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Newer tetracycline derivatives: Synthesis, anti-HIV, antimycobacterial activities and inhibition of HIV-1 integrase

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Abstract—A series of new tetracycline derivatives has been synthesized by reacting appropriate tetracyclines, formaldehyde and secondary amino (piperazino) function of fluoroquinolones using microwave irradiation with the yield ranging from 41evaluated for its anti-HIV, antimycobacterial activities and HIV-1 integrase (IN) enzyme inhibition studies. Among the synthesized compounds, compound **10** was found to be the most promising compound active against HIV-1 replication with EC_{50} of 5.2 μM and was non-toxic to the CEM cells untill 200 μM, and MIC of 0.2 μg/mL against *Mycobacterium tuberculosis*, with moderate inhibition of both 3′-processing and strand transfer steps of HIV-1 IN.

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), which is one of the most serious health problems in the world. Recently, 'highly active anti-retroviral therapy (HAART)', which involves a combination of reverse transcriptase/protease inhibitors, has dramatically improved the clinical treatment of individuals with AIDS or HIV infection.[1] However, this combination therapy using multi-types of anti-HIV drugs has not yet reached the stage of perfection owing to several serious problems including the emergence of viral strains with multi-drug resistance, significant side effects and high costs. Problems of drug toxicity and drug resistance may be reduced via the inhibition of a new HIV target. Integrase (IN) is an attractive and a validated target for anti-AIDS drug design because of its crucial role in the viral life cycle and the fact that there is no cellular homologue in humans. HIV-1 integrase is a 32 kDa protein encoded at the 3′-end of the HIV *pol* gene.[2,3] Incorporation of HIV DNA into host chromosomal DNA, which is catalyzed by HIV integrase, occurs by a specifically defined sequence of 3′-processing and strand transfer reactions.[4] A number of structurally diverse compounds have been reported to be inhibitors of HIV integrase.[5,6]

Earlier Neamati et al.[7] used the four-point pharmacophore model for HIV-1 IN inhibitors for a search of the National Cancer Institute 3D database. The 3D database search of 206,876 structures yielded a total of 179 compounds that contained the four-point pharmacophore distance pattern in one or more of their conformations. Thirty-nine compounds were manually selected for bioassay based on considerations of structural diversity and sample availability, with the most probable four-point pharmacophore centres identified and yielding a greater number of active HIV-1 IN inhibitors. Among them tetracycline exhibited IC_{50} values of 204.0 and 188.0 μM for 3′-processing and strand transfer, respectively, whereas rolitetracycline, with IC_{50} values of 28.0 and 34.1 μM for 3′-processing and strand transfer, respectively, was five times more potent than the parent compound. An even further increase in potency was observed as the bulk of the substituent on the carboxamide moiety at C-2 was increased. Three other commercially available tetracycline analogues that contain the free carboxamide group (oxytetracycline, doxycycline and methacycline) were also tested in IN inhibition assay and found to exhibit potencies in the same range as the tetracycline. In this paper, we report synthesis of some newer tetracycline derivatives with bulky aryl piperazines (fluoroquinolones), its anti-HIV and antimycobacterial activities with HIV-1 IN enzyme inhibition studies (Scheme 1).

The general procedures for the preparation[8] of target compounds **1**–**12** (Table 1) are described in the scheme.

Keywords: Tetracycline derivatives; Anti-HIV activity; Antimycobacterial activity; HIV-1 integrase.
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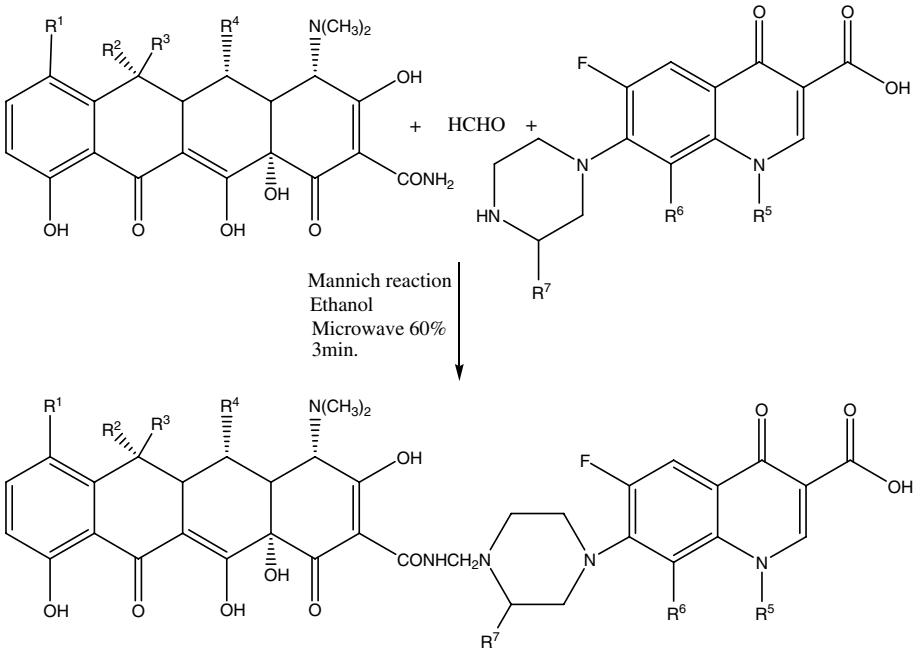
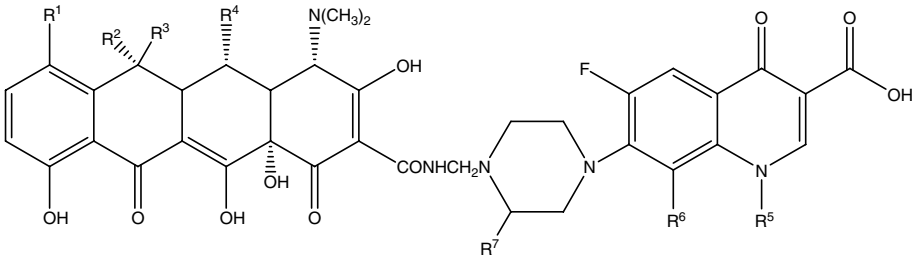


Scheme 1. Synthetic protocol of tetracycline derivatives.

Tetracycline, oxytetracycline and minocycline were reacted with formaldehyde and secondary amino (piperazino) function of fluoroquinolones like norfloxacin, lomefloxacin, ciprofloxacin and gatifloxacin to form the required Mannich bases of tetracycline, oxytetracycline and minocycline in 41–78% yield. The reaction was carried out using microwave irradiation with 60% intensity for 3 min, and the products did not require any further purification. The purity of the synthesized compounds was checked by thin-layer chromatography (TLC) and elemental analyses and the structures were identified by spectral data.[16] In general, Infra-red spectra (IR) showed CH_2 (Mannich methylene) peaks at 2860 and 2846 cm^{-1}. In the nuclear magnetic resonance spectra (^1H NMR), the signals of the respective protons of the prepared tetracycline derivatives were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ 4.1–4.3 ppm corresponding to $-NCH_2N-$ group; singlet at δ 2.7–3.1 ppm for dimethylamino group of C_4–H of tetracyclines; singlet at δ 9.6 ppm for amide NH; multiplet at δ 2.8–3.54 ppm for piperazine proton; singlet at δ 8.1 ppm for C_2–H; broad singlet at δ 14.86 ppm for COOH proton of fluoroquinolone. The elemental analysis results were within ±0.4% of the theoretical values. The absence of two broad singlets at δ 9.5 and 9.53 ppm, which is characteristic for free carboxamide proton of tetracyclines, and presence of singlet at δ 4.1–4.3 ppm (Mannich methylene proton) indicated that the active hydrogen of carboxamide function of tetracycline reacted with formaldehyde and secondary amino function of piperazine of fluoroquinolones.

The synthesized compounds were evaluated for their inhibitory effect on the replication of HIV-1 in CEM cell lines[9] and their EC_{50} (effective concentration of compound (μM) achieving 50% protection in CEM 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%) are reported in Table 2 with tetracycline and rolitetracycline as standard drugs for comparison. Briefly, the CEM cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow lab, Irvine Scotland), supplemented with 10% (v/v) heat-inactivated calf serum and 20-μg/mL gentamicin (E. Merck, Darmstadt, Germany). HIV-1 (III B) was obtained from the culture supernatant of HIV-1-infected CEM cell lines and the virus stocks were stored at −70 °C until used. Anti-HIV assays were carried out in microtitre plates filled with 100 μL of medium and 25 μL volumes of compounds in triplicate so as to allow simultaneous evaluation of their effects on HIV- and mock infected cells. Fifty microlitres of HIV at 100 $CCID_{50}$ medium was added to either the HIV-infected or mock infected part of the microtitre tray. The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Five days after, infection the viability of mock and HIV-infected cells was examined spectrophotometrically by the MTT method. Rapid glance to the obtained results revealed that seven compounds exhibited excellent anti-HIV activity with EC_{50} values less than 20 μM. Four compounds (**1**–**4**) derived from tetracycline were less toxic to CEM cells and more active against HIV-1 replication. Compounds (**5**–**8**) derived from oxytetracycline were found to be toxic to CEM cells and did not show any activity against HIV-1 replication at a concentration below their toxicity threshold. Among the synthesized compounds, compound **10** (minocycline derived) was found to be the most

Table 1. Structure and physical constants of synthesized compounds



1-12

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Yield (%)	Mp (°C)
1	H	CH_3	OH	H	$-C_2H_5$	H	H	78	220
2	H	CH_3	OH	H	$-C_2H_5$	F	$-CH_3$	54	>270
3	H	CH_3	OH	H		H	H	61	190
4	H	CH_3	OH	H		$-OCH_3$	$-CH_3$	69	230
5	H	CH_3	OH	OH	$-C_2H_5$	H	H	43	203
6	H	CH_3	OH	OH	$-C_2H_5$	F	$-CH_3$	41	145
7	H	CH_3	OH	OH		H	H	66	172
8	H	CH_3	OH	OH		$-OCH_3$	$-CH_3$	41	190
9	$-N(CH_3)_2$	H	H	H	$-C_2H_5$	H	H	62	198
10	$-N(CH_3)_2$	H	H	H	$-C_2H_5$	F	$-CH_3$	49	272
11	$-N(CH_3)_2$	H	H	H		H	H	52	201
12	$-N(CH_3)_2$	H	H	H		$-OCH_3$	$-CH_3$	58	196

Table 2. Anti-HIV, antimycobacterial activities and HIV-1 integrase inhibition of synthesized compounds

Compound	Anti-HIV activity (μm)		HIV-1 Integrase IC_{50} (μm)		MIC in μg/mL MTB
	EC_{50}[a]	CC_{50}[b]	3′-Processing	Strand transfer	
1	14.6	131	20	13	0.78
2	7.58	>200	65	44	1.56
3	8.4	141	20	12	0.39
4	20.2	140	NT	NT	0.2
5	12.2	40	21	12	0.78
6	15.1	37.4	NT	NT	0.78
7	>17.7	17.7	38	17	0.39
8	>28.9	28.9	28	13.5	0.39
9	>130.1	130.1	NT	NT	0.78
10	5.2	>200	20	18	0.2
11	>120	120	NT	NT	0.78
12	>126	126	NT	NT	0.2
Tetracycline	>40.5	40.5	204	188	>6.25
Rolitetracycline	>45.5	45.5	28	34	>6.25
Minocycline	>52.1	52.1	NT	NT	>6.25
Lomefloxacin	NT	NT	NT	NT	6.25

[a] Effective concentration of compound (μM) achieving 50% protection in CEM cell lines against the cytopathic effect of HIV-1.
[b] C_{50} cytotoxic concentration of compound (μM) required to reduce the viability of mock infected CEM cells by 50%.

potent compound with EC_{50} of 5.2 μM and was nontoxic to the CEM cells till 200 μM with a selective index (CC_{50}/EC_{50}) of >38.

Integrase inhibition studies were conducted with recombinant wild-type HIV-1 integrase and a 21-mer oligonucleotide substrate, following a previously described

procedure.[10] All the tested compounds showed moderate inhibition of both 3′-processing and strand transfer steps of HIV-1 integrase (Table 2) and were more active than tetracycline. When compared to rolitetracycline, five compounds were found to be more potent and compounds **3** and **10** emerged as the most active compounds against 3′-processing and strand transfer. These compounds also showed anti-HIV activity in cell cultures when compared to the earlier report of Neamati et al.[7] on tetracycline derivatives which though showed inhibition of HIV-1 integrase function failed to exhibit any significant activity against HIV-1 replication in cell culture.

All compounds were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* (MTB) by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards[11] for the determination of minimum inhibitory concentration (MIC). The MIC was defined as the minimum concentration of a compound required to inhibit the bacterial growth and MIC's of the compounds are reported in Table 2. Among the synthesized compounds eleven compounds inhibited MTB with MIC of less than 1 μg/mL, three compounds **4**, **10** and **12** were found to be the most active compounds with MIC of 0.2 μg/mL. The enhanced antimycobacterial activity might be due to the dual mechanism of action by inhibiting the protein synthesis by binding to the 30S subunit of ribosomes and DNA synthesis by binding to the DNA gyrase.

This study has revealed that combining tetracyclines and fluoroquinolones resulted in both anti-HIV and antitubercular activities. Worldwide, TB is the most frequent co-infection in subjects with HIV type 1 infection.[12] HIV-1 infection remains the most common risk factor for the development of active TB.[13] Both reactivation of a latent *M. tuberculosis* (MTB) infection and progressive primary TB are substantially more common in HIV-1-infected subjects.[14] Through logic and orderly thinking, it appears that an ideal drug for HIV/AIDS patients should suppress HIV replication thereby acting as anti-HIV drug and also should treat OI like TB.[15] This study revealed that compound **10** was found to be promising for the treatment of AIDS, as shown by excellent anti-HIV and antimycobacterial activity.

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16. Important spectral data of compound **10**: IR (KBr): 3100, 2860, 2846, 2800–2600, 1670, 1620, 1510, 1450 cm; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.2 (s, 3H, CH_3 of piperazine), 1.28 (t, 3H, $CH_3$of C_2H_5), 2.72 (s, 6H, –N(CH_3)$_2$ of C_4–H of minocycline), 2.8 (s, 6H, –N(CH_3)$_2$ of C_7–H of minocycline), 2.9–3.2 (m, 6H, piperazine proton), 4.1 (s, 2H, –NCH_2N–), 4.25 (q, 2H, CH_2 of C_2H_5), 6.83 (d, 1H, C_9–H of minocycline, J = 8 Hz), 7.4 (d, 1H, C_8–H of minocycline, J = 8.1 Hz) 8.1 (s, 1H, C_2–H of lomefloxacin). 9.6 (s, 1H, amide NH), 11.3 (s, 1H, C_{10}–OH) 14.82 (br s, 1H, COOH of lomefloxacin); ^{13}C NMR, δ ppm: 13.1, 16.5, 23.5, 27.3, 28.4, 36.4, 40.6, 44.4, 47.2, 49.4, 50.0, 51.9, 61.1, 69.7, 74.4, 97.7, 106.1, 109.3, 112.1, 114.5, 119.8, 120.5, 121.3, 125.4, 126.6, 135.2, 139.5, 146.0, 146.3, 148.1, 150.3, 151.1, 159.1, 166.3, 174.9, 177.5, 183.1, 193.1, 193.4.