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Pyrazole NNRTIs 1: Design and initial optimisation of a novel template

Charles E. Mowbray [a,*], Catherine Burt [a], Romuald Corbau [b], Manos Perros [b], Isabelle Tran [a], Paul A. Stupple [a], Rob Webster [c], Anthony Wood [a]

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

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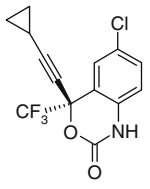
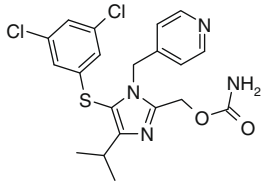
ABSTRACT

The design and synthesis of a novel series of non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs) based on a pyrazole template is described. These compounds are active against wild type reverse transcriptase (RT) and retain activity against clinically important mutants.

The first generation of clinically approved non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs) efavirenz, nevirapine and delavirdine are established as key components of highly active antiretroviral therapy (HAART).[1] These drugs block the action of the viral enzyme reverse transcriptase (RT) by binding to an allosteric binding site which is distinct from the catalytic site. In the clinic combinations containing NNRTIs with other antiretroviral agents can produce sustained reductions in viral load and slow the rate of disease progression.[2] However the first generation of approved NNRTIs are ultimately susceptible to mutations in HIV reverse transcriptase (RT) leading to drug resistance. Viruses with certain key RT mutations such as K103N can become resistant to all the first generation of approved NNRTIs.[3] In contrast the experimental NNRTI capravirine has been shown to retain excellent activity against a wide variety of viruses with clinically significant RT mutants including K103N. Furthermore two or more RT mutations are required to achieve high level resistance to capravirine.[4] Capravirine produced marked reductions in viral load in HIV infected patients and it was hoped it would also produce reductions in viral load in patients failing existing combination therapies.[5] Following the disclosure of these pre-clinical and clinical trial results for capravirine we believed that there was an excellent opportunity to identify still better NNRTIs which retain the broad spectrum of activity against clinically significant HIV with mutations in RT whilst reducing the required dose and pill burden, minimising the risk of side effects and drug–drug interactions and hence improving the ease of compliance.[6]



efavirenz



capravirine

As a starting point for our programme we wished to identify a novel NNRTI series which would retain the excellent inhibition of RT mutants demonstrated by capravirine whilst minimising the potential for rapid in vivo clearance which would necessitate a high clinical dose. The resilience of capravirine to a variety of mutations to the NNRTI binding pocket has been ascribed to a combination of three anchoring hydrogen bonds to the immutable RT backbone coupled with sufficient flexibility to allow rearrangement or repositioning of the lipophilic portions of the inhibitor which contribute the majority of binding energy within the mutated binding cavities.[7] Therefore in a new series of NNRTIs we planned to maximise hydrogen bonding to the RT backbone whilst incorporating sufficient conformational flexibility. Examination of capravirine reveals a lipophilic molecule (clog *P* 3.5) which would be expected to be prone to oxidative metabolism consistent with

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

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the short half life of only 7.5 min in our human liver microsome stability assay. The imidazole nucleus is not sufficiently basic (pK_a 4.47) for ionisation of the intrinsically lipophilic molecule to reduce the measured lipophilicity (log *D* >2.8) into the accepted range for optimal oral absorption and pharmacokinetics.[8] Furthermore we speculated that capravirine contains a biaryl sulphide and sterically unencumbered 4-pyridyl group both of which could provide likely sites for oxidative metabolism. A subsequent publication has confirmed that this does occur at least in rat liver microsomes.[9] Table 1 shows published data[10] and our own results[11] for inhibition of RT by a range of analogues of capravirine which demonstrate that within the series the pyridylmethyl group in capravirine can be removed (compare capravirine to **1**), albeit with a small loss of potency. Similarly the primary carbamate can be replaced with other moieties such as an alcohol (compare capravirine to **1a**, and **1** to **2** and **3**). In addition the 3,5-dichloro substitution on the thiophenoxy ring can be simplified to the less lipophilic 3-fluorosubstitution (compare **1** and **4**). Finally replacing the sulphur atom with a methylene linker reduces activity significantly (compare **1** and **5**).

With these observations in mind we designed (Fig. 1) the pyrazole **6** as a prototype of a new series which proved to be a moderately potent inhibitor of wild type RT (IC_{50} 1.9 μM). We were pleased with the promising activity of the pyrazole **6** and carried out extensive profiling to determine its quality as a lead with the potential to be optimised into a series capable of delivering a drug candidate. The RT inhibition of the lead **6** was shown to translate into potent antiviral activity in cell culture (EC_{50} 33 nM, EC_{90} 77 nM) in SupT1 cells infected with the RF strain of HIV. The compound was also shown to be not cytotoxic to this cell type (CC_{30} >1000 nM). Although pyrazole **6** is lipophilic (clog *P* 4.2, log *D* >4.0) it showed encouraging stability in human liver microsomes ($T_{1/2}$ 50 min cf. capravirine $T_{1/2}$ 7.5 min). The lead **6** also displayed excellent caco-2 flux (Papp A–B/B–A 41/9 cmS^{-1}). Furthermore the lead **6** did not have any profound polypharmacology, QT_c interval prolongation or drug–drug interaction liabilities as assessed in wide ligand binding, HERG channel binding and P450 inhibition studies which might limit the potential of a new series based upon this core structure. We therefore instigated a programme to optimise the potency, physicochemical and in vitro metabolic properties of this new lead.

We elected to optimise the 3-isopropyl and 5-methyl substituents of lead 6 first as these were introduced in the β-diketone at the start of our synthetic route as illustrated in Scheme 1.[10] We

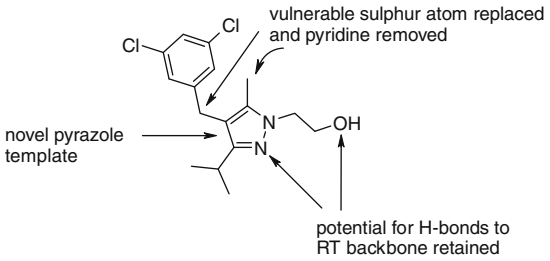
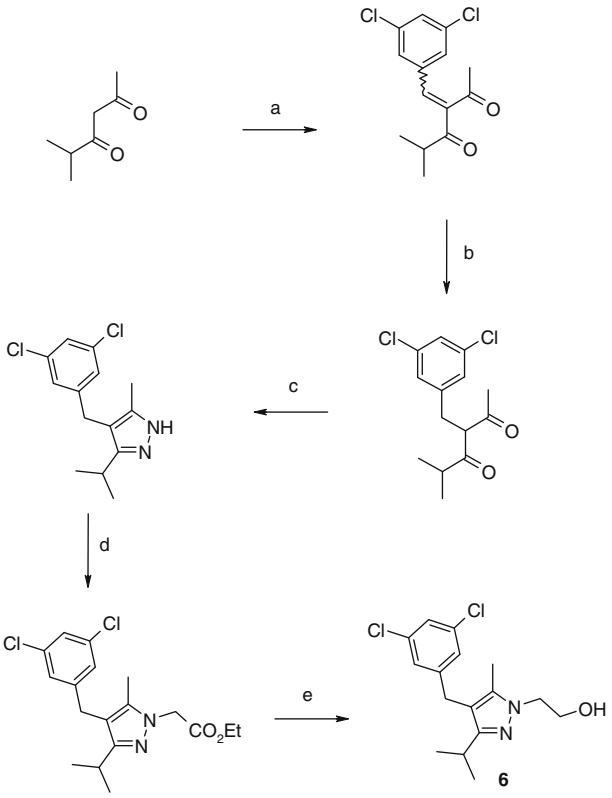
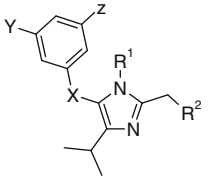


Figure 1. Design of prototype pyrazole inhibitor **6**.



Scheme 1. Synthesis of prototype pyrazole inhibitor **6**. Reagents and conditions: (a) 3,5-dichlorobenzaldehyde, acetic acid, piperidine, toluene, reflux, 50%; (b) H_2 15 psi, $Pd/BaSO_4$, EtOH, 80%; (c) hydrazine hydrate, EtOH, reflux, 80%; (d) ethyl bromoacetate, NaOEt/EtOH, 80 °C, 78%; (e) $LiAlH_4$, Et_2O, 77%.

Table 1
Wild type RT activity of truncated analogues of capravirine



Compound	X	Y	Z	R^1	R^2	IC_{50}[a] (nM)	IC_{50}[b] (nM)
Efavirenz	—	—	—	—	—	—	8.4
Caparavirine	S	Cl	Cl	4-Pyridylmethyl	$OCONH_2$	0.35	93
1	S	Cl	Cl	Me	$OCONH_2$	1.3	730
1a	S	Cl	Cl	4-Pyridylmethyl	OH	NT[c]	110
2	S	Cl	Cl	Me	OH	3	792
3	S	Cl	Cl	Me	CH_2OH	0.4	NT
4	S	F	H	Me	$OCONH_2$	0.12	NT
5	CH_2	Cl	Cl	Me	$OCONH_2$	19	NT

[a] Reported values are for inhibition of wild type RT with a poly(rA) template, (dT) 12–18 primer and dTTP as substrate.[10]
[b] Inhibition of wild type RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[11]
[c] Not tested.

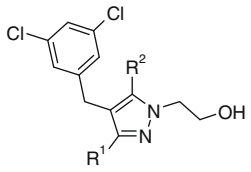
planned to subsequently investigate changes to the benzaldehyde component before varying the N-substituent and then re-optimising as necessary.

A range of pyrazoles with varied 3- and 5-substituents were prepared and compared to the lead **6** (Table 2). Reducing the 3-isopropyl group to an ethyl group slightly enhanced potency but further truncation to a methyl group was not favoured (compare **6–8**). Replacing the 5-methyl group with an ethyl group also improved potency but a larger isopropyl group was less successful (compare **8–10**). Combining the best 3- and 5-substituents gave the 3,5-diethylpyrazole **11** as the most potent compound in this early series. This compound was made from commercially available symmetrical 3,5-heptanedione which allowed straightforward synthesis without the need for separation of pyrazole N-1/N-2 isomers.

To determine whether the excellent balance of activity of capravirine versus RT with drug resistance mutations had been retained within this new series of NNRTIs we tested compounds **8** and **11**–**14** against a panel of RT enzymes with clinically relevant[12] engineered point mutations in the NNRTI binding pocket (Table 3). As we had hoped all the compounds retained excellent activity within twofold of their wild type activities against the clinically common K103N and Y181C mutations. These compounds were also active against a wider panel of RT enzymes with less clinically common NNRTI resistance mutations. Indeed all these mutant enzymes with the exception of F227L for compounds **8**, **12** and **13** remained sensitive to the compounds at concentrations within 10-fold of their wild type IC_{50}'s. In contrast although efavirenz retains activity against RT with the less clinically common mutations it is much less active versus the K103N mutant.

The metabolic stability of several members of this series was examined in human liver microsomes (Table 2). Decreasing the size of the 3-substituent reduced the half life and if this was accompanied by an increase in lipophilicity the effect was magnified (compare **6**, **8** and **10**). If the overall lipophilicity was kept constant but the 3-substituent reduced in size the half life was also reduced (compare **6** and **11**). We therefore speculated that the principal route of oxidative metabolism of these compounds was via a stabilized benzylic type radical at the pyrazole 3-position which was subsequently converted to a hydroxylated product. Formation of this radical by a P450 enzyme would be expected to be sensitive to the steric environment of the benzylic position and the overall lipophilicity of the molecule explaining the observed trends. We therefore prepared the 3-trifluoromethyl analogue **12** which should not be susceptible to this route of oxidation and we were rewarded with an increased half life, however the molecule was still metabolised demonstrating that alternative sites for oxidation exist in this template. That oxidation at more than one site was probably contributing to the metabolism of these molecules was further indicated by the increased stability of a 5-trifluoromethyl compound compared with its 5-ethyl analogue (compare **11** and **14**). The most stable member of this series, compound **12** was progressed to an isolated perfused rat liver preparation where extremely rapid glucuronidation of the alcohol moiety was detected giving an estimated unbound clearance value of approximately 10,000 mL/min/kg. This showed that in addition to removing or blocking oxidatively vulnerable groups we would need to reduce glucuronidation.

Table 2
Variation of pyrazole 3- and 5-substituents



Compound	R^1	R^2	IC_{50}[a] (μM)	$T_{1/2}$[b] (min)
Efavirenz	—	—	0.0084	—
6	Pr^i	Me	1.9	50
7	Et	Me	0.77	NT[c]
8	Me	Me	2.1	39
9	Me	Et	1.0	NT
10	Me	Pr^i	6.5	4
11	Et	Et	0.66	18
12	CF_3	Me	0.92	79
13	CF_3	Et	0.63	NT
14	Et	CF_3	0.54	32

[a] Inhibition of wild type RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[11]
[b] Half life of compound in a human liver microsome preparation.
[c] Not tested.

Table 3
IC_{50} fold-resistance of selected pyrazoles versus RT enzymes[a] bearing NNRTI resistance mutations cf. wild type

	Efavirenz	**8**	**11**	**12**	**13**	**14**
K103N	44	1.6	0.8	1.0	2.4	1.4
Y181C	2.2	2.1	1.1	1.3	1.6	1.0
F227L	0.4	16	9.0	11	16	9.3
V106A	1.8	2.7	1.8	2.4	2.2	3.4
Y188C	0.8	1.3	0.6	0.6	0.7	0.4
K101E	3.8	5.6	5.1	5.7	5.1	5.1
P236L	2.8	0.3	0.2	0.2	0.2	0.2
V108I	1.0	7.1	5.5	6.5	7.3	5.6

[a] Inhibition of wild type and mutated RT with a poly(rA) ~300 template, (dT) 16 primer and dTTP as substrate.[11]

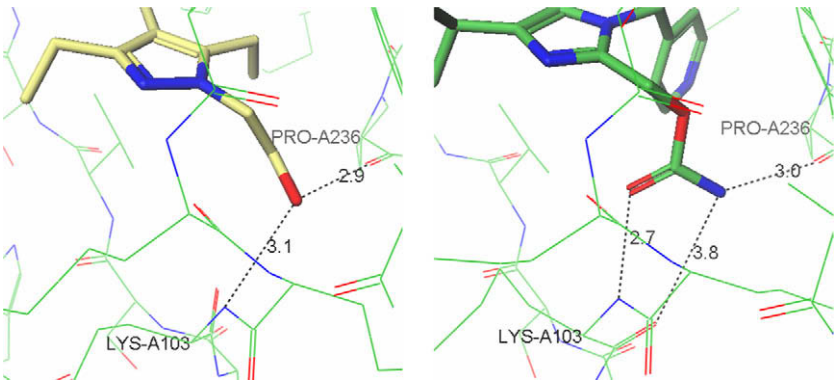


Figure 2. Model showing possible hydrogen bonding to wild type RT by alcohol **11** (shown in yellow in the left hand image) compared to the carbamate of capravirine (shown in green in the right hand image). Ligand–protein distances (Å) are shown in black and indicated with dashed lines.

From these initial investigations of RT inhibition SAR and metabolic stability it was clear that we had identified a potent series of inhibitors but to achieve further improvements in stability towards metabolism would need a reduction in lipophilicity and/or additional modification of the remaining vulnerable sites whilst further optimising potency. Further studies toward these goals are described in the following papers in this series.

Finally we suggest that the alcohol present in inhibitors such as **11** is able to make hydrogen bonds to proline-236 and lysine-103 of the RT main chain as shown in Figure 2. This is similar to that observed for the carbamate of capravirine in the published crystal structure with wild type RT.[7]

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