CONCISE ARTICLE

Discovery of novel pyridazinylthioacetamides as potent HIV-1 NNRTIs using a structure-based bioisosterism approach

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In continuation of our endeavors to develop new, potent, selective, and less toxic anti-HIV agents, we describe our structure-based bioisosterism design, synthetic strategy, and structure–activity relationship (SAR) studies that led to the identification of pyridazinylthioacetamides, a novel class of NNRTIs, isosteres of arylazolythioacetanilide derivatives. Nearly all of the tested compounds inhibited HIV-1 strain IIIB replication in the lower micromolar concentration range (EC_{50} : 0.046–5.46 μ M). Notably, the most promising compound **8k** exhibited extremely potent inhibitory activity against HIV-1 replication with an EC_{50} value of 0.046 μ M, CC_{50} of 99.9 μ M and the viral selectivity index amounted to 2149. These values were much better than those of **NVP** (EC_{50} = 0.09 μ M) and **DDC** (EC_{50} = 1.04 μ M). Compound **8k** also exhibited moderate inhibition of enzymatic activity with an IC_{50} value of 4.06 μ M, which was of the same order of magnitude as that of **NVP** (2.74 μ M). Docking calculations were also performed to investigate the binding mode of compound **8k** into the non-nucleoside binding site of HIV-1 RT and to rationalize some SARs.

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1 Introduction

Reverse transcriptase (RT) is one of the main therapeutic targets for the design of anti-HIV-1 drugs used in the treatment of acquired immunodeficiency syndrome (AIDS).^{1,2} Among RT inhibitors, the non-nucleoside reverse transcriptase inhibitors (NNRTIs), binding to an allosteric pocket located 10 Å away from the DNA polymerase active site, are a major component of the current therapeutic regimens for AIDS, namely highly active antiretroviral therapy (HAART). Nevertheless, the long-term use of NNRTIs is compounded by the emergence of drug-resistant viruses and potentially severe side effects. Therefore, to alleviate these problems, there is a substantial need for the identification of novel NNRTIs with improved activity against clinically relevant resistant mutants, and excellent pharmacokinetic and safety profiles.^{3,4}

Among the structurally diverse HIV-1 NNRTIs, substituted arylazolythioacetanilide scaffolds have been of much interest for the development of novel NNRTIs because of their high

potency and low toxicity against HIV-1 wild-type and resistant strains.^{5–11} Especially, 1,2,4-triazole derivatives **VRX-480733**¹² and **RDEA806**¹³ were selected as candidates for further studies. **RDEA806**, a promising new drug candidate undergoing phase IIa clinical trial (by Ardea Biosciences Company), was highly effective against mutant HIV-1 strains and exhibited reduced clinical adverse reactions and serum half-life for once-daily dosing (Fig. 1).

In our previous studies, we reported that a series of new arylazolythioacetanilides with structurally diverseazole scaffolds possessed potent anti-HIV activities in a cell-based replicon system.^{14–22} Among them, 1,2,3-thiadiazole derivative **ZP7** (Fig. 1) exhibited the highest anti-HIV-1 activity (EC_{50} = 36.4 nM), inhibiting HIV-1 replication in MT-4 cells with sevenfold and eightfold higher efficiency than nevirapine (**NVP**) and delavirdine (**DLV**), respectively.¹⁵

Encouraged by these promising results, and with the aim of further exploring the chemical features required for RT inhibition and identification of the new compounds as potential NNRTIs through application of the structure-based bioisosterism approach,²³ an excellent “follow-on”-based lead optimization strategy²⁴ was carried out by modifying the central core structure of the lead compounds to produce the desired potency, selectivity, and the required ADME profiles. Structural and structure–activity relationship (SAR) studies of the known arylazolythioacetanilides illustrated in the literature^{14–22} have pointed to the presence of key chemical features that correlated

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Fig. 1 Azolylthioacetanilide-based NNRTIs.

with the RT inhibitory ability, *i.e.*, the aryl group linked to the azole core fitted into the important hydrophobic pocket and the carbonyl group of the amide were able to establish a key hydrogen bond through interaction with the backbone N–H of K103. These functionalities are maintained in a spatial arrangement within the NNRTI binding pocket by positioning a heterocyclic central core in the arylazolylthioacetanilides. There are differences in the electronic and conformational contribution of the five-membered heterocyclic moiety to the binding of the inhibitors to RT.¹⁵ Additional branching moieties, such as the *N*-substituted phenyl moiety located at the protein/solvent interface near a region of the protein known to be flexible, can be present or extended to further optimize the potency and/or physicochemical properties.

Our structure-based bioisosterism design of new NNRTI chemotypes was primarily focused on the identification of additional heterocycle core frameworks with synthetic accessibility and drug-like properties, that are able to replace the central chemical template of arylazolylthioacetanilides, while keeping the above mentioned key interaction features in a spatial orientation appropriate to fit into RT.

To achieve this goal, a variety of scaffolds easy to synthesize and suitable for appropriate chemical functionalization were scrutinized by thorough bibliographic research. Herein, a series of novel 2-(4-(naphthalen-1-yl)pyridazin-3-ylthio)-*N*-arylacetamide derivatives were synthesized (Fig. 2), and their anti-HIV activities against WT HIV-1 and HIV-2, as well as their HIV RT inhibitory potency, were evaluated.

2 Results and discussion

2.1 Chemistry

An efficient preparation method for the novel 2-(4-(naphthalen-1-yl)pyridazin-3-ylthio)-*N*-acetamides (**8**) has been developed, which is outlined in Scheme 1. Using the established chemistry, a four-stage synthesis starting from commercially available 2-(naphthalen-1-yl) acetonitrile (**1**) gave 4-(naphthalen-1-yl)pyridazin-3(2*H*)-one (**6**) in high overall yield, which was the key reaction in this synthetic route.²⁵ Treatment of **6** with phosphorus pentasulfide in pyridine under reflux afforded 4-(naphthalen-1-yl)pyridazine-3(2*H*)-thione (**7**). The final pyridazinethioacetanilides (**8**) were synthesized by reaction of intermediate **7** with suitable 2-chloro-*N*-aryl-substituted acetamides (or other alkyl halides) in good yields. The 2-chloro-*N*-phenyl acetamides (or other alkyl halides) were synthesized according to the literature.²⁶ To the best of our knowledge, this is the first example of the synthesis of pyridazinethioacetanilides. The synthesized compounds were characterized by MS, IR, ¹H NMR and/or ¹³C NMR spectral data together with TLC analysis.

2.2 Biological activities

2.2.1 Anti-HIV activity evaluation. Testing of the newly synthesized pyridazinylthioacetamide derivatives for activities against HIV-1 and HIV-2 was performed in MT-4 cell cultures²⁷ infected with wild-type HIV-1 (strain IIIB)²⁸ and wild-type HIV-2

Fig. 2 The structure-based bioisosterism replacement of azoles by pyridazine.

Scheme 1 Reagents and conditions: (i) NaH, BrCH₂CH(OEt)₂, THF; (ii) KOH, EtOH–H₂O; (iii) N₂H₄–AcOH–water; (iv) Br₂, AcOH; (v) P₂S₅, pyridine; and (vi) ClCH₂CONHAr, triethylamine, THF.

(strain ROD).²⁹ The methodology of the anti-HIV assay has been previously described.^{30,31} Meanwhile, nevirapine (**NVP**), zidovudine (azidothymidine, **AZT**), dideoxycytidine (**DDC**), delavirdine (**DLV**) and efavirenz (**EFV**) were used as reference drugs. The cytotoxicity of these compounds was determined in parallel. Comparisons of inhibitory concentration (EC₅₀), cytotoxic concentration (CC₅₀), and SI (selectivity, given by the CC₅₀/EC₅₀ ratio) values for different compounds are depicted in Table 1.

As shown in Table 1, nearly all of the tested pyridazinylthioacetamide derivatives inhibited HIV-1 strain IIIB replication in the lower micromolar concentration range (EC₅₀: 0.046–5.46 μM), except for one compound **8o**, which showed an EC₅₀ value >34.51 μM. Among them, 2-(4-(naphthalen-1-yl)pyridazin-3-ylthio)-N-(2-nitrophenyl)acetamide (**8k**) was the most promising compound. It exhibited extremely potent inhibitory activity against HIV-1 replication with an EC₅₀ value of 0.046 μM, CC₅₀ value of 99.9 μM and the viral selectivity amounted to 2149, which were much better than those of **NVP** (EC₅₀ = 0.09 μM) and **DDC** (EC₅₀ = 1.04 μM). Besides compound **8k**, some other compounds, **8b**, **8f**, **8g**, **8h** and **8j**, were also endowed with the high anti-HIV-1 potencies (EC₅₀ = 0.19, 0.20, 0.13, 0.14 and 0.14 μM, respectively) and good selectivity indices (SI = 177, 929, 370, 1108 and 1438, respectively). Such results provide useful information for further development of this class of HIV-1 NNRTIs.

A brief investigation of the structure–activity relationships (SARs) revealed that the nature of the *ortho* and the *para* substitution at the phenyl ring of the anilide moiety influenced the anti-HIV activity remarkably. For instance, Table 1 also revealed the potency order of the *ortho* substitution at the phenyl ring of the anilide moiety: NO₂ (**8k**, EC₅₀ = 0.046 μM) > halogen atoms {Cl (**8b**, EC₅₀ = 0.19 μM), Br (**8e**, EC₅₀ = 0.21 μM), F (**8d**, EC₅₀ = 0.26 μM)} > Me (**8l**, EC₅₀ = 1.09 μM) > H (**8a**, EC₅₀ = 1.93 μM). Introduction of small hydrophobic substituents (methyl group or chlorine atom) at the *para* substitution in the anilide moiety of **8k** and **8b** provided compounds **8j** and **8c** with decreased anti-HIV activities,

respectively. It is worth noting that in the case of compound **8e**, introduction of the methyl, 4-acetyl and methoxy carbonyl groups at the *para* position at the phenyl ring of the anilide moiety led to **8f**, **8g** and **8h** with retained activity, whereas introduction of ethoxy carbonyl in **8e** led to **8i** with decreased activity.

Encouraged by these results, in order to find more potent inhibitors and to further explore the salient features controlling the activity, further modifications at the protein/solvent interface were performed. One *N*-substituted pyridine acetamide derivative **8m** was also synthesized with an acceptable anti-HIV-1 profile. Moreover, it is also worth mentioning that the phenylethanone derivative **8n** and the 3,4-dihydroisoquinolin-2(1*H*)-yl-ethanone derivative **8p** also demonstrated reasonable antiviral activity, presumably due to the optimum interactions with the NNRTIs binding pocket (NNIBP). The 3,4-dihydroquinolin-2(1*H*)-yl-ethanone derivative **8o** was essentially inactive, which could be a result of the unfavorable steric interactions with the enzyme. We believe that these compounds can be further optimized by more appropriate substitutions in the protein/solvent interface, and work is currently ongoing in this area.

On the whole, we found that the SAR features of the pyridazinylthioacetamides were grossly consistent with those of the previously observed arylazolythioacetanilide NNRTIs.^{14–21} These results confirm the important role of the heterocyclic core and the pyridazinylthioacetamide motif as a valuable lead series for the design of the next generation NNRTIs.

None of the pyridazinylthioacetamide derivatives inhibited the replication of HIV-2 ROD in MT-4 cells at a subtoxic concentration. Therefore, based on this fact and the above SAR conclusions, it can be concluded that these compounds were specific for HIV-1 and belonged to typical NNRTIs.

2.2.2 HIV-1 RT inhibition assay. Compound **8k** was evaluated in enzymatic tests for its ability to inhibit highly purified recombinant HIV-1 RT using poly(rC)–oligo(dG) as a template primer³² to further confirm the drug target of pyridazin-3-ylthioacetamidederivatives. The assay results of the

Table 1 Anti-HIV activity, cytotoxicity and selectivity indices of 2-(4-(naphthalen-1-yl)pyridazin-3-ylthio)-*N*-arylacetamide derivatives (**8a–8p**)

					EC ₅₀ ^a (μM)		CC ₅₀ ^b (μM)		SI ^c	
Code	X ₁	X ₂	R ₂	R ₄	HIV-1 III _B	HIV-2 ROD	HIV-1 III _B	HIV-2 ROD	HIV-1 III _B	HIV-2 ROD
8a	CH	CH	H	H	1.93	>173.91	203.0	173.91	105	<1
8b	CH	CH	Cl	H	0.19	>34.98	34.25	34.98	177	<1
8c	CH	CH	Cl	Cl	0.42	>231.63	>283.87	231.63	>684	<1
8d	CH	CH	F	H	0.26	>240.60	204.39	240.60	799	<1
8e	CH	CH	Br	H	0.21	>31.31	31.53	31.31	151	<1
8f	CH	CH	Br	Me	0.20	>215.34	182.40	215.34	929	<1
8g	CH	CH	Br	COMe	0.13	>48.54	46.31	48.54	370	<1
8h	CH	CH	Br	COOMe	0.14	>245.87	158.74	>245.87	1106	×1
8i	CH	CH	Br	COOEt	0.56	>239.28	237.36	>239.28	420	×1
8j	CH	CH	NO ₂	Me	0.14	>243.91	204.42	243.91	1438	<1
8k	CH	CH	NO ₂	H	0.046	>134.71	99.90	134.71	2149	<1
8l	CH	CH	Me	H	1.09	>150.46	141.90	150.46	130	<1
8m	N	CH	Cl	H	0.27	>164.42	156.06	164.42	573	<1
8n					5.46	>55.02	54.55	55.02	10	<1
8o					>34.51	>34.99	34.51	34.99	<1	<1
8p					1.15	>36.45	34.02	36.45	29	<1
NVP ^d					0.09		>15.02	>15.02	>168	
AZT ^d					0.021	0.0045	249.5	249.5	12221	56907
DDC ^d					1.04	1.28	>94.69	>94.69	>93	>74
EFV ^d					0.0054		>6.34	>6.34	>1187	
DLV ^d					0.03257		>36.19	>36.19	>1096	

^a EC₅₀: concentration of the compound required to achieve 50% protection of MT-4 cells against HIV-1-induced cytotoxicity, as determined by the MTT method. In bold are the values of active compounds. ^b CC₅₀: concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. ^c SI: selectivity index (CC₅₀/EC₅₀). The SI values: ×1 stands for ≥1 or <1. ^d The data were obtained from the Rega Institute for Medical Research, KU Leuven, Belgium.

compounds are summarized in Table 2. Compound **8k** exhibited moderate inhibition of enzymatic activity with an IC₅₀ value of 4.06 μM, *i.e.* the same order of magnitude as that of **NVP** (2.74 μM).

3 Molecular modeling

In order to gain a better understanding of how **8k** interacts with RT NNIBP, a hypothetical molecular model of the

Table 2 Inhibitory activity of compound **8k** against HIV-1 RT

Compd	8k	NVP
IC ₅₀ ^a (μM)	4.06	2.74

^a 50% of the inhibitory concentration of tested compounds required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into HIV-1 RT by 50%.

complex was constructed using AutodockVina [http://www.vina.scripps.edu] based on our previously reported methodology (Fig. 3). The structure of HIV RT (ID: 3DLG) was acquired from the Protein Data Bank (http://www.rcsb.org). The resulting models showed that **8k** adopted the typical “butterfly” conformation that is characteristic of many NNRTIs (Fig. 3). The binding features were similar to those of the previous hypothetical molecular models obtained with arylazolythioacetanilide derivatives (**RDEA806** and **ZP7**). The left hand side naphthalene ring of **8k** lay in a hydrophobic

cleft formed by Leu 234, Tyr188, Trp229, and had an apparent π -stacking interaction with Tyr188. The amide carbonyl of the ligand established a hydrogen bond with the backbone NH of Lys103. In addition, potential interactions were probably present between the pyridazine linker and residue Lys103. Indeed the conformational and electronic contributions of the heterocyclic linkers have a significant effect on the binding of the inhibitors in the NNIBP, which indicates the importance of central heterocycles for anti-HIV activity. Moreover, the *para* substitution in the anilide moiety (carboxyl in **RDEA806**) extends out of the hydrophobic pocket and points to the solvent-exposed regions, suggesting that there is a very large space for substitution at this position. Overall, the combination of hydrophobic interactions and π -stacking appears to govern the binding of the pyridazin-3-ylthioacetamide to RT. Consequently, the design of new arylazinythioacetanilide derivatives will be further guided by the model of interaction between the lead compound **8k** and the NNIBP of RT.

Fig. 3 (A) Predicted binding mode and molecular docking of compound **8k** into the allosteric site of HIV-1 RT (PDB code: 3DLG); (B) superimposition of the docked conformations of **8k** (white) and **ZP7** (purple) in HIV-1 RT (PDB code: 3DLG); (C) superimposition of the docked conformations of **8k** (white) and **RDEA806** (red) in HIV-1 RT (PDB code: 3DLG). The docking results are shown by PyMOL. Hydrogen bonds are indicated by dashed lines.

4 Conclusion

In our on-going efforts to identify structurally diverse compounds with HIV inhibitory activity, we have identified a novel series of 2-(4-(naphthalen-1-yl)pyridazin-3-ylthio)-*N*-arylaacetamide by the structure-based bioisosterism approach and also explored the SAR in the protein/solvent interface near a flexible region of the RT. Most active compounds showed activity in the low micromolecular range with EC₅₀ values varying from 0.046 to 5.46 μ M. The most active compound, **8k**, showed activity against wild-type HIV-1 in the low micromolar range (EC₅₀ = 0.046 μ M). As an inhibitor of the WT RT of HIV-1, compound **8k** showed inhibitory concentrations comparable to the reference drug **NVP**. Because of their excellent potency, these pyridazin-3-ylthioacetamide derivatives may have potential and should be further pursued as next generation NNRTIs. In addition, molecular modeling studies are being carried out to determine the effect of structural features on anti-HIV activity. These results have prompted further investigation into alternative heterocyclic systems which will be disclosed shortly.

Abbreviations

SAR	Structure–activity relationship;
RT	Reverse transcriptase;
NNRTIs	Non-nucleoside reverse transcriptase inhibitors;
HAART	Highly active antiretroviral therapy;
AIDS	Acquired immunodeficiency syndrome;
NNIBP	NNRTIs binding pocket.

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