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Biaryl ethers as potent allosteric inhibitors of reverse transcriptase and its key mutant viruses: Aryl substituted pyrazole as a surrogate for the pyrazolopyridine motif

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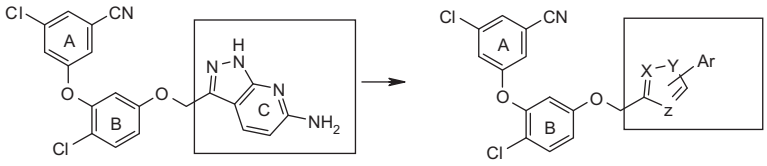
ABSTRACT

Biaryl ethers were recently reported as potent NNRTIs. Herein, we disclose a detailed effort to modify the previously reported compound **1**. We have designed and synthesized a series of novel pyrazole derivatives as a surrogate for pyrazolopyridine motif that were potent inhibitors of HIV-1 RT with nanomolar intrinsic activity on the WT and key mutant enzymes and potent antiviral activity in infected cells.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key components of current HIV therapy, HAART (Highly Active Antiretroviral Therapy), a combination of nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs, NNRTIs), and protease inhibitors (PIs). HAART can be very effective at delaying disease progression. However, due to the propensity of HIV to rapidly mutate, the efficacy and durability of HAART can be compromised. The most frequent HIV RT mutations observed in patients failing therapy with



compound **1** (MK-4965)

Scheme 1. Lead Compounds **1** and replacement of pyrazolopyridine C-ring.

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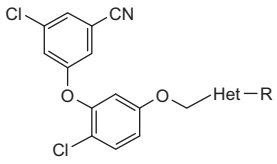
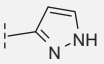
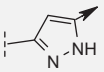
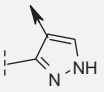
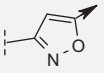
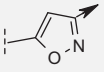
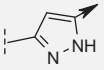
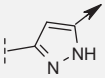
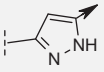
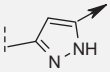
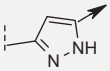
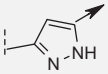
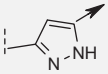
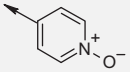
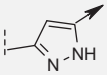
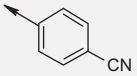
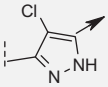
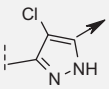
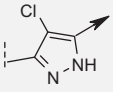
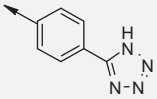
Table 1
SAR results



Compound	Het	R	RT-Pol[a]	Spread[b] (nM)		
			WT/K103N/Y181C (nM)	10% FBS/50% NHS (WT)	K103N[c]	Y181C[c]
2		—	39/179/140	922/8300	8300	—
3			0.9/2.4/12.5	222/924	924	833
4			460/>340/>3000	8300/>8300	—	—
5			2.6/5.5/20.4	926/8333	2778	833
6			31/115/763	>8300/>8300	>8333	—
7			1.6/4.6/49.5	168/307	922	178
8			0.3/10/1.5	97/922	922	833
9			0.7/4.2/3[*]	32/307	102	278
10			3.2/9.6/8.9	97/307	307	>833
11			1.1/5.2/6.7	32/307	307	278
12			0.7/4.2/4.6	25/102	102	278
13			5.3/21/46	44/307	307	500
14			0.7/3.6/12[*]	34/922	307	—
15			0.4/0.5/0.6[*]	922/922	2767	—
16			0.5/0.7/0.5[*]	97/307	102	278
17			0.3/0.7/0.6[*]	2778/>8333	8333	—

[a] Compounds were evaluated in a standard SPA assay.
[b] CIC95 (cell culture inhibitory concentration) is defined as the concentration at which the spread of virus is inhibited by >95% in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing either 10% fetal bovine serum (FBS) or 50% normal human serum (NHS). The antiviral activity of compound against wild-type ABI R8 virus was measured. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean.
[c] In the presence of 10% FBS.
[*] Compounds were evaluated in a standard ECL assay.

first generation NNRTIs are K103N and Y181C. Therefore, new agents with better activity profiles against mutant HIV-1 RT are needed.

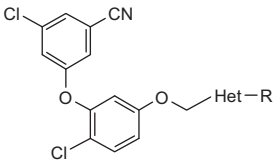
The current study was initiated with the previously reported biaryl ethers lead compound **1** (Scheme 1).[1–4] In this Letter, we report the SAR study results of a structural alternative, substituted pyrazole, as a replacement of pyrazolopyridine (the C-ring), that exhibited excellent potency against wild-type (WT) reverse transcriptase (RT) and key clinically observed RT mutants.

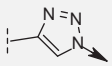
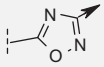
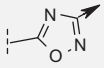
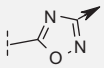
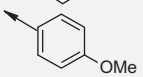
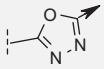
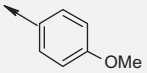
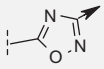
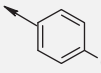
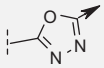
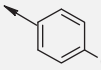
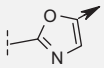
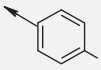
The previous reported compound **1** showed excellent potencies and had good oral bioavailablity in preclinical species (Scheme 1).[2] In an effort to increase the structural diversity of our compounds, we focused on the replacement of the pyrazolopyridine C-ring. We speculated that the pyrazolopyridine moiety (*a.k.a.* fused aryl pyrazole) could be replaced with aryl substituted pyrazole. To test this hypothesis, the truncated analogue (**2**) was synthesized (Table 1). Gratifyingly, compound **2** maintained potency against WT RT and its key mutants in the enzyme assay (RT_Pol).[5] However, its activity in the cell based assay (Spread assay) was significantly reduced.[6] We were encouraged that introduction of a phenyl group at the 5-position of the pyrazole (compound **3**) improved the potency in both enzyme and cell based assays and decreased the sera shift between 10% FBS and 50% NHS. The isomeric 4-phenyl pyrazole analogue (compound **4**) was inactive. It is known that the isoxazolopyridine can be an effective isostere for the pyrazolopyridine.[1] Replacement of pyrazole with isoxazoles led to compounds **5** and **6**. The 5-phenyl isoxazole analogue (**5**) exhibited good intrinsic potencies in the enzyme assay, but it lost potency in the Spread assay. The isomeric 3-phenyl isoxazole (**6**) was less potent than **5**. Replacement of benzene with thiophene, furan, and methylated pyrazole produced compounds **7**–**9** that had improved potency, especially in cell based assay, compared with **3**. While a significant shift between the sera was frequently observed, compound **9** showed excellent activities in both enzyme and the cell based assays against WT and key mutant enzymes. The amino group and the pyrido nitrogen atom in pyrazolopyridine were reported to be critical for both the physiochemical properties and the potency of compound **1**.[2] We speculated that introduction of a pyridine moiety would decrease the lipophilicity of compounds and hence reduce the effects of protein binding in the Spread assay. Therefore, a pyridine moiety was installed. All three pyridine analogues (**10**–**12**) showed potent intrinsic activity and improved potency in the Spread assay versus benzene analogue (**3**). Of the three pyridine analogues prepared, the 4-pyridyl derivative (**12**) had the best overall profile. It showed excellent intrinsic antiviral activity against WT, K103N, and Y181C mutants. Compound **12** also exhibited excellent activity in the cell base assay against the same panel of mutants with small shift in activity between 10% FBS and 50% NHS. Oxidation of **12** led to the N-oxide **13** was accompanied by a significant loss of activity, especially against the mutants. Replacement of pyridine with 4-cyanobenzene led to analogue **14** that was less potent. Incorporation of a chloride atom in the pyrazole ring yielded subnanomolar compounds (**15**–**17**) against WT and mutant enzymes in the RT_Pol assay, but they were significantly less potent in the cell based assay, indicating the influence of protein binding.

The other structural diverse ring systems that were also investigated are shown in Table 2. Nearly all the compounds prepared (**18**–**25**) exhibited good potency (⩽30 nM) against the WT RTs in the enzyme assay. Compounds **22**, **24**, and **25** showed the potency around or less than 10 nM against WT and K103N mutant viruses. However, these compounds proved much less active in the cell based assay, presumably due to their high protein binding. The related other ring systems, such as, thioazole, imidazole, triazole, and the other oxazoles, showed lower activity (data not shown).

Compound **3** showed promising pharmacokinetic profiles, low clearance, good volume distribution, and long half life in both rats and dogs following *iv* coadministration (rat: Cl: 7.3 mL/min/kg, V_d: 5.2 L/kg, AUC_{iv}: 2.6 μM h, $t_{1/2}$: 10.4 h; dog: Cl: 2.2 mL/min/kg, V_d: 0.5 L/kg, AUC_{iv}: 4.4 μM h, $t_{1/2}$: 9.5 h) (Table 3). Replacement of benzene ring with thiophene (**7**), 4-pyridine (**12**), and 4-N-oxide pyridine (**13**) led to higher clearance in rat. However, the 2-pyridine analogue (**10**) showed improved clearance in rat and longer $t_{1/2}$

Table 2
SAR results



Compound	Het	R	RT-Pol[a] WT/ K103N/ Y181C (nM)	Spread[b] (nM) 10% FBS/50% NHS (WT)
18			68/946/ >10,000	—
19			15/56/240	—
20			29/69/120	>8300/>8300
21			2.1/30/170	>8300/>8300
22			5.2/9.3/85	926/926
23			31/130/ 3100	>8300/>8300
24			4.9/11/91	2778/830
25			2.1/4.4/170	926/277

The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean.

[a] Compounds were evaluated in a standard SPA assay.

[b] CIC95 (cell culture inhibitory concentration) is defined as the concentration at which the spread of virus is inhibited by >95% in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing either 10% fetal bovine serum (FBS) or 50% normal human serum (NHS). The antiviral activity of compound against wild-type ABI R8 virus was measured.

Table 3
Pharmacokinetics of selected compounds[a]

Compound #	Rat				Dog			
	AUC	Cl	V_d	$t_{1/2}$	AUC	Cl	V_d	$t_{1/2}$
3	2.6	7.3	5.2	10.4	4.4	2.2	0.5	9.5
7	0.9	22.9	2.9	4.5	4.0	2.5	0.4	3.5
10	5.0	3.9	1.3	5.5	1.1	4.5	1.6	13.3
12	0.4	44.7	3.2	1.2	—	—	—	—
13	0.6	30.5	12.8	5.4	—	—	—	—
15	1.0	17.9	4.9	10.2	—	—	—	—
16	0.4	50.5	3.9	1.5	0.2	48.2	3.7	1.5

[a] iv cassette administration (co-administration with other analogues). AUC: μM h, CL: mL/min/Kg, V_d: L/kg, $t_{1/2}$: hours. Vehicle: DMSO. Sprague–Dawley rats, 0.5 mg/Kg. Beagle dog: 0.25 mg/Kg. Interanimal variability was less than 20%.

Table 4
Antiviral potency of compounds **12** against clinically isolated mutant viruses (NL4-3 isolates)[a]

Mutantion	Compound **12**	
	IC_{50}[b] (μM)	IC_{50} fold shift
WT	0.0045	1
L100I	0.0219	4.9
K103N	0.0197	4.4
Y181C	0.0362	8.0
Y188L	>1.000	>222
G190A	0.0223	5.0
G190S	0.1607	35.7
K103N/V179E	0.0902	20.0
K103N/Y181C	>1.000	>222
K103N/G190A	0.1196	26.6
Y181C/G190A	0.1309	29.1
K103N/P225H	0.0324	7.2
K101E/G190A	0.1181	26.2
K101E/Y181C/G190A	0.3315	73.7
K103N/Y181C/G190A	>1.000	>222
V106A/G190A/F227L	>1.000	>222

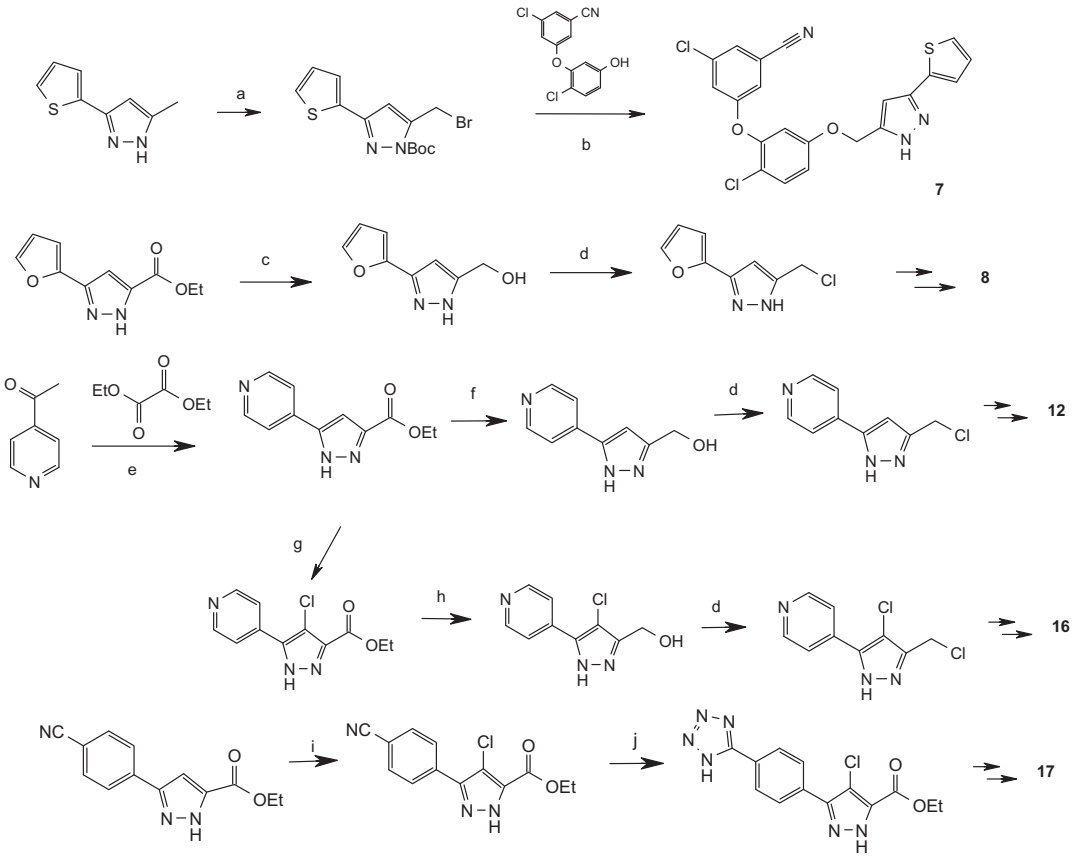
[a] Phenoscreen assay, monogram bioscience, in presence of 40% NHS. For details, see Ref. 7.
[b] The IC_{50} is defined as the concentration of compound in cell culture required to inhibit 50% of viral replication.

in dog. Introduction of chlorine atom at the pyrazole ring increased clearance significantly in both rat and dog.

To assess the antiviral activities of compounds in this series against a panel of clinically relevant mutants, the pyrazole compound **12** was selected for a PhenoScreen assay performed by Monogram Bioscience.[7] It showed good antiviral activities against a broader panel of clinically relevant mutant HIV-1 viruses (Table 4). These results further suggest that the aryl substituted pyazole is an effective surrogate for the indazole/pyrazolopyridine moieties.

Compounds described in this letter were prepared straightforwardly according to the method reported previously.[1,2] The key intermediate, halogenated methyl pyrazoles/oxazoles, were synthesized according to the route depicted in Scheme 2. Either radical bromination of substituted methyl pyrazoles/oxazoles with NBS in the presence of benzoyl peroxide or reduction of corresponding pyrazole ester with LAH following by treatment with thionyl chloride afforded the desired halogenated methyl pyrazoles/oxazoles (the intermediates for **3**–**9**). The final product (**7**) was then prepared by the coupling of the bromomethyl pyrazole with the 3-chloro-5-(2-chloro-5-hydroxyphenoxy)benzonitrile under basic conditions. The other analogues were prepared in an analogous manner. The pyrazole ester required for analogue **12** was prepared from 1-(4-pyridinyl)ethanone. Addition of the enolate to diethyl oxalate following by the cyclization with hydrazine delivered the desired ester. The chlorinated pyrazoles were synthesized from the corresponding pyrazole alcohol with NCS under basic conditions (the intermediate for **16**). The ester intermediate for the tetrazole analogue (**17**) was prepared from the corresponding cyano derivative by the action of sodium azide in the presence of zinc bromide.

In summary, the pyrazole **12** was identified in an effort to modify the previously reported compound **1**. We have designed and synthesized a series of novel pyrazole derivatives as a surrogate for pyrazolopyridine motif that were potent inhibitors of HIV-1 RT with nanomolar intrinsic activity on the WT and key mutant enzymes and potent antiviral activity in infected cells. This com-



Scheme 2. Reagents and conditions: (a) (1) Boc_2O, DMAP, TEA, CH_3CN, 84%; (2) NBS, benzoyl peroxide, CCl_4, reflux, 66%; (b) (1) 3-chloro-5-(2-chloro-5-hydroxyphenoxy)benzonitrile, Cs_2CO_3, NMP; (2) HCl, EtOAc, 0 °C to rt, 38%; (c) LAH, THF, 86%; (d) $SOCl_2$, neat, >95%; (e) (1) NaOEt, THF, then diethyl oxalate; (2) hydrazine·HCl, water, 36% for two steps; (f) LAH, THF, 68%; (g) NCS, NaOH, THF, 59%; (h) LAH, THF, 37%; (i) NCS, NaOH, THF, 81%; (j) NaN_3, $ZnBr_2$, IPA/water, 90 °C, 94%.

pound possesses broad activity against a number of clinically significant mutant viruses.

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5. Experimental protocols for the HIV-1 RT polymerase SPA assay are described in the following patent application: Saggar, S. A.; Sisko, J. T.; Tucker, T. J.; Tynebor, R. M.; Su, D.-S.; Anthony, N. J. US Patent Appl. US 2007/021442A1, 2007. Efavirenz was tested in this assay as a control (K_i (nM): 0.4/8.5/0.3 (WT/K103N/Y181C)).
6. The antiviral potency of compounds against wild-type (H9IIIB) virus or H9IIIB with K103N or Y181C mutations was measured in a multiple-cycle replication assay in MT2 cells. Cells were infected overnight (moi $\approx$ 0.01) in the absence of compound, washed, and cultured for 3 days in varying compound concentrations. Viral replication was assessed by measuring as p24 in culture supernatants, and the CIC_{95} is the lowest concentration of compound inhibiting replication by $\geqslant$95%. Mutants K103N, Y181C and K103N/Y181C are prepared by the Advanced Biotechnology, Inc. Also see, Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zygay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 4096. Efavirenz was tested in this assay as a control (CIC_{95} (nM): 4.3/38 (WT, 10% FBS/50% NHS), 232 (K103N, 10% FBS), 8.0 (Y181C, 10% FBS), and 272 (K103N/Y181C, 10% FBS)).
7. Assays performed by Monogram Bioscience, San Francisco, CA. Values are the average of two determinations. Detailed assay protocols are available at: http://www.monogramhiv.com/assays/hcp/phenolHIVTechnology.aspx.