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Bioorganic & Medicinal Chemistry Letters 16 (2006) 2127–2129
Bioorganic & Medicinal Chemistry Letters

Abacavir prodrugs: Microwave-assisted synthesis and their evaluation of anti-HIV activities

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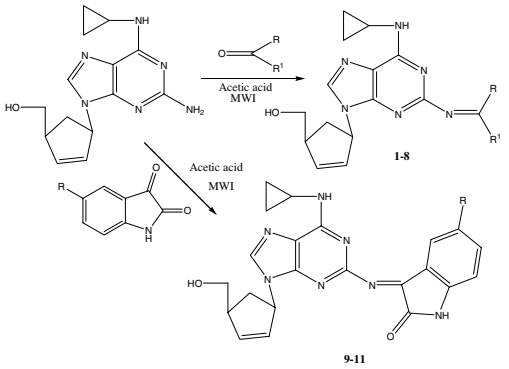
Received 25 November 2005; revised 2 January 2006; accepted 16 January 2006
Available online 3 February 2006

Abstract—The synthesis of a new series of abacavir prodrugs involving N^2-substitution with various substituted benzaldehyde and ketone derivatives is described. The in vitro anti-HIV activities indicated that compound (3-(2-(4-methylaminobenzylideneamino)-6-(cyclopropylamino)-9*H*-purin-9-yl)cyclopentyl)methanol (**3**) was found to be most potent compound with EC_{50} of 0.05 μM and CC_{50} of >100 μM with selectivity index of >2000. Compound **3** was found to be 32 times more potent than the parent drug (EC_{50} of 1.6 μM). At pH 7.4, 37 °C, the hydrolytic $t_{1/2}$ ranged between 120 and 240 min.

Acquired immunodeficiency syndrome (AIDS) is a degenerative disease of the immune and central nervous system caused by the human immunodeficiency virus (HIV). Invasion of the central nervous system (CNS) by the HIV, the causative agent of AIDS, leads to serious neurological disorders and may be a factor in the development of persistent HIV infections[1]. Many investigations have focused on the development of agents which can more readily penetrate the CNS by crossing the blood–brain barrier. These studies involved modification of the 5′-hydroxyl group of the parent anti-HIV nucleoside, for example, zidovudine[2,3], stavudine[4], abacavir[5], and 3′-azido-2′,3′-dideoxyuridine[6], or modification of the nucleoside base with lipophilic functional groups[7,8] or the phosphate groups of nucleotides[9]. These investigations aimed at increasing lipid solubility, since the correlation between lipophilicity, membrane permeability, and CNS penetration has long been established[10]. In this work, 11 abacavir Schiff bases were prepared and evaluated for their anti-HIV activity and also in vitro hydrolytic stability study was reported.

Esterification of the 5′-hydroxyl group of anti-HIV 2′,3′-dideoxynucleosides has been used extensively in attempts to improve brain uptake and efficacy[11]. In this paper, we modified the 2-amino group of (4-(2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl)cyclopent-2-enyl)methanol (abacavir). The synthesis of abacavir prodrugs proceeded smoothly by condensing parent drug with an equimolar ratio of carbonyl compounds in the presence of glacial acetic acid to form the Schiff's bases (**1**–**11**) which were *E* isomers (see Scheme 1). The reaction utilizes the microwave irradiation in an unmodified domestic microwave oven[12] at 80% intensity with 30 s/cycle for 3 min and set aside. The resultant solid was washed with dilute ethanol, dried, and recrystallized from ethanol–chloroform mixture. Yield: 64–89%. Unlike conventional methods (duration—3 h)[13], microwave-assisted reactions were very facile (2–3 min.), the product not requiring any further purification. The purity of the synthesized



Scheme 1. Synthesis of Schiff bases of Abacavir.

Keywords: Abacavir; Prodrugs; Anti-HIV activity; Schiff bases.
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doi:10.1016/j.bmcl.2006.01.050

compounds was checked by TLC and by elemental analyses, and the compounds of this study were identified by spectral data. In general, IR spectra showed C=N (azomethine) peak at 1640 cm^{-1} and NH-str at 3368 cm^{-1}. In the ^1H NMR spectra, the signals of the respective protons of the prepared prodrugs were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra showed a D_2O exchangeable broad singlet at δ 7.5 ppm corresponding to –NH– group; multiplet at δ 0.55–0.68 ppm for CH_2-cyclopropyl proton; double doublet for 5′ CH_2-group at δ 3.6 ppm (J = 5.4 Hz) and triplet at δ 5.2 ppm (J = 8.0 Hz) for OH group. All these compounds do not contain broad singlet at δ 5.01 ppm indicates that the primary amino group of abacavir reacts with the carbonyl compounds. The elemental analysis results were within ±0.4% of the theoretical values.

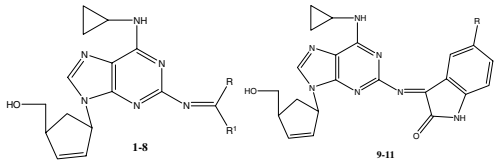
The synthesized compounds were evaluated for their inhibitory effect on the replication of HIV-1 in CEM cell lines[8] and their EC_{50} (effective concentration of compound (μM) achieving 50% protection in MT-4 cell lines against the cytopathic effect of HIV-1), and CC_{50} (cytotoxic concentration of compound (μM) required to reduce the viability of mock infected CEM cells by 50%), are reported in Table 1 with abacavir as standard drug for comparison. Rapid glance at the obtained results revealed that the compounds **1**–**11** exhibited excellent anti-HIV activity. Among the synthesized compounds, six compounds (**1**–**6**) were found to be more active than abacavir. Compound (3-(2-(4-methylaminobenzylideneamino)-6-(cyclopropylamino)-9*H*-purin-9-yl)cyclopentyl)methanol (**3**) was found to be most potent compound with EC_{50} of 0.05 μM and CC_{50} of >100 μM with selectivity index (CC_{50}/EC_{50}) of >2000. Compound **3** was found to be 32 times more potent than parent drug (EC_{50} of 1.6 μM). Schiff bases derived from substituted benzaldehyde were found to be more active than ketones. Among the substituted benzaldehyde series the order of activity is $–CH_3$ > $–N(CH_3)_2$ > $–OCH_3$ > NO_2. Nitro-substituted benzaldehyde derivatives were less active at HIV-1 replication, at the same time they are highly toxic to the CEM cell line.

The lipophilicity ($c\log P$) of the synthesized compounds increased remarkably compared with the parent drug, abacavir (Table 1). This may render them more capable of penetrating various biomembranes[14], consequently improving their permeation properties through viral cell membranes. The results showed that most of the compounds showed an improvement in anti-HIV activity compared to the parent drug.

The significance of the present study is that the Schiff bases have improved $\log P$ and thus expected to cross the blood–brain barrier effectively, as the sanctuary of HIV is the brain. Hence, these compounds could be more effective than the parent compound, Abacavir, in penetrating the brain.

The usefulness of the prodrugs of abacavir 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 at pH 7.4, 37 °C[2]. The data in Table 1 indicated that the various prodrugs of lamivudine were susceptible to hydrolysis with $t_{1/2}$ in the range of 120–240 min.

Table 1. Physical constants and anti-HIV activity of Abacavir Schiff bases



Compound	R	R^1	Mp (°C)	Yield (%)	$c\log P$[a]	EC_{50}[b] (μM)	CC_{50}[c] (μM)	$t_{1/2}$[d] (min)
1	H	2-Nitro phenyl	165	69	2.42	0.48	13	ND
2	H	4-Nitro phenyl	176	66	2.42	0.52	12	ND
3	H	4-Methyl phenyl	212	64	3.38	0.05	>100	150
4	H	4-Methoxy phenyl	232	70	2.77	0.36	>100	240
5	H	4-Dimethylamino phenyl	167	89	3.18	0.06	>100	120
6	H	2-Hydroxy-4-methoxy phenyl	172	68	2.38	1.2	100	ND
7	CH_3	4-Hydroxy phenyl	201	71	2.07	1.9	62	ND
8	C_6H_6	4-Bromo phenyl	223	68	5.18	3.7	45	ND
9	H	—	215	82	1.45	5.0	48	ND
10	F	—	158	87	1.61	6.2	56	ND
11	CH_3	—	184	78	1.94	2.1	78	ND
Abacavir	—	—	—	—	0.22	1.6	83	—

[a] Calculated using Chemdraw ultra 8.0 program.
[b] Effective concentration of compound achieving 50% protection in CEM cell lines against the cytopathic effect of HIV-1.
[c] Cytotoxic concentration of compound required to reduce the viability of mock infected CEM cells by 50%.
[d] At pH 7.4 at 37 °C, and 'ND' indicates not determined.

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