Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 304–308
Bioorganic & Medicinal Chemistry Letters

Substituted 2-oxo-azepane derivatives are potent, orally active γ-secretase inhibitors

Eric A. Kitas,[a,*] Guido Galley,[a] Roland Jakob-Roetne,[a] Alexander Flohr,[a] Wolfgang Wostl,[a] Harald Mauser,[a] André M. Alker,[b] Christian Czech,[c] Laurence Ozmen,[c] Pascale David-Pierson,[d] Dieter Reinhardt[c] and Helmut Jacobsen[c]

[a]*Pharma Division, Discovery Chemistry, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*
[b]*Pharma Division, Molecular Structure Research, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*
[c]*Pharma Division, Preclinical CNS Research, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*
[d]*Pharma Division, Safety Technical Services, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*

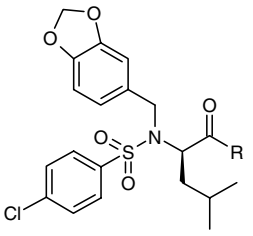
Received 21 September 2007; revised 18 October 2007; accepted 22 October 2007
Available online 5 November 2007

Abstract—A hydroxamic acid screening hit **1** was elaborated to 5,5-dimethyl-2-oxoazepane derivatives exhibiting low nanomolar inhibition of γ-secretase, a key proteolytic enzyme involved in Alzheimer's disease. Early ADME data showed a high metabolic clearance for the geminal dimethyl analogs which could be overcome by replacement with the bioisosteric geminal difluoro group. Synthesis and structure–activity relationship are discussed and in vivo active compounds are presented.

Alzheimer's disease (AD) is characterized by the deposition in the brain of amyloid in extracellular plaques and intracellular neurofibrillary tangles. The amyloid plaques are composed of Aβ peptides (mainly 40- and 42-amino-acid length) which originate from β-amyloid precursor protein (APP) by a series of proteolytic cleavage steps involving enzymes β-secretase and γ-secretase. Whereas β-secretase is a typical aspartyl protease, γ-secretase proteolytic activity consists of several proteins including the presenilins, nicastrin, aph1, and pen-2.[1] Among the known substrates of γ-secretase are the APP and the proteins of the Notch receptor family.[2,3] According to the amyloid hypothesis of AD, the production and deposition of Aβ is the ultimate cause of the disease.

In our program to identify potent and selective inhibitors of γ-secretase, a micromolar screening hit **1** was identified from our propriatory compound library. Hydroxamic acid **1** was elaborated to the primary amide **2**, a nanomolar inhibitor of amyloid synthesis as determined by our cellular Aβ lowering assay (Table 1).[4]

Table 1. HTS hit **1** and representative early modifications



Compound	R	Aβ lowering IC_{50} (μM)
1	NHOH	2.6
2	NH_2	0.008

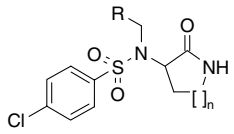
The poor physicochemical properties (e.g., compound **2**: solubility <1 μg/ml; log *D* > 3; low PAMPA permeability[5]) and relatively high clearance (e.g., compound **2**: human/mouse 34/282 μl/min/mg protein) observed for primary amides in this class led us to consider ring constrained structures (Table 2). Cyclic lactams **3**–**6** were prepared, thus fusing the vectors corresponding to the isobutyl and amide groups in **2**. The azepinone ($n = 3$) ring size was found to be optimal in this series with sulfonamide **5** having a modest inhibition of IC_{50} 0.40 μM.[6]

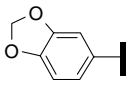
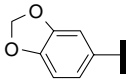
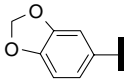
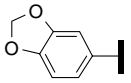
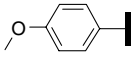
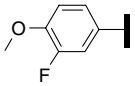
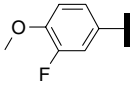
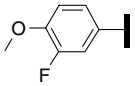
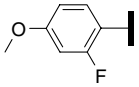
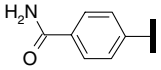
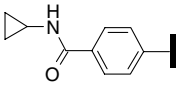
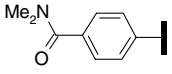
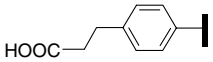
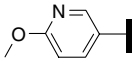
Alternatives to the piperonyl northern vector were examined and phenyl ring substitutions (Me, OMe

Keywords: Alzheimer's disease; Amyloid Aβ lowering; γ-Secretase; Proteolytic activity; Metabolism; In vivo.
* Corresponding author. Tel.: +41 61 688 76 18; fax: +41 61 688 64 59; e-mail: eric_a.kitas@roche.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.074

Table 2. Lactam derivatives **3–16**



Compound	*n*	R	Aβ lowering IC_{50} (μM)
3	1		>20
4	2		14
5	3		0.40
6	4		9
7	3		0.32
8	3		0.23
9 [R isomer]	3		0.17
10 [S isomer]	3		17
11	3		0.15
12 [R isomer]	3		0.08
13	3		0.17
14	3		2
15 [R isomer]	3		0.14
16	3		0.9

OCF_3, Cl, F, data not shown) indicated that larger para-substituents were tolerated (**12–15**) and inhibitory activities could be improved by further addition of F to the phenyl ring (**8–11**). The preferred *R*-stereochemistry was established by preparing compounds **9** and **10** starting from (*R*)-3-amino-azepan-2-one and (*S*)-3-amino-azepan-2-one, respectively. Furthermore, phenyl to pyridyl replacement such as in compound **16** maintained sub-micromolar affinity for γ-secretase. Minimal variations to the aryl western vector were attempted as *para*-chlorophenyl was a well-established pharmacophore in sulfonamide or sulfone classes of γ-secretase inhibitors.[7] Nevertheless, some variations will be discussed later (vide infra, Table 4).

Next we considered the SAR at the azepinone ring in an attempt to improve the modest inhibition seen so far. *N*-Methyl substitution of the lactam gave inactive compounds (data not shown).

An overlay of lactam **12** with the most potent acyclic analog **2** (Fig. 1) shows the high similarity of both classes. Interestingly, the structural comparison suggests that the lactams do not occupy all available space (indicated by the cyan sphere). Thus, additional substitution in 4- or 5-position was considered for improving binding affinity.

Indeed the introduction of an isopropyl- or CF_3-substituent (Table 3, compounds **17** and **18**) in position 5 led

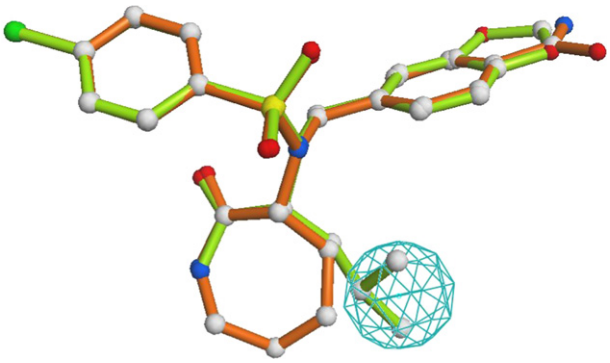
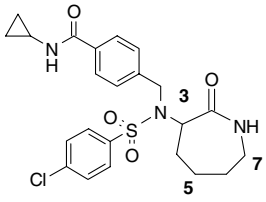


Figure 1. Overlay of acyclic HTS derivative **2** and lactam **12**. The X-ray structure[8] of **15** of the lactam series was taken as reference for a flexible alignment of **2** using the program Moloc (Gerber, P.; www.moloc.ch, 2007). For clarity the propionic acid side chain was replaced by a primary amide leading to compound **12**.

Table 3. Substituted 2-oxoazepane derivatives



Compound	Lactam subst.	Aβ lowering IC_{50} (μM)	Cl (human/mouse microsomes)[a]
17	5-*i*-Pr[Rac]	0.04	51/203
18	5-CF_3 [Rac]	0.02	19/39
19	5-*t*-Bu[Rac]	0.12	93/110
20	5,5-Me_2	0.006	45/191
21	7,7-Me_2	>20	—
22	6,6-Me_2	3.5	—
23	4,4-Me_2	0.39	—
24 [R isomer]	5,5-Me_2	0.015	36/655
32 [R isomer]	5,5-F_2	0.004	1.9/2.2
33 [R isomer]	6,6-F_2	0.30	0.0/5.4

[a] Microsomal clearance (μl/min/mg protein).

to stronger inhibition of γ-secretase activity compared to the corresponding unsubstituted derivative **13**. This effect was less pronounced for the larger *t*-butyl derivative **19**. A very potent compound could be obtained with geminal dimethyl substitution **20** and also simplified the structure by the removal of one chiral center. Starting from **20** as the least complex structure, a 'gem.dimethyl walk' around the caprolactam (compounds **20**–**23**) showed that optimal substitution was in fact the 5-position.

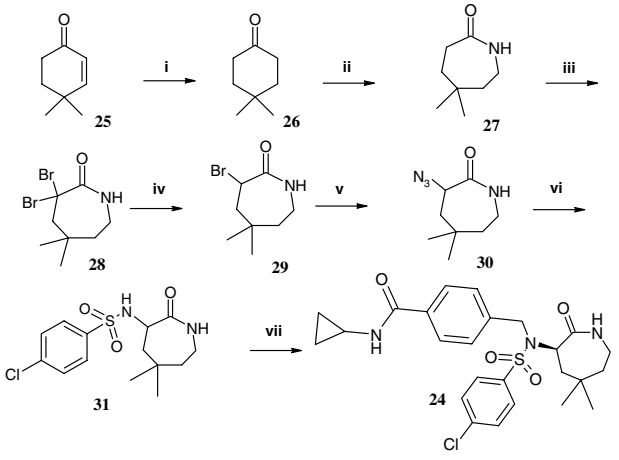
The synthesis of **24** (the active *R*-isomer of **20**) is described in Scheme 1 and began with the hydrogenation of α,β-unsaturated cyclohexenone **25** to yield **26** which was subjected to a Beckmann rearrangement using hydroxylamine-o-sulfonic acid in formic acid.[9] Selective dibromination in position 3 of the azepinone followed by monodebromination under hydrogenolytic conditions afforded **30**.[10] Sodium azide displacement of Br and reduction of azide to amine was followed by preparation of sulfonamide **31** as a mixture of 1:1 enantiomers which were separated by chiral chromatography. Further derivatization with 4-chloromethyl-*N*-cyclopropyl-benzamide under basic conditions gave compound **24**. The active isomer was tentatively assigned with the *R*-configuration by comparing Aβ lowering activities of the enantiomer pair **9** and **10**.

With the first potent γ-secretase inhibitors in hand, we initiated early ADME profiling. Human/mouse microsomal clearance was relatively high for 5-alkyl substituted derivatives **17**, **19**, **20**, when compared to **18**, suggesting that alkyl substituents were labile to microsomal oxidation. Our attempts at blocking this liability led us to the surprising result that the 5,5-difluoro and 6,6-difluoro analogs yielded highly bioavailable inhibitors **32** and **33**, respectively, with **32** matching the potency of the chiral 5,5-dimethyl analog **24** in the Aβ lowering assay (Table 3).

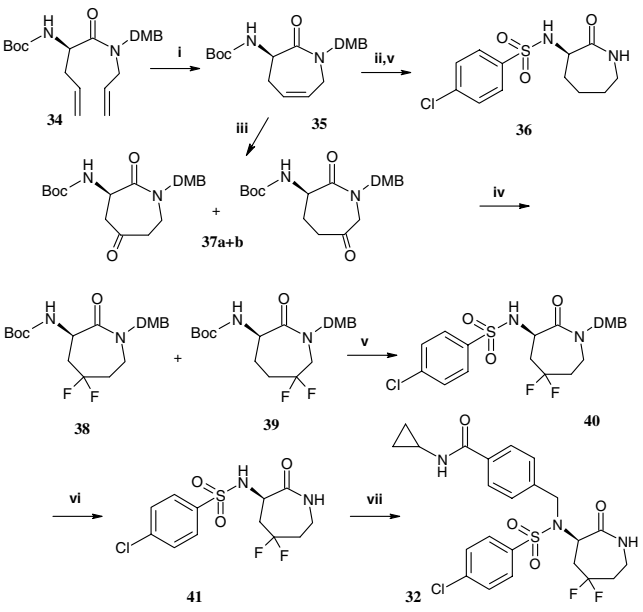
The synthesis of fluoro ring-substituted 3-amino-azepan-2-ones is described in Scheme 2. (*R*)-*N* Boc-allylglycine was coupled to allyl-(2,4-dimethoxybenzyl)amine to yield the bis-allyl intermediate **34**.[12] Ring closure metathesis reaction preferably using the 2nd generation Grubb's catalyst (0.1 equiv) proceeded efficiently without the need for high dilution affording **35** in 80% yield. The chiral integrity of the cyclization product was assessed by its further reaction to yield known compound **36** where respective optical rotations could be compared,[13] confirming the mildness of the olefin metathesis conditions. Alkene **35** was oxidized to the 5,6-oxo regioisomers using a palladium complex in a saturated oxygen environment.

The regioisomeric mixture **37a,b** was used directly in the further transformation to geminal difluorinated compounds using DAST (3 equiv) in a modest 45% overall yield and following silica gel chromatography the preferred isomer **38** was isolated in a 22% yield. In a straightforward manner, further homologation of **38** yielded the potent inhibitor **32**.[14]

Compound **32** was profiled in vivo in a transgenic mouse model for inhibition of γ-secretase activity[15] and was found to be inactive after 3×20 mg/kg po and ip administration. We attributed this finding to possible low brain penetration due to the presence of two amides in its structure. We therefore went back to methoxyaryl northern substituents that we already

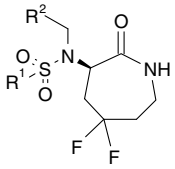


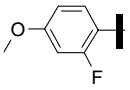
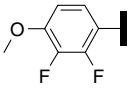
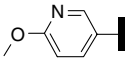
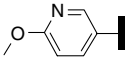
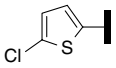
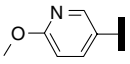
Scheme 1. Reagents and conditions: (i) H_2, Pd–C in EtOH, 95%; (ii) hydroxylamine-o-sulfonic acid in formic acid, reflux, 2 h, 55%; (iii) PCl_5, $ZnCl_2$, Br_2 4 h, 67 %; (iv) H_2 Pd–C, NaOAc in AcOH, 71%; (v) NaN_3 in DMSO, 80 °C, 1.5 h, 21%; (vi) H_2 Pd–C then 4-chlorobenzenesulfonyl chloride, DIEA, 1.5 h 67%; (vii) chiral separation on Chiralpak AD 20% isopropanol, 80% heptane, then 4-chloromethyl-*N*-cyclopropyl-benzamide, K_2CO_3 KI, 65 °C 2.5 h, 31%.[11]



Scheme 2. Reagents and conditions: (i) Grubb's II cat., reflux, 1.5 h, $MeCl_2$, [0.03 M] 80%; (ii) H_2 Pd–C, EtOH then TFA; (iii) O_2, $PdCl_2$,CuCl, DMF/water, 50 °C, 2 d, 72%; (iv) DAST, $MeCl_2$, 5 h, 45% overall; (v) separation from **39** then HCl/1,4-dioxane, 2 h then 4-chlorobenzene-sulfonyl chloride, DIEA in $MeCl_2$, 2 h, 60%; (vi) TFA/triethylsilane/water, 3 d, 40%; (vii) 4-chloromethyl-*N*-cyclopropyl-benzamide, K_2CO_3, KI in DMF 65 °C, 1.5 h.

Table 4. 5,5-Difluoro-substituted 2-oxoazepane derivatives



Compound	R^1	R^2	Aβ lowering IC_{50} (μM)	Cl (human/mouse microsomes)[a]
42	pCl-Ph		0.02	13/105
43	pCl-Ph		0.002	19/121
44	pCl-Ph		0.03	9/72
45	$CF_3(CH_2)_2$		0.15	—
46			0.11	—

[a] Microsomal clearance (μl/min/mg protein).

found active with the non-fluorinated caprolactams (vide supra, Table 2). The methoxyphenyl derivative **42** was less potent than **32** but inhibitory activity could be further improved by addition of another fluorine (Table 4). In fact, compounds **43** and **44** were found to be active in vivo at a minimal effective dose (MED) of 20 mg/kg po. This relatively high dose may be explained by the low brain exposure (typically brain/plasma ratio <0.1) determined from the in vivo experiments.[16]

A few examples at replacing the *para*-chlorophenyl moiety were prepared and trifluoropropyl **45** and the 5-chlorothiophene **46** derivatives were identified as potential alternatives.

In conclusion, we have identified a series of 5,5-difluoro-substituted 2-oxoazepane derivatives which are potent inhibitors of γ-secretase. Difluoro-substitution was found to be a key factor to ensure both potency and metabolic stability of the compounds. Further variation of substituents yielded compounds **43** and **44** which were orally active in a transgenic mouse model for AD. Low brain exposure possibly related to observed P-glycoprotein interactions for representative compounds,[17] is a remaining issue that needs to be addressed for this otherwise attractive series.

Acknowledgments

The excellent technical assistance of Patrick Studer, Betty Hennequin, Annick Goergler, Roman Hutter, Peter Weber, Joseph Wuhrlin, Daniel Zimmerli, Fabienne Goepfert, Patrick Biry, Remy Wybrecht, Marie Elise Brun, Vittorio Bona, Christophe Schweitzer, and Markus Haenggi is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.074.

References and notes

1. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
2. (a) Radtke, R. et al. *Nat. Immunol.* **2004**, *5*, 247; (b) Radtke, F.; Clevers, H. *Science* **2005**, *307*, 1904.
3. Shih, Ie-Ming; Wang, Tian-Li *Cancer Res.* **2007**, *67*, 1879.
4. Description of Aβ lowering assay: HEK293 cells which express a human βAPP cDNA were treated for 16–18 h with compound. The supernatant was harvested and the Aβ 40 peptide concentrations were determined. Ninety-six well ELISA plates (e.g., Nunc MaxiSorb) were coated with monoclonal antibody which specifically recognizes the C-terminal end of Aβ40 (Brockhaus et al., *NeuroReport* **1998**, 9, 1481). After blocking of non-specific binding sites with, e.g., 1% BSA and washing, the culture supernatants were added in suitable dilutions together with a horseradish peroxidase-coupled Aβ-detection antibody (e.g., antibody 4G8, Senetek, Maryland Heights, MO) and incubated for 5–7 h. Subsequently the wells of the microtiter plate were washed extensively with Tris-buffered saline containing 0.05% Tween 20 and the assay was developed with tetramethylbenzidine/H_2O_2 in citric acid buffer. After stopping the reaction with one volume 1 N H_2SO_4 the reaction was measured in an ELISA reader at 450 nm wavelength. In repeated measurements the stan-

dard deviation for the calculated IC_{50} was 30%. Measured data were also in agreement with cell-free-assay data obtained for selected compounds according to the procedure by Li (*Proc. Natl. Acad. Sci. U.S.A.* **2000**, 97, 6138).
5. For a description of the PAMPA (parallel artificial membrane permeability assay) model, a prediction assay for oral absorption Kansy, M.; Fischer, H.; Kratzat, K.; Senner, F.; Wagner, B.; Parrilla, I. *Helv. Chim. Acta* **2000**, 447.
6. Other recent publications on *N*-(oxoazepanyl) benzenesulfonamides and related derivatives as γ-secretase inhibitors for treating Alzheimer's disease: Neitzel, M.; Dappen, M. S.; Marugg, J. WO Patent WO2005042489, 2005; Parker, M. F.; Bronson, J. J.; Barten, D. M. et al. *Bioorg. Med. Chem. Lett.* **2007**, 17, 5790.
7. (a) Further literature exemplifying the sulfonamide and sulfone classes of γ-secretase inhibitors: Smith, D. W. WO Patent WO2000050391, 2000; (b) Pineiro J. L.; Churcher I.; Dinnell K. et al. WO patent WO2002081435, 2002; (c) Parker M. F.; McElhone K. E.; Mate R. A. et al. WO patent WO20-03053912, 2003; (d) Josien H. B. WO patent WO2003013527, 2003; (e) Churcher, I.; Harrison, T.; Kerrad, S. et al. WO patent WO2004031137, 2004; (f) Brands K. M.; Davies A. J.; Oakley P. J. et al. WO2004013090, 2004; (g) Churcher, I.; Beher, D.; Best, J. D., et al. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 280; (h) Asberom, T.; Zhao, Z.; Bara, T. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 511.
8. Full crystallographic data for compound **15** have been deposited with the Cambridge Crystallographic Data Center (CCDC Reference No. 661530). Copies of the data can be obtained free of charge via the internet at http://www.ccdc.cam.ac.uk.
9. Olah, G. A.; Fung, A. P. *Synthesis* **1979**, 537.
10. Shirota, F.; Nagasawa, H. T.; Elberling, J. A. *J. Med. Chem.* **1977**, *20*, 1623.
11. For detailed experimental conditions, see: Galley, G.; Jakob-Roetne, R.; Kitas, E. A. WO Patent WO2006005486, 2006.
12. Analogous RCM in synthesis of 3-aminoazocan-2-one derivatives Creighton, C. J.; Leo, G. C.; Du, Y.; Reitz, A. B. *Bioorg. Med. Chem.* **2004**, *12*, 4375.
13. Compound **36** $[\alpha]_D$ −130.6° ($c = 0.64$, $CHCl_3$) was also prepared using commercial (*R*)-3-amino-azepan-2-one: $[\alpha]_D$ −130.9° ($c = 0.89$, $CHCl_3$).
14. For detailed experimental conditions, see: Flohr, A.; Galley, G.; Jakob-Roetne, R.; Kitas, E. A.; Wostl, W. US Patent US20070037789, 2007.
15. Compounds were profiled in vivo in the PS2APP transgenic mouse which expresses the human APPsw cDNA and a human FAD presenilin 2 cDNA. See also (a) Richards, J. G.; Higgins, G. A.; Quagazzal, A. M.; Ozmen, L.; Kew, J. N.; Bohrmann, B.; Malherbe, P.; Brockhaus, M.; Loetscher, H.; Czech, C.; Huber, G.; Bluethmann, ; Jacobsen, H.; Kemp, J. A. *J. Neurosci.* **2003**, *23*, 8989; (b) Inhibition of γ-secretase was assessed by accumulation of its substrate, the C-terminal fragment (CTFβ) of APP released by the previous β-secretase cleavage. The accumulation of CTFβ in total brain was quantified by Western blot (see reference above Richards et al. for details). Compounds were first tested at 20 mg/kg for efficacy followed by a dose–response with 0.5–3–10–30 mg/kg, three animals per dose. The well-characterized γ-secretase inhibitor LY411575 was used as reference compound. The CTFβ band was quantified by Chemo-luminescence and normalized to an endogenous control protein band. In the PS2APP transgenic mouse the expression of the human APP transgene is driven by a neuron-specific form of the Thy1 promoter. The peripheral level of APP and the plasma level of Aβ peptide are too low to allow a meaningful determination of γ-secretase inhibition; The use of CTFβ accumulation as a sensitive marker for γ-secretase inhibition has been described by others (c) Zhao, Guojun; Cui, Mei-Zhen; Mao, Guozhang; Dong, Yunzhou; Tan, Jianxin; Sun, Longsheng; Xu1, Xuemin *J. Biol. Chem.* **2005**, *280*, 37689; (d) Walsh, D. M.; Fadeeva, J. V.; LaVoie, M. J.; Paliga, K.; Eggert, S.; Kimberly, W. T.; Wasco, W.; Selkoe, D. J. *Biochemistry* **2003**, *42*, 6664; (e) De Strooper, B.; Saftig, P.; Craessaerts, K.; Vanderstichele, H.; Guhde, G.; Annaert, W.; Von Figura, K.; Van Leuven, F. *Nature* **1998**, *391*, 387.
16. Compound **44** was dosed at 20 mg/kg po and the brain/plasma ratio was determined at 2 h: plasma conc. = 1046 g/ml, brain conc. = 60 ng/g; $b/p = 0.06$; $N = 3$.
17. The permeability and the P-gp substrate properties of **44** were assessed by a bi-directional transport assay in an epithelial cell monolayer system, using LLC-PK1 cells exogenously expressing human P-glycoprotein (P-gp), MDR1. A high passive permeability of around 280 nm/s was estimated based on the observed apical to basolateral and reverse permeabilities in the different experiments where active transport was inhibited. Directional transport (at a concentration of 1 μM) was observed in cells expressing the human MDR1 transporter with an export permeability ratio of 2.2, while in presence of an inhibitor (Elacridar, 5 μM) this transport was fully inhibited (export ratio = 1). These results indicate that **44** is a substrate for human MDR1 P-glycoprotein.