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Synthesis and biological evaluation of *N*-acetyl-β-aryl-1,2-didehydroethylamines as new HIV-1 RT inhibitors in vitro

Pi Cheng,[a,d] Zhi-Yong Jiang,[a,c] Rui-Rui Wang,[b] Xue-Mei Zhang,[a,c] Qian Wang,[b] Yong-Tang Zheng,[b,*] Jun Zhou[a,c] and Ji-Jun Chen[a,c,*]

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming, Yunnan 650204, PR China*
[b]*Laboratory of Molecular Immunopharmacology, Key Laboratory of Animal Models and Human Diseases Mechanisms, Kunming Institute of Zoology, The Chinese Academy of Sciences, Kunming, Yunnan 650223, PR China*
[c]*The Joint-Laboratory of Antiviral Natural Medicines, Kunming Branch, The Chinese Academy of Sciences, Kunming, Yunnan 650204, PR China*
[d]*Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China*

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Abstract—A variety of *N*-acetyl-β-aryl-1,2-didehydroethylamines were synthesized by direct reduction–acetylation of β-aryl-nitroolefins and assayed as HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the first time. Compound **7a** exhibited a TI value of >13.2 with CC_{50} value of >0.787 mM in C8166 cells. This structure–activity relationship (SAR) study provided a new lead for design and discovery of more potent and selective analogues act as NNRTIs.

Due to the AIDS crisis, development of new, selective and safe non-nucleoside reverse transcriptase inhibitors (NNRTIs) still remains a high priority for medical research.[1] Structure simplification of natural product has provided us an efficient way to find new and less toxic anti-HIV lead compounds.[2] *N*-Acetyl-β-aryl-1,2-didehydroethylamine (Fig. 1) is the characteristic and basic structure unit present in some β-aryl-1,2-didehydroethylamine alkaloids isolated from marine species, some alkaloids of this family display a wide spectrum of biological activities.[3] For example, ziziphines (Fig. 1) possessing this skeleton are a series of cylopeptide alkaloids isolated from marine species *Ziziphus oenophila*,[4] among which ziziphines N and Q show significant in vitro activities against *Plasmodium falciparum* with IC_{50} values of 3.9 and 3.5 μg/mL, respectively. Hamigeroxalamic acid, an alkaloid isolated from Mediterranean sponge *Hamigera hamigera*, shows deterrent activity in a fish feeding assay.[5] Although there has been no report about the anti-HIV-1 activity of the β-aryl-1,2-didehydroethylamine alkaloids, they have the similar biological source as the anti-HIV-1 RT tetrahydroisoquinoline alkaloid.[6] In our previous work, we discovered that simple tetrahydroisoquinonine alkaloid possessed moderate activity against HIV-1 RT.[7] Thus, we were interested in the study of the anti-HIV-1 activity of β-aryl-1,2-didehydroethylamine. *N*-Acetyl-β-aryl-1,2-didehydroethylamine can be viewed as a simplified β-aryl-1,2-didehydroethylamine alkaloid. Therefore, to explore the range of biological activities of *N*-acetyl-β-aryl-1,2-didehydroethylamines, we have synthesized a series of title compounds which were evaluated as NNRTIs of HIV-1 in vitro.

N-Acetyl-β-aryl-1,2-didehydroethylamines have been the subject of intensive synthetic studies for long time.[8] Among these synthetic strategies are the Beckmann rearrangement of benzylideneacetone oxime[9] and the addition reaction of arylvinylisocyanates.[10] However, these two methodologies are limited to the special oximes or the water-sensitive isocyanates, furthermore, strong acid or basic treatment is generally required. Laso and colleagues found that the conjugated nitro group can be reduced to amine and further acetylated by refluxing a mixture of nitroalkene, acetic acid, acetic

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* Corresponding authors. Tel.: +86 871 5223265; fax: +86 871 5223265; e-mail: chenjj@mail.kib.ac.cn

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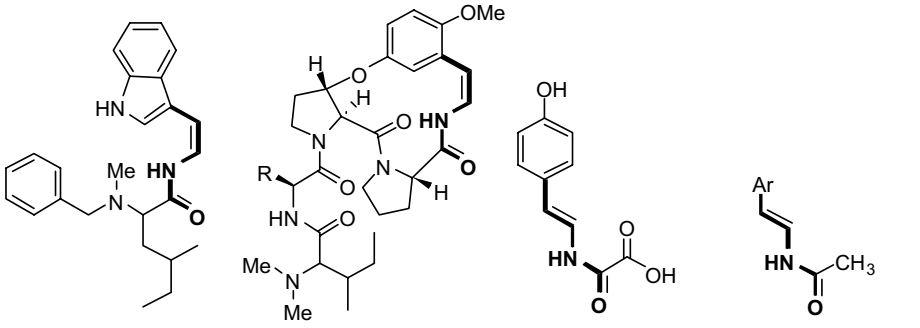
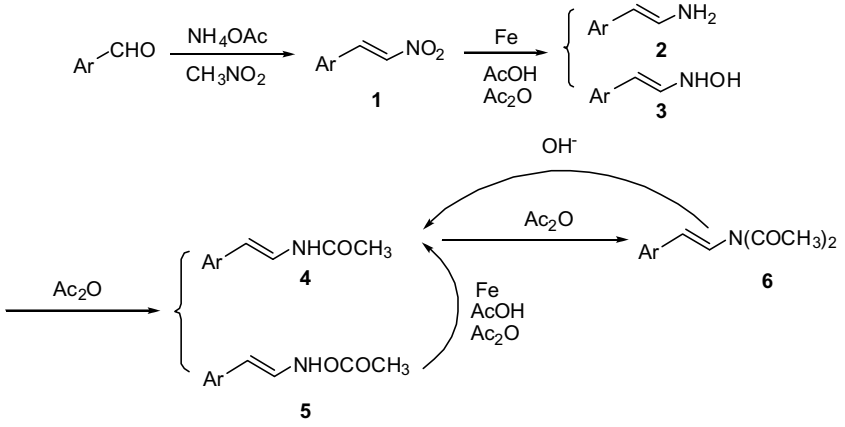


Figure 1. Structures of some naturally occurring bioactive β-aryl-1,2-didehydroethylamine alkaloids and their basic structure unit.

anhydride and iron powder under nitrogen in a one-pot procedure.[11] β-Aryl-nitroolefin (**1**, Scheme 1) contains a conjugated nitro group and can be prepared by treating aromatic aldehyde with ammonium acetate in boiling nitromethane.

Therefore, we decided to synthesize *N*-acetyl-β-aryl-1,2-didehydroethylamines (**4**, Scheme 1) through selective direct reduction–acetylation of β-aryl-nitroolefin derivatives based on Laso's method and assessed the enamides (**4**, Scheme 1) as inhibitors of HIV-1 RT. As outlined in Scheme 1, β-aryl-nitroolefin **1** was first reduced to compound **3** and/or compound **2**. Compound **3** would be trapped in the presence of acetic anhydride to form oxime acetate **5**, and so would be compound **2** to yield the desired *N*-acetyl-β-aryl-1,2-didehydroethylamine **4**. Oxime acetate **5** should be in turn reduced to **4** in the reaction condition. Compound **4** might be further acetylated to the *N*-acetyl-arylvinylacetamide **6**, which could be reversed by treatment with methanolic potassium hydroxide. In this procedure, target compounds **4** were obtained in moderate yields.[12]

Surprisingly, the *trans*-conformational β-phenylnitroolefin (**1a**, Table 1) displayed 99.67% inhibitory ratio against HIV-1 RT at 200 μg/mL with a CC_{50} value of 13.6 μM against C8166 cells. To our knowledge, this is the first report about the anti-HIV-1 RT activity of the cytotoxic nitroolefin. These findings, therefore, urged us to prepare its analogues with a variety of substituents and with other aromatic moieties to explore the structure–activity relationship (SAR). The activities of nitroolefin intermediates against HIV-1 RT (IC_{50} values) are given in Table 1. The potency of inhibition against HIV-1 RT was dependent on the positions of the substituents on the phenyl for compounds **1a**–**1s**, it clearly appeared that the *ortho* monosubstituted-phenyl compounds possessed higher enzymatic activity. For example, *ortho* bromo substituted compound **1b** exhibited 95.74% inhibitory ratio against HIV-1 RT at 200 μg/mL (IC_{50} = 0.2024 mM), while its *meta* and *para* substituted analogues **1c** and **1d** were inactive. *ortho* chloro substituted compound **1e** exhibited 99.28% inhibitory ratio (IC_{50} = 0.3609 mM), but its analogues **1f** and **1g** were significantly less active. The bulkier 2,4,5-trimethylsubstituted compound **1l** only showed 31.53% inhibitory ratio, significantly lower than that of the *ortho* methyl substituted analogue **1j** (IC_{50} = 0.2614 mM). The *ortho* methoxy substituted compound **1o** possessed 99.69% inhibitory ratio (IC_{50} = 0.2246 mM), while the bulkier 2,4,5-trimethoxylsubstituted compound **1r** only exhibited 49.12% inhibitory ratio (IC_{50} > 0.8368 mM). Compared to those *ortho* monosubstituted phenyl compounds, naphthyl analogues (**1t**



Scheme 1. Synthesis of *N*-acetyl-β-aryl-1,2-didehydroethylamine **4**.

Table 1. Structures, anti-HIV-1 RT activities and cytotoxicities of β-aryl-nitroolefin **1**[a]

Compound	Ar	HIV-1 RT % inhibition[b]	IC_{50}[c] (mM)	CC_{50}[d] (mM)
1a	(*E*)-Phenyl	99.67	0.0879	0.0136
1b	(*E*)-2-Bromophenyl	95.74	0.2024	0.00057
1c	(*E*)-3-Bromophenyl	13.42	nt[e]	nt
1d	(*E*)-4-Bromophenyl	20.30	nt	nt
1e	(*E*)-2-Chlorophenyl	99.28	0.3609	0.003
1f	(*E*)-3-Chlorophenyl	49.76	>1.093	0.0046
1g	(*E*)-4-Chlorophenyl	19.83	nt	nt
1h	(*E*)-2-Fluorophenyl	95.42	0.3646	0.0028
1i	(*E*)-4-Fluorophenyl	22.87	nt	nt
1j	(*E*)-2-Methylphenyl	99.24	0.2614	0.0074
1k	(*E*)-4-Methylphenyl	36.86	nt	0.0152
1l	(*E*)-2,4,5-Trimethylphenyl	31.53	nt	0.0069
1m	(*E*)-2-Hydroxyphenyl	98.22	0.54	0.0188
1n	(*E*)-4-Hydroxyphenyl	96.38	0.5533	0.0158
1o	(*E*)-2-Methoxyphenyl	99.69	0.2246	0.005
1p	(*E*)-4-Methoxyphenyl	93.51	0.5612	0.0063
1q	(*E*)-2,4-Dimethoxyphenyl	86.35	0.5534	0.0054
1r	(*E*)-2,4,5-Trimethoxyphenyl	49.12	>0.8368	0.0047
1s	(*E*)-4-Trifluoromethylphenyl	29.38	nt	nt
1t	(*E*)-1-Naphthyl	81.41	0.786	0.0396
1u	(*E*)-2-Naphthyl	58.84	0.9347	0.0406
1v	(*E*)-3-Thienyl	86.23	0.7092	0.0255
1w	(*E*)-2-Furyl	91.84	0.4652	0.0289
PFA		96.46	0.0013	nt

[a] All data represent mean values for at least two separate experiments.
[b] Compounds were tested at 200 μg/mL, the positive control agent phosphonoformic acid (PFA) was tested at 20 μg/mL, the inhibition of recombinant HIV-1 RT activity was performed with a commercially available ELISA kit (Boechringer Mannheim, Germany) according to the instructions of the manufacturer.[13]
[c] IC_{50} = effective concentration that inhibited 50% HIV-1 RT.
[d] CC_{50} = toxic concentration in C8166 cells that caused the reduction of viable cells by 50%.
[e] nt, not tested.

and **1u**), thienyl (**1v**) and furyl (**1w**) analogues were less active. However, nitroolefin intermediates were quite cytotoxic, for example, **1b** showed a CC_{50} value of 0.57 μM in C8166 cells.

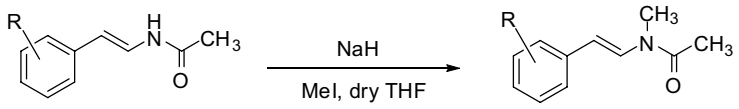
Nitroolefins were converted to amides **4**, the structures and enzymatic activities of **4** are summarized in Table 2. Compounds with *ortho* substituted bromo (**4b**), chloro (**4e**), fluoro (**4h**), methyl (**4j**) and *meta* substituted bromo groups (**4c**) displayed stronger activity than unsubstituted analogue **4a**. However, the *ortho* hydroxyl or methoxy substituted compounds **4m** and **4o** exhibited no activity. Naphthyl compounds **4t** and **4u** showed higher inhibitory ratio against HIV-1 RT than **4a**. Compounds (**4v**–**x**), containing the heterocycles, exhibited no activity.

Interestingly, the SAR of compounds **4** against RT is similar to that of nitroolefins **1**: the *ortho* monosubstituted-phenyl compounds possess higher enzymatic activity. This suggests that compounds **1** and **4** probably have similar mechanism of action with RT. In some newly developed NNRTIs such as the oxindole[14] and benzimidazole-2-one[15] derivatives, NH unit was a proton-donating group in the hydrogen bond formation process between the inhibitor and HIV-1 RT based on detailed docking studies, *N*-alkyl analogues would suffer dramatic loss of activities. In our experiment, compounds **4a**–**x** were less active compared with their synthetic precursors β-phenylnitroolefins **1a**–**w**, indicating

Table 2. Structures and anti-HIV-1 RT activities of *N*-acetyl-β-aryl-1,2-didehydroethylamine **4**[a]

Compound	Ar	Yield (%)	HIV-1 RT % inhibition [b]
4a	(*E*)-Phenyl	45	22.25
4b	(*E*)-2-Bromophenyl	51	54.35
4c	(*E*)-3-Bromophenyl	43	41.48
4d	(*E*)-4-Bromophenyl	50	na[c]
4e	(*E*)-2-Chlorophenyl	43	34.02
4f	(*E*)-3-Chlorophenyl	43	10.74
4g	(*E*)-4-Chlorophenyl	49	na
4h	(*E*)-2-Fluorophenyl	45	36.19
4i	(*E*)-4-Fluorophenyl	44	na
4j	(*E*)-2-Methylphenyl	50	40.15
4k	(*E*)-4-Methylphenyl	64	16.62
4l	(*E*)-2,4,5-Trimethylphenyl	52	25.45
4m	(*E*)-2-Hydroxyphenyl	47	10.10
4n	(*E*)-4-Hydroxyphenyl	33	na
4o	(*E*)-2-Methoxyphenyl	46	na
4p	(*E*)-4-Methoxyphenyl	56	na
4q	(*E*)-2,4-Dimethoxyphenyl	44	na
4r	(*E*)-2,4,5-Trimethoxyphenyl	35	na
4s	(*E*)-4-Trifluoromethylphenyl	49	na
4t	(*E*)-1-Naphthyl	40	26.47
4u	(*E*)-2-Naphthyl	50	30.05
4v	(*E*)-3-Thienyl	26	na
4w[d]	(*Z*)-3-Thienyl	12	12.02
4x	(*E*)-2-Furyl	38	na

[a] All data represent mean values for at least two separate experiments.
[b] Compounds were tested at 200 μg/mL.
[c] na, not active.
[d] **4w** was produced combined with **4v** by reduction of (*E*)-3-(2-nitrovinyl)thiophene (**1v**).



4b: R = 2-Br
4e: R = 2-Cl
4h: R = 2-F
4j: R = 2-Me
4c: R = 3-Br
4d: R = 4-Br

7a: R = 2-Br
7b: R = 2-Cl
7c: R = 2-F
7d: R = 2-Me
7e: R = 3-Br
7f: R = 4-Br

Scheme 2. Synthesis of *N*-methyl analogues **7a**–**f**.

Table 3. Enzymatic activities (IC_{50}), syncytium reduction (EC_{50}) and cytotoxicity (CC_{50}) assay of selected compounds[a]

Compound	HIV-1 RT % inhibition[b]	IC_{50}[c] (mM)	EC_{50}[d] (mM)	CC_{50}[e] (mM)	TI[f]
4b	54.35	nt	0.1338	0.241	1.8
4c	41.48	nt	0.0893	0.134	1.5
4e	34.02	nt	0.1238	0.297	2.4
4h	36.19	nt	0.3830	0.341	<1
4j	40.50	nt	0.3790	0.385	1.0
4u	30.05	nt	0.0497	0.154	3.1
7a	85.27	0.339	0.0598	>0.787	>13.2
7b	75.22	0.434	0.0959	0.441	4.6
7c	61.69	0.477	0.1191	0.667	5.6
7d	79.80	0.487	0.1158	0.609	5.2
7e	45.42	0.838	0.1284	0.409	3.2
7f	na	nt	nt	nt	nt
AZT[g]	nt	nt	1.08×10^{-5}	>5.092	>471,481

[a] All data represent mean values for at least two separate experiments.
[b] Compounds were tested at 200 μg/mL.
[c] IC_{50} = effective concentration that inhibited 50% HIV-1 RT.
[d] EC_{50} = effective concentration required to protect C8166 cells against the cytopathogenicity of HIV-1_{IIIB} by 50%.[16]
[e] CC_{50} = cytostatic concentration required to reduce C8166 cell proliferation by 50%.[17]
[f] Therapeutic index: CC_{50}/EC_{50} ratio.
[g] AZT was used as positive control.

that the hydrogen atom of NH unit was probably not crucial to potent activity. To examine whether there is hydrogen bond between **4** and the enzyme, the *N*-methyl analogues of **4b**, **4e**, **4h** and **4j** were synthesized (Scheme 2). Compounds **7a**–**d** showed 85.27%, 75.22%, 61.69% and 79.80% inhibitory ratio (Table 3) against HIV-1 RT at 200 μg/mL, respectively, increased nearly onefold from **4b**, **4e**, **4h** and **4j**. This suggested that NH unit in compounds **4** was not involved to form a hydrogen bond with HIV-1 RT. On the contrary, the *N*-methyl analogues were proved to have stronger interaction with the HIV-1 RT active site and increased enzymatic activity accordingly. Furthermore, *N*-methyl analogues (**7e**, **7f**) of **4c** and **4d** were prepared (Scheme 2) and evaluated for their inhibitory abilities to RT, respectively. As outlined in Table 3, **7e** and **7f** lost significant enzymatic activity compared with **7a**, which gave us an information that *ortho*-substituted *N*-methyl derivatives still possessed higher enzyme activities.

Compounds **4b**, **4c**, **4e**, **4h**, **4j**, **4u** and **7a**–**e** with HIV-1 RT inhibitory ratio above 30% were tested for their anti-HIV-1 activities (EC_{50}) and cytotoxicties (CC_{50}) in cell-based assays, in addition the therapeutic index (TI) was calculated (Table 3). The antiviral assay was performed based on a protocol described in our previous reports.[16,17] Besides, enzymatic activities (IC_{50}) of compounds **7a**–**e** were evaluated. The following is to be noted regarding the anti-HIV-1 activity data with the tested compounds: (i) the cytotoxicities of selected compounds significantly decreased compared with nitroolefin intermediates; (ii) compounds **4h** and **4j** were inactive ($EC_{50} > 200.0$ μM, TI $\leqslant 1$), whereas the other compounds exhibited weak (**4b**, **4c**, **4e**, **4u**), moderate (**7b**–**e**) activity; (iii) the most active compound was (*E*)-*N*-(2-bromostyryl)-*N*-methylacetamide (**7a**, TI > 13.2); (iv) compound **7a** showed higher enzymatic activity (IC_{50}) and TI value than its isomer **7e**, which suggested that higher enzymatic inhibitory ratio would lead to increased anti-HIV activity in cell-based assay.

Although the TI values of the tested compounds are suboptimal, they possess a naturally occurring structure unit and the SAR exploration provided us a guide on further anti-HIV-1 study of the derivatives in this family. Furthermore, the anti-HIV-1 assay of the natural β-aryl-1,2-didehydroethylamine alkaloids can be initiated based on our findings. In summary, we have described a convenient synthesis of the *N*-acetyl-β-aryl-1,2-didehydroethylamine derivatives, this class of compounds were studied as inhibitors of HIV-1 replication against RT for the first time, representing a new lead for the design and synthesis of more potent and selective analogues that act as NNRTIs. Further structure modification on the nitrogen atom of compounds **4** is underway.

Acknowledgments

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12. Procedure for preparing compound **4a**: a suspension of iron powder (120 mmol) in acetic acid (1.2 mL) and acetic anhydride (12 mL) was heated to reflux for 30 min under a nitrogen atmosphere, then the β-phenylnitroolefin (**1a**, 6 mmol) was added to the mixture in half hour and the reaction system was kept refluxing for another 4 h until the disappearance of starting material monitored by TLC check. The mixture was then cooled and poured to ice cooled water and filtered, the filtrate was evaporated to give a residue which was dissolved in 10 mL methanol, the pH was adjusted to about 12 using the methanolic KOH. Filtration through Celite followed by evaporation of methanol and purification on silica gel chromatography afforded the target compounds. In this procedure, (*E*)-*N*-(styryl)acetamide **4a** was obtained as white solid, mp 104–106 °C; ^{1}H NMR ($CDCl_3$) δ: 7.52 (d, J = 14.4 Hz, 1H, NCH), 7.32–7.16 (m, 5H, Ar–H), 6.09 (d, J = 14.4 Hz, 1H, Ar–CH), 2.11 (s, 3H, $COCH_3$); IR ν_{max} (KBr) cm^{-1}: 3287 (NH) 1669 (CO); EI-MS *m*/*z* (%): 161 [M]$^+$ (35), 147 (49), 118 (100), 105 (85), 91 (77), 77 (47).
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