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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, biological screening and ADME prediction of benzylindole derivatives as novel anti-HIV-1, anti-fungal and anti-bacterial agents

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Abstract Present study is focused on design, synthesis, and biological evaluation of substituted benzylindole derivatives as anti-HIV, anti-fungal, and anti-bacterial agents. Out of the reported compounds, compound **B1** and **B2** showed potent Anti-HIV activity, whereas compound **B1**–**B4** showed good anti-fungal and anti-bacterial activity. ADME properties of benzylindol analogs were analyzed using Qikprop 2.5 tool of Schrodinger.

Keywords HIV-1 · Reverse transcriptase inhibitor · Benzylindol · ADME prediction

Introduction

It has been recently reported that more than 33 million of people were infected worldwide with human immunodeficiency virus (HIV-1). The overall number of people living with HIV has increased as a result of new infections and the beneficial effects of the more widely available highly active anti-retroviral therapy (HAART), which employs a combinational use of drugs (Este and Cihlar, 2010).

Currently, FDA-approved anti-HIV drugs belong to seven different groups: nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), co-receptor inhibitors (CRIs), and integrase inhibitors (INIs) (Mehellou and De Clercq, 2010).

Although the HAART has brought about a substantial decrease in the death rate, changing AIDS from a rapidly lethal disease into a chronic manageable condition, the retroviral infection can be only temporarily controlled but not eradicated since, HIV-1 becomes almost undetectable in the plasma for more than 2 years, persisting in reservoirs.

Furthermore, the HAART efficacy has been limited by the emergence of drug-resistant viral strains, drug-toxicity, and the poor ability of patients to adhere to the prescribed therapy and costs, so a refining of the current therapies and the developing of new therapeutic paradigms are still warranted (Sechi *et al*., 2009).

NNRTIs are a structurally diverse group of compounds which binds to the viral enzyme RT, where it interacts with a specific allosteric non-substrate binding pocket site. NNRTIs resistance mutations arise rapidly and most often directly in contact with the NNRTI molecule. Therefore, attempts have been made to develop NNRTIs that are active against resistance mutations. These attempts have led to the identification of a number of compounds that are active against resistance mutations. Currently, drugs which are used to treat AIDS under NNRTIs are Nevirapine (**1**), Efavirenz (**2**), Etravirine (**3**), Tenofovir desoproxil (**4**), Delaviridine (**5**), and Rilpivirine (**6**) (Fig. 1) (Balzarini *et al*., 2004).

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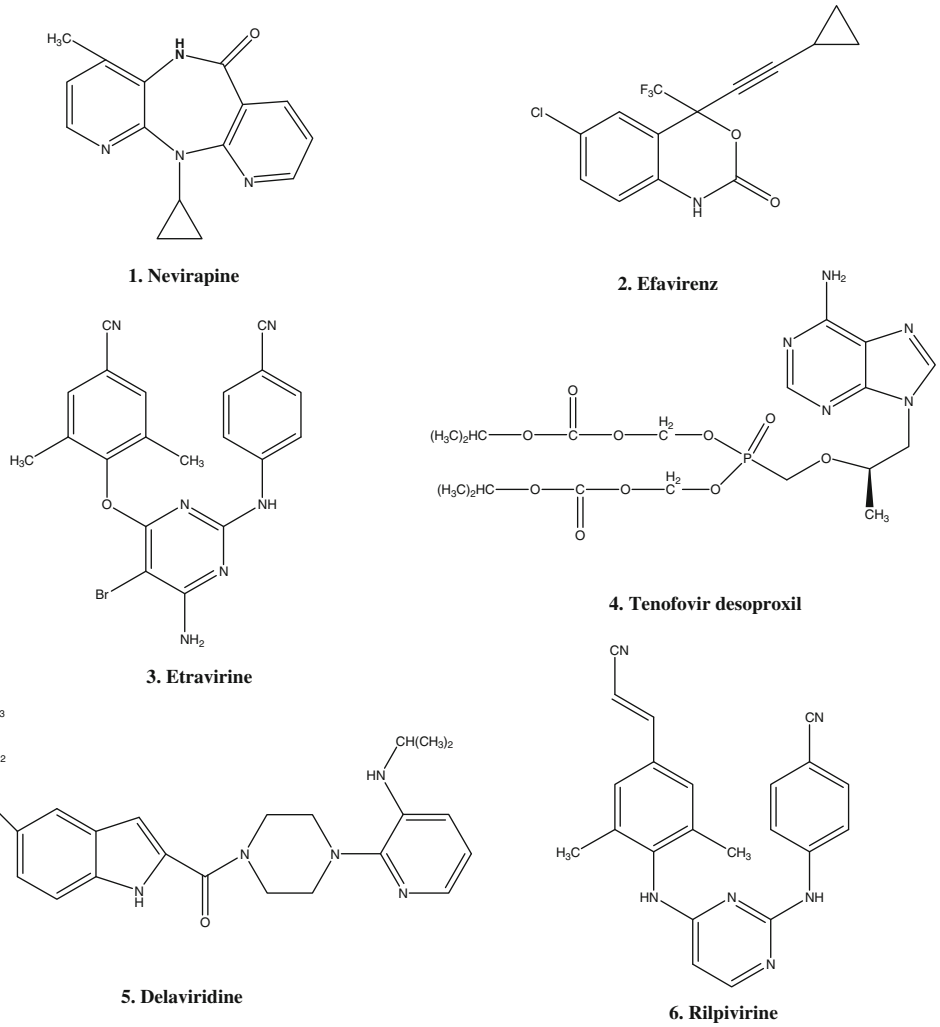
Fig. 1 Clinically used NNRTIs



From the literature survey, it was observed that the butterfly-like shape is an important factor in the binding of the first generation NNRTIs. Despite their chemical diversity, they assume very similar butterfly-like shape. The butterfly structure has a hydrophilic centre as a 'body' and two hydrophobic moieties representing the 'wings'.

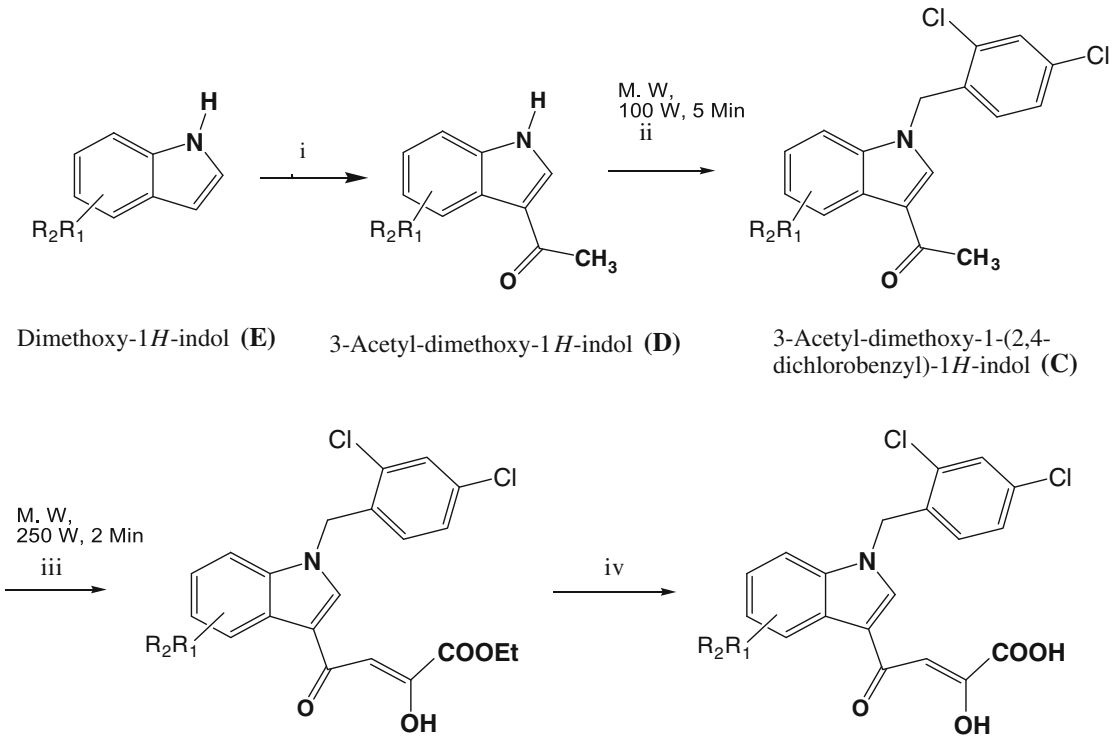
Based on the above observation, we thought it was logical to design a new molecular scaffold containing benzylindol. Due to the importance of their indole back bone, the compound showed a variety of biological activities such as anti-bacterial (Daisley and Shah, 1984; Varma and Nobles, 1967), anti-fungal (Varma and Nobles, 1967; Piscapo *et al.*, 1987), anti-HIV (Pandeya *et al.*, 1999, 2000), and anti-convulsant activity (Bhattacharya and Chakraborti, 1998). Owing to the broad spectrum of chemotherapeutic properties, it appears as an ideal drug for AIDS treatment which suppresses HIV replication there by acting as an anti-HIV drug as well as possesses efficacy against opportunistic infections associated with AIDS like tuberculosis, hepatitis, and other bacterial diseases.

In this study, we have focused on a new series of benzylindole derivatives with various substituents at the benzene-fused ring, also to ensure drug-like pharmacokinetic profile of the designed NCEs, the ADME properties were calculated.

Result and discussion

Chemistry

The synthetic route employed to obtain the title compounds is depicted in Scheme 1. Dimethoxyindoles **E1**–**E4** were 3-acetylated by a Vilsmeier Haack reaction and *N*-alkylated by treatment with 2,4-dichlorobenzyl chloride, in the presence of a catalytic amount of sodium hydride to give intermediates **C1**–**C4**. Successively, diketo esters **B1**–**B4**, obtained by coupling with diethyl oxalate, were converted in the final derivatives **A1**–**A4** by hydrolysis in basic medium. The introduction of a nitro



Dimethoxy-1*H*-indol **(E)**

3-Acetyl-dimethoxy-1*H*-indol **(D)**

3-Acetyl-dimethoxy-1-(2,4-dichlorobenzyl)-1*H*-indol **(C)**

4-(1-(2,4-Chlorobenzyl)-dimethoxy-1*H*-indol-3-yl) -2-hydroxy-4-oxobut-2-enoates **(B)**

4-(1-(2,4-Chlorobenzyl)-dimethoxy-1*H*-indol-3-yl)-2-hydroxy-4-oxobut-2-enoic acid **(A)**

Scheme 1 Synthetic scheme of designed compounds (**A1**–**A4**, **B1**–**B4**) Reagents: (*i*) phosphoryl chloride, dimethylacetamide, 4 N NaOH; RT 12 h; (*ii*) DMF, sodium hydride, 2,4-dichlorobenzyl chloride; 100 W, 5 min, 50 °C; (*iii*) diethyl oxalate, $NaOCH_3$, THF; 250 W, 2 min, 50 °C (*iv*) 2 N NaOH; RT 1.5 h

group on 2,3-Dimethoxybenzaldehyde afforded a mixture of 5- and 6-nitro derivatives which were separated by flash chromatography on silica gel eluting with 30 % EtOAc/cyclohexane (Fukuyama *et al*., 1998). The 2,3-dimethoxy-6-nitrobenzaldehyde was converted into the corresponding 2,3-dimethoxy-6,b-dinitrostyrene by refluxing with nitromethane in the presence of glacial acetic acid and ammonium acetate. Finally, 4,5-dimethoxyindole (**E1**) was obtained by a Henry's reductive cyclization (Henry, 1895).

5,7-Dimethoxyindole was synthesized according to Condie's procedure, obtaining high yields. A solution of 3,5-dimethoxybenzaldehyde in nitromethane was refluxed to afford 3,5-dimethoxy-b-nitrostyrene which was converted in the nitro derivative and then into the desired product by reductive cyclization (Condie *et al*., 2005).

A different synthetic pathway, according to a modification of Rege's procedure, was followed to obtain the dimethoxy substitution at the 6-C and 7-C positions of the indole nucleus. The treatment of commercially available 3-methoxy-4-hydroxybenzaldehyde with acetic anhydride provided the *O*-acyl protected intermediate which, after nitration, was hydrolyzed under basic conditions. Methylation of the hydroxyl group with dimethyl sulfate followed by reaction with nitromethane and reductive cyclization, resulted in 6,7-dimethoxyindole (Rege *et al*., 2006). For the designed NCE_S, yield obtained for each product and their physicochemical properties are mentioned in Table 1.

Biological activity

The standard nevirapine and test compounds (**A1**–**A4**, **B1**–**B4**) were powdered finely and suspended in DMSO. The RT inhibition assay was performed by using an RT assay kit (Roche). The procedure for performing RT inhibition assay was carried out as described in the kit protocol (Table 2).

It has been observed from in vitro screening that newly synthesized compounds possess RT inhibitory activity. Among the synthesized compounds, **B1**–**B4** showed a more significant RT inhibitory activity. The compound in which acid group was substituted at C3 position on the benzylindol ring showed lesser RT inhibitory activity in comparison with ester at C3 position except B3. The hydrophobic groups are important for RT inhibitory activity at fused benzene ring.

Table 1 Characterization of synthesized compounds (**A1**–**A4**, **B1**–**B4**)

Compound ID	Derivatives (R_1R_2)	Molecular weight	% yield	M.P. (°C)	Rf value	Solvent system
A-1	4,5-Dimethoxy	450.25	45.76	120–122	0.69	*n*-Hexane: ethyl acetate (9:1)
A-2	4,6-Dimethoxy		56.98	163–165	0.71	
A-3	4,7-Dimethoxy		28.66	193–195	0.59	
A-4	5,6-Dimethoxy		60.12	175–177	0.61	
B-1	4,5-Dimethoxy	478.32	21.34	210–212	0.65	
B-2	4,6-Dimethoxy		20.00	133–135	0.76	
B-3	4,7-Dimethoxy		17.88	171–173	0.53	
B-4	5,6-Dimethoxy		27.12	240–242	0.59	

Table 2 In vitro reverse transcriptase assay data of synthesized derivatives (**A1**–**A4**, **B1**–**B4**)

Compound ID	Optical density	% RT inhibition
Control	0.879	0.11
A-1	0.192	78.18
A-2	0.183	79.20
A-3	0.151	82.84
A-4	0.188	78.63
B-1	0.108	87.72
B-2	0.109	87.61
B-3	0.119	86.47
B-4	0.168	80.90
Nevirapine	0.008	99.92

However, while comparing more and less bulky substituents at this position, the methoxy group showed increased activity. This indicates that the ring substituents are required for RT inhibiting activity at C4 and C7 position.

Compounds **B1**–**B4** showed good anti-fungal and anti-bacterial activity. Compound **B1** and **B3** has shown excellent activity against the *Aspergillus niger* and *Penicillum notatum*. Compound **B3** and **B4** has shown comparable activity with ketoconazole for *Aspergillus fumigatus*, whereas against the *Candida albicans* activity was found to excellent than ketoconazole. Compound **B2** has shown excellent activity against *Candida albicans* and *Penicillum notatum* as compared to ketoconazole.

Compound **B1** and **B4** has shown excellent activity against *Bacillus substilis* and *S.typhi* as compared to ciprofloxacin. Compound **B2** showed better anti-bacterial activity against *Bacillus substilis*, whereas **B3** showed good activity against *S.typhi* as compared to ciprofloxacin (Figs. 2 and 3).

ADME properties

Total 44 descriptors and pharmaceutically relevant properties of benzylindol analogs were analyzed using Qikprop. Significant descriptors required for predicting the drug-like properties of molecules are reported here. These properties are as follows,

1. Molecular weight (mol_MW) (150–650)
2. Octanol/water partition coefficient (LogP (o/w)) (−2 to 6.5)
3. Aqueous solubility (QPlogS) (−6.5 to 0.5)
4. Apparent MDCK cell permeability (QPPMDCK) (<25 poor, >500 great)
5. Brain/blood partition coefficient (QPlogBB) (−3.0 to 1.2)
6. Percent human oral absorption (C80 % is high, B25 % is poor)

All the structures showed significant values for the properties analyzed (Table 3) and showed drug-like characteristics based on Lipinski's rule of 5. The ADME values of design compound inhibitors are given in Table 3.

The first three properties are based on Lipinski rule of five, molecular weight (mol_MW) less than 650, partition coefficient between octanol and water (logP (o/w)) between −2 and 6.5, and solubility (QPlogS) greater than −7.

Brain/blood partition coefficient (QPlogBB) parameter indicated the ability of the drug to pass through the blood–brain barrier which is mandatory for inhibition of HIV infection. Whereas QPPMDCK predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood–brain barrier.

Higher the value of MDCK cell, higher the cell permeability. All designed compounds had shown ADME properties in acceptable range.

Conclusions

We have designed, synthesized, and evaluated 4-[1-(2,4-dichlorobenzyl)-dimethoxy-1*H*-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid for reverse transcriptase inhibition

Table 3 Prediction of ADMET properties of synthesized compounds (**A1–A4**, **B1–B4**)

Compound ID	Molecular weight	LogP (o/w)	LogS	LogBB	PMDCK	Violation of rule of 5	% Oral absorption
A-1	450.20	4.881	−5.79	−1.355	143.841	0	88.194
A-2	450.20	4.826	−5.956	−1.496	114.201	0	85.893
A-3	450.20	4.839	−5.896	−1.475	113.699	0	86.184
A-4	450.20	4.727	−5.941	−1.571	97.473	0	84.219
B-1	478.32	5.554	−6.642	−1.042	1,410.7	1	100.00
B-2	478.32	5.552	−6.805	−1.126	1,221.2	1	96.511
B-3	478.32	5.579	−6.851	−1.132	1,209.1	1	96.772
B-4	478.32	5.415	−6.634	−1.139	1,140.1	1	95.466

activity. All the title compounds possess RT inhibitory activity. The $-OCH_3$ group of benzylindol ring helps to form the hydrogen bonding with in hydrophobic pocket of enzyme and thus increases the affinity of compounds toward RT enzyme inhibition. It is further concluded that compounds with benzylindol ring having ester group substitution on C3 position and combining hydrophobic group like dichloro benzyl ring substitution on 1H-indol position showed higher activity. This may form the possible butterfly-like conformation of 1,3 substitutions on benzylindol ring required for RT inhibition. The presence of methoxy group at fused benzene position may lead to change in the conformation of butterfly structure.

Materials and methods

Melting points were determined on Veego VMP-D digital melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a FTNMR VARIAN MERCURY YH-300 spectrometer (300 MHz) with TMS as internal standard and DMSO as a solvent. Mass spectra were recorded on time of flight mass spectrometer. FT-IR spectra were recorded on JASCO FT-IR 4100 using KBr powder. The lab system multiscan microplate reader was used to determine absorbance of the sample to calculate the % inhibition.

General procedure for the synthesis of 3-acetyl-dimethoxy-1*H*-indole

Phosphoryl chloride (0.92 mL, 10 mmol) was added to ice cold dimethylacetamide (2.79 mL, 30 mmol) with stirring and cooling in ice. The suitable dimethoxyindole derivative (177 mg, 1 mmol) was added and reaction mixture was stirred at room temperature for 12 h, then poured over ice and basified with a 4 N aqueous sodium hydroxide solution. The mixture was extracted with ethyl acetate and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was powdered by treatment with diethyl ether and recrystallized from dichloromethane.

*3-Acetyl-4,5-dimethoxy-1H-indole (**D1**)*

White solid (166 mg, 76 %); IR (KBr) (ν, cm^{-1}) 3,260, 1,720, 1,593, 1,497, 1,315, 1,217, 1,153, 1,042, 820, 805.

*3-Acetyl-4,6-dimethoxy-1H-indole (**D2**)*

White solid (101 mg, 46 %); IR (KBr)) (ν, cm^{-1}) 3,265, 1,728, 1,595, 1,522, 1,347, 1,214, 1,178.

*3-Acetyl-4,7-dimethoxy-1H-indole (**D3**)*

IR (KBr) (ν, cm^{-1}) 3,250, 1,727, 1,591, 1,470, 1,150, 768.

*3-Acetyl-5,6-dimethoxy-1H-indole (**D4**)*

White solid (176 mg, 81 %); IR (KBr) (ν, cm^{-1}) 3,300, 1,730, 1,630, 1,590, 1,110, 980.

General procedure for the synthesis of 3-acetyl-dimethoxy-1-(2,4-dichlorobenzyl)-1*H*-indole

The appropriate 3-acetyl-dimethoxy-1*H*-indole derivative (219 mg, 1 mmol) was dissolved in DMF (1 mL) at 0 °C and dry sodium hydride (120 mg, 5 mmol) was added. The mixture was stirred for 2 min. 2,4-Dichlorobenzyl chloride (293 mg, 1.5 mmol) was added drop wise, and the resulting solution was placed in a cylindrical quartz tube (diam. 2 cm). The reaction mixture was then stirred and irradiated in a microwave oven at 100 W and at continuous temperature (50 °C) for 5 min. A saturated $NaHCO_3$ solution was added. The reaction mixture was extracted with ethyl acetate and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was powdered by treatment with diethyl ether and crystallized from a mixture of diethyl ether and dichloromethane.

*3-Acetyl-4,5-dimethoxy-1-(2,4-dichlorobenzyl)-1H-indole (**C1**)*

Oil (255 mg, 78 %); IR (KBr) (ν, cm^{-1}) 3,250, 1,760, 1,583, 1,494, 1,316, 1,267, 1,205, 1,148, 1,051, 825, 726.

*3-Acetyl-4,6-dimethoxy-1-(2,4-dichlorobenzyl)-1H-indole (**C2**)*

White solid (242 mg, 74 %): IR (KBr) (v, cm^{-1}) 3,310, 1,790, 1,487, 1,320, 760, 614.

*3-Acetyl-4,7-dimethoxy-1-(2,4-dichlorobenzyl)-1H-indole (**C3**)*

IR (KBr) (v, cm^{-1}) 3,410, 3,318, 1,780, 1,294, 925, 710.

*3-Acetyl-5,6-dimethoxy-1-(2,4-dichlorobenzyl)-1H-indole (**C4**)*

White solid (219 mg, 67 %): IR (KBr) (v, cm^{-1}) 3,380, 3,260, 1,690, 1,160, 839, 695.

General procedure for the synthesis of 4-[1-(2,4-dichlorobenzyl)-dimethoxy-1*H*-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate

A mixture of suitable 3-acetyl-dimethoxy-1-(2,4-dichlorobenzyl)-1*H*-indole (378 mg, 1 mmol) diethyl oxalate (219 mg, 1.5 mmol) and a catalytic amount of $NaOCH_3$ in anhydrous THF (2 mL) was placed in a cylindrical quartz tube (diam. 2 cm), then stirred and irradiated at continuous temperature, in a microwave oven for two subsequent periods in the same conditions (250 W, 2 min, 50 °C). The solvent was evaporated under reduced pressure; the collected solid was washed with ether and crystallized from ethanol/diethyl ether.

*Ethyl 4-[1-(2,4-dichlorobenzyl)-4,5-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate (**B1**)*

Yellow solid (410 mg, 96 %): IR (KBr) (v, cm^{-1}) 3,410, 320, 1,698, 1,335, 735; ^{1}H NMR ($CDCl_3$): d ¼ 1.20 (t, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.09 (q, 2H, CH_2), 5.35 (s, 2H, CH_2), 6.47 (s, 1H, CH), 6.94 (d, 1H, ArH), 7.11e7.17 (m, 3H, ArH), 7.25–7.30 (m, 2H, ArH), 7.80 ppm (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ ppm: 190 (C=O), 187 (C–OH), 169 (COOH), 138–128 (Aromatic), 114 (ethylene), 56–54 (aliphatic, CH).

*Ethyl 4-[1-(2,4-dichlorobenzyl)-4,6-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate (**B2**)*

Yellow solid (419 mg, 98 %): IR (KBr) (v, cm^{-1}) 3,230, 1,725, 1,495, 130, 710, 630; ^{1}H NMR ($CDCl_3$): d ¼ 1.22 (t, 3H, CH_3), 3.73 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.09 (q, 2H, CH_2), 5.32 (s, 2H, CH_2), 6.23 (s, 1H, CH), 6.53-6.61 (m, 1H, ArH), 7.11–7.29 (m, 4H, ArH), 7.51 (d, 1H, ArH), 8.50 ppm (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ ppm: 187 (C=O), 184 (C–OH), 171 (COOH), 140–128 (aromatic), 110 (ethylene), 56–54 (aliphatic, CH).

*Ethyl 4-[1-(2,4-dichlorobenzyl)-4,7-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate (**B3**)*

Yellow solid (408 mg, 94 %): IR (KBr) (v, cm^{-1}) 3,240, 2,720, 1,760, 1,140, 930, 705; ^{1}H NMR ($CDCl_3$): d ¼ 1.22 (t, 3H, CH_3), 3.73 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.09 (q, 2H, CH_2), 5.32 (s, 2H, CH_2), 6.23 (s, 1H, CH), 6.53–6.61 (m, 1H, ArH), 7.11–7.29 (m, 4H, ArH), 7.51 (d, 1H, ArH), 8.50 ppm (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ ppm: 188 (C=O), 185 (C–OH), 170 (COOH), 140–128 (aromatic), 112 (ethylene), 56–54 (aliphatic, CH).

*Ethyl 4-[1-(2,4-dichlorobenzyl)-5,6-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate (**B4**)*

Yellow solid (384 mg, 90 %): IR (KBr) (v, cm^{-1}) 3,120, 2,640, 1,760, 1,453, 920, 716; ^{1}H NMR ($CDCl_3$): d ¼ 1.32 (t, 3H, CH_3), 3.82 (s, 6H, OCH_3), 4.19 (q, 2H, CH_2), 5.47 (s, 2H, CH_2), 6.29 (s, 1H, CH), 7.15 (s, 1H, ArH), 7.20–7.26 (m, 2H, ArH), 7.39–7.43 (m, 2H, ArH), 7.95–7.98 ppm (m, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ ppm: 189 (C=O), 185 (C–OH), 171 (COOH), 140–131 (aromatic), 109 (ethylene), 56–53 (aliphatic, CH).

General procedure for the synthesis of 4-[1-(2,4-dichlorobenzyl)-dimethoxy-1*H*-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid

A methanol solution (5 mL) of derivatives (478 mg, 1 mmol) was treated with 2 N NaOH (5 mL, 50 mmol) and stirred at room temperature for 1.5 h. Then the reaction mixture was acidified with conc. HCl to afford a solid that was collected and recrystallized from ethanol/diethyl ether.

*4-[1-(2,4-Dichlorobenzyl)-4,5-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid (**A1**)*

Yellow solid (199 mg, 50 %): IR (KBr) (v, cm^{-1}) 3,360, 2,930, 1,760, 1,715, 920, 739, 695; ^{1}H NMR ($CDCl_3$): d ¼ 3.76 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 5.46 (s, 2H, CH_2), 7.08–7.19 (m, 3H, ArH–CH), 7.29–7.39 (m, 4H, ArH), 8.67 (s, 1H, ArH), 13.74 (bs, 1H, OH), 15.83 ppm (bs, 1H, OH); ^{13}C NMR (DMSO-d_6) δ ppm: 189 (C=O), 187 (C–OH), 165 (COOH), 148–138 (aromatic), 111 (ethylene), 60–53 (aliphatic, CH). MS: m/z 450.05 (M, 100), 249.05 (55).

*4-[1-(2,4-Dichlorobenzyl)-4,6-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid (**A2**)*

Yellow solid (199 mg, 50 %): IR (KBr) (v, cm^{-1}) 3,250, 2,830, 1,720, 1,453, 1,160, 710, 670; ^{1}H NMR ($CDCl_3$): d ¼ 3.76 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.45 (s, 2H,

CH_2), 6.41 (s,1H, CH), 6.76 (s,1H, ArH), 7.17–7.37 (m, 5H, ArH), 8.39 ppm (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ ppm: 190 (C=O), 188 (C–OH), 167 (COOH), 138–128 (aromatic), 113 (ethylene), 56–53 (aliphatic, CH). MS: *m/z* 450.07 (M, 100), 249.07 (55).

*4-[1-(2,4-Dichlorobenzyl)-4,7-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid (**A3**)*

Yellow solid (82 %): IR (KBr) (v, cm^{-1}) 3,310, 2,830, 1,720, 1,320, 910, 710; ^1H NMR ($CDCl_3$): d ¼ 3.76 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 5.45 (s, 2H, CH_2), 6.41 (s,1H, CH), 6.76 (s,1H, ArH), 7.17–7.37 (m, 5H, ArH), 8.39 ppm (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ ppm: 188 (C=O), 186 (C–OH), 164 (COOH), 138–128 (aromatic), 113 (ethylene), 56–53 (aliphatic, CH). MS: *m/z* 450.09 (M, 100), 249.09 (55).

*4-[1-(2,4-Dichlorobenzyl)-5,6-dimethoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid (**A4**)*

Yellow solid (183 mg, 56 %): IR (KBr) (v, cm^{-1}) 3,360, 2,810, 1,720, 1,490, 1,130, 740, 610; ^1H NMR ($CDCl_3$): d ¼ 3.78 (s, 6H, OCH_3), 5.46 (s, 2H, CH_2), 6.93 (s, 1H, CH), 7.14–7.20 (m, 2H, ArH), 7.25 (s, 1H, ArH), 7.42–7.46 (m, 2H, ArH), 7.69 (s, 1H, ArH), 8.77 ppm (s, 1H, ArH); ^{13}C NMR (DMSO-d_6) δ ppm: 191 (C=O), 188 (C–OH), 169 (COOH), 138–128 (aromatic), 112 (ethylene), 56–53 (aliphatic, CH); MS: *m/z* 450.01 (M, 100), 249.01 (55).

In vitro anti-HIV activity

The standard nevirapine (1) and test compounds (**A1**–**A4** and **B1**–**B4**) were powdered finely and suspended in DMSO. Finally, 20 µg sample was used for in vitro assay. The RT inhibition assay was performed by using an RT assay kit (Roche). The procedure for RT inhibition assay was performed as described in the kit protocol. Briefly, the reaction mixture consists of template primer complex, dNTPs, and RT enzyme in the lysis buffer with or without inhibitors. After 1 h incubation at 37 °C, the reaction mixture was transferred to streptavidine-coated microtiter plate (MTP). The biotin-labeled dNTPs that are incorporated in the template due to activity of RT were bound to streptavidine. The unbound dNTPs were washed using wash buffer, and anti-DIG-POD was added to the MTP. The DIG-labeled dNTPs incorporated in the template were bound to anti-DIG-POD Antibody. The unbound anti-DIG-POD was washed and the peroxide substrate (ABST) was added to the MTP. A colored reaction product was produced during the cleavage of the substrate catalyzed by a peroxide enzyme. The absorbance of the sample was determined as optical density (OD) at 405 nm using micro titer plate ELISA reader. The % inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{OD at } 405\,\text{nm with inhibitor}}{\text{OD at } 405\,\text{nm without inhibitor}}\right) \times 100$$

Anti-microbial assay

The present study has focused on the determination of minimum inhibitory concentration (MIC) of benzylindol derivative series. MIC is important in diagnostic laboratories to confirm resistance of microorganisms to an anti-microbial agent and also to monitor the activity of new anti-microbial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an anti-microbial agent against an organism.

MIC is the lowest concentration of an anti-microbial that will inhibit the visible growth of a microorganism after

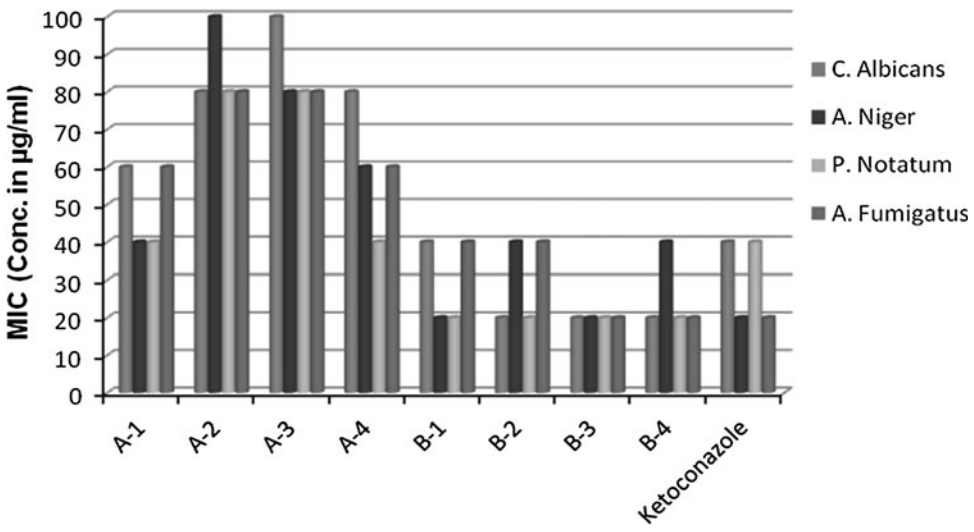


Fig. 2 MIC of compounds against *Candida albicans, Aspergillus niger, Penicillium notatum,* and *Aspergillus fumigates*

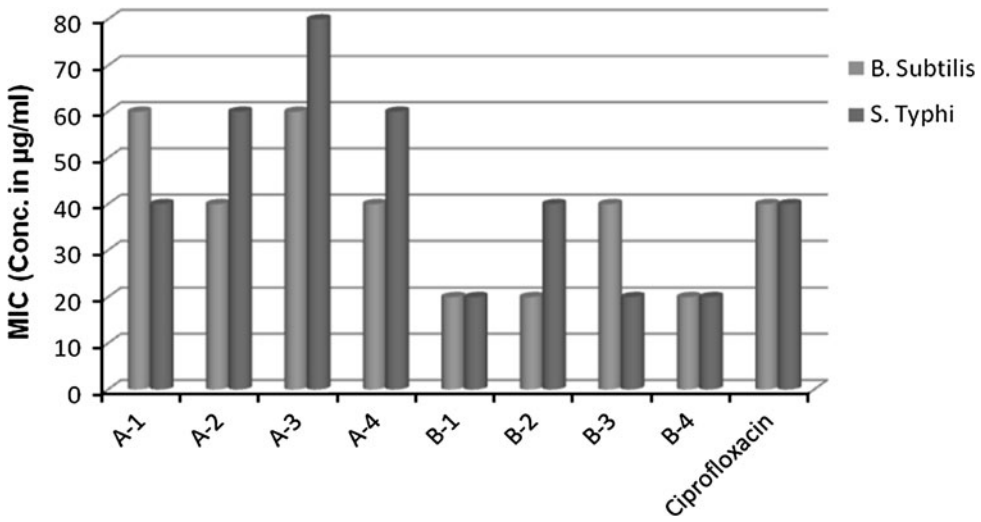


Fig. 3 MIC of compounds against (**A1**–**A4**, **B1**–**B4**) *Bacillus subtilis* and *S. typhi*

overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. This procedure is a standard assay for anti-microbial.

ADME prediction

ADME properties were calculated using Qikprop 2.5 tool of Schrodinger. It predicts both physicochemically significant descriptors and pharmacokinetically relevant properties. QikProp provides ranges for comparing a particular molecule's properties with those of 95 % of known drugs. Qikprop evaluates the acceptability of analogs based on Lipinski's rule of five (Lipinski *et al*., 1997), which is essential to ensure drug-like pharmacokinetic profile while using rational drug design. All the analogs were neutralized before being used by Qikprop.

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