (Table 4). Compound **18** exhibited an encouraging pharmacokinetic profile with a combination of very low clearance, low volume of distribution, long half-life and very high bioavailability. Based on these data, compound **18** was predicted to exhibit good pharmacokinetics in humans.

To summarise, we have described the discovery of novel amides derived from the 3-amino-pyrrolidine template we previously reported[6] as potent SNRIs. More specifically, compound **18** exhibited good SNRI activity in combination with good selectivity against the dopamine transporter and no significant off-target pharmacology. Compound **18** also exhibited a very encouraging ADME profile and pharmacokinetics potentially compatible for bid dosing.

As a result compound **18** was selected for further evaluation in preclinical disease models. The results of these studies will be reported in future publications.

Acknowledgements

We Carol Bains, Gerwyn Bish, Helena Barker, Timothy Buxton, Edelweiss Evrard, Arnaud Lemaitre, Debbie Lovering, Edward Pegden, Bhairavi Patel, and Melanie Skerten for compound synthesis. We are also grateful to Stephen Phillips and Donald Newgreen and their teams (Discovery Biology) for screening data, and to Neil Attkins, Anthony Harrison, Christopher Kohl and Nicola Lindsay (PDM) for ADME studies.

References and notes

1. (a) Jann, M. W.; Slade, J. H. *Pharmacotherapy* **2007**, *11*, 1571; (b) Montgomery, S. *Int. J. Psychol. Clin. Pract.* **2006**, *10*(Suppl. 2), 5; (c) Jackson, S. *Curr. Med. Res. Opin.* **2005**, *21*, 1669.
2. (a) Wernicke, J. F.; Iyengar, S.; Ferrer-Garcia, M. D. *Curr. Drug Ther.* **2007**, *2*, 161; (b) Bauer, M.; Moeller, H-J.; Schneider, E. *Expert Opin. Pharmacother.* **2006**, *7*, 421.
3. (a) Kajdasz, D. K.; Iyengar, S.; Desaiah, D. *Clin. Ther.* **2007**, *29*, 2536; (b) Thor, K. B.; Kirby, M.; Viktrup, L. *Int. J. Clin. Pract.* **2007**, *61*, 1349.
4. (a) Mariappan, P.; Alhasso, A.; Ballantyne, Z.; Grant, A.; N'Dow, J. *Eur. Urol.* **2007**, *51*, 67; (b) Steers, W. D.; Herschorn, S.; Kreder, K. J.; Moore, K.; Strohbehn, K.; Yalcin, I.; Bump, R. C. *BJU Int.* **2007**, *100*, 337.
5. (a) Fray, M. J.; Bish, G.; Brown, A. D.; Fish, P. V.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4345; (b) Fray, M. J.; Bish, G.; Fish, P. V.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4349.
6. Fish, P. V.; Fray, M. J.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2022.
7. Whitlock, G. A.; Blagg, J.; Fish, P. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 596.
8. Fish, P. V.; Deur, C.; Gan, X.; Greene, K.; Hoople, D.; MacKenny, M.; Para, K. S.; Reeves, K.; Ryckmans, T.; Stiff, C.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2562.
9. Lee, J. T.; Kroemer, H. K.; Silberstein, D. J.; Funck-Brentano, C.; Lineberry, M. D.; Wood, A. J.; Roden, D. M.; Woosley, R. L. *New Engl. J. Med.* **1990**, *322*, 100.
10. The assays were a modification of those described by Blakely, R. D.; Clark, J. A.; Rudnick, G.; Amara, S. G. *Anal. Biochem.* **1991**, *194*, 302. HEK293 cells expressing a single human amine transporter protein (7500 cells/well in Millipore 96-well filter bottom plates) were pre-incubated at 25 °C for 5 min with assay buffer containing vehicle (DMSO in water) or test compound. Uptake of neurotransmitter into the cells was initiated by the addition of tritiated 5-HT (50 nM), NA (200 nM) or DA (200 nM) substrates, the samples were shaken in an incubator at 25 °C for 5 min (5-HT, DA) or 15 min (NA). The assays were stopped by an ice-cold buffer wash followed by filtration. The filters were then dried before measuring the amount of radioactivity taken up into the cells by scintillation counting. Potency of test compounds was quantified as IC_{50} values, that is, concentration required to inhibit the specific uptake of radiolabelled substrate into the cells by 50% relative to maximum (vehicle only) over a 10-point dose–response range.
11. Mahar Doan, K. M.; Humphreys, J. E.; Webster, L. O.; Wring, S. A.; Shampine, L. J.; Serabjit-Singh, C. J.; Adkison, K. K.; Polli, J. W. *J. Pharm. Exp. Ther.* **2002**, *303*, 1029.
12. Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Discov.* **2007**, *6*, 881.
13. *CaCO-2:* Human colon adenocarcinoma cell line. Flux across cells was measured at 10 μM substrate concentrations. Figures quoted correspond to the flux rates ($P_{app} \times 10^{-6}$ cm s^{-1}) for apical to basolateral (AB) and basolateral to apical (BA) directions. See: Van de Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. *J. Med. Chem.* **2001**, *44*, 1313 and references therein.
14. (a) Fermini, B.; Fossa, A. A. *Nat. Rev. Drug Discov.* **2003**, *2*, 439; (b) Keating, M. T.; Sanguinetti, M. C. *Cell* **2001**, *104*, 569.