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Synthesis of stavudine amino acid ester prodrugs with broad-spectrum chemotherapeutic properties for the effective treatment of HIV/AIDS

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Abstract—A series of prodrugs of stavudine were synthesized in an effort to enhance spectrum of chemotherapeutic properties for the effective treatment of HIV/AIDS. The 5′-OH function of stavudine was esterified with ciprofloxacin, norfloxacin, isoniazide, pyrazinamide, piperazine and dimethylamine acetic acid. The anti-HIV-1 activity of the esters was determined in CEM cell line and stavudine ester bearing piperazine acetic acid was found to be the most potent compound with a selective index of $>15{,}723$. Stavudine prodrug bearing ciprofloxacin and norfloxacin acetic acid showed 100% inhibition against *Mycobacterium tuberculosis* $H_{37}Rv$ at 6.25 μg/mL. The prodrugs also exhibited antibacterial activity against 24 pathogenic bacteria. In vitro hydrolysis of the various esters in human plasma indicated that these agents were relatively stable toward plasma esterases with $t_{1/2}$ ranging from 20–240 min.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is caused by the retrovirus, human immunodeficiency virus (HIV).[1] The HIV infection, which targets monocytes expressing surface CD4 receptors, eventually produces profound defects in cell-mediated immunity.[2] Overtime, infection leads to severe depletion of CD4 T-lymphocytes (T-cells) resulting in opportunistic infections like tuberculosis (TB), fungal, neurological and neoplastic diseases and ultimately death. Tuberculosis is making a worldwide resurgence. Although only about 10% of people infected with *Mycobacterium tuberculosis* develop active TB during their lifetime, when compromised by HIV infection as many as 50% of people infected with *Mycobacterium tuberculosis* would develop active TB during shortened lifetime.[3] In the meantime, resistance to many currently available antimicrobial agents continues to grow.[4] 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 opportunistic infections like tuberculosis and other bacterial infections.[5] As a result, we undertook a study to prepare and rapidly evaluate amino acid ester prodrugs of stavudine in an effort to identify compounds, which could suppress HIV-replication and also inhibit the opportunistic microorganisms.

2. Chemistry

Esters have dominated prodrug research because they have ideal characteristics, exhibiting reasonable chemical stability in vitro, which allowed them to be formulated with adequate shelf lives. In addition, by virtue of their ability to function as esterase substrates, esters are suitable labile in vivo.[6] Esterification of the 5′-hydroxyl group was a common approach to enhance brain uptake and in vivo efficacy of anti-HIV nucleoside derivatives.[7] The reaction sequence used for the preparation of amino acid ester prodrugs was achieved in two steps (Scheme 1). The precursor stavudine chloroacetate (**2**) was obtained by refluxing stavudine (**1**) with chloroacetyl chloride in methylene chloride using

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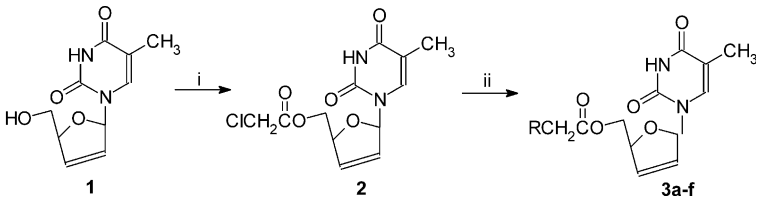


Scheme 1. (i) $ClCOCH_2Cl$, pyridine, methylene dichloride; (ii) amine, amide, acid hydrazide, methylene dichloride.

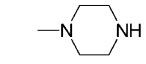
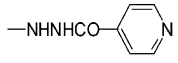
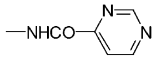
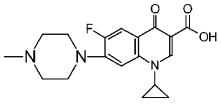
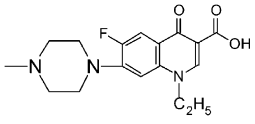
pyridine as an acid scavenger. Subsequent condensation of the intermediate (**2**) with the respective secondary amine, amide, and acid hydrazide at room temperature gave the titled compounds **3a–f** in 68–82% yield (Table 1). The purity was assessed by TLC; and the assignments of the structures were based on elemental and spectroscopic methods of analysis. The IR spectral data revealed the characteristic carbonyl stretching vibration bands at 1730–1750 cm^{-1} of the ester group in addition to the ether bands at approximately 1160 and 1250 cm^{-1}. In the ^1H NMR spectra the signals of the respective protons of the prepared esters were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a doublet signal at $\delta \sim 1.88$ ppm correlated to be the methyl group located at C-5 of stavudine; NH proton of thymidine nucleus appeared at $\delta \sim 9.56$ ppm in addition to a singlet signal at $\delta \sim 3$ ppm corresponding to the $COCH_2N$ group. Elemental analysis results were within $\pm 0.4\%$ of the theoretical values.

3. Anti-microbial activity

The antiviral activity of stavudine and its prodrugs against HIV-1 was determined in vitro in T4 lymphocytes (CEM cell line)[8] (Table 1). Compound **3a** was found to be more potent with EC_{50} of <0.0636 μM, CC_{50} of >1000 μM and selective index (SI) of $>15{,}723$. The enhanced activity of prodrug **3a** over stavudine might be due to improved delivery of stavudine more efficiently via the active transport system,[9] to target cells at higher concentration than that which can be achieved by stavudine alone.

Some of the compounds were screened against *Mycobacterium tuberculosis* strain $H_{37}Rv$ at a single concentration, 6.25 μg/mL in BACTEC 12B medium using the BACTEC 460 radiometric system.[10] Among the four esters (**3c–f**) tested, (Table 1) compounds containing fluoroquinolone moiety (**3e** and **f**) showed 100% inhibition and isoniazid containing prodrug showed 90% inhibition.

Table 1. Biological properties and stability of various prodrugs of stavudine

Compd	R	Anti HIV-1 activity (μM)			Antimycobacterial[d] activity (% inhibition)	In vitro hydrolysis $t_{1/2}$ (min)[e]
		EC_{50}[a]	CC_{50}[b]	SI[c]		
3a		<0.0636	>1000	>15,723	NT	20
3b	$-N(CH_3)_2$	0.343	456	1329	NT	30
3c		0.402	>200	>497	90	60
3d		0.520	567	1090	0	60
3e		61.80	695	11.24	100	240
3f		100.0	210	2.1	98	>240
Stavudine	—	0.09	>100	>1111	0	—

NT indicates not tested.
[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] At the dose of 6.25 μg/mL.
[e] In human plasma.

Table 2. Biological testing results from antibacterial screening: MIC's[a] in μg/mL

Microorganisms	3c	3d	3e	3f	Norfloxacin	Ciprofloxacin
1. *Klebsiella ozaenae*	9.76	1250	1.22	1.22	0.0381	0.0381
2. *Klebsiella pneumoniae*	1250	1250	1.22	1.22	0.0381	0.0095
3. *Shigella sonnei*	156.25	312.5	4.88	9.76	0.0381	0.0095
4. *Shigella boydii*	4.88	312.5	0.61	0.61	0.0381	0.0095
5. *Plesiomonas shigelloides*	312.5	312.5	0.61	9.76	0.0763	0.0190
6. *Morgonella morganii*	156.25	312.5	0.61	9.76	0.0763	0.0095
7. *Staphylococcus aureus*	156.25	1250	4.88	9.76	0.1526	0.0095
8. *Pseudomonas aeruginosa*	1250	1250	1.22	1.22	0.0763	0.0381
9. *Vibrio mimicus*	4.88	78.12	0.30	0.61	0.1526	0.0095
10. *Vibrio fluvialis*	1250	1250	0.61	9.76	0.0763	0.0095
11. *Vibrio cholerae* 0139	156.25	1250	0.61	0.61	0.0763	0.0095
12. *Vibrio cholerae* 01	1250	1250	0.30	0.61	0.0763	0.0095
13. *Vibrio parahaemolyticus*	1250	1250	0.30	1.22	0.0763	0.0095
14. *Escherichia coli* NCTC 10418	156.25	1250	0.30	1.22	0.0381	0.0190
15. *Edwardsiella tarda*	156.25	1250	4.88	9.76	0.0381	0.0095
16. *Proteus vulgaris*	312.5	1250	4.88	9.76	0.0381	0.0190
17. *Proteus mirabilis*	2.44	1250	0.30	1.22	0.0763	0.0095
18. *Salmonella typhimurium*	1250	1250	0.30	9.76	0.0763	0.0095
19. *Salmonella paratyphi A*	9.765	1250	0.30	1.22	0.0381	0.0190
20. *Salmonella typhi*	156.25	1250	0.30	9.76	0.0763	0.0190
21. *Salmonella enteritidis*	4.88	1250	0.61	9.76	0.0381	0.0095
22. *Citrobacter ferundii*	1250	1250	0.61	2.44	0.0381	0.0095
23. *Enterobacter*	312.5	39.06	0.30	0.30	0.0763	0.0190
24. *Bacillus megatherium*	2.44	39.06	0.30	0.30	0.0763	0.0095

[a] Concentration required to inhibit the growth of microorganisms.

These four compounds (**3c–f**) were also screened for in vitro antibacterial activity against 24 pathogenic bacteria by agar dilution technique,[11] and the results are summarized in Table 2. All the tested esters were found to be less active than the standard drugs ciprofloxacin and norfloxacin. Compound **3e** inhibited all the 24 bacteria with minimum inhibitory concentration less than 5 μg/mL.

4. In vitro stability studies

The usefulness of the prodrugs of stavudine 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 esters were therefore determined in human plasma.[12] The data in Table 1 indicated that the various esters of stavudine were susceptible to the action of plasma esterases with $t_{1/2}$ in the range of 20 to 240 min.

5. Conclusion

In the present study we have demonstrated that incorporation of antimicrobial agents into the stavudine as prodrugs lead to broad-spectrum chemotherapeutic activities. Thus these prodrugs would be beneficial for the effective treatment of HIV/AIDS. Furthermore this logic could be tried out with other potential anti-HIV drugs like nevirapine, zidovudine, etc., in the future.

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