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Brief Article

Synthesis and Biological Evaluation of Aryl-phospho-indole as Novel HIV-1 Non-nucleoside Reverse Transcriptase Inhibitors

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A novel series of 3-aryl-phospho-indole (API) non-nucleoside reverse transcriptase inhibitors of HIV-1 was developed. Chemical variation in the phosphorus linker led to the discovery of 3-phenyl-methyl-phosphinate-2-carboxamide **14**, which possessed excellent potency against wild-type HIV-1 as well as viruses bearing K103N and Y181C single mutants in the reverse transcriptase gene. Chiral separation of the enantiomers showed that only *R* enantiomer retained the activity. The pharmacokinetic, solubility, and metabolic properties of **14** were assessed.

Introduction

The reverse transcriptase (RT[a]) of human immunodeficiency virus type 1 (HIV-1) plays an essential role in the life cycle of the virus. At the time this work was initiated, three non-nucleoside reverse transcriptase inhibitors (NNRTIs) had been approved by the FDA for treatment in highly active antiretroviral therapy (HAART), namely nevirapine, delavirdine, and efavirenz (EFV).[1] More recently a fourth compound, etravirine (TMC125),[2] a second-generation NNRTI, became available for the treatment of HIV-1. The emergence of resistance to these drugs led us to search for a new NNRTI with a higher genetic barrier against clinically relevant mutant strains.[3]

We report herein a new series of aryl phospho indole (API) molecules as novel NNRTIs.

At the beginning of this endeavor to identify new NNRTIs, we examined various known scaffolds.[4−6] All NNRTIs bind in the non-nucleoside inhibitor binding pocket (NNIBP) which is situated in the palm domain of the p66 chain and has been well characterized by X-ray crystallography.[7] As illustrated with efavirenz in Figure 1, NNRTIs are making at least three key interactions with the reverse transcriptase; one involves a hydrogen donor with the backbone carbonyl of K101 and there are two interactions with conserved residues W229 and F227 in a hydrophobic/aromatic pocket formed by Y181, Y188, W229, and F227.[8]

On the basis of the above structural model and previously known scaffolds,[6] we selected the aryl-phospho-indole (API) scaffold for a novel NNRTI series.[9] The substituted bicyclic hydrogen donor scaffold could interact with K101 and F227 (Figure 1), and the branching phosphorus tetrahedral linker would direct the substitution toward the lipophilic W229 region. This approach was validated by synthesizing and determining the antiviral activities of compounds containing 5-chloro-indole-2-carboxamide linked to a simple phenyl group via a phosphorus linker.

Chemistry. The synthesis of API is illustrated in Scheme 1. Key intermediate **3** was synthesized in two steps from commercially available indole **1**. *N*-Protection with phenylsulfonyl chloride and NaH in DMF generated indole **2**, which was brominated at position 3 using bromine in DMF to afford **3** in good yield. Introduction of phosphorus at position 3 was performed using metal−halogen exchange chemistry. After several unsuccessful attempts with Grignard reagents, halogen−lithium exchange was then performed at low temperature to generate the nonisolated 3-lithiated indole species which reacted with the various phenylchloro-phosphorylated compounds.[9,10]

Phosphinates **4**, **5**, and **8** were synthesized from indole **3** and chlorophenylphosphonate (PhP(O)ClOR, with R = Et, Bn and Me), which were obtained from reaction of phenylphosphonyl dichloride with one equivalent of the corresponding alcohols.[11] Phosphinamidate **6** was obtained in a similar manner but from chlorophosphinamidate derived from phenylphosphonyl dichloride treated with one equivalent of dimethylamine.[12]

Phosphine oxide **7** was synthesized from lithiated-**3** and diphenylphosphinic chloride, while phosphine oxide **9** was obtained by reaction of lithiated-**3** with one equivalent phenylphosphonyl dichloride followed by reaction with methylmagnesium bromide in a one-pot procedure. Finally the *N*-protection group was removed and the ester moiety of phosphorylated indoles was transformed into a carboxamide under the action of ammonia in methanol at 50 °C. Phosphinic acid **16** was obtained by hydrogenolysis of **11**. Phosphinothioate **17** was prepared by reaction of **14** with Lawesson's reagent.

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[a] Abbreviations: API, aryl-phospho-indole; HIV-1, human immunodeficiency virus type 1; NNRTI, non-nucleoside reverse transcriptase inhibitor; RT, reverse transcriptase; NNIBP, nonnucleoside inhibitor-binding pocket; HAART, highly active antiretroviral therapy; WT, wild type; SFC, supercritical fluid chromatography.

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In Vitro Results. The antiviral activities (EC_{50}) of APIs **10**−**17** were evaluated in a cell-based assay against the HIV-1 wild type (WT) and against NNRTI-resistant strains K103N, Y181C, and K103N−Y181C.[13] As seen in Table 1, several compounds where R^3 is a small alkyl or alkoxy (**10**, **14**, **15**, and **17**) showed low nanomolar or subnanomolar activity against the HIV-1 WT strain. Inhibitors bearing benzyloxy (**11**) and *N*-dimethylamine (**12**) R^3 groups showed weaker activity (14−34 nM) against the WT virus. Activity was lost for **16** having a phosphinic moiety (R^3 = OH) and **13**, having a phosphine oxide bearing two phenyl groups. Against the K103N and Y181C single mutant viruses, only compounds **10** and **14** showed reasonable activity (7−16 nM). Finally, only phosphinate **14** showed any activity toward the K103N/Y181C double mutant virus and was thus selected for further evaluation. In addition to cell-based assay, activities against the RT enzyme were also evaluated and IC_{50} was found to be 722, 233, 103, and 349 nM for **10**, **14**, **15**, and **17**, respectively (EFV: IC_{50} = 63 nM), confirming that those compounds are acting as NNRTIs.

As **14** was synthesized and tested as a racemic mixture, the chiral separation of **14** into the enantiomers **18** and **19** was performed using supercritical fluid chromatography (SFC) conditions (Scheme 2).

Testing against HIV-1 revealed that enantiomer **18** exhibited better antiviral activity against WT and mutant viruses than racemic **14** (Table 2), whereas **19** was 1800-fold less active against WT virus and completely inactive against NNRTI-resistant viruses (Table 2).

The stereochemistry of the active molecule **18** was hypothesized to be *R* according to molecular modeling studies; when both enantiomers were docked in the NNRTI bind ing pocket (1FK9), the most potent enantiomer, **18**, has a binding energy of −23 kcal/mol, compared to −18 kcal/mol for the less active enantiomer. The *R* stereochemistry was subsequently confirmed by an X-ray structure of the active enantiomer of a later compound in this series (IDX899/GSK2248761) that was ultimately selected for clinical development.

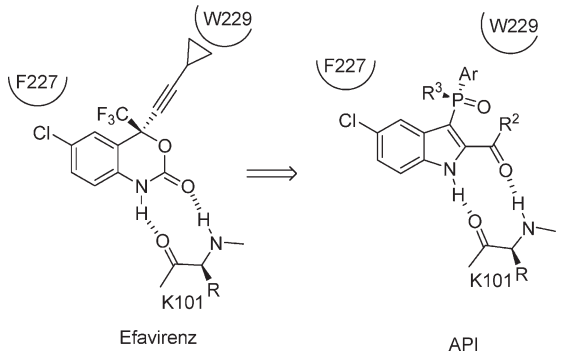


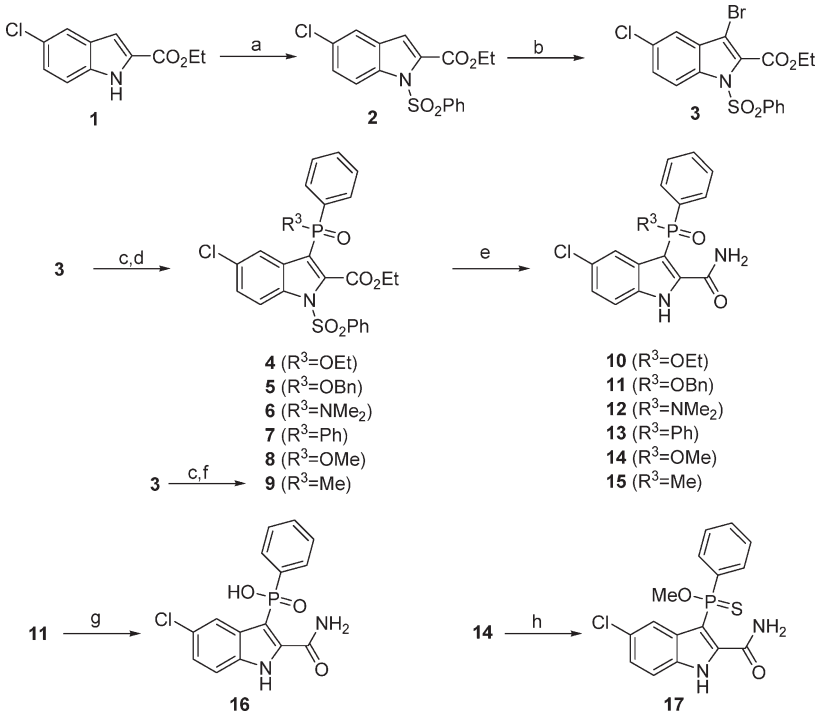
Figure 1. Selection of 3-aryl-phospho-indole (API) as NNRTIs.

Table 1. Cell-Based Assay Antiviral Activity (in nM) of **10**−**17** against HIV-1[a]

compd	R^3	WT EC_{50}	K103N EC_{50}	Y181C EC_{50}	K103N/Y181C EC_{50}
10	OEt	1.2	16.4	13.7	>1250
11	OBn	33.7	>1250	>1250	>1250
12	NMe_2	13.9	>1250	467	>1250
13	Ph	>1250	>1250	>1250	>1250
14	OMe	0.7	6.8	10.1	446.6
15	Me	0.8	1382	266	>1250
16	OH	>1250	>1250	>1250	>1250
17	OMe (*P* = S)	0.4	89.9	41.4	>1250

[a] EC_{50} is the concentration of compound that inhibits HIV-1 production by 50%.

Scheme 1[a]



[a] Reagents and conditions: (a) $PhSO_2Cl$, NaH, DMF, room temperature, 93%; (b) Br_2, DMF, room temperature, 90%; (c) *n*-BuLi, THF, −100 °C; (d) $PhP(O)R^3Cl$, THF, −80 °C to room temperature; (e) NH_3, MeOH, 50 °C; (f) (i) $PhP(O)Cl_2$ 1.1 equiv, THF, −100 °C, (ii) CH_3MgBr, THF, −60 °C to rt; (g) cyclohexene, Pd/C, MeOH, microwave; (h) Lawesson's reagent, toluene, microwave.

Scheme 2. Chiral Separation of the Enantiomers of (±)-**14** under SFC Conditions

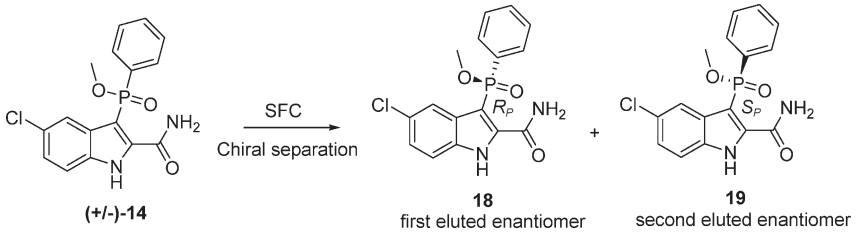


Table 2. In Vitro Activity of Separated Enantiomers (in nM) of **14**[a]

compd	WT EC_{50}	K103N EC_{50}	Y181C EC_{50}	K103N/Y181C EC_{50}
(R_P)-**18**	0.1	1.2	3.6	137.4
(S_P)-**19**	181.7	>1250	>1250	>1250

[a] EC_{50} is the concentration of compound that inhibits HIV-1 production by 50%.

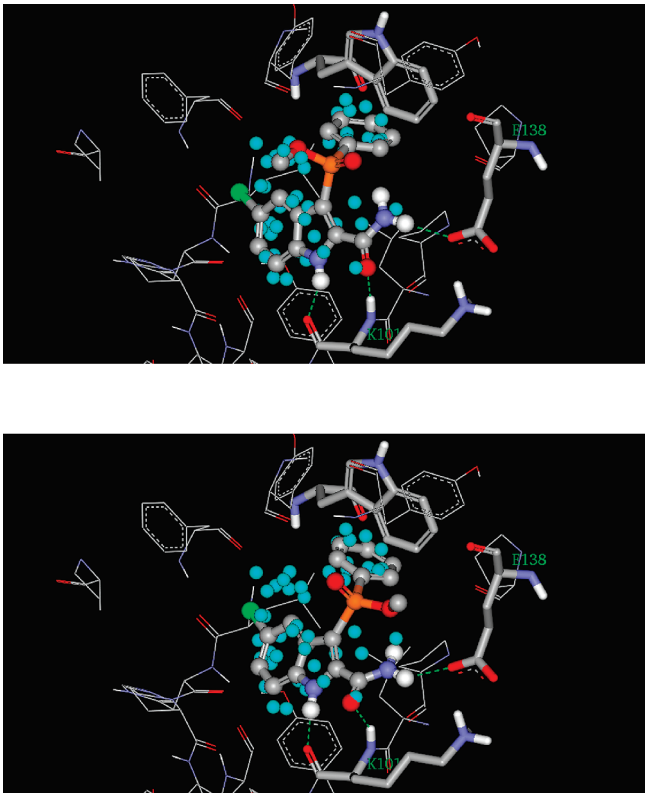


Figure 2. Enantiomers of **14** docked in 1FK9 showing best docked poses of the potent enantiomer, **18** (top) and the less active enantiomer **19** (bottom). Both ligands are shown in ball and stick form; some residues of interest are also in stick depiction. The cyan spheres indicate lipophilic interaction sites that define a potential location of interaction with the ligand.

An interaction map for the binding pocket was derived based on the site-finding algorithm from Ludi (available with the Discovery Studio 2.1 suite) and is presented in Figure 2. Cyan spheres in the figure represent lipophilic interaction sites. The lipophilic methoxy moiety in the most active enantiomer **18** (top panel) overlays lipophilic cyan spheres, improving its binding interaction. In contrast, the methoxy group in the less active enantiomer **19** (bottom) points away from this region into a nonlipophilic area and thus does not favor its binding energy.

Table 3. Pharmacokinetics of Racemic **14** in Rat[a]

compd	*F* (%)	Cl (mL/min/kg)	$t_{1/2}$ (h)
14	107	2.85	1.19

[a] Oral dosing at 5 mg/kg, IV dosing at 1 mg/kg. IV and PO formulation: suspension in PEG400.

Table 4. Metabolism of (±)-**14** in Hepatocytes (% Remaining after 4 h)

compd	monkey	human	dog	rat
14	49	99	100	100

We next evaluated the pharmacokinetic potential of racemic **14**. As shown in Table 3, **14** achieved bioavailability in rats of approximately 100%.

Compared to EFV, **14** possessed much higher solubility at various pH (90 μM at pH = 7 for **14**, $<0.5\,\mu$M for EFV). Furthermore, **14** exhibited limited in vitro metabolism in hepatocytes from different species as shown in Table 4.

Conclusion

In summary, we designed and synthesized a series of 3-phenylphosphinate-2-carboxamide indoles as novel NNRTIs. Variation in the phosphorus linker led to the discovery of **14**, which possessed excellent potency against wild-type HIV-1 and K103N and Y181C single mutants. Chiral separation of the enantiomers showed that the *R* configuration on the phosphorus linker was necessary for activity. The encouraging pharmacokinetic, solubility, and metabolic properties of **14** prompted us to further evaluate this series. Structure−activity relationships of further aryl phosphoindoles having a 3-methylphosphinate linker led to the discovery of the promising NNRTI IDX899, *P*-[2-(aminocarbonyl)-5-chloro-1*H*-indol-3-yl)]-*P*-[3-[(1*E*)-2-cyanoethenyl]-5-methyl-phenyl]-(*R*)-phosphinic acid methyl ester, that has been licensed to ViiV Healthcare (as GSK2248761) and is currently in phase II clinical development for the treatment of HIV infection. The discovery of this molecule will be reported in detail in the near future.

Experimental Methods

Synthesis of Aryl Phospho Indoles. The synthesis of (±)-**14** only is described here, while the synthesis of all other compounds is described in the Supporting Information. Purity of all compounds was determined to be >95% by analytical HPLC using a WATERS unit (Alliance 2695, photodiode array detector 2996) equipped with a reverse phase analytical column Waters Novapack C18 4 μm 150 mm × 3.9 mm. The compound to be analyzed was eluted using a linear gradient of 5−95% acetonitrile over a 20 min period with a flow rate of 1 mL/min, and the chromatogram was recorded using an UV detection from 210 to 400 nm (PDA Max Plot).

Ethyl 5-Chloro-3-[methoxy(phenyl)phosphoryl]-1-(phenylsulfonyl)-1*H*-indole-2-carboxylate, (±)-8. This is also the typical procedure for the synthesis of **4**, **5**, and **6**. To a stirred and cooled (−90 °C) solution

of ethyl 3-bromo-5-chloro-1-(phenylsulfonyl)-1*H*-indole-2-carboxylate, **3** (0.50 mmol), in anhydrous THF (2.5 mL) under N_2, was added *n*-BuLi (2.5 M in hexanes, 0.24 mL, 0.60 mmol) dropwise. After 5 min at −90 °C, methyl phenylchlorophosphonate (0.60 mmol) in THF (0.5 mL) was added dropwise at the same temperature. The reaction was allowed to warm up to room temperature over 3 h (TLC monitoring, eluant dichloromethane/EtOAc 9/1). Water was then added (5 mL). Extraction with EtOAc (3 × 20 mL) drying and evaporation led to a crude oil that was purified by chromatography on silica gel to afford compound (±)-**8**; colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (t, J = 7.2 Hz, 3H), 3.80 (d, J = 11.4 Hz,3H), 4.54 (q, J = 7.2 Hz, 2H), 7.36 (dd, J = 2.1 and 9.0 Hz, 1H), 7.47−7.67 (m, 6H), 7.84−7.96 (m, 4H), 8.09−8.12 (m, 2H). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 26.7. MS (ESI) m/z = 518 $(M + H)^+$.

Methyl 2-(Aminocarbonyl)-5-chloro-1*H*-indol-3-yl-(phenyl)-phosphinate, (±)-14. This is also the typical procedure for the synthesis of **10**−**15**. Compound (±)-**8** (258 mg, 0.50 mmol) was dissolved in a saturated solution of ammonia in methanol (5 mL) in a pressure tube. The tube was heated under microwave irradiation under pressure at 50 °C (maximum power input 100W, CEM discover apparatus) for 2 h. After evaporation of solvents, purification by chromatography on silica gel (eluant: dichloromethane/MeOH 95/5 to 9/1) afforded desired carboxamide indole (±)-**14** in 58% yield; pale-yellow powder. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.85 (d, J = 11.4 Hz, 3H), 6.08 (broad s, 1H), 7.30 (dd, J = 2.0 and 9.0 Hz, 1H), 7.36−7.56 (m, 4H), 7.68 (d, J = 1.8 Hz, 1H), 7.73−7.81 (m, 2H), 10.78 (broad s, 1H), 10.03 (broad s, 1H). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 33.3. MS (ESI) m/z = 349 $(M + H)^+$.

HPLC Chiral Separation. Enantiomers of (±)-**14** were separated by supercritical fluid chromatography preparative method at Chiral Technologies. Preparative column: Chiralpak AD-H (250 mm × 20 mm) with CO_2/MeOH + 1% diethylamine 80/20 as the mobile phase, flow rate 60 mL/min. Analytical column: Chiralcel OD-H (250 mm × 4.6 mm) with *n*-heptane/ethanol/diethylamine 70/30/0.1 as the mobile phase, flow rate 1.0 mL/min. First eluting enantiomer **18**, t_R = 8.89 min, second eluting enantiomer **19**, t_R = 7.96 min, orders of elution refers to those observed on the preparative column. Compounds **18** and **19** were repurified by column chromatography on silica gel (eluant: dichloromethane/MeOH 95/5 to 9/1) before biological testing.

Methods for Molecular Docking. Docking was done with CDOCKER (CHARMm based molecular dynamic scheme to dock ligands into a receptor binding site) from the Discovery Studio 2.1 suite to obtain 10 poses per compound. Docked poses were then minimized with the GBSW implicit solvent model. The binding energy was calculated also with the GBSW model.

HIV-1 Infected Cells Inhibition Experiments. The antiviral activity of compounds was measured by the inhibition of virus-induced cytopathogenicity in MT-4 cells using the HIV strain BH10 wild-type or the resistant viruses Y181C, K103N, and Y181C/K103N as described in ref 14.

Supporting Information Available: Synthetic procedures and characterization data of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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