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Synthesis and in vitro anti-HIV activity of *N*-1,3-benzo[*d*]thiazol-2-yl-2-(2-oxo-2*H*-chromen-4-yl)acetamide derivatives using MTT method

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ABSTRACT

A series of novel *N*-1,3-benzo[*d*]thiazol-2-yl-2-(2-oxo-2*H*-chromen-4-yl)acetamide derivatives has been synthesized. All the newly synthesized compounds were evaluated for their anti-HIV activity using MTT method. Most of these compounds showed moderate to potent activity against wild-type HIV-1 with an EC_{50} ranging from >7 EC_{50} [μg/ml] to <100 EC_{50} [μg/ml]. Among them, *N*-1,3-benzo[*d*]thiazol-2-yl-2-(2-oxo-2*H*-chromen-4-yl)acetamide **6v** was identified as the most promising compound (EC_{50} = <7 μg/ml). Among all the compounds, three compounds **6m**, **6v** and **6u** have been exhibits potent anti-HIV activity against MT-4 cells.

In the present era, acquired HIV (Human Immunodeficiency Virus) is the most fatal disorder for which no completely successful chemotherapy has been developed so far. A variety of anti-HIV agents rely on inhibition of key enzymes involved with the virus life cycle. Over 20 anti-retroviral medications have now been approved for the treatment of HIV-infected individuals.[1] The current therapy against the human immunodeficiency virus type 1 (HIV-1), which is the etiological agent of acquired immunodeficiency syndrome (AIDS), is based on six of categories drugs: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs); nonnucleoside reverse transcriptase inhibitors (NNRTIs); protease inhibitors (PIs); cell entry inhibitors [fusion inhibitors (FIs) and co-receptor inhibitors (CRIs)]; and integrase inhibitors (INIs).[2] HAART (Highly active anti-retroviral therapy) has demonstrated that targeting multiple enzymes provides one means of circumventing this problem, with combination therapies directed against both the HIV reverse transcriptase and the protease enzymes showing good results in reducing viral loads in HIV-1 infected patients.[3] However, the benefits of HAART as the standard AIDS chemotherapy are compromised by high cost and severe toxicity,[4,5] both resulting from using multiple drugs.

The nonnucleoside reverse transcriptase inhibitors (NNRTIs) are a structurally and chemically dissimilar group of anti-retrovirals that are potent and highly selective inhibitors of HIV-1 reverse transcriptase.[6] NNRTIs[7–11] bind to the nonnucleoside binding site (NNBS), an allosteric hydrophobic pocket located about 10 Å far from the polymerase active site. However, the above inhibitors failed in monotherapy, combination protocols were designed with both RTIs and PRIs in order to increase the clinical efficacy and reduce the emergence of resistant variants. Rapid development of drug resistance and toxicity problems make urgent the need to investigate new targets in the replicative cycle of HIV-1 to develop inhibitors different from RTIs and PRIs.[12,13] Apart from RT and PR, another target useful for chemotherapeutic intervention is the HIV-1 integrase (IN), an enzyme which catalyzes the insertion of the viral DNA into the genome of the host cell through a multistep process.[14,15] However, these drugs have only limited or transient clinical benefit due to their side effects and the development of drug-resistant viral strains.[16] Therefore, current searches for new anti-HIV agents are focused on discovering compounds with novel structures and different mechanisms of action.

Numerous different classes of compounds[17,18] have been reported as potent anti-HIV-1 agent. Coumarins (2-oxo-2*H*-chromen) have been found to exhibit a wide range of biological and controlled therapeutic activities in view of their extensive occurrence in nature and wide range of toxicity. (+)-Calanolide,[19,20] a natural dipyranocoumarin, currently undergoing anti-AIDS clinical trials, has also proven to be an effective anti-mycobacterial against drug-sensible and drug-resistant Mycobacterium tuberculosis strains. Several heterocyclic substituted and fused coumarin derivatives are widely used in drugs. The anti-HIV-1 compounds such as 4-phenyl

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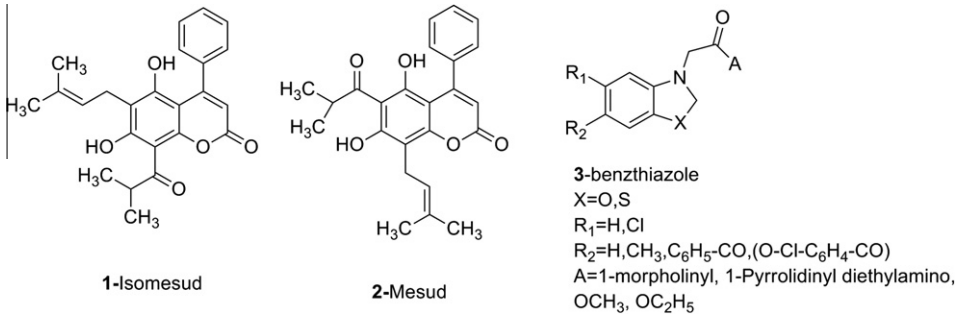


Figure 1. Biologically active anti-HIV molecules.

coumarins (Fig. 1, **1-2**), which could serve as lead compounds for the development of additional therapeutic approaches against AIDS. Benzothiazole containing five member heterocyclic compounds and several benzothiazole derivatives show considerable biological activities. Moreover, benzothiazoles (Fig. 1, **3**) showed inhibitors of HIV-1 protease with improved potency and anti-viral activities.[21]

In our efforts to discover novel anti-HIV agents[22–25] and synthesis of various biologically active heterocycles,[26–29] we have synthesized diverse *N*-1,3-benzo[*d*]thiazol-2-yl-2-(2-oxo-2*H*-chromen-4-yl)acetamides by the condensation of coumarin-4-acetic acids and respected benzothiazoles (scheme 1). All the synthesized *N*-1,3-benzo[*d*]thiazol-2-yl-2-(2-oxo-2*H*-chromen-4-yl)acetamides were screened for their in vitro anti-HIV activity against human HIV cell line. These compounds required to achieve 50% protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method.

To synthesize substituted coumarin-4-acetic acid (scheme 1) a mixture of citric acid monohydrate (0.1 M) and 28 ml of concd sulphuric acid was stirred at room temperature for 60 min and then slowly heated to 70 °C. After 30 min, the evolution of carbon monoxide had slackened and the clear yellow colored solution was rapidly cooled to 0 °C. To this stirred solution, phenol (0.08 M) was added and 11.2 ml of concd sulphuric acid, each in three equal portions, at a rate that the temperature does not exceed 10 °C. The resulting solution was stored at 0 °C for 16 h, poured into ice and the resulting crystalline precipitate was filtered off and washed with water. It was then treated with 10% sodium bicarbonate solution and filtered out. The filtrate, on acidification gave substituted coumarin-4-acetic acid. The physical data of all the synthesized compounds are cited in Table 1.

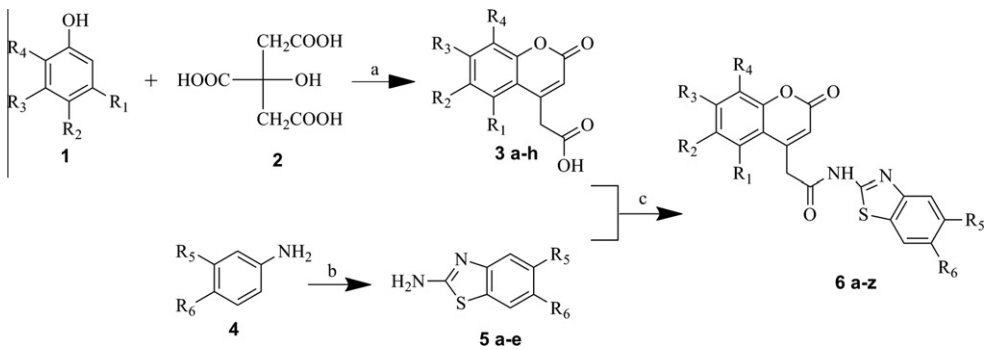
MT-4 cells were obtained from institutions such as MRC and NIH. 'Complete medium': RPMI-1640 medium with 20 mM HEPES buffer, supplemented with 10% (v/v) heat-inactivated FCS, 2 mM L-glutamine, 0.1% sodium bicarbonate and 20 mg/ml gentamicin. Titration of HIV was performed in MT-4 cells by the standard limiting dilution method (dilution 1:2, four replica wells per dilution) in 96-well plates. The infectious virus titer was determined by light microscope scoring of syncytia after four days of incubation. Virus titers were expressed as CCID50/mL.

A 20 ml of MTT (7.5 mg/ml) solution (warmed to 37 °C) was added to each well of the microtiter plates using the titertek multidrop dispenser. The trays were incubated at 37 °C in a CO_2 incubator for 1 h. A constant volume of medium (e.g., 150 ml) was removed from each cup using a multichannel pipette or a microplate washer without disturbing the MT-4 cell clusters containing the formazan crystals. The fomazan crystals were solubilized by addition 100 ml of the acidified triton X-100 isopropanol solution to each cup using the microplate washer. It was dissolved completely using vibrating platform shaker for 10 min. The absorbance was examined in an eight-channel computer-controlled multiskan ascent reader and stacker at two wavelengths (540 and 690 nm). The absorbance measured at 690 nm from the absorbance at 540 nm was subtracted to eliminate the effects of scattering by cell debris.

CC_{50} is the concentration of compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration (μg/ml) required to achieve 50% protection in HIV-infected cells was calculated by the following formula.

$$EC_{50} = \frac{(OD_T)_{HIV} - (OD_C)_{HIV}}{(OD_C)_{MOCK} - (OD_C)_{HIV} \times 100}$$

where (ODT)HIV is the OD measured with a given concentration of the test compound in the HIV-infected cells; (ODC)HIV is the OD measured for the control untreated, HIV-infected cells, which stands for 100% infection-related CPE; and (ODC)mock is the OD measured for the control untreated, mock-infected cells, which stands for 0% infection-related CPE. A strict scheme of cell cultivation (subcultivating the cells every 3–4 d, seeding at 6–105 cells per ml for MT-4 cells) was necessary to avoid overgrowth of the uninfected cells and decrease of cellular viability after five day incubation.



Scheme 1. Synthesis of various *N*-1, 3-benzo[*d*]thiazol-2-yl-2-(2-oxo-2*H*-chromen-4-yl)acetamides. Reagents and condition: (a) concd sulfuric acid; (b) KCNS, Br_2, CH_3COOH, 0 °C; (c) THF, pyridine, 0-5 °C, $SOCl_2$.