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Pyrazole NNRTIs 3: Optimisation of physicochemical properties

Charles E. Mowbray [a,*], Romuald Corbau [b], Michael Hawes [a], Lyn H. Jones [a], James E. Mills [a], Manos Perros [b], Matthew D. Selby [a], Paul A. Stupple [a], Rob Webster [c], Anthony Wood [a]

[a] Department of Discovery Chemistry, Pfizer Global Research and Development, Sandwich, Kent CT 13 9NJ, UK
[b] Department of Discovery Biology, Pfizer Global Research and Development, Sandwich, Kent CT 13 9NJ, UK
[c] Department of Pharmacokinetics, Dynamics and Metabolism Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT 13 9NJ, UK

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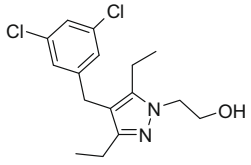
ABSTRACT

Our efforts to reduce overall lipophilicity and increase ligand-lipophilicity efficiency (LLE) by modification of the 3- and 5-substituents of pyrazole **1**, a novel non-nucleoside HIV reverse transcriptase inhibitor (NNRTI) prototype were unsuccessful. In contrast replacement of the substituted benzyl group with corresponding phenylthio or phenoxy groups resulted in marked improvements in potency, ligand efficiency (LE) and LLE.

In our previous papers we described the initial design and subsequent optimisation of a new series of pyrazole NNRTIs.[1,2] The early lead compound in this series alcohol **1** was an inhibitor of wild type (WT) and drug resistant mutant HIV reverse transcriptase (RT) but was relatively lipophilic (clog *P* 4.3) and consequently suffered rapid metabolism in human liver microsomes. Our efforts to date had not managed to improve potency and increase metabolic stability and we recognized the need to improve ligand-lipophilicity efficiency (LLE).[3] Following our initial survey of the SAR in the pyrazole series we made a more in depth study of the 3- and 5-substituents on the pyrazole core (Table 1). The majority of these compounds were designed to be less lipophilic than the leads **1** and **2** and we anticipated that they would have improved metabolic stability. However all these compounds were significantly weaker inhibitors of HIV RT and in many cases were essentially inactive.



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We were frustrated by our lack of success in improving potency and physical properties through modification of the substituents around the periphery of the benzylpyrazole and so resolved to make more synthetically challenging[5] modifications to the core template itself. Whilst developing this more challenging chemistry we were able to probe whether we could disconnect the relationship between lipophilicity and potency by preparing some simple 3,5-dimethyl pyrazoles shown in Table 2.

We were very excited to discover that phenylthiopyrazole **25** was about five times more potent as an HIV RT inhibitor than the corresponding benzylpyrazole **24**. This improved activity against the isolated enzyme also resulted in improved antiviral activity. We recognized that this improvement in potency and ligand efficiency came at the cost of an increase in lipophilicity and so did not offer an improvement in LLE. Oxidation of the new linking sulfur atom to give the corresponding sulfoxide **26** or sulfone **27** produced significant losses in potency consistent with introducing polar substituents in a region of the enzyme which had so far favoured lipophilic groups. The phenoxypyrazole **28** proved to be the most interesting member of this set of structurally similar compounds being slightly more potent as an HIV RT inhibitor than the parent benzylpyrazole **24** and also lacking the metabolically vulnerable, doubly benzylic carbon atom. Removal of this site for possible oxidative metabolism resulted in increased stability in human liver microsomes (compare **24** to **28**) as shown in Table 5. Although the ligand efficiency (LE) and LLE of this ether **28** are not significantly different from those of the parent compound **24** we felt that the observed improved potency and metabolic stability coupled with the opportunity to explore a wider range of phenyl group substitution patterns by employing parallel chemistry

* Corresponding author. Tel.: +44 1304 648427; fax: +44 1304 651821.
E-mail address: Charles.Mowbray@Pfizer.com (C.E. Mowbray).

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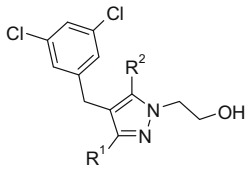
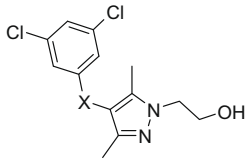
Table 1
Variation of pyrazole 3- and 5-substituents



Compound	R^1	R^2	*c*log *P*	IC_{50}[a] (μM)
Efavirenz	—	—	3.7	0.0084
1	Et	Et	4.3	0.66
2	Pr^i	Me	4.2	1.9
3	NMe_2	Me	3.5	36
4	Me	OMe	3.2	6.3
5	Me	OEt	3.8	54
6	CO_2Et	Me	3.7	27
7	Me	CO_2Et	3.7	>10
8	CH_2OCH_3	Me	2.8	>10
9	CO_2H	Me	2.2	>100
10	Ph	Me	4.9	>10
11	Me	Ph	4.9	>10
12	NH_2	Me	2.2	>10
13	$CONH_2$	Me	1.6	>10
14	Et	NH_2	2.7	>50
15	Et	$NHCH_2CH_2OMe$	3.6	>20
16	Et	NH_2CONH_2	2.2	>100
17	Et	NHCOMe	2.1	>100
18	Et	$NHCO_2Et$	3.4	77
19	Et	NHCOEt	2.7	>100
20	Et	NME_2	4.0	9.8
21	Et	$NHSO_2Me$	2.1	>100
22	Et	NHCO-3-pyridyl	2.6	>100
23	Et	$NHCOCH_2OMe$	1.9	55

[a] Inhibition of wild type HIV RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[4]

Table 2
Initial variation of biaryl linker



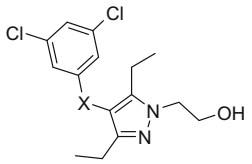
Compound	X	*c*log *P*	IC_{50}[a] (μM)	LE	LLE	AV_{50}[c] (nM)
24	CH_2	3.3	2.1	0.42	2.41	1100
25	S	4.0	0.36	0.47	2.47	80
26[b]	SO	2.1	>10	—	—	—
27	SO_2	2.3	15	0.32	2.55	—
28	O	3.6	1.1	0.44	2.33	157

[a] Inhibition of wild type HIV RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[4,6]
[b] Racemic.
[c] Antiviral activity in cell culture in SupT 1 cells infected with the RF strain of HIV.

warranted further exploration. This decision was further supported by synthesis and profiling of the homologous set of 3,5-diethylpyrazoles **29**–**33** shown in Table 3.

The improvements seen for thioether **25** and ether **28** were magnified in this series of diethylpyrazoles. The phenylthiopyrazole **32** and phenoxypyrazole **33** were approximately 10 times and five times more potent HIV RT inhibitors than the benzylpyrazole **1**, respectively. These improvement in HIV RT inhibition again delivered compounds with correspondingly improved antiviral activity in cell culture. Other linking groups such as ketone, methoxymethylene and hydroxymethylene were explored in compounds **29**–**31** and although modest potency was retained for some of these, none of them seemed to offer the promise of ether **33**.

Table 3
Further variation of biaryl linker

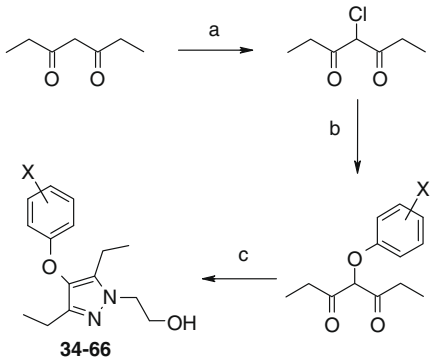


Compound	X	*c*log *P*	IC_{50}[a] (μM)	LE	LLE	AV_{50}[c] (nM)
1	CH_2	4.3	0.66	0.41	1.86	14
29	CO	3.9	2.0	0.36	1.77	94
30[b]	CHOMe	3.6	3.0	0.34	1.89	341
31[b]	CHOH	2.8	>100	—	—	—
32	S	5.0	0.065	0.48	2.17	0.63
33	O	4.7	0.12	0.46	2.23	3.3

[a] Inhibition of wild type HIV RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[3,6]
[b] Racemic.
[c] Antiviral activity in cell culture in SupT 1 cells infected with the RF strain of HIV.

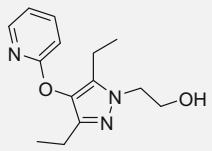
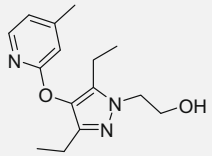
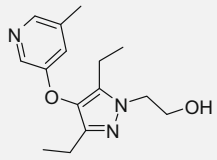
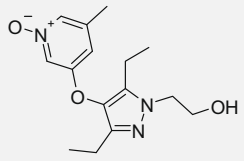
The metabolic stability of ether **33** was evaluated and compared to the parent benzylpyrazole **1** (Table 5). The ether **33** demonstrated improved resistance to oxidative metabolism when assessed in human liver microsomes. However in human hepatocytes ether **33** was rapidly metabolized by glucuronidation of the primary alcohol as previously demonstrated for members of the benzyl pyrazole series.[1] As our earlier efforts to modify or replace the alcohol side chain in lead **1** had generally resulted in a loss of activity[2] we believed that the best design strategy to reduce the rate of glucuronidation of lead ether **33** was to reduce the overall lipophilicity of the compound.[7] We recognized the dominant contribution of the 3,5-dichlorophenyl group to the overall lipophilicity of ether **33** and resolved to seek alternative, less lipophilic replacements. To enable this exploration of SAR we developed a synthetic route amenable to parallel chemistry[6] shown in Scheme 1 and using readily available phenols we subsequently prepared compounds **34**–**66** bearing a wide variety of substitution patterns with reduced lipophilicities shown in Table 4.

A few of the substitution patterns tested in this study such as 3-Cl, 2,5-diF, 2,6-diF, 3,5-diF and 3,5-diMe retained submicromolar activity against HIV RT but were weaker inhibitors of HIV RT than the parent **33** and still had *c*log *P* values in the range 3.2–4.3. In contrast the 3-CN compound **52** was only a threefold weaker inhibitor of HIV RT and crucially was two log units less lipophilic than the parent **33**. This marked improvement in lipophilic efficiency can also be seen in the plot of −log(RT IC_{50}) against *c*log *P* for the pyrazole series (Fig. 1) and the jump in LLE from 2.23 for parent compound **33** to 3.77 for the new 3-cyanophenoxypyrazole **52**.



Scheme 1. General synthesis of ethers **34**–**36**. Reagents and conditions: (a) Bu_4NBr, TMS-Cl, DMSO, MeCN; (b) Cs_2CO_3, appropriate phenol, acetone; (c) 2-hydroxyethylhydrazine, AcOH.

Table 4
Modification of pyrazole 4-substituent

Compound	X	clog *P*	IC_{50}[a] μM	LE	LLE
33	3,5-diCl	4.7	0.12	0.46	2.23
34	2-Cl	3.7	3.8	0.38	1.68
35	3-Cl	4.0	0.56	0.44	2.28
36	4-Cl	4.0	>50	—	—
37	2,3-diCl	4.3	4.3	0.36	1.03
38	2,4-diCl	4.5	>90	—	—
39	2,5-diCl	4.5	1.2	0.39	1.46
40	2,6-diCl	4.2	6.1	0.35	0.98
41	3,4-diCl	4.6	31	0.3	−0.06
42	2-F	3.2	3.3	0.38	2.28
43	3-F	3.4	1.2	0.41	2.52
44	4-F	3.4	>100	—	—
45	2,3-diF	3.3	2.6	0.37	2.31
46	2,4-diF	3.4	>40	—	—
47	2,5-diF	3.4	0.81	0.41	2.74
48	2,6-diF	3.2	0.96	0.4	2.87
49	3,4-diF	3.5	>50	—	—
50	3,5-diF	3.6	0.60	0.41	2.67
51	3,5-diMe	4.3	0.51	0.42	2.03
52	3-CN	2.7	0.35	0.43	3.77
53	4-CN	2.7	>50	—	—
54	3-$CONH_2$	1.8	>100	—	—
55	3-F-4-CN	2.8	>100	—	—
56	4-F-3-Me	3.9	24	0.31	0.72
57	4-F-3-CN	2.8	35	0.28	1.62
58	2,3-DiF-4-CN	2.7	>100	—	—
59	2-Cl-4-CN	3.2	31	0.29	1.33
60	3-Cl-4-CN	3.3	>100	—	—
61	2,6-diMe-4-CN	3.7	3.2	0.33	1.8
62	3,5-diMe-4-CN	3.8	>100	—	—
63		1.8	>100	—	—
64		2.3	>100	—	—
65		2.3	4.2	0.38	3.12
66		1.7	>100	—	—

[a] Inhibition of wild type HIV RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[6]

We were keen to evaluate any improvement in stability towards oxidative metabolism and glucuronidation of this less lipophilic lead. The apparent stability of 3-cyanophenoxyprazole **52** in human liver microsomes was actually worse than the parent 3,5-dichlorophenoxypyrazole **33** ($T_{1/2}$ = 27 versus 89 min) however we believe that this is actually due to a reduction in microsomal binding as seen in Table 5. This reduction in microsomal binding is likely to be a further consequence of the reduction of lipophilicity. We were very pleased to observe a dramatic reduction in the rate of glucuronidation of 3-cyanophenoxyprazole **52** compared to 3,5-dichlorophenoxypyrazole **33** resulting in a 20-fold lower clearance rate in human hepatocytes. These results vindicated our design goal of lowering overall lipophilicity to reduce the rate of glucuronidation in this series.

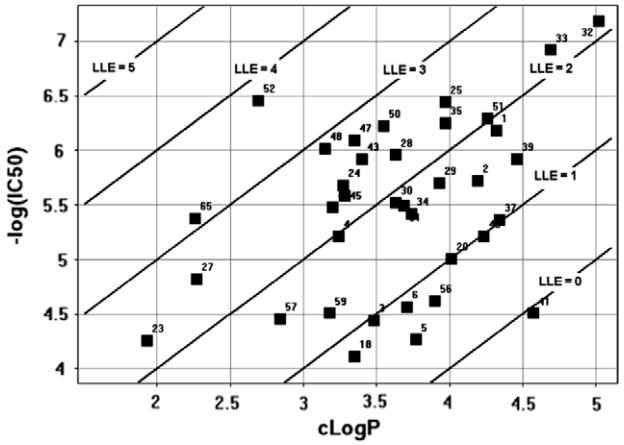


Figure 1. Plot of −log(RT IC_{50}) against clog *P* for pyrazole series. The 45° lines indicate equal values of LLE.

In our initial design of the early pyrazole NNRTIs we had managed to retain excellent activity against HIV RT bearing important drug resistance mutations from clinically significant drug resistant viruses.[1] Throughout the development of this series of inhibitors we regularly checked to make sure that new leads retained this broad spectrum of activity as is shown for selected compounds in Table 6. It can be seen that the excellent spectrum of activity of early lead **1** is retained as the atom linking the phenyl group to the pyrazole core is changed from carbon to sulfur (compounds **25** and **32**) or oxygen (compounds **33** and **52**).

Table 5
Microsomal stability and binding of key compounds

Compound	HLM $T_{1/2}$[a] (min)	Predicted Cl_u from human hepatocytes[a,b] (mL/min/kg)	Microsomal free fraction[a] (%)
1	18	—	24
24	39	—	—
28	92	—	—
33	89	1000	13
52	27	50	80

[a] The methods for determination of in vitro metabolism and microsomal binding have recently been separately published.[8]
[b] Cl_u is unbound clearance.

Table 6
IC_{50} fold-resistance of selected pyrazoles versus HIV RT enzymes[a] bearing NNRTI resistance mutations cf. wild type

	Efavirenz	**1**	**25**	**32**	**33**	**52**
K103N	44	0.8	2.5	1.3	1.3	2.3
Y181C	2.2	1.1	1.7	1.5	3.8	1.5
F227L	0.4	9.0	—	13	7.1	4.7
V106A	1.8	1.8	—	2.9	3.8	15
Y188C	0.8	0.6	—	0.7	0.2	0.3
K101E	3.8	5.1	—	4.6	4.6	9.4
P236L	2.8	0.2	—	0.9	0.3	0.4
V108I	1.0	5.5	—	5.0	5.9	11
L100I	—	—	—	10	—	2.9
L234I	—	—	—	11	—	11

[a] Inhibition of wild type and mutated HIV RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[4,6]

At this stage in our work we felt that the profile of the nitrile **52** represented a significant step towards our goal of identifying a novel NNRTI combining excellent antiviral activity with a high quality pharmacokinetic, pharmaceutical and safety profile. We aimed to further improve both antiviral activity and the pharmacokinetic profile of this series and our efforts towards this goal are described in the next paper in this series.

Acknowledgements

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References and notes

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2. The second paper in this series 'Pyrazole NNRTIs 2: Exploring the Dependency of Potency on Lipophilicity': Burt, C.; Corbau, R.; Mills, J.; Mowbray, C. E.; Perros, M.; Tran, I.; Price, D.A.; Selby, M. D.; Stupple, P. A.; Webster, R.; Wood, A. Manuscript in preparation.
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5. Synthetic routes to compounds described in this paper have already been outlined[4,6] and will be discussed in more detail elsewhere.
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