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Research Letter

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Comparison of the Non-Nucleoside Reverse Transcriptase Inhibitor Lersivirine with its Pyrazole and Imidazole Isomers

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Lersivirine is a potent non-nucleoside reverse transcriptase inhibitor with exceptional mutant resilience. Here, we compare the pharmacological and pharmacokinetic profile of lersivirine with its pyrazole and imidazole isomers and briefly explore the profile of these series. This work establishes lersivirine as the outstanding molecule in this set.

Key words: drug design, drug discovery, structure-based drug design

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Reverse transcriptase (RT) is a DNA polymerase that is essential to the infectious life cycle of HIV, and inhibition of this enzyme has shown utility in the treatment of HIV. Non-nucleoside RT inhibitors (NNRTIs) are non-competitive in that they bind to an allosteric site close to, but distinct from the catalytic site of the enzyme. NNRTIs hinder the protein domain movements required by RT to efficiently synthesize new DNA (1). Because this site is not present in the RT enzyme of normal cells, NNRTIs are more specific for the viral enzyme. NNRTIs are particularly vulnerable to the development of viral resistance caused by mutations in RT that can retain viable enzymatic function.

The NNRTI lersivirine has recently emerged as an exciting clinical candidate for the treatment of HIV that possesses excellent efficacy against NNRTI-resistant viruses (2,3). The crystal structure of lersivirine with the RT enzyme can help rationalize the relationship between protein–ligand molecular interactions and mutant resilience (Figure 1). Key features include an edge-to-face π-interaction between the dicyanophenyl ring in lersivirine and the immutable Trp229 residue (4–6) and hydrogen bonding from the hydroxyl group to the peptide back bone of RT (7,8). Additionally, the Y181 residue adopts the more unusual 'down' position, similar to that present in the apo-structure, that may explain the resilience of lersivirine to the clinically relevant Y181C mutation (3,6).

These key interactions suggest other templates that are able to present these essential fragments in an appropriate geometry may retain the impressive potency and mutant profile of lersivirine. We have previously described the creation of the imidazole isomer **1** of lersivirine (Figure 2), which retains these important structural elements and preserves the desirable potency for the RT enzyme and activity against the clinically relevant mutations K103N and Y181C (9,10).

The pyrazole isomer of lersivirine (**2**) was also prepared as there would appear to be little difference in the appropriate presentation of the optimized interacting fragments (Figure 2). Moreover, the N1 pyrazole atom does not make any specific interactions with the enzyme, as expected, and we therefore presumed that moving this atom to other locations in the five-membered ring would be tolerated. The preparation of **2** progressed smoothly *via* the unoptimized route shown in Scheme 1 in just 4 steps. Commercially available

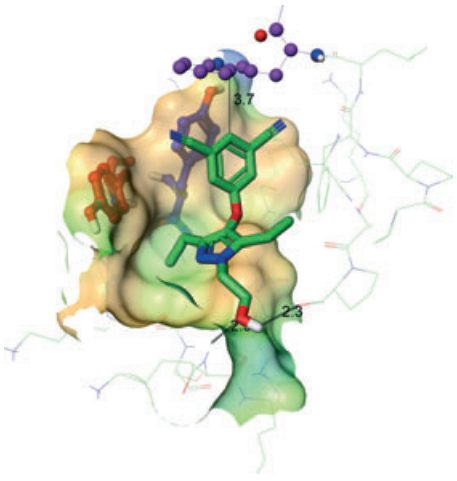


Figure 1: Crystal structure of lersivirine with wild-type reverse transcriptase (PDB 2WON). Key interactions highlighted (distances in Å). Residues Y181 (red), Y188 (dark blue) and W229 (purple) are shown (3).

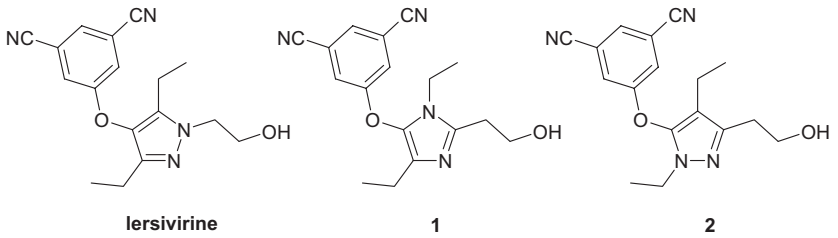
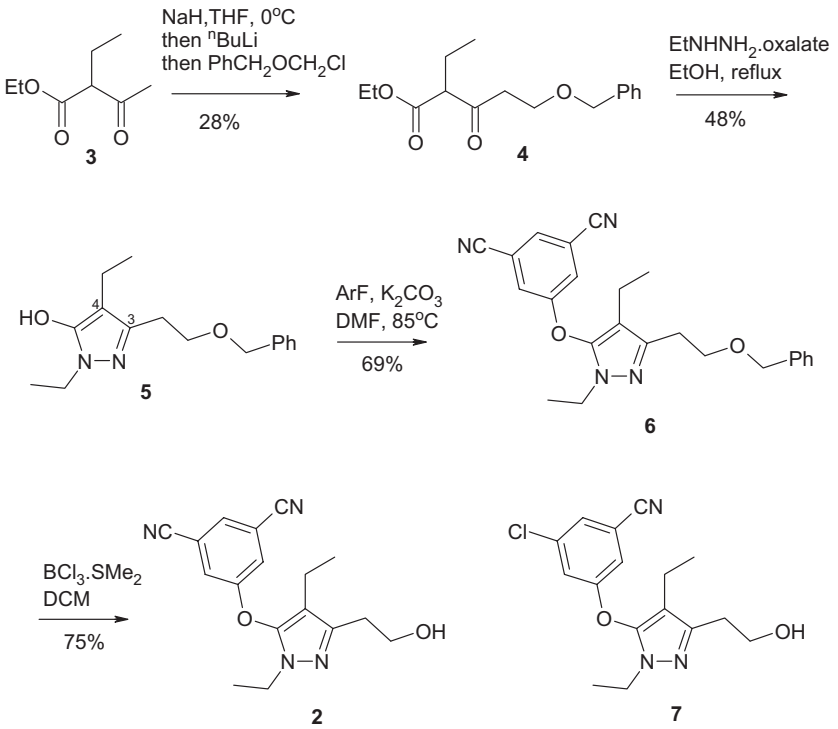


Figure 2: NNRTI isomers lersivirine, imidazole **1** and pyrazole **2**.



Scheme 1: Preparation of pyrazole **2** and structure of congener **7**.

ketoester **3** was doubly deprotonated and reacted with benzylchloromethyl ether to provide **4** that was cyclized with ethyl hydrazine to furnish **5**. The key step was arylation of hydroxy-pyrazole **5** using 3,5-dicyano fluorobenzene, a disconnection similar to that used to prepare **1** and other NNRTIs in our laboratory.[6,9,10]

The structure of **2** was unambiguously determined using ^{1}H-^{13}C HMBC NMR correlation experiments (see Experimental). Interestingly, similar pyrazole NNRTIs were reported by Roche, although compound **2** was not exemplified.[a] The synthetic route to these molecules also used the cyclization of hydrazines with β-ketoesters, and then Vilsmeier conditions installed a C4 formyl group with concomitant chlorination of the hydroxy-pyrazole. Phenol displacement of the chloride, conversion of the aldehyde to the C4 alkyl and C3 modification provided a variety of pyrazole NNRTIs. For example, compound **7** (Scheme 1) was prepared in 10 steps from sodium diethyloxaloacetate. Therefore, our short route would appear to be an attractive alternative to the preparation of these derivatives.

The RT and antiviral (AV) potency for the three isomers is shown in Table 1. Although all isomers are potent inhibitors of RT, lersivirine possesses the highest AV potency and when normalized for lipophilicity, it also has the highest lipophilic efficiency (LipE = $-\text{Log}IC_{50}$ – LogD) (11,12). All three compounds exhibited activity against the K103N and Y181C mutations, which were within tenfold of their wild-type potencies (2,3,9,10).

Figure 3 shows an overlay of the heterocyclic rings of the three isomers: lersivirine (from the cocrystal structure with wild-type RT) and the energy minimized structures of imidazole **1** and pyrazole **2**.[b] The similarity of the conformations, and no doubt similar interactions with the RT enzyme, is reflected in the comparable potency and mutant resilience of these isomers.

Table 1: Pharmacology and pharmacokinetics of lersivirine, **1** and **2**

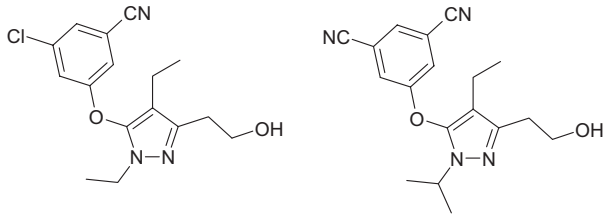
	Lersivirine (2,3)	**1**	**2**
LogD	1.8	1.6	1.8
HLM $T_{1/2}$ (mins)	89	>120	>120
HHeps $T_{1/2}$ (mins)	>120	>120	>120
wt RT IC_{50} (nM)[a]	119	78	250
wt AV IC_{50} (nM)[b]	4	10	29
AV LipE	6.6	6.4	5.7
Rat Cl_u (mL/min per Kg)[c]	101	99	216
Rat V_{du} (L/Kg)[d]	13.8	4.3	6.4
Rat $T_{1/2}$ (h)[e]	1.6	0.50	0.34
Solubility (mg/mL)	0.15	0.25	0.22
Reactive metabolite	Negative	Positive	Negative

AV, antiviral; HLM, human liver microsomes; RT, Reverse transcriptase.
[a]Geomean for $n = 3$.
[b]Lersivirine, **1** and **2** were not cytotoxic, $CC_{50} > 100\ \mu M$.
[c] rat iv pk, 2 mg/kg dose, mean for $n = 2$, Cl_u = unbound clearance.
[d]V_{du} = unbound volume of distribution. [e]$T_{1/2}$ = half-life.

Figure 3: Overlay of cocrystal structure of lersivirine (green) from Figure 1 (protein removed for clarity) with minimized structures of imidazole **1** (cyan) and pyrazole **2** (pink).

The polarity of these compounds translates to impressive aqueous solubility and metabolic stability, as demonstrated by half-life in human liver microsomes (HLM) and human hepatocytes (HHeps, Table 1). We have previously reported that imidazole **1** formed a reactive metabolite conjugate with glutathione following its incubation with HLM (9), a feature that has been linked with hepato- and idiosyncratic toxicity (13). It is noticeable that the pyrazoles lersivirine and **2** do not form reactive metabolite conjugates in this manner.

Intravenous rat pharmacokinetic experiments demonstrate clear differences between the isomers (Table 1). The high clearance of pyrazole **2** confers a short half-life in rat. Imidazole **1** has lower clearance, but a low volume of distribution translates to only a moderate improvement in half-life over **2**. However, lersivirine combines low clearance with a high volume, resulting in a significantly longer half-life in rat over **1** and **2** (14). These data clearly establish lersivirine as the superior NNRTI isomer.



7
wt AV IC_{50} 2.9 nM
LogD 2.8
LipE 5.7
HLM $T_{1/2}$ 51 min
HHeps $T_{1/2}$ 25 min

8
wt AV IC_{50} 3.9 nM
LogD 2.2
LipE 6.2
HLM $T_{1/2}$ 52 min
HHeps $T_{1/2}$ 105 min

Figure 4: Aryloxy-pyrazole Non-nucleoside RT inhibitors **7** and **8**.

For completeness, we used the hydroxy-pyrazole arylation chemistry shown in Scheme 1 to prepare the chloro and isopropyl derivatives **7** and **8,** respectively (Figure 4) because structure–activity relationships from the imidazole series suggest these congeners could be more potent than **2** (9). Structural information also guides these changes because both the ethyl- and cyano-substituents reside in hydrophobic pockets that can accommodate a moderate increase in size and fragmental lipophilicity. As expected, these derivatives possess superior potency to **2**, and although **8** possesses a superior lipophilic efficiency to **2** and **7**, the higher lipophilicity also drives poorer metabolic stability, thus ruling out further progression of these specific compounds. We have recently reviewed the aromatic chloride to nitrile medicinal chemistry transformation, and the result here (no improvement in LipE from **2** to **7**) is not surprising (15). However, we believe further elaboration of this series, and the general learnings outlined above could provide exciting starting points in the creation of mutant resilient inhibitors.

Conclusions

In conclusion, we have prepared potent NNRTI isomers that possess a balanced profile against clinically relevant mutant enzyme. Pyrazole **2** was prepared in four steps and satisfied a key feature of our design in the NNRTI area to keep synthetic complexity to a minimum so as not to negatively impact cost of goods and to facilitate analogue generation. A comparison of the pharmacological and pharmacokinetic profiles of the three isomers establishes lersivirine to be the exceptional molecule in this set. Interestingly, subtle structural differences to the NNRTI template for these derivatives translate to significantly different medicinal chemistry profiles. Initial elaboration of the pyrazole series exemplified by **2**, **7** and **8** suggests that this series warrants further investigation. Ongoing clinical trials are investigating the potential of lersivirine for the treatment of HIV-infected patients (16).

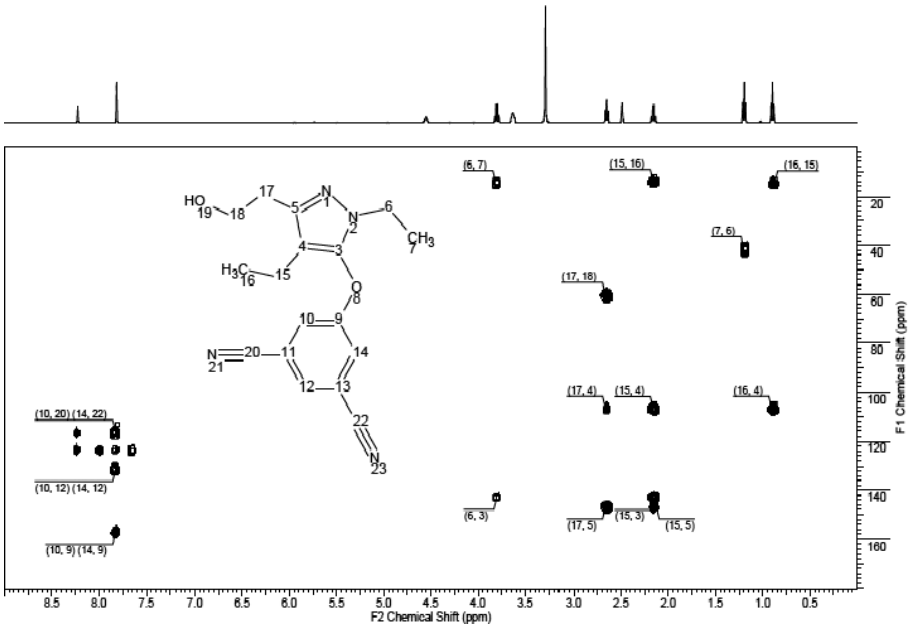


Figure 5: HMBC experiment of **2**.

Experimental

General

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed precoated Merck silica gel (60 F254) plates, and FCC (flash column chromatography) was carried out using 40- to 63-μm silica gel. NMR spectra were carried out on a Varian Mercury 400 and a Varian Inova 500 spectrometer in the solvents specified. LC/MS were recorded on a Waters Micromass ZQ using atmospheric pressure chemical ionization (APCI). Other abbreviations are used in conjunction with standard chemical practice.

5-[2,4-diethyl-5-(2-hydroxy-ethyl)-2H-pyrazol-3-yloxy]-isophthalonitrile (2)

NaH (60% in oil, 660 mg, 16.5 mmol) was suspended in THF (6 mL) and cooled to -5 °C before the dropwise addition of commercially available ketoester **3** (2.4 g, 15 mmol) dissolved in THF (10 mL). After 10 min, ^{n}BuLi (1.6 M, 10.3 mL, 16.5 mmol) was added dropwise and after a further 10 min, benzylchloromethyl ether (2.1 mL, 15 mmol) in THF (10 mL) was added. After 2 h, the reaction mixture was quenched with brine, acidified with 2N HCl aq. and extracted with diethyl ether, dried ($MgSO_4$) filtered, concentrated under reduced pressure and the residue purified using FCC (10% EtOAc/pentane) to yield **4** as a colourless oil (1.4 g, 28%); ^{1}H NMR (400 MHz, $CDCl_3$) 7.30 (m, 5H), 4.49 (s, 2H), 4.15 (q, 2H), 3.75 (t, 2H), 3.39 (t, 1H), 2.81 (t, 2H), 1.92 (m, 2H), 1.31 (t, 3H), 0.92 (t, 3H); APCI MS *m/z* 279 $[M+H]^+$.

4 (1.4 g, 5.3 mmol) was dissolved in ethanol (40 mL), ethylhydrazine oxalate (960 mg, 6.3 mmol) was added, and the mixture was heated under reflux for 9 h. The solvent was removed under reduced pressure, and the residue dissolved in EtOAc, washed with water, dried ($MgSO_4$) filtered and concentrated under reduced pressure to yield **5** as a white solid (690 mg, 48%), which was used without further purification: APCI LCMS *m/z* 275 $[M+H]^+$ (>95% pure). **5** (340 mg, 1.2 mmol) was dissolved in dimethylformamide (DMF) (3 mL) potassium carbonate (170 mg, 1.2 mmol); then, 3,5-dicyanofluorobenzene (210 mg, 1.44 mmol) was added, and the mixture was stirred at 85 °C for 30 min. Water was added and extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (20% EtOAc/pentane) to yield **6** as a colourless oil that crystallized on standing (155 mg, 69%): mp 83–85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 7.68 (s, 1H), 7.40 (s, 2H), 7.35 (m, 4H), 7.31 (m, 1H), 4.58 (s, 2H), 3.91 (q, 2H), 3.80 (t, 2H), 2.92 (t, 2H), 2.20 (q, 2H), 1.31 (t, 3H), 0.98 (t, 3H); APCI MS *m/z* 401 $[M+H]^+$.

6 (155 mg, 0.39 mmol) was dissolved in dichloromethane (DCM) (5 mL) cooled to 0 °C, $BCl_3.SMe_2$ (1 M in DCM, 0.39 mL, 0.39 mmol) was added dropwise and allowed to warm slowly to room temperature. The mixture was then cooled to 0 °C; 2N NaOH aq (10 mL) was added and stirred for 30 min. The mixture was extracted with DCM, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was triturated with diisopropylether to yield **2** as a white crystalline solid (90 mg, 75%); mp 120–122 °C; Found C, 65.69; H, 5.82; N, 17.95. calc. for $C_{17}H_{18}N_4O_2$: C, 65.79; H, 5.85; N, 18.05; ^{1}H NMR (400 MHz, $CDCl_3$) 7.68 (s, 1H), 7.42 (s, 2H), 4.00 (t, 2H), 3.89 (q, 2H), 2.83 (t, 2H), 2.20 (q, 2H), 1.35 (t, 3H), 1.00 (t, 3H); APCI MS *m/z* 311 $[M+H]^+$; HMBC (Figure 5); chemical stability of **2**: pH/% remaining 1.2/95, 4.8/99, 7.2/94, 9.8/36, 12.7/0.

Acknowledgments

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Notes

[a]Dunn, J.P., Hogg, J.H., Mirzadegan, T., Swallow, S. (2004) Preparation of pyrazole derivatives as non-nucleoside reverse transcriptase inhibitors for the treatment of HIV disorders and compositions thereof. WO/2004/074257.

[b]The unconstrained minimisations were performed using a limited-memory Truncated-Newton minimiser with a termination energy threshold of 0.5 kcal/mol.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. The syntheses of compounds **7** and **8**.

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