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Synthesis and anti-HIV activity of some 3-acetyl/acetoacetyl-4-hydroxy benzopyran-2-ones: An *in vitro* evaluation

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ABSTRACT

In the present investigation, ten set of compounds were synthesized and characterized by NMR, IR, mass spectroscopy and CHN(O) analysis. The compounds were screened for their *in vitro* anti-HIV activity against HIV-1(IIIB) and HIV-2(ROD) virus strains. The results are summarized.

KEYWORDS

3-Acetyl/
Acetoacetyl coumarins;
HIV-1/HIV-2 screening.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is one of the greatest challenges to humankind. AIDS and HIV infection represent global health hazards, complex scientific puzzles, obvious targets for drug discovery and vaccination and both have enormous social, economical and ethical ramifications[1-3].

Three different classes of chemotherapeutic agents are actually used to inhibit the replication of HIV-1, the etiological agent of AIDS: the nucleoside(NRTI) and non-nucleoside (NNRTI) reverse transcriptase inhibitors, the protease inhibitors (PR) and the inhibitors of the fusion of the virus with host cell[4].

The highly active anti-retro viral therapy (HAART), which is based on the use of a combination of a cited drugs, effectively inhibit the replication cycle of HIV-1. The advent of HAART has made possible the suppression of the HIV-1 replication to such an extent that the virus becomes undetectable in the blood of infected persons. However, HAART fails to irradicate viral replications, which persist at a low level in cellular reservoirs, despite the chemotherapy[5].

The ability of HIV-1 to evolve drug resistance and the toxicity of HAART regimens make integrase (IN), which is the third virally encoded enzyme required for HIV-1 replication, a legitimate target for the development of new drugs. Moreover, IN has no cellular counterparts, and thus came into sight 10 years ago as a new therapeutic opportunity[6-9]. Hopefully, integrase inhibitors will become a potential additive to HAART or a salvage therapy for patients resistant to currently available anti-HIV drugs[9].

Numerous scaffolds have been reported as IN inhibitors[9-10] of which most important class is typified by an aryl β-diketo acid motif(DKAs)[11]. (Compounds

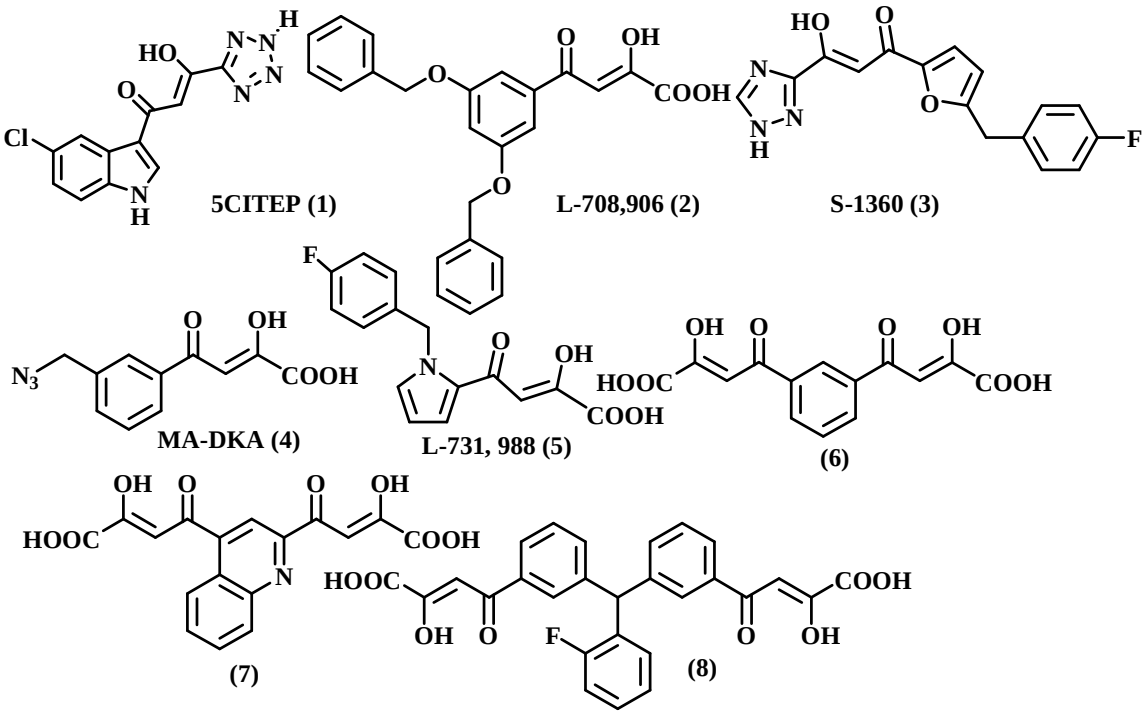


Figure 1 : Diketo acid (DKAs) anti-HIV analogues

1-5, Figure 1)

DKAs selectively inhibit the ST reaction of IN and exhibit antiviral activity against HIV-1 infected cells in a manner consistent with inhibition of integration. These compounds are also useful tools to explore the molecular mechanism[11,12,13].

More recently, some bifunctional DKAs (BDKAs) were reported, which are characterized by the presence of two diketo acid chains in the skeleton of the IN inhibitors(Compounds **6-8**, Figure 1)[14-16].

The molecular binding of DKA to integrase complex has been a focus of research because of the importance of DKAs and DKA-like derivatives as antiviral lead compounds and their unique mechanism of action[9].

EXPERIMENTAL

All the starting materials were purchased from Spectrochem, Mumbai and used without further purification. Elemental analysis of the compounds was carried out on Elementar Vario EL III Carlo Erba 1108 model.NMR spectra were recorded on bruker avance II 200MHz, spectrometer in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on shimadzu GCMS-QP2010 and IR spectra were recorded on a shimadzu FTIR-8400 using KBr optics.

Chemistry

General method for synthesis of substituted 3-acetyl-4-hydroxychroman-2-one(2a-e)

It was synthesized according to reported method[17].

A solution of substituted 4-hydroxycoumarin (3.0g, 0.019 mol) in dry pyridine(24mL) and piperidine (1-2drops) was cooled to 0-5^{0}C. Then acetylchlo ride (2.17g, 0.028mol) was added and the reaction mixture was stirred for 48 h at 20^{0}C. The dark red reaction mixture was then poured onto crushed-ice and the product brought to pH 1-2 with 2M HCl. The precipitate was filtered and washed with water until neutral pH and dried.(Yield 71-77%)

Synthesis of 1-(4-hydroxy-2-oxo-2H-substituted chromen-3-yl)butane-1,3-dione(3a-e)

Potassium tert butoxide(5.0g, 45mmol) was suspended in dry toluene (125ml) under nitrogen atmosphere. The suspension was cooled down to 0^{0}C and a solution of substituted 4-hydroxy-3-acetylcoumarin (5.0g) in dry toluene was added and kept it at 0^{0}C. Ethyl acetate was added dropwise through dropping funnel at 0^{0}C and stirr the reaction mixture at room tem-

perature of 12h, there was no progress of reaction, then the reaction mixture was heated at 60^0C for an appropriate time. The reaction was monitored by TLC. After the completion of reaction as indicated by TLC (ethylacetate:hexane:6:4), toluene was removed and the resulting crude product was extracted with ethyl acetate and washed with brine and dried over anhydrous sodium sulphate to leave crude product. Finally, the product was purified on silica gel column chromatography (60-100mesh) using ethyl acetate/hexane as eluents.

3-Acetyl-4-hydroxyl-2H-chromen-2-one (2a)

^{1}H-NMR($CDCl_3$), δppm: 2.42(s, 3H, -$COCH_3$), 4.2(brs, 1H, -OH), 7.71-7.25(m, 4H, Ar-H); EI-MS(m/z): 204(m^+ 60), 189(58.2), 168(100), 140 (52.9), 89(28.3), 77(22.8); IR(KBr, cm^{-1}): 3424, 2890, 1731, 1540, 1360, 832. Anal.Calcd. For $C_{11}H_8O_4$: C; 64.73, H; 3.92. Found: C;64.73; H; 3.88

3-Acetyl-4-hydroxy-6-methyl-2H-chromen-2-one(2b)

^{1}H-NMR($CDCl_3$), δppm: 2.46(s, 3H, -CH_3), 2.78 (s, 3H, -$COCH_3$), 4.10(brs, 1H, -OH), 7.25-7.54 (m, 3H, Ar-H); EI-MS(m/z): 218(m^+ 62), 204(24), 190 (28), 162(72), 148(100), 120(14); IR(KBr, cm^{-1}): 3413, 3082, 2926, 1717, 1606,1364; Anal.Calcd. For $C_{12}H_{10}O_4$: C; 66.06, H; 4.59, Found: C, 66.06, H; 4.62.

3-Acetyl-4-hydroxy-5,7-dimethyl-2H-chromen-2-one (2c)

^{1}H-NMR($CDCl_3$), δppm: 2.21(s, 3H, -CH_3), 2.41(s, 3H, -CH_3), 2.71(s, 3H, -$COCH_3$), 4.05(brs, 1H, -OH), 7.51(d, 1H, J=1.5, Ar-H), 7.62(d, 1H, J=1.2, Ar-H); EI-MS(m/z): 232(m^+ 100), 218(59.4), 217(25.2), 204(33.9), 190(72.1), 178(19.5), 148(14.7), 122(12.3), 120(26.2), 91(33.5); IR (KBr, cm^{-1}): 3429, 3070, 2925, 1710, 1610, 1338; Anal. Calcd. For $C_{13}H_{12}O_4$: C; 67.24, H; 5.17, Found: C, 67.19, H; 5.21.

3-Acetyl-4-hydroxy-5,8-dimethyl-2H-chromen-2-one (2d)

^{1}H-NMR($CDCl_3$), δppm: 2.23(s, 3H, -CH_3), 2.27(s, 3H, -CH_3), 2.77(s, 3H, -$COCH_3$), 4.21(brs, 1H, -OH), 7.51(d, 1H, J=8.5, Ar-H), 7.65(d, 1H, J=9.1); EI-MS(m/z): 231(m^+ 52), 219(6.1), 217 (22.2), 203(22.6), 176(19.5), 175(25.7), 135(36.7), 134(50.2); IR (KBr, cm^{-1}): 3441, 3080, 2933, 1732, 1604, 1335; Anal. Calcd. For $C_{13}H_{12}O_4$: C; 67.24, H; 5.17, Found: C, 67.25, H; 5.18.

3-Acetyl-4-hydroxy-7,8-dimethyl-2H-chromen-2-one (2e)

^{1}H-NMR($CDCl_3$), δppm: 2.01(s, 3H, -CH_3), 2.19(s, 3H, -CH_3), 2.93(s, 3H, -$COCH_3$), 3.99(brs, 1H, -OH), 7.35(d, 1H, J=8.4, Ar-H), 7.56(d, 1H, J=7.9); EI-MS(m/z): 232(m^+ 68), 218(35.5), 190 (42.5), 182(100), 148(88.3), 120(20.8), 91(38.5), 77 (22.5); IR (KBr, cm^{-1}): 3418, 3095, 2920, 1726, 1615, 1344; Anal. Calcd. For $C_{13}H_{12}O_4$: C; 67.24, H; 5.11, Found: C, 67.28, H; 5.12.

1-(4-hydroxy-2-oxo-2H-chromen-3-yl)butane-1,3-dione (3a)

^{1}H-NMR ($CDCl_3$), δppm: 4.15(brs, 1H, -OH), 2.44(s, 3H, -CH_3), 4.19(s, 2H, -CH_2), 7.81-6.35(m, 4H, Ar-H); EI-MS (m/z): 246(m^+54), 231(29.8), 229 (23.6), 205(25.3), 204(25.9) 189(100), 162(45.1), 44 (10.6); IR (KBr, cm^{-1}): 3419, 3100, 2930, 1710, 1604, 1333; Anal. Calcd. For $C_{13}H_{10}O_5$: C; 63.41, H; 4.07, Found: C, 63.38, H; 4.05.

1-(4-hydroxy-6-methyl-2-oxo-2H-chromen-3-yl)butane-1,3-dione (3b)

^{1}H-NMR ($CDCl_3$), δppm: 4.04(brs, 1H, -OH), 2.57(s, 3H, -CH_3), 2.95(s, 3H, -$COCH_3$) 4.25(s, 2H, -CH_2), 7.81-6.75(m, 3H, Ar-H); EI-MS (m/z): 260(m^+54), 233(11.2), 232(26.6), 218(17.6), 208 (9.3), 190(27.6), 165(35.8), 148(100), 147(15.6), 134 (65.1), 91(33.1); IR (KBr, cm^{-1}): 3420, 3090, 2929, 1715, 1605, 1332; Anal.Calcd. For $C_{14}H_{12}O_5$: C; 64.62, H; 4.62, Found: C, 64.58, H; 4.59.

1-(4-hydroxy-5,7-dimethyl-2-oxo-2H-chromen-3-yl)butane-1,3-dione(3c)

^{1}H-NMR ($CDCl_3$), δppm: 4.09(brs, 1H, -OH), 2.21(s, 3H, -CH_3), 2.35(s, 3H, -CH_3), 2.95(s, 3H, -$COCH_3$), 4.25(s, 2H, -CH_2) 7.35(d, J=1.9, 1H, Ar-H), 7.65(d, J=1.7, Ar-H); EI-MS (m/z): 274(m^+28.2), 257(15.6), 232(54.3), 217(22.3), 190(45.8), 148(12.3), 120(60.2), 91(9.1), 44(100); IR (KBr, cm^{-1}): 3425, 3072, 2935, 1721, 1600, 1338; Anal. Calcd. For $C_{15}H_{14}O_5$: C; 65.69, H; 5.11, Found: C, 65.71, H; 5.09

1-(4-hydroxy-5,8-dimethyl-2-oxo-2H-chromen-3-yl)butane-1,3-dione(3d)

^{1}H-NMR ($CDCl_3$), δppm: 4.12(brs, 1H, -OH), 2.25(s, 3H, $-CH_3$), 2.41(s, 3H, $-CH_3$), 3.1(s, 3H, $-CH_3$), 4.10(s, 2H, $-CH_2$), 7.35(d, J= 8.2, 1H, Ar-H), 7.68(d, J=8.1, 1H, Ar-H) EI-MS (m/z): 274(m^+28), 257(7.1), 232(18.9), 217(15.6), 190(20.3), 148 (55.2), 120(100), 91(52.5), 44(27.3); IR (KBr, cm^{-1}): 3423, 3130, 2931, 1725, 1621, 1329; Anal. Calcd. For $C_{15}H_{14}O_5$: C; 65.69, H; 5.11, Found: C, 65.67, H; 5.09

1-(4-hydroxy-7,8-dimethyl-2-oxo-2H-chromen-3-yl)butane-1,3-dione (3e)

^{1}H-NMR ($CDCl_3$), δppm: 4.17(brs, 1H, -OH), 2.25(s, 3H, $-CH_3$), 2.29(s, 3H, $-CH_3$) 2.95(s, 3H, $-COCH_3$), 7.68(d, J=8.2, 1H, Ar-H), 7.87(d, J=7.8, 1H, Ar-H); EI-MS (m/z): 274(m^+38), 259(15.2), 232 (18.5), 217(15.3), 190(20.2), 148(55.4), 120(100), 91(52.5), 44(27.3); IR (KBr, cm^{-1}): 3430, 3127, 2928, 1722, 1605, 1327; Anal. Calcd. For $C_{15}H_{14}O_5$: C; 65.69, H; 5.11, Found: C, 65.65, H; 5.10

Biology

***In vitro* antiviral assays**

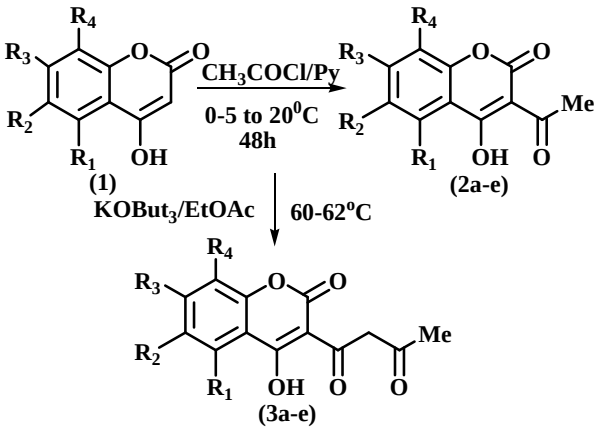
Evaluation of the antiviral activity of the compounds against HIV-1(strain III_B) and HIV-2 (strain ROD) in MT-4 cells was performed using the MTT assay as previously described[18]. Stock solutions(10x final concentration) of test compounds were added in 25μL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 2000 robot(Beckman Instruments, Fullerton,CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(III_B)[9] or HIV-2 (ROD)[20] stock (50μL) at 100-300$CCID_{50}$(cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of the test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells[21] were centrifuged for 5min at 1000rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and an amount of 50μL volumes was transferred to the microtiter tray wells. Five days after infection, the viability of mock-and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan ascent reader, Labsystems, Helsinki, Finland), at two wavelengths(540 and 690nm). All data were calculated using the median OD(optical density) value of three wells. The 50% cytotoxic concentration(CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection against the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

RESULTS AND DISCUSSION

In the current paper, ten different 3-acetyl and acetoacetyl 4-hydroxy benzopyrans-2-ones were synthesized having various electron withdrawing and electron releasing group on the benzenoid part of the coumarin moiety. The acetylation of the 4-hydroxy coumarins takes place exclusively on 3-position of the



SCHEME 1

Full Paper

TABLE 1 : Physical data of 3-acetyl and 3-acetoacetyl-4-hydroxycoumarin

Comp. code	Substitution				MP/^{0}C	Yield/%
	R_1	R_2	R_3	R_4		
2a	H	H	H	H	120-122	69
2b	H	CH_3	H	H	145-147	71
2c	CH_3	H	CH_3	H	150-152	65
2d	CH_3	H	H	CH_3	150-151	69
2e	H	H	CH_3	CH_3	135-137	75
3a	H	H	H	H	138-140	78
3b	H	CH_3	H	H	147-148	62
3c	CH_3	H	CH_3	H	135-136	66
3d	CH_3	H	H	CH_3	122-124	71
3e	H	H	CH_3	CH_3	165-167	67

TABLE 2 : *In vitro* anti-HIV activity

Code	Strain	EC_{50} (μg/ml)	EC_{90} (μg/ml)	CC_{50} (μg/ml)	SI	Max protection
2a	IIIB	>41	>41	40.5	<1	2
2b	IIIB	>13	>13	12.7	<1	9
2c	IIIB	>9	>9	8.7	<1	0
2d	IIIB	>10	>10	9.8	<1	10
2e	IIIB	>12	>12	11.9	<1	3
3a	IIIB	>2	>2	2.3	<1	0
3b	IIIB	>61	>61	61.1	<1	0
3c	IIIB	>9	>9	9.4	<1	0
3d	IIIB	>14	>14	14	<1	1
3e	IIIB	>32	>32	32.1	<1	5
3a	ROD	>13	>13	12.8	<1	8
3b	ROD	>11	>11	12.9	<1	5
3c	ROD	>11	>11	12.7	<1	2
AZT (Zidovudine)	ROD	0.0018	-	11.50	6453	-

IIIB(HIV 1 strain), ROD (HIV-2 strain), EC_{50}=50% Effective Concentration, EC_{90}=90% Effective Concentration, CC_{50}=50% Cytotoxic Concentration, SI=Selectivity Index =ratio of CC_{50} to EC_{50}

coumarin ring, this is due to the keto-enol tautomeric forms of 4-hydroxycoumarin. For the possible anti-HIV activity of these compounds were screened for HIV-1(ROD) and HIV(III B). Screening was carried out according to standard protocol reported in the *in vitro* anti viral assay. Compounds **(2c),(3a)** and**(3c)** show EC_{50} 9, 2 and 9 respectively. CC_{50} of all the compounds are almost same to the EC_{50}. Ideally EC_{50} should be low as to indicate the selectivity of the compounds for anti-HIV activity. Comparing the activity of these molecules against particular HIV strain, **(2c)** was found to be most active. Selectivity index of all compounds were found to be <1 which clearly indicate the toxicity of the compounds against the cell lines.

CONCLUSION

From the findings, we conclude that, all the newly synthesized keto compounds did not show promising antiviral activity. The prime reason for the same is the ketonic form of the 4-hydroxycoumarins and hence not acts as hydroxy acid like structures.

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