Bioorganic & Medicinal Chemistry Letters 21 (2011) 6456–6460

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of pyrazoles as novel FPR1 antagonists

Andrew D. Morley [a,*], Andrew Cook [a], Sarah King [a], Bryan Roberts [a], Sarah Lever [a], Richard Weaver [b], Cathy MacDonald [b], John Unitt [c], Malbinder Fagura [c], Tim Phillips [c], Richard Lewis [a], Mark Wenlock [a]

[a] AstraZeneca R&D Charnwood, Chemistry, Bakewell Road, Loughborough LE11 5RH, UK
[b] DMPK, Bakewell Road, Loughborough LE11 5RH, UK
[c] BioScience, Bakewell Road, Loughborough LE11 5RH, UK

ARTICLE INFO

Article history:
Received 18 July 2011
Revised 15 August 2011
Accepted 17 August 2011
Available online 6 September 2011

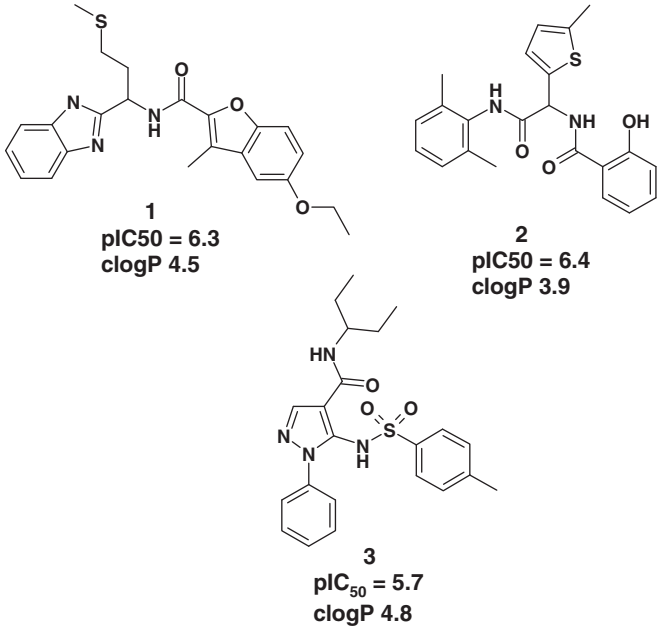
Keywords:
FPR1 antagonist
Hit-to-lead
Small molecule

ABSTRACT

A series of pyrazole inhibitors of the human FPR1 receptor have been identified from high throughput screening. The compounds demonstrate potent inhibition in human neutrophils and attractive physicochemical and in vitro DMPK profiles to be of further interest.

N-Formyl peptide receptors (FPR1, 2 and 3) represent a family of G protein-coupled receptors that form a key element in mounting an innate immune response and in response to products of cell damage.[1–3] FPR1 is functionally expressed on a variety of cell types ranging from inflammatory cells like neutrophils and macrophages to nonhematopoietic cells, such as lung epithelial cells, platelets, osteoblasts, and hepatocytes,[4–7] suggesting a wider role of FPR1 beyond antibacterial host defense. Most importantly activation of FPR1 leads to stimulation of proinflammatory neutrophil functions, critical to the detection, and efficient clearance of bacterial pathogens. Indeed the presence of *N*-formyl peptides and FPR1 expressing cells has been associated with inflammatory disease progression, in particular neutrophilic diseases like chronic obstructive pulmonary disease (COPD), where bacterial infection and potentially smoke-driven lung cell damage (e.g., mobilisation of host mitochondrial *N*-formyl peptides[8–10]) maybe linked to exacerbation of symptoms and chronic lung damage. Hence there is a strong therapeutic desire to antagonise FPR1 receptor function to potentially reduce inflammatory lung function and long term damage in diseases such as COPD.

We have previously reported the characterised two hit chemical series (i.e., benzimidazole **1** and diamide **2**) as suitable tool compounds for exploring FPR1 biology.[11] These compounds provided useful in vitro tools but tended to be too lipophilic to possess the desired physicochemical and DMPK characteristics to be of value for a drug discovery programme. In addition to these series, we also identified a series of pyrazoles (e.g., **3**) from the HTS.

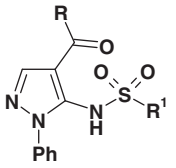


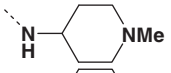
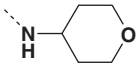
Compound **3** was part of a collection enhancement library of ~350 compounds having two points of diversity at the amide and sulphonamide. Some of the SAR from this library is shown in Table 1. Compounds were tested for FPR1 antagonism in a human neutrophil FPR1 Fluorescence Imaging Plate Reader (FLIPR)[12] assay.

* Corresponding author.
E-mail address: andymorley@live.com (A.D. Morley).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.08.085

Table 1
Historic Library SAR



Compd	R	R^1	FPR1 pIC_{50}[a]	*c*log*P*	LE[b]	LLE[c]
3	NHCH(Et)Et	4-MePh	5.7	4.8	0.19	0.9
4	NHMe	4-MePh	NA	2.9	—	—
5	NH*c*-Hexyl	4-MePh	5.4	4.9	0.17	0.6
6	NMe*c*-Hexyl	4-MePh	5.8	4.2	0.18	1.6
7	$NHCH_2Ph$	4-MePh	5	4.9	0.16	0.1
8	$NHCH_2$(2,4-Me)Ph	4-MePh	6.1	5.8	0.18	0.3
9	NHCH(nPr)Et	4-MePh	6.5	5.9	0.2	0.6
10		4-MePh	NA	3.0	—	—
11		4-MePh	5	2.5	0.16	2.5
12	NHCH(Et)Et	2-MePh	5.2	4.8	0.17	0.4
13	NHCH(Et)Et	Ph	5.3	4.3	0.18	1
14	NHCH(Et)Et	*c*-Pentyl	5.3	4.1	0.19	1.2

[a] Inhibition of fMLF stimulated intracellular calcium mobilisation in human neutrophils. pIC_{50} values are the means of at least two experiments. NA, not active (<25% inhibition) at 50 μM.
[b] LE, ligand efficiency.[13] Values are calculated as pIC_{50}/heavy atom count
[c] LLE, lipophilic ligand efficiency.[14] Values are calculated as pIC_{50}-clogP

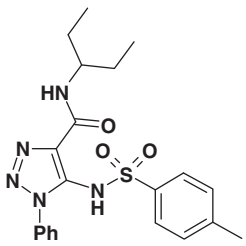
Data suggests FPR potency correlates with lipophilicity for R and although some analogues had pIC50 values of ~6.5, their ligand efficiencies (LE)[13] and lipophilic ligand efficiencies (LLE)[14,15] were modest. The sulphonamide substituents (R^1) that were included in the original library were all relatively simple and contained only simple functional groups as substituents, but both aliphatic and aromatic analogues showed similar levels of potency, indicating some scope to explore further.

Attempts to modify or replace the sulphonamide and amide motifs were largely unsuccessful. Alkylation of the sulphonamide completely abolishes FPR1 activity, as did replacement of the sulphonamide with amide. This suggests the acidic group is necessary for FPR potency, although conformational effects of these changes can not be ruled out. The amide group can be successfully modified to maintain FPR1 activity, but only by incorporating less polar alternatives. In the cases where potency is maintained the physicochemical and in vitro DMPK profiles are significantly inferior to **3**, offering no overall advantage. Other heterocyclic scaffolds were evaluated as part of the SAR exploration for FPR1 and as a broader assessment of overall profiles. Triazole **15** and imidazole **16**, with the same substitution vectors maintained potency, whereas exploration of different regions/vectors (e.g., **17**) resulted in a loss of FPR1 activity.

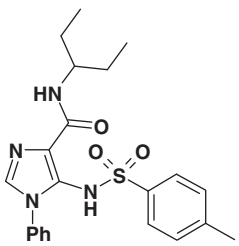
SAR exploration of the substituent attached via the nitrogen of the pyrazole was limited, with no improvements in potency observed. Ortho substitution (e.g., **18**) reduces FPR1 activity and introduction of polar groups (e.g., **19**) has a detrimental impact on biological activity. Substitution with heteroaromatics (**20**) had a similar effect, though improved LLEs were observed for some of these examples. Direct *N*-aryl attachment to the pyrazole is not essential for potency, with the benzyl analogue (**21**) showing a similar profile to phenyl. Smaller aliphatic substituents such as isopropyl (**22**) were tolerated, but less active, showing slightly reduced LE but marginally increased LLE. The *N*-methyl substituent (23) was inactive. Broader expansion of the sulphonamide substituent showed SAR to be quite flat. Potency and LLEs could be significantly improved through introduction of polar substituents at the 4 position of the aryl ring, such as compound **25**, but these observations were only with lipophilic tertiary amide analogues (compound **24** being significantly less active) and also came at the expense of increasing molecular weight to >500. These results are summarised in Table 2.

Introduction of a methyl substituent at the 3-position of the pyrazole scaffold produced a modest increase in potency (**26** vs **3**). Despite detailed evaluation, attempts to introduce polar motifs in this region were not compatible with FPR1 activity. Analysis indicated that lipophilic substituents were still required, but increasing the steric bulk close to the amide nitrogen gave potency improvements. This SAR wasn't observed to the same extent when the 3-substituent is hydrogen as can be seen when comparing **30** and **31**. This substitution pattern also brought about improvements in LLE. Effects appear specific with a need for both lipophilic components in order to achieve potent FPR1 activity, as shown by the significantly weaker potency of **27** and **33** compared with **34** and **35**. These latter motifs appeared to be useful amide substituents for FPR1 with enhanced potency, LE and LLEs. In both these cases the individual enantiomers were prepared and tested. No significant enantiomeric FPR1 potency differences were observed for **34**, where as for the isomers of **35**, the (*S*)-enantiomer (**36**) was preferentially active over the (*R*)-enantiomer (**37**) by ~sevenfold. This data is summarised in Table 3.

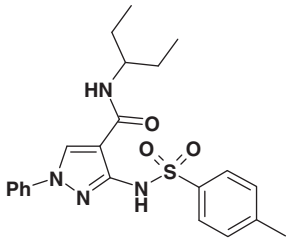
In order to try and understand the variation in SAR by introduction of the 3-methyl substituent, NMR evaluation of the DMSO-d_6 spectra of matched pairs were undertaken. These studies showed almost identical chemical shift for the regions of interest, suggesting the introduction of the 3-methyl group is not affecting internal hydrogen bonding capabilities between the amide and sulphonamide units under these conditions. Preliminary molecular modelling studies and analysis alongside the NMR spectra failed to support any particular hypothesis and subtle conformation effects can not be ruled out in effecting SAR. Additional substituents were introduced at the 3-position of the pyrazole, but with the exception of a CF_3 motif (e.g., **38**) all other variations resulted in reduced FPR1 potency.



15
pIC_{50} = 5.4
clogP 4.3

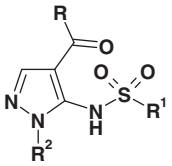


16
pIC_{50} = 5.8
clogP 5.6



17
pIC_{50} = 5.0
clogP 4.8

Table 2
Sulphonamide and pyrazole substituent SAR



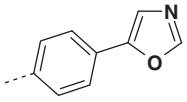
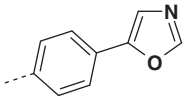
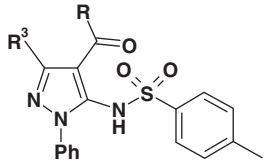
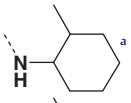
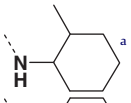
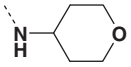
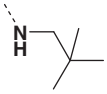
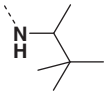
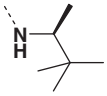
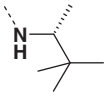
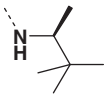
Compd	R	R^1	R^2	FPR1 pIC_{50}	*c*log*P*	LE	LLE
18	NHCH(Et)Et	4-MePh	2-ClPh	5	5.1	0.16	−0.1
19	NHCH(Et)Et	4-MePh	4-CNPh	4.8	4.4	0.15	0.4
20	NHCH(Et)Et	4-MePh	2-Pyridyl	4.7	3.6	0.16	1.1
21	NHCH(Et)Et	4-MePh	CH_2Ph	5.3	4.8	0.17	0.5
22	NHCH(Et)Et	4-MePh	*i*-Pr	5	3.7	0.18	1.3
23	NHCH(Et)Et	4-MePh	Me	NA	2.8	–	–
24	NHCH(Et)Et		Ph	5.3	4.1	0.16	1.2
25	NE*t*c-hexyl		Ph	7.1	4	0.19	3.1

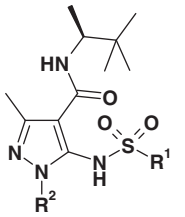
Table 3
Amide SAR and comparison of effect of 3-substituent

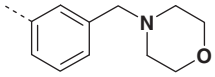


Compd	R	R^3	FPR1 pIC_{50}	*c*log*P*	LE	LLE
3	NHCH(Et)Et	H	5.7	4.8	0.19	0.9
26	NHCH(Et)Et	Me	6.3	4.7	0.20	1.6
27	NH*i*-Pr	Me	5.2	3.7	0.18	1.5
28	NHc-hexyl	Me	6.1	4.9	0.19	1.2
29	NMec-hexyl	Me	5.5	4.4	0.17	1.1
30	 [a]	H	6.0	5.3	0.19	0.7
31	 [a]	Me	6.8	5.4	0.21	1.2
32		Me	5.3	4.1	0.16	1.2
33		Me	5.7	4.7	0.19	1.0
34		Me	6.8	4.2	0.23	2.6
35		Me	6.9	5.0	0.22	1.9
36		Me	7.1	5.0	0.22	2.1
37		Me	6.3	5.0	0.20	1.3
38		CF_3	7.6	4.9	0.21	2.7

[a] Diasterisomeric mixture

Table 4
Follow up sulphonamide and pyrazole substituent SAR



Compd	R^1	R^2	FPR1 pIC_{50}	*c*log*P*	LE	LLE
39		Ph	NA	4.2	—	—
40	*c*-hexyl	Ph	6.0	4.9	0.19	1.1
41	3-pyridyl	4-FPh	6.0	4.2	0.19	1.8
42	4-CNPh	4-FPh	7.6	4.7	0.22	2.9
43	4-CF_3Ph	4-FPh	7.9	6.0	0.22	1.9

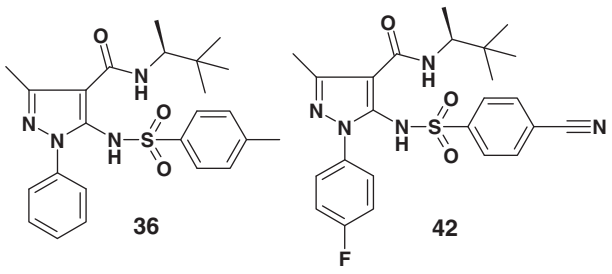
As the introduction of the 3-methyl group had altered the amide SAR, we revisited evaluation of the *N*-pyrazole and sulphonamide groups. In the case of analogues derived from **34**, no improvements in potency were generated. Expansion of **35** showed SAR around the *N*-pyrazole aryl group appeared broadly to track with previous observations, whereas the sulphonamide SAR now became more specific with aryl analogues being significantly more potent than alkyl (e.g., **36** vs **40**). Some key observations are shown in Table 4. Despite extensive efforts, attempts to introduce basic functions at any site around the pyrazole template resulted in completely inactive analogues, with compounds **39** and **10** exemplifying these observations. The para position of both aryl groups gave the best opportunity to improve FPR1 activity, with compounds **42** and **43** being the most potent examples identified in this phase and subsequently possess some of the best LE and LLEs for the series.

Broader evaluation of the profiles of **36** and **42** are shown in Table 5. The compounds show good potency, behaving as FPR1 antagonists, with no effects in an artefact assay. They also demonstrate good potency in a CD11 whole blood assay. The series, in general, showed no activity against a broad panel of receptors, kinases and enzymes (<20% effect @ 10 μM). Compounds shows little cross over for inhibiting rat, mouse, or guinea pig FPR1, which is consistent with our findings with other unrelated series for this receptor. Physicochemical and in vitro DMPK parameters are generally good, giving confidence that the series could provide a foundation for broader evaluation.

A generic synthetic route to the analogues is shown in Scheme 1. The key pyrazole core **46** was synthesized either by reaction of substituted hydrazines with **44**, or by reaction of hydrazidoyl halides **45** with ethyl cyanoacetate according to literature procedures.[21,22] Intermediate **46** was then sulfonylated with the appropriate sulfonyl chloride using sodium hydride to deprotonate the amino-pyrazole. Target compounds **48** were obtained either by hydrolysis of the carboxylic acid ester **47** to the corresponding carboxylic acid and subsequent coupling of the acid with amines using standard coupling agents or more conveniently by direct reaction of **47** with an excess of the aminomagnesium halide prepared by pre-mixing amine and *iso*-propyl magnesium chloride in THF (Bodroux reaction).[23] It should be noted that using the later method yields were generally significantly higher.

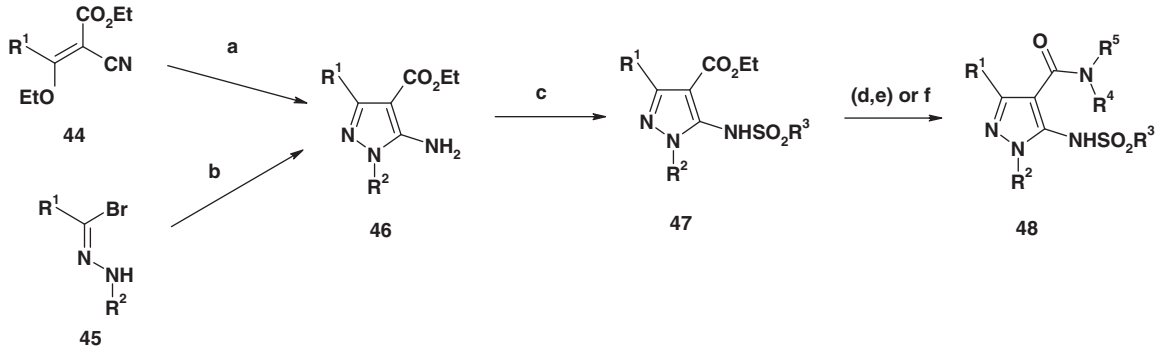
We have identified a novel series of antagonists, for a challenging GPCR target and evaluated SAR to generate significant increases in potency from the original HTS hit. The associated profiles of these initial compounds make the series significantly more attractive than other published FPR1 antagonists and provide a useful start point for more detailed evaluation of the series, which will subsequently be reported.

Table 5
Profiles of **36** and **42**



Parameter	Lead criteria[16]	36	42
FPR1 pIC_{50}	pIC_{50} >7	7.1	7.6
FPR1 artefect		NA	NA
Whole blood CD11 pA2		6.4	6.9
*c*log*P*/log*D*	<3.0 (log*D*)	5/1.4	4.7/0.5
Mol Wt	<450	455	483
LE		0.22	0.22
LLE		2.1	2.9
Solubility μM[17]	>100	>1000	>1000
Hu/Rat Prot Binding	% Free	1.1/4.2	6/10
Hu/Rat Mics[a] (μl/min/mg)	<30	40/53	5/13
Hu/Rat Heps[b] (μl/min/10^6cells)	<15	7/12	<3/4
Chem stability[18]	$t_{1/2}$ >100 hr	>1000	>1000
Cyp inhib pIC_{50}[c]	pIC_{50} <5	<5 (5/5)	<5 (5/5)

pIC_{50} and pA2 values are the means of at least three experiments.
[a] Human microsome metabolism intrinsic clearance (μL/min/mg).[19]
[b] Rat Sprague–Dawley hepatocyte metabolism intrinsic clearance (μL/min/10^6 cells).[20]
[c] Inhibition of cytochrome P450 isoforms: 1A2, 2C9, 2C19, 2D6, and 3A4



Scheme 1. Synthesis of target derivatives. Reagents and conditions: (a) R^2NHNH_2, EtOH/water (20:1), reflux, 3–15 h. (b) Ethyl cyanoacetate, EtOH, NaOEt, 25 °C, 3–12 h. (c) R^3SO_2Cl, THF, NaH, 25 °C, 3–12 h. (d) $LiOH{\cdot}H_2O$, EtOH, 70 °C, 5–12 h. (e) HNR^4R^5, HATU, DIEA, DCM or DMF, 25 °C, 4–20 h. (f) HNR^4R^5, *iso*-propyl magnesium chloride, THF, 25 °C, 1–3 h.

Acknowledgments

We acknowledge Becky Holford and Kathy Dodgson for developing the FPR1 FMAT assay and running the HTS and Jadeen Christie, Matthew Perry and Andrew Baxter and chemistry input.

References and notes

1. Ye, R. D.; Boulay, F.; Wang, J. M.; Dahlgren, C.; Gerard, C.; Parmentier, M.; Serhan, C. N.; Murphy, P. M. *Pharmacol. Rev.* **2009**, *61*, 119.
2. Schiffmann, E.; Corcoran, B. A.; Wahl, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1059.
3. Schiffmann, E.; Showell, H. V.; Corcoran, B. A.; Ward, P. A.; Smith, E.; Becker, E. L. *J. Immunol.* **1975**, *114*, 1831.
4. McCoy, R.; Haviland, D. L.; Molmenti, E. P.; Ziambaras, T.; Wetsel, R. A.; Perlmutter, D. H. *J. Exp. Med.* **1995**, *182*, 207.
5. Czapiga, M.; Gao, J.; Kirk, A.; Lekstrom-Himes, J. *Exp. Hematol.* **2005**, *33*, 73.
6. Rescher, U.; Danielczyk, A.; Markoff, A.; Gerke, V. *J. Immunol.* **2002**, *169*, 1500.
7. Shin, M. K.; Jang, Y. H.; Yoo, H. J.; Kang, D. W.; Park, M. H.; Kim, M. K.; Song, J. H.; Kim, S. D.; Min, G.; You, H. K.; Choi, K. Y.; Bae, Y. S.; Min do, S. *J. Biol. Chem.* **2011**, *286*, 17133.
8. Rabiet, M. J.; Huet, E.; Boulay, F. *Eur. J. Immunol.* **2005**, *35*, 2486.
9. Carp, H. *J. Exp. Med.* **1982**, *155*, 264.
10. Zhang, Q.; Raoof, M.; Chen, Y.; Sumi, Y.; Sursal, T.; Junger, W.; Brohi, K.; Itagaki, K.; Hauser, C. J. *Nature* **2010**, *464*, 104.
11. Unitt, J.; Fagura, M.; Phillips, T.; King, S.; Perry, M.; Morley, A.; MacDonald, C.; Cook, A.; Baxter, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2991.
12. Schroeder, K. S.; Neagle, B. D. *J. Biomol. Screen.* **1996**, *1*, 75.
13. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430. Values in this manuscript are calculated and reported as pIC_{50}/Heavy atom count.
14. Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Disc.* **2007**, *6*, 881.
15. *c*log*P*, Version 4.3, BioByte (www.biobyte.com).
16. Baxter, A.; Cooper, A.; Kinchin, E.; Moakes, K.; Unitt, J.; Wallace, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 960.
17. Wenlock, M. C.; Barton, P.; Potter, T.; Austin, R. P. *J. Assoc. Lab. Autom.* **2011**, *5*, 1–10. doi:10.1016/j.jala.2010.10.002.
18. MacFaul, P. A.; Ruston, L.; Wood, J. M. *Med. Chem. Commun.* **2011**, *2*, 140.
19. McGinnity, D. F.; Parker, A. J.; Soars, M.; Riley, R. J. *Drug Metab. Dispos.* **2000**, *28*, 1327.
20. Soars, M. G.; Grime, K.; Sproston, J. L.; Webborn, P. J. H.; Riley, R. J. *Drug Metab. Dispos.* **2007**, *35*, 859.
21. (a) Tanaka, K.; Suzuki, T.; Maeno, S.; Mitsuhashi, K. *J. Het. Chem.* **1986**, *23*, 1535; (b) Abunada, N. M.; Hassaneen, H. M.; Kandile, N. G.; Miqdad, O. A. *Molecules* **2008**, *13*, 1501.
22. (a) Sutherland, H. S.; Blaser, A.; Kmentova, I.; Franzblau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Palmer, B. D.; Denny, W. A.; Thompson, A. M. *J. Med. Chem.* **2010**, *53*, 855; (b) Das, J.; Moquin, R. V.; Dyckman, A. J.; Li, T.; Pitt, S.; Zhang, R.; Shen, D. R.; McIntyre, K. W.; Gillooly, K.; Doweyko, A. M.; Newitt, J. A.; Sack, J. S.; Zhang, H.; Kiefer, S. E.; Kish, K.; McKinnon, M.; Barrish, J. C.; Dodd, J. H.; Schieven, G. L.; Leftheris, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6886; (c) Beck, J. R.; Gajewski, R. P.; Lynch, M. P.; Wright, F. L. *J. Het. Chem.* **1987**, *24*, 267.
23. (a) Bodroux, F. *Bull. Soc. Chim. Fr.* **1905**, *33*, 831; (b) Bodroux, F. *Bull. Soc. Chim. Fr.* **1906**, *35*, 519.