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# Synthesis and anti-HIV activity of nevirapine prodrugs

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The synthesis, *in vitro* anti-HIV activity and stability studies of the N-Mannich bases of nevirapine are reported. Among the synthesized compounds, 5-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}-11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[2,3-e:3′,2′-*b*][1,4]diazepin-6-one (**3**) was found to be the most potent compound with $EC_{50}$ of 0.0159 μM against HIV-1 replication and $CC_{50}$ of >1000 μM against CEM cell lines with selectivity index of >62893. Compound **3** was five times more active than nevirapine ($EC_{50}$ of 0.09 μM). *In vitro* hydrolysis of the Mannich bases in phosphate buffer (pH 7.4) indicated that these agents were relatively stable with $t_{1/2}$ ranging from 15 to 240 min.

## 1. Introduction

The non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent a class of structurally diverse, potent and highly-selective anti-HIV agents that were first discovered in the early 1990's as a result of large scale compound library screening in antiviral and/or biochemical assays, followed by extensive chemical lead optimization (Pauwels 2004). Nevirapine (Hargrave et al. 1991) was the first NNRTI to receive regulatory approval for the treatment of HIV infection and is currently used in combination with nucleoside reverse transcriptase inhibitors (NRTI) such as zidovudine, stavudine, didanosine and abacavir (Luzuriaga et al. 1997). Nevirapine is a non-competitive inhibitor that binds allosterically to an asymmetric and hydrophobic cavity, about 10 Å away from the catalytic site of the HIV-1 RT (Kohlstaedt et al. 1992). As a result of NNRTI binding, certain RT domains that actively participate in DNA synthesis are restricted in flexibility and mobility which, in turn, leads to a dramatic reduction in catalytic enzyme efficiency (Shen et al. 2003). Among the anti-HIV prodrug approaches, many investigations were aimed at increasing lipid solubility, since the correlation between lipophilicity, membrane permeability, and CNS penetration has long been established (Tan et al. 1999; Parang et al. 2000). Recently we reported that cellular permeability and spectrum of activity of this drug can be modulated by derivatization to a bioreversible form, namely a prodrug (Sriram et al. 2005a). In this study a new series of prodrugs of nevirapine has been synthesized in an effort to enhance the uptake of the prodrugs by the HIV-1 infected cells.

## 2. Investigations, results and discussion

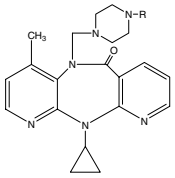
### *2.1. Synthesis*

The general procedures for the preparation of target compounds **1**–**9** (Table) are described in the Scheme. The lactam hydrogen of nevirapine reacts with formaldehyde and various piperazines to form the required Mannich bases of nevirapine in a microwave oven with 68–92% yield. Unlike conventional methods (duration up to 24 h) (Sriram et al. 2005b), microwave assisted reactions are very facile (2–3 min), the product does not require any further purification. The purity of the synthesized compounds was checked by TLC and elemental analyses and the structures were identified by spectral data. In general, IR spectra showed a $CH_2$ (Mannich methylene) peak at 2860 and 2846 $cm^{-1}$. In the $^1H$ NMR spectra the signals of the respective protons of the prepared nevirapine derivatives were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ 4.03–4.1 ppm corresponding to the $-NCH_2N-$ group; multiplet at δ 2.8–3.6 ppm for the piperazine proton; multiplet at δ 0.28–1.35 ppm for the cyclopropyl proton; and singlet at 2.37 for a C-4 methyl group. The elemental analysis results were within ±0.4% of the theoretical values.

### *2.2. Biological investigation and discussion*

The synthesized compounds were evaluated for their inhibitory effect on the replication of HIV-1 in CEM cell lines and their $EC_{50}$ (effective concentration of compound (μM) achieving 50% protection in MT-4 cell lines against the cytopathic effect of HIV-1), and $CC_{50}$ (cytotoxic concentration of compound (μM) required to reduce the viability of mock infected CEM cells by 50%), were reported in the Table with nevirapine as standard drug for comparison. Rapid glance to the obtained results revealed that compounds **1**–**9** exhibited good anti-HIV activity with $EC_{50}$ ranging from 0.0159–0.523 μM. Four compounds (**1**–**4**), ($EC_{50}$ ranging from 0.0159–0.0636 μM) were found to be more potent than nevirapine ($EC_{50}$ of 0.09 μM). Among the

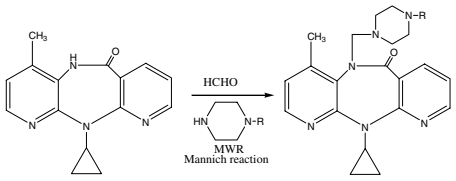
**Table: Physical constants and anti-HIV activities of the synthesized compounds**



| Compd. | R | Yield (%) | M.p. (°C) | cLog P | $EC_{50}$[a] (μM) | $CC_{50}$[b] (μM) | SI[c] | $t_{1/2}$[d] min |
|---|---|---|---|---|---|---|---|---|
| **1** | Phenyl | 91 | 194 | 4.39 | 0.0636 | >200 | >3144 | ND |
| **2** | 3-Chloro phenyl | 69 | 241 | 4.95 | 0.0318 | >200 | >6289 | ND |
| **3** | 4-Chloro phenyl | 89 | 236 | 4.95 | 0.0159 | >1000 | >62893 | 45 |
| **4** | 4-Fluoro phenyl | 78 | 246 | 4.55 | 0.0318 | >200 | >6289 | ND |
| **5** | 4-Nitro phenyl | 92 | 221 | 4.19 | 0.12 | >200 | >1666 | ND |
| **6** | 4-Methoxy phenyl | 86 | 229 | 4.26 | 0.12 | >200 | >1666 | ND |
| **7** | Benzyl | 83 | 182 | 4.05 | 0.523 | >200 | >382 | 240 |
| **8** | Methyl | 80 | 165 | 2.31 | 0.44 | >200 | >454 | ND |
| **9** | H | 68 | 138 | 1.94 | 0.62 | >200 | >322 | 15 |
| Nevirapine | – | – | – | 2.05 | 0.09 | >200 | >2222 | – |

[a] Effective concentration of compound achieving 50% protection in CEM cell lines against the cytopathic effect of HIV-1. [b] Cytotoxic concentration of compound required to reduce the viability of mock infected CEM cells by 50%, [c] Selectivity index or ratio of $CC_{50}$ to $EC_{50}$, [d] hydrolysis half life at pH 7.4 and 37 °C, ND indicates not determined

**Scheme**




synthesized compounds, 5-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}-11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[2,3-e:3′,2′-*b*][1,4]diazepin-6-one (**3**) was found to be the most potent compound with $EC_{50}$ of 0.0159 μM against HIV-1 replication and $CC_{50}$ of >1000 μM against CEM cell lines with selectivity index ($CC_{50}/EC_{50}$) of >62893. Compound **3** was five times more active than parent nevirapine. With respect to the structure-activity relationship, introduction of a halogen substituted phenyl ring in the piperazine moiety enhanced the activity, whereas unsubstituted, benzyl and methyl groups decreasesed activity. The lipophilicity (ClogP) of the synthesized compounds increased remarkably compared with the parent drug, nevirapine (Table). This may render them more capable of penetrating various biomembranes (Moss and Bundgaard 1990), consequently improving their permeation properties through viral cell membranes. The results showed that there was an improvement in anti-HIV activity compared to the parent drug.

### *2.3. Hydrolytic stability studies*

The usefulness of the prodrugs of nevirapine should depend not only on the stability of the prodrug for its transport across the cell membrane but also upon its reversion to the parent compound intracellularly, especially in the virally infected cells. The half-lives ($t_{1/2}$) of hydrolysis of the prodrugs were therefore determined in phosphate buffer pH 7.4 at 37 °C. The data in the Table indicated that the *in vitro* hydrolysis of the various Mannich bases at pH 7.4 indicated that these agents were relatively stable with $t_{1/2}$ ranging from 15–240 min.

It was earlier reported for nevirapine derivatives, that maximum activity is obtained with compounds containing a 4-methyl substituent in the absence of substitution at the lactam nitrogen (N-5); however, 5-methyl substitution is preferred if the 4-position is not substituted (Hargrave et al. 1991). This combination of a 4-methyl group and an unsubstituted lactam is present in all of the most active compounds. In view of the above structural requirement, the better activity of these reported compounds can be explained by deaminomethylation of nevirapine prodrugs *in vivo*.

## 3. Experimental

### *3.1. Chemistry*

Melting points were determined in one end open capillary tubes on a Büchi 530 melting point apparatus and are uncorrected. IR and $^1$H NMR spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Bruker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. Elemental analyses (C, H, and N) were undertaken with Perkin-Elmer model 240C analyzer. All the results were in an acceptable range. The homogeneity of the compounds was monitored by ascending TLC on silicagel-G (Merck) coated aluminium plates, visualized by iodine vapour. Developing solvents were chloroform-methanol (9 : 1). A domestic microwave oven with the following specifications had been used: Make LG; Input 220 V∼50 Hz, 980 W, 4.7 A; Frequency 2450 MHz.

### *3.2. General procedure for the preparation of Mannich bases*

To a suspension of 11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[2,3-e:3′,2′-*b*][1,4]diazepin-6-one (0.02 mol) in ethanol was added appropriate aryl piperazines (0.02 mol) and 37% formaldehyde (0.5 ml) and irradiated in a microwave oven at an intensity of 80% with 30 s/cycle. The number of cycles in turn depended on the completion of the reaction, which was checked by TLC. The reaction timing varied from 1.5–3 min. The filtrate was evaporated under reduced pressure and the residue recrystallized from the mixture of ether : ethanol gave the compounds requested. Their physical and spectral data are given as follows.

### *3.3. 11-Cyclopropyl-4-methyl-5-[4-phenylpiperazin-1-yl]methyl}-5,11-dihydro-6H-dipyrido[2,3-e : 3′,2′-b][1,4]diazepin-6-one* (**1**)

Yield: 91%; m.p.: 194 °C; IR (KBr): 3326, 2860, 2850, 1129, cm$^1$; $^1$H NMR ($d_6$-DMSO) δ (ppm): 0.28–0.53 (m, 4 H, $CH_2$ of cyclopropyl); 1.34 (s, 3 H, $CH_3$), 2.37 (m, 1 H, CH of cyclopropyl), 2.5–3.5 (m, 8 H, -piperazine-H), 4.03 (s, 2 H, $-NCH_2N$), 6.5–8.36 (m, 10 H, ArH).
$C_{26}H_{28}N_6O$

### *3.4. 5-{[4-(4-Chlorophenyl)piperazin-1-yl]methyl}-11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[2,3-e : 3′,2′-b][1,4]diazepin-6-one* (**3**)

Yield: 78%; m.p.: 246 °C; IR (KBr): 3320, 2860, 2846, 1129, cm$^1$; $^1$H NMR ($d_6$-DMSO) δ (ppm): 0.28–0.53 (m, 4 H, $CH_2$ of cyclopropyl); 1.36 (s, 3 H, $CH_3$), 2.37 (m, 1 H, CH of cyclopropyl), 2.56–3.45 (m, 8 H, -piperazine-H), 4.03 (s, 2 H, $-NCH_2N$), 6.52–8.36 (m, 9 H, ArH).
$C_{26}H_{27}ClN_6O$

### *3.5. 11-Cyclopropyl-5-{[4-(4-methoxyphenyl)piperazin-1-yl]methyl}-4-methyl-5,11-dihydro-6H-dipyrido[2,3-e : 3′,2′-b][1,4]diazepin-6-one* (**6**)

Yield: 86%; m.p.: 229 °C; IR (KBr): 3320, 2860, 2850, 1129, $cm^1$; $^1H$ NMR ($d_6$-DMSO) δ (ppm): 0.28–0.55 (m, 4 H, $CH_2$ of cyclopropyl); 1.36 (s, 3 H, $CH_3$), 2.37 (m, 1 H, CH of cyclopropyl), 2.54–3.40 (m, 8 H, -piperazine-H), 3.73 (s, 3 H, $-OCH_3$) 4.10 (s, 2 H, $-NCH_2N$), 6.56–8.4 (m, 9 H, ArH).
$C_{27}H_{30}N_6O_2$

### *3.6. 5-[4-Benzylpiperazin-1-yl]methyl}-11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[2,3-e : 3′,2′-b][1,4]diazepin-6-one* (**7**)

Yield: 83%; m.p.: 182 °C; IR (KBr): 3326, 2860, 2850, 1129, $cm^1$; $^1H$ NMR ($d_6$-DMSO) δ (ppm): 0.28–0.53 (m, 4 H, $CH_2$ of cyclopropyl); 1.34 (s, 3 H, $CH_3$), 2.37 (m, 1 H, CH of cyclopropyl), 2.5–3.5 (m, 8 H, -piperazine-H), 3.62 (s, 2 H, $-CH_2$ of benzyl), 4.03 (s, 2 H, $-NCH_2N$), 6.5–8.36 (m, 10 H, ArH).
$C_{27}H_{30}N_6O$

### *3.7. 11-Cyclopropyl-4-methyl-5-[4-methylpiperazin-1-yl]methyl}-5,11-dihydro-6H-dipyrido[2,3-e : 3′,2′-b][1,4]diazepin-6-one* (**8**)

Yield: 80%; m.p.: 165 °C; IR (KBr): 3320, 2860, 2852, 1129, $cm^1$; $^1H$ NMR ($d_6$-DMSO) δ (ppm): 0.28–0.53 (m, 4 H, $CH_2$ of cyclopropyl); 1.35 (s, 3 H, $CH_3$), 2.20 (s, 3 H, $CH_3$ of piperazinyl) 2.37 (m, 1 H, CH of cyclopropyl), 2.46 (m, 8 H, -piperazine-H), 4.03 (s, 2 H, $-NCH_2N$), 6.52–8.36 (m, 5 H, ArH).
$C_{21}H_{26}N_6O$

### *3.8. Anti-HIV screening*

Candidate agents were dissolved in dimethylsulfoxide, and then diluted 1 : 100 in cell culture medium before preparing serial half-log10 dilutions. T4 lymphocytes (CEM cell-line) were added and after a brief interval HIV-1 was added, resulting in a 1 : 200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls. Cultures were incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT was added to all the wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitative formazan production, and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity.

### *3.9. In vitro stability study*

To 990 µl of phosphate buffer (pH 7.4) was added 10 µl of a solution of compound (10 mg/ml in dimethylsulfoxide) and the mixture was incubated at 37 °C in a water bath. At various time intervals (0–4 h), 100 µl of the samples were withdrawn and added immediately to ice-cold methanol (400 µl). The samples were centrifuged and the supernatants were filtered through nylon 66 filters (0.45 µm) and analyzed according to the method described by Bundgaard and Johansen (1980).

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