Bioorganic & Medicinal Chemistry Letters 20 (2010) 26–30

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Exploring SAR features in diverse library of 4-cyanomethyl-pyrazole-3-carboxamides suitable for further elaborations as CB1 antagonists

Martin Cooper, Jean-Marie Receveur, Emelie Bjurling, Pia K. Nørregaard, Peter Aadal Nielsen, Niklas Sköld, Thomas Högberg *

7TM Pharma A/S, Fremtidsvej 3, DK-2970 Hørsholm, Denmark

ARTICLE INFO

Article history:
Received 24 October 2009
Revised 10 November 2009
Accepted 12 November 2009
Available online 15 November 2009

Keywords:
CB1 antagonists
Obesity
CNS
Blood–brain-barrier
Chemical library
Plate synthesis

ABSTRACT

A chemically diverse library of secondary and tertiary 4-cyanomethyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamides was synthesized to enable mapping of the SAR, in the eastern amide region, with regard to CB1 antagonist activity, This study was initiated as a prelude to the design and synthesis of possible CB1 antagonists that do not readily pass the blood–brain-barrier. In general a range of modifications were found to be tolerated in this part of the molecule, although polar and especially charged groups did to a degree reduce the CB1 antagonistic activity. Several compounds with single-digit or even sub-nanomolar potency, suitable for further elaboration of the nitrile moiety, were identified.

Endogenous cannabinoids (CB) or endocannabinoids, such as anandamide, are signaling substances derived from arachidonic acid that are functionally related to Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychotropic constituent in marijuana (Fig. 1).[1,2] Two types of cannabinoid receptors have been identified and cloned. The CB1 receptor is widely expressed in the brain and to a lower extent in several peripheral organs such as liver, adipose tissue, gastrointestinal tract, vagal nerves, pancreas and skeletal muscle.[1–3] CB2 receptors exist mainly in the immune system, although recent publications have reported their existence also in the CNS.[1–3] The endocannabinoids play a pivotal role, through the CB1 receptor, in regulating food intake and energy expenditure, in synergy with other anorexigenic and orexigenic regulatory pathways.[1,2] CB1 receptor-deficient mice are resistant to diet-induced obesity even though their total caloric intake is similar to that of wild-type littermates.[4] Correspondingly CB1 antagonists/inverse agonists, such as rimonabant and taranabant (Fig. 1), have been shown to inhibit food intake and reduce body weight in obese animals and humans.[1,2,5] These effects are likely to be mediated by a combination of central and peripheral target organ actions.[2,6] Pharmacological blockade of the peripheral CB1 system can elicit beneficial effects on other parameters of the metabolic syndrome that are independent of eating behavior and body weight.[2,6] Thus clinical trials with rimonabant showed improvement in glycemic control and lipid profile in type 2 diabetic patients (SERENADE) as well as loss of visceral and hepatic fat in abdominally obese patients (ADAGIO).[6,7] These long term peripheral effects could arise from, for example, reduced hepatic lipogenesis, enhanced lipid oxidation, increased adipose lipolysis, and reduced insulin resistance. Recently, blockade of the CB1 receptor has also been shown, in rodents, to hold promise as a therapy for chronic liver diseases such as liver fibrosis.[8]

An issue associated with this drug class is the induction of central nervous system (CNS) side-effects such as anxiety and depression.[2,9] Effects evidently linked to functional CB1 receptors in brain areas such as the frontal cortex, hippocampus and amygdala.[10] A plausible working hypothesis, to avoid the CNS effects seen with rimonabant and taranabant but still achieve effects on body weight and metabolic parameters, would be to design compounds not able to pass the blood–brain-barrier (BBB).[2,11] One can envisage several possible ways to achieve this such as by considerably increasing polar surface area and lowering log *D*, compared to the centrally acing drugs, or by invoking efflux transporters located in the BBB. As a first step to obtaining more polar compounds, we needed to expand the known SAR for rimonabant-like compounds so as to better understand which features drive potency and identify regions where polar groups can be introduced without adversely affecting CB1 antagonistic effects.

The rimonabant template offers several possibilities for modifications and we have explored several of these. In this report we present a strategy of introducing a nitrile group to the 4-methyl

* Corresponding author. Tel.: +45 3925 7760; fax: +45 3925 7776.
E-mail address: th@7tm.com (T. Högberg).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.11.047

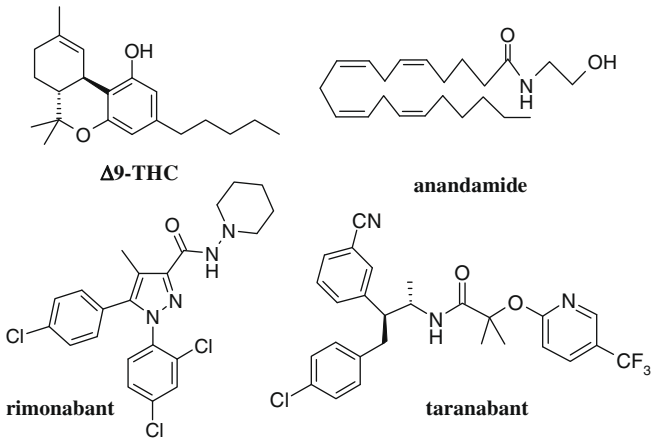


Figure 1. Chemical structures of CB1 agonists (Δ^9-THC and anandamide) and two representative CB1 antagonists progressed to clinical development.

group of the pyrazole core together with the exploration of a wide range of eastern amide modifications (**IV**). The nitrile was chosen as it can be elaborated into a variety of polar functional groups that are either neutral (amides, amidoximes), positively charged (amidines) or negatively charged (carboxylic acids, tetrazoles) at physiological pH. The two chloro substituents on the phenyl rings were chosen so as to maximise potency while limiting log P in the core system. The changes in calculated log *P* values and polar surface areas (PSA) of these target motifs are shown in relation to a nitrile compound in Figure 2. Rimonabant displays the corresponding values of *c* log *P* 6.47 and PSA 50.2 Å^2 which reflects a favorable BBB penetration. The pyrazole intermediate **II** was obtained from 2-chlorophenylhydrazine and the diketo compound **I,** according to the literature procedure outlined in Scheme 1.[12] The key intermediate **III** was obtained by radical induced bromination of **II** directly followed by crown ether facilitated substitution with cyanide in acetonitrile at reflux overnight, or under the milder conditions with cyanide in ethanol/water, and a final hydrolysis of the ester. Finally the amides **IV** were obtained by coupling the resulting acid **III** with amines under standard conditions, such as 1-hydroxy-benzotriazole and 1-ethyl-3(3-dimethyl-aminopropyl)-carbodiimide in dichloromethane. Many of the amides were made by plate chemistry using polystyrene (PS)-supported reagents and a diverse set of amine reagents and parallel purification techniques.[13]

The compounds were screened against the human CB1 receptor using a GTPγS antagonist assay with CP55940 as agonist and membranes produced from CHO-K1 cells stably transfected with recombinant human CB1 receptor. Selected compounds were also investigated for inverse agonism by running the same assay without the CP55940 agonist. As seen in Table 1, a wide range of amide functionalities are tolerated in the eastern amide position; thus the anilinic amides **1**-**6** have potencies in the range of 10–60 nM. It is notable that the 3-pyridyl derivative **2** loses potency compared

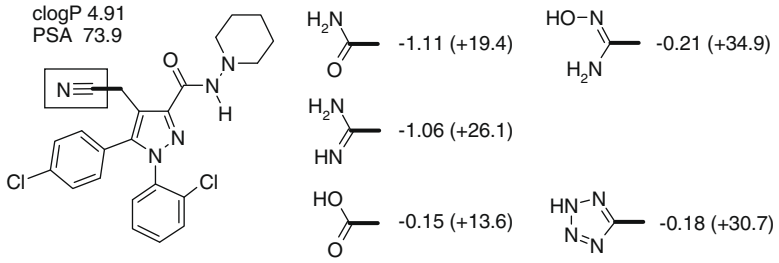
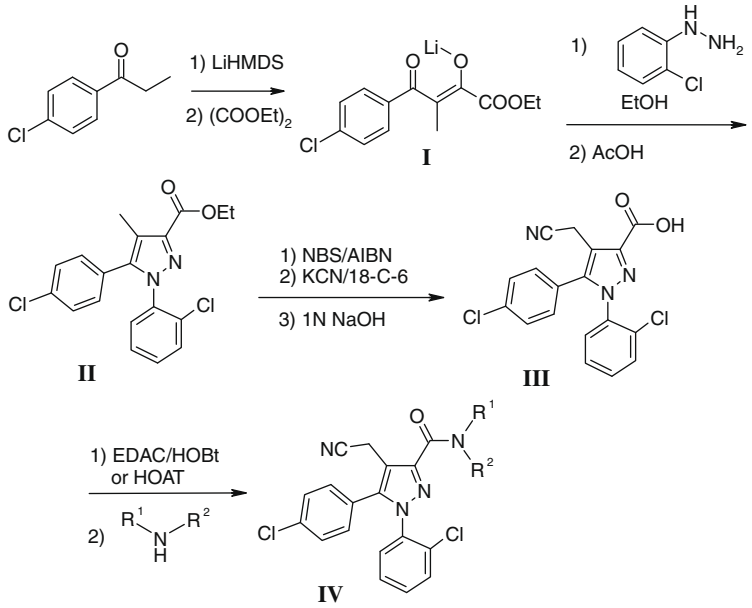
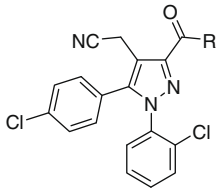


Figure 2. Differences in *c* log *P* and PSA (in parentheses) by modifications of the nitrile group in **14** into amides, amidoximes, amidines, carboxylic acids, and tetrazoles.



Scheme 1.

Table 1
Antagonism on hCB1 and lipophilicity of secondary and tertiary 4-cyanomethyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamides



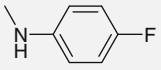
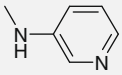
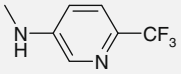
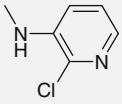
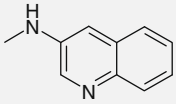
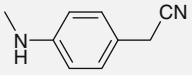
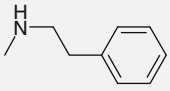
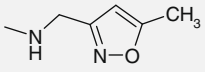
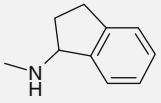
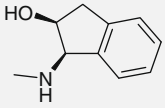
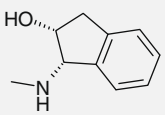
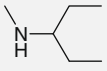
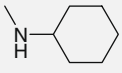
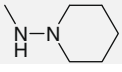
No.	R	*c* log *P* (log *D*)[a]	IC_{50} GTPγS[b] (nM)
	Rimonabant	6.5 (4.7)	4.5
1[14]		5.6 (4.2)	11
2		4.6 (3.3)	57
3		5.6	14
4		5.3 (4.4)	18
5		6.0 (4.0)	19
6		4.9 (3.8)	55
7		5.8	16
8		3.9	15
9		6.0	0.41
10		4.9	7.8
11		4.9	1.7
12		5.5	5.8
13		5.7 (3.5)	1.9
14		4.9 (3.6)	7.5

Table 1 (*continued*)

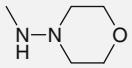
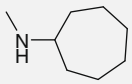
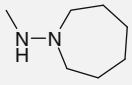
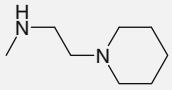
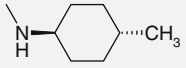
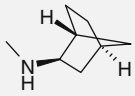
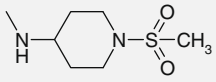
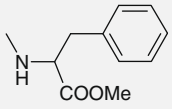
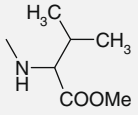
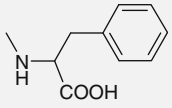
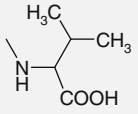
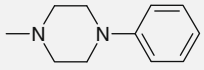
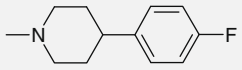
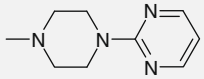
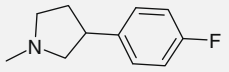
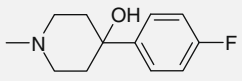
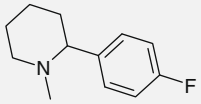
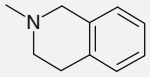
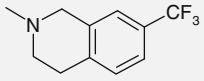
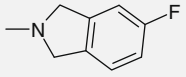
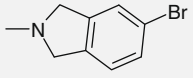
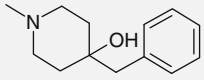
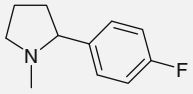
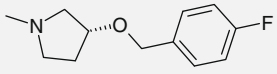
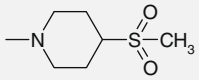
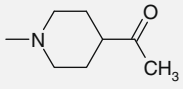
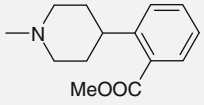
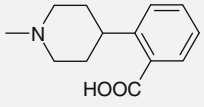
No.	R	*c* log *P* (log *D*)[a]	IC_{50} GTPγS[b] (nM)
15		4.4	71
16		6.2	0.49
17		5.5	0.52
18		5.3 (2.9)	1110
19		6.2	1.6
20		6.0	2.2
21		2.9	31
22		5.4	1.2
23		4.9	7.3
24		5.2	4450
25		4.7	6250
26		5.8	13
27		6.3	1.0
28		4.0	3.8
29		5.9	9.2
30		4.7	3.0

Table 1 (*continued*)

No.	R	*c* log *P* (log *D*)[a]	IC_{50} GTPγS[b] (nM)
31		7.1	23
32		6.1	5.7
33		7.0	2.1
34		5.9	5.7
35		6.5	0.22
36		4.8	3.4
37		6.6	27
38		5.8	20
39		3.8	17
40		3.3	199
41		6.2	2.2
42		4.8	2810

[a] *c* log *P* was calculated and log *D* chromatographically measured according to Ref. 15.

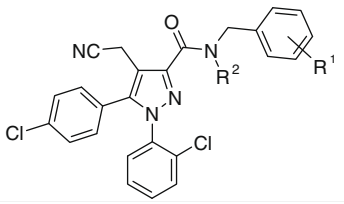
[b] Antagonistic activity in GTPγS assay using CP55940 as agonist and membranes produced from CHO-K1 cells stably transfected with hCB1 receptor. All compounds displayed efficacy above 100% indicating inverse agonist activity and all values are mean of at least two determinations.

to **1** (previously described in Ref. 14) but the activity can be regained by the introduction of a trifluoromethyl (**3**) or chloro (**4**) group in *para* or *ortho* position, resepctively. The phenyl moiety can be moved further by insertion of an ethylene linker as in **7** without loss in activity compared to **1**. Also the more polar isoxazole compound **8** has a comparable activity. However, the rigid indan system in **9** leads to high potency. Interestingly, one of the enantiomeric hydroxyindan derivatives, that is, **11,** displays activity comparable to **9** whereas the other (**10**) drops in activity. A number of aliphatic amines were also investigated and the direct comparator **14** to rimonabant displays comparable activity (4.5 vs 7.5 nM) despite the drop in log *P* and log *D* induced by the nitrile moiety. Removal of the hydrazine nitrogen (**13**) leads to an increase in potency, whereas the introduction of oxygen leads to a 10-fold less active morpholine **15**. Ring expansion with or without hydrazine gives compounds with sub-nanomolar potencies (**16, 17**). Notably, the insertion of an ethylene linker that was allowed in the phenyl case (**7**) is not allowed in the piperidine case (**18**). The more bulky amines **19** and **20** show potencies equal to the cyclohexylamine **13**, but the more polar sulfonamide **21** loses activity. Thus, there are several examples where the introduction of additional acceptor sites on the eastern amide moiety leads to a reduction in activity. To explore this aspect further, carboxylic acids **24** and **25** were included in the library and these were found to have considerably reduced potencies compared to the corresponding esters **22** and **23.**

The library also contained a set of tertiary amides (**26–42**), which provide complementary SAR information. The piperazinylpyrimidine **28**, containing several acceptor sites, displays high potency comparable with the piperidinylphenyl compounds, either with or without hydroxyl groups, that is compounds **30** and **27**, respectively. However, the piperazine **26** is less active than the more polar **28**. Linkage with pyrrolidine (**29**) seems less attractive while appending the phenyl system in the 2-position of the pyrrolidine (**37**) or piperidine (**31**) leads to a greater loss in potency. Some ring annelated compounds show pronounced potency with a positive influence from lipophilic electron withdrawing groups (cf. **32** vs **33** and **34** vs **35**). The methylsulfone **39** is comparable to **26** whereas the more polarized acetyl derivative **40** is not tolerated. We also confirmed the finding of the deleterious effect of a carboxyl moiety in this series (**41** vs **42**).

The substituent effects on a set of benzylic amides were explored (Table 2). Compounds with a single halogen substituent, either *para* (**43**–**45**), *meta* (**46**) or *ortho* (**47**), were found to be comparable. Unexpectedly the 2,4-dichloro substituted compound **49** showed no potency advantage over **44,** whereas the 2,6-dichloro substituted compound **50** was worse — presumably due to steric effects. However, certain other disubstitution patterns (**51**, **52**) were found to be more favourable, with the significant difference in potency between the *para* bromo compounds **45** and **51** being attributable to the *ortho* fluoro group. In terms of more polar motifs, it was noteworthy to observe that the *para* cyano compound

Table 2
Antagonism on hCB1 and lipophilicity of 4-cyanomethyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamides



No.	R^1	R^2	*c* log *P* (log *D*)[a]	IC_{50} GTPγS[b] (nM)
43	4-F	H	5.8 (4.1)	2.1
44	4-Cl	H	6.3 (4.4)	1.2
45[14]	4-Br	H	6.5 (4.4)	5.8
46	3-Cl	H	6.3 (4.4)	2.6
47	2-F	H	5.8 (4.1)	4.1
48	4-CN	H	5.0 (3.8)	4.2
49	2,4-Cl_2	H	7.0 (4.6)	1.6
50	2,6-Cl_2	H	7.0 (4.6)	7.0
51	2-F, 4-Br	H	6.6 (4.5)	0.51
52	4-F, 3-Br	H	6.6 (4.4)	0.70
53	4-F	CH_3	5.9 (4.2)	5.6

[a] and [b] as in Table 1. Values are single or mean of double determinations.

48 had the same potency as the halogen derivatives. Finally, comparison of **43** and **53** implied that the amide hydrogen is not an essential interaction feature.

These compounds also act as inverse agonists, for example, the bromo derivative **45** has an IC_{50} of 1.2 nM in the GTPγS assay. A selectivity of greater than 1000-fold for CB1 over CB2 was demonstrated, for a selection of compounds, by testing against the CB2 sub-type receptor in a GTPγS antagonist assay using CP55940 as agonist (data not shown).

In conclusion, we have explored the CB1 antagonist SAR features for a chemically diverse library of secondary and tertiary 4-cyanomethyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamides. In general a range of amide substitutions are tolerated, but polar and especially charged groups in this part of the molecule reduce the CB1 antagonistic activity. Some very potent eastern modifications have been identified which will form the basis of further work to identify less lipophilic compounds that do not readily pass the blood–brain-barrier.

Acknowledgements

The authors thank Rokhsana Andersen and Helle Iversen for excellent technical assistance.

References and notes

1. Endocannabinoid reviews: (a) Di Marzo, V. *Pharmacol. Res.* **2009**, *60*, 77; (b) De Petrocellis, L.; Di Marzo, V. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 1; (c) Di Marzo, V.; Ligresti, A.; Cristino, L. *Int. J. Obesity* **2009**, *33*, S18; (d) deKloet, A. D.; Woods, S. C. *Endocrinology* **2009**, *150*, 2531.
2. CB1 reviews: (a) Janero, D. R.; Makriyannis, A. *Expert Opin. Emerging Drugs* **2009**, *14*, 43; (b) Di Marzo, V. *Drug Discovery Today* **2008**, *13*, 1026; (c) Akbas, F.; Gasteyger, C.; Sjödin, A.; Astrup, A.; Larsen, T. M. *Obes. Rev.* **2009**, *10*, 58.
3. (a) Di Marzo, V. *Diabetologia* **2008**, *51*, 1356; (b) Mackie, K. *Neuroendocrinology* **2008**, *20*, 10.
4. Ravinet Trillou, C.; Delgorge, C.; Menet, C.; Arnone, M.; Soubrie, P. *Int. J. Obes.* **2004**, *28*, 640.
5. Medicinal chemistry reviews: (a) Lee, H.-K.; Choi, E. B.; Pak, C. S. *Curr. Top. Med. Chem.* **2009**, *9*, 482; (b) Jagerovic, N.; Fernandez-Fernandez, C.; Goya, P. *Curr. Top. Med. Chem.* **2008**, *8*, 205; (c) Muccioli, G. C.; Lambert, D. M. *Curr. Med. Chem.* **2005**, *12*, 1361.
6. Scheen, A. J.; Paquot, N. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 103.
7. (a) Rosenstock, J.; Hollander, P.; Chevalier, S.; Iranmanesh, A. *Diabetes Care* **2008**, *31*, 2169; (b) Després, J. P. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 416; (c) Després, J. P. *Curr. Pharm. Des.* **2009**, *15*, 553.
8. Caraceni, P. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 65.
9. (a) Moreira, F. A.; Grieb, M.; Lutz, B. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 133; (b) Viveros, M. P.; deFonseca, F. R.; Bermudez-Silva, F. J.; McPartland, J. M. *Endocr. Metab. Immune. Disord. Drug Targets* **2008**, *8*, 220.
10. Viveros, M. P.; Marco, E. M.; Llorente, R.; López-Gallardo, M. *Neural Plast.* **2007**, *2007*, 52908.
11. Kunos, G.; Osei-Hyiaman, D.; Bátkai, S.; Sharkey, K. A.; Makriyannis, A. *Trends Pharmacol. Sci.* **2009**, *30*, 1.
12. Lan, R.; Liu, O.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769.
13. The parallel library synthesis was conducted according to either of two protocols (a) or (b), as follows. (a) Larger scale using 9 parallel tubes: 1-Hydroxybenzotriazole (HOBt, 47 mg, 1.3 equiv) and 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDAC, 77 mg, 1.5 equiv) were added to a stirred solution of acid **III** (100 mg, 0.27 mmol, 1 equiv) in 15 ml CH_2Cl_2. After 20 min, the appropriate amine (1.2 equiv) and diispropylethylamine (DIPEA, 77 mg, 1.3 equiv) were added and stirring continued overnight. Saturated $NaHCO_3$ solution was added and the reaction mixture extracted with CH_2Cl_2. The organic phase was washed with brine, dried ($MgSO_4$) and evaporated by vacuum centrifuge. Final purification was made using semi-preparative reverse-phase HPLC or normal-phase column chromatography over silica; (b) Plate chemistry using 1.4 ml tubes in 96 well format: Acid **III** (0.075 ml of 0.1 M CH_2Cl_2 solution, 1.5 equiv), PS-DCC (8.1 mg, 2 equiv), HOBt (0.085 ml of 0.1 M CH_2Cl_2 solution, 1.7 equiv) and PS-DIPEA (2.6 mg, 2 equiv) were mixed in 0.80 ml CH_2Cl_2. The plate was shaken for 25 min, the appropriate amines (0.050 ml of 0.1 M *N*-methylpyrrolidone solution, 1 equiv) were added and shaking continued for 3 days. Additional CH_2Cl_2 (0.15 ml) and PS-trisamine (6.0 mg, 5 equiv) were added to each tube and shaking continued for a further 24 h. The resins were filtered and washed with CH_2Cl_2. Combined liquors were evaporated and residues redissolved in DMSO (0.25 ml). Following LC–MS analysis, the solutions were diluted with further DMSO so as to provide 10 mM stock solutions for screening.
14. Amengual, R.; Marsol, C.; Mayeux, E.; Sierra, M.; Wagner, P. Patent Appl. PCT Int. Appl. WO 2006133926, 2006.
15. Valko, K.; Du, C. M.; Bevan, C.; Reynolds, D. P.; Abraham, M. H. *Curr. Med. Chem.* **2001**, *8*, 1137.