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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL (2-OXO-3-(ARYLIMINO) INDOLIN-1-YL)-*N*-ARYL PROPANAMIDES AS ANTI-HUMAN IMMUNODEFICIENCY AGENTS

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ABSTRACT

Objective: Acquired immunodeficiency syndrome (AIDS) was first identified in the Western world in 1981. Since then, AIDS has been increasingly wide spreading, its rapid worldwide dissemination brought about by modern mass tourism. Isatin (1 H-indole-2, 3-Dione), an endogenous compound identified in many organisms, shows a wide range of biological activities. In view of the above details, we wish to report the synthesis and evaluation of novel isatin analogs, as promising anti-human immunodeficiency (HIV) agents.

Methods: A series of novel isatin analogs (3a-3p) were synthesized, and their chemical structures were confirmed by nuclear magnetic resonance:^{1}H, ^{13}C, ESI-MS spectral data, and CHNS.

Results: The compounds were evaluated as inhibitors of HIV type-1 in MT-4 cell cultures. Of these sixteen compounds, only 5 compounds showed potent anti-HIV activity.

Conclusion: Evaluation of compound properties *in silico* showed that they possess significant drug-like characteristics.

Keywords: Isatin analogs, Synthesis, Human immunodeficiency virus, Biological screening, Absorption, distribution, metabolism and elimination.

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INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) was first identified in the Western world in 1981 [1]. Since then, AIDS has been increasingly wide spreading, and its rapid worldwide dissemination brought about by modern mass tourism. In many developing countries, infection rates are increasing exponentially, despite many efforts to educate and assist populations in adopting a safer lifestyle. Human immunodeficiency virus (HIV) is the causative agent for AIDS which is continuously evolving and rapidly spreading throughout the world as a global infection. HIV is a member of lentivirus genus, which belongs to retrovirus family and is a single-stranded, positive-sense, enveloped RNA virus [2]. The HIV infection targets the monocytes expressing surface CD4 receptors and produces the profound defects in cell-mediated immunity [3]. Overtime infection leads to rigorous depletion of CD4 T-lymphocytes (T-cells), resulting in opportunistic infections such as tuberculosis, fungal, viral, protozoal, and neoplastic diseases and ultimately death [4]. Reverse transcriptase (RT), integrase, and protease are the three enzymes which are used as drug targets for the HIV treatment [2]. The RT of the HIV virus type 1 (HIV-1) has been well known as an important viral target for the discovery and development of anti-HIV therapeutic agents. Accordingly, two functionally distinct classes of HIV-1 RT inhibitors (nucleoside and non-nucleoside) have been developed and are being used clinically [5].

Highly active antiretroviral therapy (HAART) is the standard of care for AIDS patients. The majority of HIV-infected individuals are currently taking RT inhibitors as a critical part of HAART. The HAART has decreased the number of AIDS cases significantly [6]. However, HAART is not able to eradicate HIV-1 from patients absolutely. On the other hand, the toxicity of current available anti-HIV drugs makes it difficult to retain patients' observance to antiretroviral therapy [7]. The predictable appearance of drug-resistant mutants, especially multidrug-resistant mutants, in response to antiretroviral therapies makes things worse [8]. The rates of success of HAART are predicated to decrease gradually with the increase in the emergence of drug-resistant strains. Therefore, a continuous development of novel anti-HIV agents which can efficiently restrain the existing drug-resistant viral strains is the call of the day.

Chemically, isatin may be characterized as the lactam of *o*-aminobenzoyl formic acid. Isatin is an endogenous compound identified in humans and rat tissues for the 1st time in 1988 [9,10]. Isatins are an important group of heterocyclic compounds due to the importance of indole backbone which is biologically active and of significant importance in medicinal chemistry.

Isatin (1 H-indole-2,3-dione), an endogenous compound identified in many organisms, shows a wide range of biological activities [11,12]. The isatin pharmacophore has attracted and still attracts much attention from medicinal chemists because of its structural resemblance to various moieties present in vitamins, proteins, and nucleic acids. From worldwide reported literature, the various isatin analogs are known to possess a range of biological properties, including antibacterial and antifungal [13-17], antiviral [18-20], anti-HIV [21,22], antiglycation [23], anticonvulsant, sedative-hypnotic [24,25], diuretic [26], anti-Alzheimer's activity [27], and anti-inflammatory [28] activities. Among the structurally diverse NNRTIs, isatin scaffolds have demonstrated high potency against HIV-1 drug-resistant strains [29]. Thus, isatin appears as an ideal moiety for the development of anti-HIV agents for AIDS treatment which suppresses HIV replication and also possesses broad-spectrum chemotherapeutic properties [14,30-33]. In view of the above details, we wish to report the synthesis and evaluation of novel isatin analogs, as promising anti-HIV agents.

METHODS

All the chemicals and solvents for synthesis were purchased from Sigma, Spectrochem. Unless otherwise mentioned, the solvents were used without purification. Reactions were monitored by TLC on pre-coated silica gel plates (Kieselgel 60 F 254, Merck), and the spots were detected under UV light (254 nm). Melting points were determined using Optimelt™ (Stanford Research Systems, Sunnyvale, CA 94089) by capillary method and are uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Varian 400 MHz instrument DMSO-d_6 as a solvent. Chemical shifts are reported in parts per million (d) downfield with respect to tetramethylsilane (TMS) as internal standard. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet). Coupling constants (J) are given in hertz. ^{13}C NMR spectra were recorded on a Bruker advance Spectrophotometer 100 MHz using $CDCl_3$ as a solvent. Mass spectra were recorded by the ESI-MS electrospray ionization technique. Elemental analysis was performed on a Vario Micro cube CHNS analyzer of elementar, and the values were within the acceptable ±0.4 % limit of the calculated values. (N-2 (2-Hydroxyothyl) piperazine-N'-(2-ethanesufonic acid), (N, N'-Dimethylformamine) (DMF), (3,(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide) (MTT), penicillin, Streptomycin sulfate, and glutamine were purchased from Sigma; 2-(2-Mercaptoethanol) was purchased from Bio-Rad. RPMI-1640 and fetal bovine serum (FBS) were purchased from Gibco. *In silico* studies were carried out using absorption, distribution, metabolism, and elimination (ADME) software Maestro-8.5 (Schrodinger LLC) [34].

General procedure for synthesis of intermediates 3-(4-arylimino) 1, 3-dihydro-indol-2-one (1ia-1ib) [17,35,36]

Equimolar quantities of isatin (0.004 mole) and the substituted aniline (0.004 mole) were dissolved in 10–15 mL of warm ethanol in the presence of 2–3 drops of glacial acetic acid and refluxed for 2–3 h. After standing for approximately 24 h at room temperature, the crystalline yellow product was obtained (1_ia-1_ib), which was separated by filtration, further vacuum dried, and recrystallized from ethanol [17,35,36].

General procedure for preparation of 3-chloro-N-phenyl propanamide derivatives (2_ia-2_ih) [37]

Substituted aniline (3.01 mL, 0.033 mole) was dissolved in 12.5 ml glacial acetic acid. 3-chloropropionyl chloride (3.56 mL, 0.037 mole) was added drop wise to this solution while cooling in ice bath. The reaction mixture was stirred in ice-bath for 1 h and then stirred for 2 h in room temperature. Next, the mixture was poured into saturated sodium acetate solution. The precipitate was filtered, washed with cold water, and recrystallized from ethanol:water mixture (2_ia-2_ih).

General preparation of synthesis of 3-(2-oxo-3-(arylimino) indolin-1-yl)-N-arylpropanamide (3a-3p)

To the appropriate Schiff bases of isatin (10 mmol) in 8–10 mL of anhydrous DMF, K_2CO_3 (15 mmol) was added and stirred at room temperature for 1 h. After completion of 1 h, the solution turned red brown in color. Appropriate propranamide (10 mmol) and KI (2 mmol) were then added to this solution drop wise and heated at 60°C for 3–12 h. After conforming the end of reaction by TLC (EtAc:Hxn, 40:60), the mixture was poured into ice cold water. Precipitated crude product was filtered and washed thoroughly with cold water (3×200 mL). Compounds were recrystallized from ethanol water mixture.

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-phenylpropanamide (3a)

Yield: 66 %, mp: 183–185°C, IR (KBr, cm−^{1}): 3316 (NH stretching), 3141, 3088 (Ar-CH stretching), 1745, 1673 (C=O stretching), 1607 (C=N stretching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.736 (t, 2H, *J*=7.2, 6.4 Hz, -CH_2-), 4.049 (t, 2H, *J*=7.2, 6.8, -CH_2-), 6.405 (d, 1H, *J*=7.2 Hz, Ar-H), 6.828 (t,1H, *J*=8, 6.8 Hz, Ar-H), 6.957–6.981 (m, 1H, Ar-H), 7.012–7.049 (m, 1H, Ar-H), 7.099–7.128 (m, 1H, Ar-H), 7.207–7.332 (m,4H, Ar-H), 7.425-7.467 (m, 1H, Ar-H), 7.482-7.549 (t, 3H, Ar-H),10.044(s, 1H,-NH-). MS (ESI) m/z=404.0 $(M+1)^+$; calcd for,$C_{23}H_{18}ClN_3O_2$: 403.86. Elemental Analysis: Calcd C, 68.40; H, 4.49; N, 10.40; Found: C, 68.45; H, 4.50; N, 10.42.

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-o-tolylpropanamide (3b)

Yield: 70 %, mp:140–142°C, IR (KBr, cm^{-1}): 3247 (NH stretching), 3054,2960 (Ar-CH stretching), 1744, 1708 (C=O stretching), 1601 (C=N stretching), ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.109 (s, 3H, -CH_3), 2.759 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 4.061 (t, 2H, *J*=7.2, 6.4 Hz, -CH_2-), 6.418 (d, 1H, *J*=7.2 Hz, Ar-H), 6.842 (t,1H, *J*=7.6, 7.6 Hz, Ar-H), 6.938–6.975 (m, 1H, Ar-H), 7.041–7.258 (m, 5H, Ar-H), 7.297–7.347 (m, 2H, Ar-H), 7.436–7.558 (m, 2H, Ar-H), 9.430 (s,1H,-NH). ^{13}C NMR (100 MHz, δ, ppm, $CDCl_3$):17.87, 35.40,37.25,110.62,115.55,116.07,118.05,123.14,123.86,125.45,125.89,126.45,126.83,130.72, 130.81, 135.02,135.36,147.37, 158.44,168.53. MS (ESI) m/z=418.0 $(M+1)^+$; calcd for, $C_{24}H_{20}ClN_3O_2$: 417.89, Elemental analysis: Calcd C, 68.98; H, 4.82; N, 10.06; Found: C, 68.95; H, 4.85; N, 10.10.

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-p-tolylpropanamide (3c)

Yield: 60 %, mp: 148–150°C, IR (KBr, cm^{-1}): 3312 (N–H stretching), 3190, 2933 (Ar–CH stretching), 1734, 1690 (C=O stretching), 1608 (C=N stretching),^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.235 (s, 3H, -CH_3), 2.710 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 4.037 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 6.401 (d, 1H, *J*=7.6 Hz, Ar-H), 6.825 (t,1H, *J*=8, 7.6 Hz, Ar-H), 6.968 (d, 1H, *J*=9.2 Hz, Ar-H), 7.062-7.126 (m, 3H, Ar-H), 7.231 (d, 1H, *J*=8.4 Hz, Ar-H), 7.322 (d, 1H, *J*=8 Hz, Ar-H), 7.377–7.443 (m, 3H, Ar-H), 7.463–7.521 (m, 1H, Ar-H), 9.952 (s, 1H,-NH-). MS (ESI) m/z=418.0 $(M+1)^+$; calcd for, $C_{24}H_{20}ClN_3O_2$: 417.89. Elemental analysis: Calcd C, 68.98; H, 4.82; N, 10.06; Found: C, 69.01; H, 4.84; N, 10.09.

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3-methoxyphenyl) propanamide (3d)

Yield: 59%, mp:120–122°C, IR (KBr, cm−^{1}): 3310 (N–H stretching), 3158,3104 (Ar–CH stretching), 1733, 1677 (C=O stretching), 1603 (C=N stretching).^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.630 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 3.692 (s,3H, -OCH_3), 4.022 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 6.384 (d, 1H, *J*=6.8 Hz, Ar-H), 6.584–6.611 (m,1H, Ar-H), 6.809 (t, 1H, *J*=8, 6.8 Hz, Ar-H), 6.916-6.963 (m, 1H, Ar-H), 7.019–7.106 (m, 2H, Ar-H), 7.112-7.176 (m, 1H, Ar-H), 7.187-7.291 (m, 2H, Ar-H), 7.301 (d, 1H, *J*=8 Hz, Ar-H), 7.407-7.629 (m, 2H, Ar-H), 10.017 (s, 1H,-NH-). MS (ESI) m/z=434.0 $(M+1)^{+;}$ calcd for, $C_{24}H_{20}ClN_3O_3$: 433.89. Elemental analysis: Calcd C, 66.44; H, 4.65; N, 9.68; Found: C, 66.48; H, 4.61; N, 9.70

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(4-methoxyphenyl) propanamide (3e)

Yield: 73 %, mp: 192–194°C. IR (KBr, cm^{-1}): 3298 (N–H stretching), 3057, 2955 (Ar–CH stretching), 1732, 1665(C=O stretching), 1603 (C=N stretching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.691 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 3.708 (s,3H, -OCH_3), 4.037 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 6.405 (d, 1H, *J*=7.2 Hz, Ar-H), 6.809–6.868 (m,3H, Ar-H), 6.969 (d, 1H, *J*=9.2 Hz, Ar-H), 7.098–7.238 (m, 2H, Ar-H), 7.325 (d, 1H, *J*=8 Hz, Ar-H), 7.393–7.523 (m, 4H, Ar-H), 9.904 (s, 1H,-NH-). MS (ESI) m/z=434.0 $(M+1)^+$; calcd for, $C_{24}H_{20}ClN_3O_3$: 433.89. Elemental analysis: Calcd C, 66.44; H, 4.65; N, 9.68; Found: C, 66.41; H, 4.69; N, 9.72.

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2,4-dimethylphenyl) propanamide (3f)

Yield: 69 %, mp: 209–211°C. IR (KBr, cm^{-1}): 3272 (N–H stretching), 3063, 3030 (Ar–CH stretching), 1744,1654 (C=O stretching), 1606 (C=N stretching).^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.205 (s, 3H, -CH_3), 2.225 (s, 3H, -CH_3), 2.728 (t, 2H, *J*=7.2, 6.4 Hz, -CH_2-), 4.048 (t, 2H, *J*=7.2, 6.4 Hz, -CH_2-), 6.414 (d, 1H, *J*=7.6 Hz, Ar-H), 6.840 (t,1H, *J*=7.6, 7.2 Hz, Ar-H), 6.916–6.980 (m, 2H, Ar-H), 7.096 (t, 1H, *J*=6.8, 2 Hz, Ar-H), 7.138–7.219 (m, 3H, Ar-H), 7.317–7.347 (m, 1H, Ar-H), 7.432–7.531

(m, 2H, Ar-H), 9.354 (s, 1H,-NH-). MS (ESI) m/z=432.0 $(M+1)^{+;}$ calcd for, $C_{25}H_{22}ClN_3O_2$: 431.91. Elemental analysis: Calcd C, 69.52; H, 5.13; N, 9.73; Found: C, 69.55; H, 5.17; N, 9.75.

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2,5-dimethyl phenyl) propanamide (3g)

Yield: 77 %, mp: 193–195°C. IR (KBr, cm^{-1}): 3280 (N–H stretching), 3040, 3026 (Ar–CH stretching), 1734, 1665 (C=O stretching), 1601 (C=N stretching). ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.050 (s, 3H, -CH_3), 2.211 (s, 3H, -CH_3), 2.740 (t, 2H, *J*=7.2, 6.4 Hz, -CH_2-), 4.053 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 6.419 (d, 1H, *J*=7.2 Hz, Ar-H), 6.825–6.880 (m,2H, Ar-H), 6.960 (d, 1H, *J*=8 Hz, Ar-H), 7.014–7.104 (m, 3H, Ar-H), 7.127–7.218 (m, 1H, Ar-H), 7.332 (d, 1H, *J*=8.4 Hz, Ar-H), 7.454 (t,1H, *J*=8, 7.2 Hz, -Ar-H), 7.492–7.555 (m, 1H, Ar-H), 9.374 (s, 1H,-NH-). MS (ESI) m/z=432.0 $(M+1)^+$; calcd for, $C_{25}H_{22}ClN_3O_2$: 431.91. Elemental analysis: Calcd C, 69.52; H, 5.13; N, 9.73; Found: C, 69.55; H, 5.17; N, 9.69.

3-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3,4-dimethyl phenyl) propanamide (3h)

Yield: 80 %, mp: 155–157°C. IR (KBr, cm^{-1}): 3271 (N–H stretching), 3056, 2932 (Ar–CH stretching), 1732, 1683(C=O stretching), 1609 (C=N stretching).^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.126 (s, 6H, merged 2× -CH_3), 2.681 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 4.013 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 6.383 (d, 1H, *J*=7.6 Hz, Ar-H), 6.809 (t,1H, *J*=8,7.2 Hz, Ar-H), 6.949 (d, 1H, *J*=8.8 Hz, Ar-H), 6.984–7.014 (m, 1H, Ar-H), 7.073–7.096 (m,1H, Ar-H), 7.110–7.176 (m, 1H, Ar-H), 7.198–7.330 (m,3H, -Ar-H), 7.406–7.532 (m, 2H, Ar-H), 9.864 (s, 1H,-NH-). MS (ESI) m/z=432.0 $(M+1)^+$; calcd for, $C_{25}H_{22}ClN_3O_{2;}$ 431.91. Elemental analysis: Calcd C, 69.52; H, 5.13; N, 9.73; Found: C, 69.54; H, 5.16; N, 9.70.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-phenyl propanamide (3i)

Yield: 71%, mp: 187–189°C. IR (KBr, cm^{-1}): 3322 (N–H stretching), 3184, 3067 (Ar–CH stretching), 1749, 1687 (C=O stretching). 1602 (C=N stretching), ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.732 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 4.047 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 6.483 (d, 1H, *J*=8 Hz, Ar-H), 6.829 (t,1H, *J*=7.6,7.2 Hz, Ar-H), 7.039 (d, 3H, *J*=8.8 Hz, Ar-H), 7.219–7.356 (m,4H, Ar-H), 7.436 (t,1H, *J*=8, 7.6 Hz, -Ar-H), 7.531 (d, 3H, *J*=8.8 Hz, Ar-H), 10.040 (s, 1H,-NH-). MS (ESI) m/z=404.0 $(M+1)^+$; calcd for,$C_{23}H_{18}ClN_3O_2$: 403.86. Elemental analysis: Calcd C, 68.40; H, 4.49; N, 10.40; Found: C, 68.42; H, 4.52; N, 10.37.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-o-tolylpropanamide (3j)

Yield: 49 %, mp: 200–202°C, IR (KBr, cm^{-1}): 3252 (N–H stretching), 3224, 3101 (Ar–CH stretching), 1724,1670 (C=O stretching), 1604 (C=N stretching),^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.102 (s, 3H, -CH_3), 2.755 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 4.059 (t, 2H, *J*=7.2, 6.4 Hz, -CH_2-), 6.496 (d, 1H, *J*=7.2 Hz, Ar-H), 6.845 (t,1H, *J*=7.6, 7.2 Hz, Ar-H), 7.022–7.074 (m, 2H, Ar-H), 7.126–7.256 (m, 3H, Ar-H), 7.298 (d, 1H, *J*=8 Hz, Ar-H), 7.364 (d, 1H, *J*=8 Hz, Ar-H), 7.447 (t, 1H, *J*=8, 6.8 Hz, Ar-H),7.539(d, 2H, *J*=8.4 Hz, Ar-H), 9.426 (s, 1H,-NH-). MS (ESI) m/z=418.0 $(M+1)^+$; calcd for, $C_{24}H_{20}ClN_3O_2$: 417.89. Elemental analysis: Calcd C, 68.98; H, 4.82; N, 10.06; Found: C, 68.95; H, 4.85; N, 10.08.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-p-tolylpropanamide (3k)

Yield: 64%, mp: 193–195°C, IR (KBr, cm^{-1}): 3304 (N–H stretching), 3158, 2940 (Ar–CH stretching), 1747, 1678 (C=O stretching), 1601 (C=N stretching).^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.235 (s, 3H, -CH_3), 2.708 (t, 2H, *J*=7.2, 6.4 Hz, -CH_2-), 4.037 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 6.481 (d, 1H, *J*=7.6 Hz, Ar-H), 6.829 (t,1H, *J*=7.6, 7.2 Hz, Ar-H), 7.028–7.193 (m, 4H, Ar-H), 7.222 (d, 1H, *J*=7.6 Hz, Ar-H), 7.335-7.455 (m, 3H, Ar-H),7.531(d, 2H, *J*=8.8 Hz, Ar-H), 9.951 (s, 1H,-NH-). MS (ESI) m/z=418.0 $(M+1)^+$; calcd for, $C_{24}H_{20}ClN_3O_2$: 417.89. Elemental analysis: Calcd C, 68.98; H, 4.82; N, 10.06; Found: C, 68.95; H, 4.85; N, 10.09.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3-methoxyphenyl) propanamide (3l)

Yield: 59 %, mp: 164–166°C, IR (KBr, cm^{-1}): 3310 (N–H stretching), 3210, 3068 (Ar–CH stretching), 1743, 1690 (C=O stretching), 1602 (C=N stretching).^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.703 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 3.691 (s,3H, -OCH_3), 4.021 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 6.464 (d, 1H, *J*=7.6 Hz, Ar-H), 6.599 (d,1H, *J*=8 Hz, Ar-H), 6.811 (t, 1H, *J*=7.6, 7.6 Hz, Ar-H), 7.011–7.106 (m, 3H, Ar-H), 7.144–7.214 (m, 2H, Ar-H), 7.322 (d, 1H, *J*=8.8 Hz, Ar-H), 7.398–7.440 (m, 1H, Ar-H), 7.500–7.543 (m, 2H, Ar-H),10.018 (s, 1H,-NH-). MS (ESI) m/z=434.0 $(M+1)^+$; calcd for, $C_{24}H_{20}ClN_3O_3$: 433.89. Elemental analysis: Calcd C, 66.44; H, 4.65; N, 9.68; Found: C, 66.48; H, 4.68; N, 9.70.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(4-methoxyphenyl) propanamide (3m)

Yield: 69%, mp: 225–227°C, IR (KBr, cm^{-1}): 3281 (N–H stretching), 3064, 3022 (Ar–CH stretching), 1747,1685 (C=O stretching), 1601 (C=N stretching),^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.686 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 3.706 (s,3H, -OCH_3), 4.035 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 6.482 (d, 1H, *J*=8 Hz, Ar-H), 6.811–6.866 (m,3H, Ar-H), 7.039 (d, 2H, *J*=8.8 Hz, Ar-H), 7.217 (d, 1H, *J*=8 Hz, Ar-H), 7.395–7.454 (m, 3H, Ar-H), 7.531 (d, 2H, *J*=8.4 Hz, Ar-H), 9.9 (s, 1H,-NH-). MS (ESI) m/z=434.0 $(M+1)^+$; calcd for, $C_{24}H_{20}ClN_3O_3$: 433.89. Elemental analysis: Calcd C, 66.44; H, 4.65; N, 9.68; Found: C, 66.47; H, 4.61; N, 9.71.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2,4dimethylphenyl) propanamide (3n)

Yield: 66 %, mp: 239–241°C, IR (KBr, cm^{-1}): 3282, (N–H stretching), 3056, 2960 (Ar–CH stretching), 1738,1678 (C=O stretching), 1608 (C=N stretching), ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.051 (s, 3H, -CH_3), 2.225 (s, 3H, -CH_3), 2.727 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 4.047 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 6.493(d, 1H, *J*=7.6 Hz, Ar-H), 6.844 (t,1H, *J*=8,7.6 Hz, Ar-H), 6.924 (d,1H, *J*=7.2 Hz, Ar-H),), 6.977(s,1H, Ar-H), 7.021–7.059 (m, 2H, Ar-H), 7.142 (d, 1H, *J*=8 Hz, Ar-H), 7.2 (d, 1H, *J*=8 Hz, Ar-H), 7.364 (d,1H, *J*=8.8 Hz, -Ar-H), 7.444 (t, 1H, *J*=8, 8 Hz, Ar-H), 7.547 (t, 2H, *J*=7.2. 1.6 Hz, Ar-H), 9.352 (s, 1H,-NH-). MS (ESI) m/z=432.0 $(M+1)^{+;}$ calcd for, $C_{25}H_{22}ClN_3O_2$: 431.91, Elemental Analysis: Calcd C, 69.52; H, 5.13; N, 9.73; Found: C, 69.55; H, 5.17; N, 9.70.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2,5-dimethyl phenyl) propanamide (3o)

Yield: 73 %, mp: 260–262°C, IR (KBr, cm^{-1}): 3264 (N–H stretching), 3061, 3041 (Ar–CH stretching), 1730, 1673 (C=O stretching), 1606 (C=N stretching).^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.042 (s, 3H, -CH_3), 2.210 (s, 3H,-CH_3), 2.737 (t, 2H, *J*=6.8, 6.4 Hz, -CH_2-), 4.051 (t, 2H, *J*=7.2, 6.8 Hz, -CH_2-), 6.497 (d, 1H, *J*=7.2 Hz, Ar-H), 6.865 (t,2H, *J*=7.2. 5.6 Hz, Ar-H), 7.043 (t,3H, *J*=11.2, 8.8 Hz, Ar-H),), 7.20 (d,1H, *J*=8 Hz, Ar-H), 7.36 (d, 1H, *J*=8 Hz, Ar-H), 7.437 (d, 1H, *J*=7.6 Hz, Ar-H), 7.541 (d, 2H, *J*=8 Hz, Ar-H), 9.371 (s, 1H,-NH-). MS (ESI) m/z=432.1 $(M+1)^{+;}$ calcd for, $C_{25}H_{22}ClN_3O_2$: 431.91, Elemental Analysis: Calcd C, 69.52; H, 5.13; N, 9.73; Found: C, 69.50; H, 5.18; N, 9.79.

3-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3,4-dimethylphenyl) propanamide (3p)

Yield: 53 %, mp: 208–210°C, IR (KBr, cm^{-1}): 3290 (NH stretching), 3062, 2939 (Ar–CH stretching), 1740, 1673 (C=O stretching), 1601 (C=N stretching). ^1H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.125 (s, 3H, -CH_3), 2.144 (s, 3H, -CH_3), 2.679 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 4.011 (t, 2H, *J*=6.8, 6.8 Hz, -CH_2-), 6.464 (d, 1H, *J*=8 Hz, Ar-H), 6.810 (t,1H, *J*=7.6, 7.2 Hz, Ar-H), 7.009 (t,3H, *J*=8.4, 7.2 Hz, Ar-H),), 7.190–7.274 (m,2H, Ar-H), 7.321 (d, 1H, *J*=8.4 Hz, Ar-H), 7.419 (t, 1H, *J*=7.2, 7.2 Hz, Ar-H), 7.51 (d, 2H, *J*=8.4 Hz, Ar-H), 9.863 (s, 1H,-NH-).^{13}C NMR (100 MHz, δ, ppm, $CDCl_3$): 19.28,19.94,35.52,37.22,110.66,115.59,117.97,119.55, 120.78,121.76, 123.01,126.19,128.77,129.74,129.93,131.04,132.87,134.92,135.58,13 7.15,147.33,

148.38, 154.82, 163.80, 168.44, 168.4, MS (ESI) m/z=432.1 $(M+1)^+$; calcd for, $C_{25}H_{22}ClN_3O_2$: 431.91. Elemental Analysis: Calcd C, 69.52; H, 5.13; N, 9.73; Found: C, 69.55; H, 5.14; N, 9.77.

Cells and viruses

C8166, H9 cells, and HIV-1_{IIIB} were kindly donated by the Medical Research Council, AIDS Regent Project, UK. The cells were maintained at 37°C in 5% CO_2 in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FBS) (Gibco). HIV-1_{IIIB} was prepared from the supernatants of H9/HIV-1_{IIIB} cells. The 50% HIV-1 tissue culture infectious dose ($TCID_{50}$) in C8166 cells was determined and calculated by Reed and Muench method. Virus stocks were stored in small aliquots at −70°C. The titer of virus stock was 1.0×10^8 $TCID_{50}$/mL [38].

Cytotoxicity assay

The cellular toxicity of compounds on C8166 was assessed by MTT colorimetric assay [39]. Briefly, 100 µl of 4×10^5 cells was plated into 96-well plates, and 100 µl of various concentrations of compounds was added and incubated at 37°C in a humidified atmosphere of 5% CO_2 for 72 h. Discard 100 µL supernatant, MTT reagent was added and incubated for 4 h, and 100 µl of 50% DMF-15% SDS was added. After the formazan was dissolved completely, the plates were analyzed by a Bio-Tek ELx 800 ELISA reader at 570 nm/630 nm. CC_{50} was calculated.

Inhibition of syncytia formation

The inhibitory effect of samples on acute HIV-1 infection was measured by the syncytia formation assay. In the presence or absence of various concentrations of samples, 4×10^4 C8166 cells were infected with HIV-1 at a multiplicity of infection of 0.04 and cultured in 96-well plates at 37°C in 5% CO_2 for 3 days. AZT was used as a positive control. At 3 days' post-infection, cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cell) in each well of 96-well plates under an inverted microscope [40]. The inhibitory percentage of syncytia formation was calculated by the percentage of syncytia number in treated sample compared to that in infected control. EC_{50} was calculated.

According to the method described by Reed and Muench [41], CC50 and EC_{50} were determined from dose-response curve. TI of anti-HIV activity is CC_{50}/EC_{50}.

Cell viability (% of control)=$(OD_{test}-OD_{blk})/(OD_{ctrl}-OD_{blk})\times100$

CPE inhibition (%)=$(1-CPE_{test}/CPE_{ctrl})\times100$

Computational studies

In silico ADME evaluation

In silico ADME evaluation of compounds was performed by ADME software QikProp v 3.0 [42]. Structures of the compounds were saved in the mol format using Chem Office software. Then, mol files of compounds were uploaded into the ADME predictor software for further evaluation. All descriptors were estimated at pH 7.4

RESULTS AND DISCUSSION

Chemistry

A library of 16 isatin analogs (3a-3p) was synthesized following the reaction outlined in Scheme 1. The synthesis of the title compounds was realized in 3 steps.

First, 1, 3-dihydro-indol-2-one (1_ia-1_ib) was synthesized, following the method reported for the synthesis of isatin [17,35,36]. For this, isatin (1) and substituted anilines were dissolved in warm ethanol in the presence of 2–3 drops of glacial acetic acid and refluxed for 2–3 h**.** After standing for approximately 24 h at room temperature (r.t), product was obtained. Next, the substituted anilines and 3-chloropropionyl chloride (2) were reacted in the presence of glacial acetic acid in ice cold condition leading to the formation of chloro propionamides (2_ia-2_ih) [36,37]. Then, the chloro propranamides (2_ia-2_ih) were treated with Schiff bases of isatin (1_ia-1_ib) to yield the title compounds (3a-3p) [Scheme 1].

The ^{1}H NMR spectra of the title compounds (3a-3p) were recorded in DMSO-d_6 solution which entirely concorded with the predictable resonance signals in terms of chemical shifts and integrations [36]. ^{1}H NMR spectra of the title compounds (3a-3p) showed a broad singlet of 1 proton assigned to NH proton at δ 9.00–10.82. Depending on the nature of the substituent's and substitution patterns on the *N*-phenyl ring, the aromatic protons of certain compounds (3a-3p) were observed in distinct chemical shifts with expected splitting patterns as doublets, triplets, or multiplets integrating more than one proton due to the close chemical shifts ranging from δ 6.365 to 7.582. In the aliphatic region, a broad singlet of four protons (two protons for each $-CH_2$) assigned to the methylenic proton of N-CH_2-CH_2-CO at range δ 2.568–4.676 was observed for the compounds (3a-3p). A broad singlet of three protons assigned to methoxy protons of OCH_3 at δ 3.706–3.722 was observed for the substituents at the *N* -phenyl ring of the compounds 3d, 3e, 3l, and 3m, respectively. A broad singlet of three protons assigned to methyl protons of -CH_3 at δ 1.993–2.286 was observed for the substituents at the *N* -phenyl ring of the compounds 3b, 3c, 3f, 3g, 3h, 3j, 3k, 3n, 3o, and 3p. The NMR data of the title compounds (3a-3p) are summarized in experimental sections. In^{13}C NMR spectra, aromatic carbons were observed in the region of 112.88–161.28 ppm and aliphatic carbon was observed (-CH_3) in the region of 17.22–21.11 ppm. The characteristic carbon for C=O was observed in the region of 164.81–164.00 ppm and $-CH_2$ was observed 35.22–37.68 ppm. The structural confirmations of these compounds (3a-3p) were determined using ESI-MS.

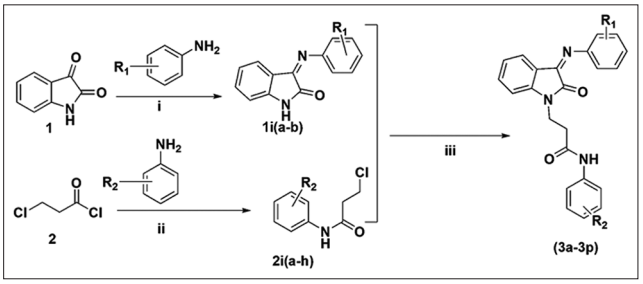
Biological activity

The novel isatin analogs (3a-3p) were tested for their cytotoxicities and anti-HIV-1 activities in C8166 cells infected by the HIV-1_{IIIB}. The activity data were interpreted in CC_{50} values (cytotoxic concentration), EC_{50} (effective concentration), and TI (therapeutic index, given by the CC50/EC50 ratio). The results are summarized in Table 1 and compared to zidovudine (AZT) as reference drugs. The experimental results indicated that most of the newly synthesized compounds showed inhibitory activity against HIV-1 RT and prevented the CPE of HIV-1_{IIIB} at uM concentrations and in some cases had very low cytotoxicity against MT-4 cells, thus resulting in good TI.

ADME properties *in silico*

Modern drug development procedure using computers involves building a 3D virtual compound library (database) for virtual screening. Some libraries consist of compounds with activities against several diseases, for example, ZINC database while others are activity focused library, for example, naturally occurring plant-based anticancer compound activity-target database [43-45]. Before any serious time-consuming analysis is performed, the library is usually filtered to eliminate unworthy molecules through a concept referred to as REOS – "Rapid Elimination of Swill." REOS aids to identify molecules with poor ADME properties. REOS is justified by the fact that many drugs fail to make it to market, after many resources have been invested, because of poor ADME performance [46].

The principal goal of the *in silico* calculation of ADME properties of compounds is the prediction of their *in vivo* biokinetics as potential drugs [47,48]. ADME Predictor QikProp v 3.0 was applied to predict ADME properties of the compounds under consideration [49]. An oral administration is a commonly used route for drugs and a required



Scheme 1: Synthetic route for compounds 3a-3p

one for new agents. Absorption of drugs after oral administration is a very complicated process, and a number of parameters for its prediction are used. Relatively simple parameters are molecular weight (M) and polar surface area (PSA). They are included in the Lipinski's rule of five [50]. The data presented in Table 2 show that all considered descriptors are in the recommended range (the number of unfulfilled rules=0). Two other parameters included in the Oprea's criteria: The number of rotatable bonds (nRB) and PSA possess also the recommended values (nRB <10; PSA<120 Å) [47]. This shows that the compounds under consideration possess strong drug-like properties.

A more sophisticated model of absorption prediction takes into account the values of apparent permeability (Papp) for Madin-Darby Canine Kidney cells. The data collected in Table 2 show that, for all compounds, medium permeability in the range is acceptable (<25 poor, >500 great) [51]. Good native water solubility (S) for all compounds is estimated and also acceptable range (–6.5–0.5) [52-54]. The predicted log BB (logarithm of the brain/blood partition coefficient) that were both calculated. According to the data collected in Table 2, all studied compounds show low brain penetration (log BB –3.0–1.2), and they rather do not across BBB [55-57]. This implied that

Table 1: Cytotoxicity and anti-HIV activity of newly synthesized compounds

Compound	Experiment	Method	First time		Second time		
			CC_{50} (mM)	EC_{50} (mM)	CC_{50} (mM)	EC_{50} (mM)	TI
3a	CA	MTT	26.23	—	36.08	—	2.2
	ISF	CPE	—	12.08	—	16.09	
3b	CA	MTT	164.11	—	—	—	7.4
	ISF	CPE	—	22.06	—	—	
3c	CA	MTT	33.31	—	31.51	—	1.1-1.7
	ISF	CPE	—	31.25	—	19.05	
3d	CA	MTT	21.26	—	—	—	2.7
	ISF	CPE	—	7.77	—	—	
3e	CA	MTT	56.25	—	—	—	3.1
	ISF	CPE	—	18.09	—	—	
3f	CA	MTT	166.30	—	—	—	9.3
	ISF	CPE	—	17.87	—	—	
3g	CA	MTT	29.60	—	—	—	2.2
	ISF	CPE	—	13.27	—	—	
3h	CA	MTT	29.55	—	—	—	3.8
	ISF	CPE	—	7.76	—	—	
3i	CA	MTT	70.19	—	—	—	8.2
	ISF	CPE	—	8.52	—	—	
3j	CA	MTT	34.49	—	—	—	2.2
	ISF	CPE	—	15.91	—	—	
3k	CA	MTT	74.55	—	—	—	3.7
	ISF	CPE	—	20.15	—	—	
3l	CA	MTT	74.59	—	129.98	—	4.9-8.3
	ISF	CPE	—	15.17	—	15.74	
3m	CA	MTT	175.70	—	193.46	—	7.2-9.4
	ISF	CPE	—	18.60	—	26.99	
3n	CA	MTT	216.16	—	203.82	—	5.9-10.2
	ISF	CPE	—	36.40	—	20.00	
3o	CA	MTT	>231.53	—	>231.53	—	>8.6
	ISF	CPE	—	21.51	—	26.90	
3p	CA	MTT	214.53	—	—	—	6.8
	ISF	CPE	—	31.40	—	—	
AZT	CA	MTT	4830.86	—	—	—	5×105
	ISF	CPE	—	0.0094	—	—	

Table 2: Prediction of ADME properties of newly synthesized compounds

Comp.	nRB	MW	LogPo/w	PlogS	PlogBB	PMDCK	% HOA	PSA	Rule of five
3a	6	403.867	4.506	−6.257	−0.79	1150.341	100	78.784	0
3b	6	417.894	4.813	−6.618	−0.719	1362.555	100	76.728	0
3c	6	417.894	4.814	−6.827	−0.82	1150.209	100	78.786	0
3d	7	433.893	4.603	−6.431	−0.852	1206.357	100	87.038	0
3e	7	433.893	4.581	−6.413	−0.872	1149.274	100	87.078	0
3f	6	431.921	5.122	−7.189	−0.747	1363.14	100	76.727	1
3g	6	431.921	5.125	−7.198	−0.748	1363.175	100	76.73	1
3h	6	431.921	5.085	−7.248	−0.835	1149.125	100	78.784	1
3i	6	403.867	4.507	−6.258	−0.79	1151.717	100	78.782	0
3j	6	417.894	4.814	−6.62	−0.719	1362.414	100	79.732	0
3k	6	417.894	4.816	−6.832	−0.821	1151.795	100	78.781	0
3l	7	433.893	4.604	−6.432	−0.851	1207.409	100	87.042	0
3m	7	433.893	4.581	−6.414	−0.871	1150.661	100	87.074	0
3n	6	431.921	5.109	−7.09	−0.728	1381.529	100	76.518	1
3o	6	431.921	5.113	−7.1	−0.729	1381.694	100	76.521	1
3p	6	431.921	5.086	−7.249	−0.835	1150.457	100	78.779	1

ADME: Absorption, distribution, metabolism and elimination

oral bioavailability of the all designed compounds was good and all the compounds (3a-3p) showed ADME properties in acceptable range.

CONCLUSION

To sum up, we have obtained and characterized a series of 1-*H*-indole-2, 3-dione (Isatin) as a new group of heterocyclic compounds. They were prepared in the multi-step novel efficient synthesis procedure. The compounds displayed diverse anti-HIV activities against cell lines.

In summary, through rational design, we have generated the 16 novel isatin analogs (3a-3p) as inhibitors of HIV in comparison with AZT. Interestingly, the biological studies revealed that several isatin analogs showed comparable HIV inhibitory activity with low cytotoxicity. SAR studies suggested that mostly isatin analogs with electron-releasing substituents in the phenyl ring attached to the isatin nucleus by the linker moiety -CH_2CH_2CONH- showed highest therapeutic index, though it was much less than that of AZT.

The compounds 3f, 3i, 3m, 3n, and 3o displayed moderate anti-HIV activity. These results appear to confirm that the isatin moiety would be beneficial to improve the antiviral activity. It is worth mentioning that the TI values of the derivatives for inhibiting HIV are in the comparable ranges. Due to their excellent potency, the biological and the structural data would serve as a valuable guide to the further optimization of the anti-HIV activity of the Schiff bases of isatin family. Furthermore, the compounds possess strong drug-like properties and good pharmacokinetics as well as low toxicity is predicted for them *in silico*.

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AUTHORS' CONTRIBUTIONS

All authors have made considerable contributions to the work reported in the manuscript.

CONFLICTS OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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