Synthesis, antitubercular and anticancer activity of new Baylis–Hillman adduct-derived N-cinnamyl-substituted isatin derivatives

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Abstract Baylis–Hillman adduct-derived N-cinnamyl-substituted isatin derivatives were synthesized and evaluated for their antitubercular activity on Mycobacterium tuberculosis H37Rv strain ATCC 27294 by agar dilution method. Anticancer activity for the same compounds was also screened on four different cell lines: Chinese hamster ovary (CHO cells), Colo 205 (human colon cancer), Sup-T1 (human lymphoma) and C6 glioma (rat glioma) by MTT assay method. The compounds (3j–l) have shown significant activity against Mycobacterium strain and the compound 3l has shown specific cytotoxic activity.

Keywords Baylis–Hillman bromides · Isatins · Antitumor agents · Cytotoxicity

Introduction

Isatin (1H-indole-2,3-dione) is a privileged natural product found in various plants including those of the genus Isatis (Guo and Chen, 1986) and has also been found in humans as a metabolic derivative of adrenaline (Ischia et al., 1988). The synthetic flexibility of isatin has led to the synthesis of an array of substituted derivatives displaying a broad spectrum of biological properties and the derivatives have been developed for therapeutic applications. The various biological properties of isatin include, antibacterial/antifungal (Varma and Nobles 1975), antiviral (Varma and Nobles 1967), antitumor (Matesic et al., 2008), anti-HIV/antitubercular (Sriram et al., 2006), antiprotozoal (Raj et al., 2012, Imam and Varma 1975), antihelminthic (El-Sawi et al., 1998) and other biological activities (Silva et al., 2001, Pandeya et al., 2005).

On the other hand substituted isatin derivatives have been reported to possess DNA gyrase inhibitor (Oblack et al., 2005), human rhinovirus 3C protease inhibitor (Webber et al., 1996), SARS corona virus 3C-like protease inhibitor (Zhou et al., 2006) and caspase-3 inhibitor activities (Chu et al., 2005). Investigation of the structure–activity relationships of isatin derivatives has revealed that 5-halogenated (Sriram et al., 2005) and N-alkylated isatin (Chen et al., 2005; Singh et al., 2011) derivatives are proved to be showing marked rise in biological activity. For example compound 5-fluoro-3-substituted-2-oxindole (SU11248) has been approved by FDA for the treatment of gastrointestinal stromal tumours and advanced renal cell carcinoma (Prenen et al., 2006; Motzer et al., 2006).

Inspired with the biological profile of isatins and their increasing importance in pharmaceutical and biological fields, and in connection with our research on the design and synthesis of biologically active and pharmacologically important new heterocycles (Narender et al., 2006; Ravinder et al., 2010, 2012), it was thought worthwhile to synthesize the title compounds with a view to obtain certain new chemical entities in order to prepare molecules
having potentially enhanced biological activities and to have them evaluated for their bioactivity. Herein, we report the synthesis of Baylis–Hillman adduct-derived N-cinnamyl-substituted isatin derivatives and screening of their antitubercular and anticancer activity.

**Results and discussion**

**Chemistry**

The starting substrates, 5-halo-isatin derivatives (2a–d) were obtained commercially whereas Baylis–Hillman bromides (1a–c) were prepared by treating Baylis–Hillman adducts (Basavaiah et al., 2010; Sing and Batra 2008) with conc. H2SO4/aq. HBr at 0 °C in DCM solvent (Buchholz and Hoffmann 1991). During the optimization studies, we focused our efforts on searching suitable reaction conditions for the preparation of target compounds. Initially, the reaction was carried out between Baylis–Hillman bromide (1a) and isatin (2a) using different bases such as NaH, KOH, K2CO3, NEt3, NaOMe and in various solvents such as DMF, acetonitrile and MeOH (Scheme 1). In our study, it was observed that K2CO3 in acetonitrile was suitable to promote and complete the reaction in which the product (3a) was obtained in good yield (87 %). From mechanistic view, the nucleophile can attack on allyl bromide of Baylis–Hillman adduct in two fashions (SN2 and SN20) leading to the formation of two products (I and II) as shown in Scheme 2, literature also revealed the formation of two types of products while treating the Baylis–Hillman bromides (Buchholz and Hoffmann 1991) with nucleophile; but in our study, in this reaction only SN2-type product was obtained exclusively rather than SN2‘-type product. We reasoned that bulky isatin nucleophile fails to attack at β-position of the Baylis–Hillman bromide (SN2‘ path) due to steric repulsion gained by aromatic group, hence it is attacking at steric-free allyl carbon having bromide group via SN2 path and leads to the compound I exclusively which is confirmed by spectral analysis. A series of compounds (3b–I) were synthesized with this optimized conditions in good yields (80–93 %). All the products synthesized were well characterized by spectroscopic techniques. Thus the synthesized products were screened for their anticancer activity against four different cell lines by MTT assay method and antituberculosis activity against *Mycobacterium tuberculosis* H37Rv strain ATCC 27294 by agar dilution method.

**Pharmacology**

**Antitubercular studies**

All the synthesized compounds (3a–I) were screened for their in vitro antitubercular activity against MTB (*M. tuberculosis* H37Rv strain ATCC 27294) by agar dilution method for the determination of MIC in duplicate. The minimum inhibitory concentration (MIC; µg/mL) was determined for each compound. The MIC is defined as the minimum concentration of compound required to completely inhibit the bacterial growth. Rifampicin, Isoniazid, Ethambutol and Pyrazinamide were used as reference drugs. The results of in vitro antitubercular activities (MIC in µg/mL) along with the standard drugs for comparison are summarized in Table 1. All the screened compounds have shown moderate to good in vitro activity against *M. tuberculosis*, with MIC in the range of 1.56–25
All the compounds are more potent than Pyrazinamide drug (50.77 µg/mL) and less potent than Rifampicin (0.1 µg/mL). The compounds 3f, 3g and 3i are showing better activity than Ethambutol (7.64 µg/mL). The results clearly indicate that the antitubercular activity of the synthesized compounds depend on the substituent’s present on both moieties (isatin, cinnamyl). The compound (3a) that does not have any substituent on both the moieties as well as the compounds that are having one halo substituent either on isatin (3b–d) or cinnamyl moiety (3e) are displaying poor activity except the compound 3i having nitro group on phenyl ring. The compounds that are having different halogen substituents on both the moieties (3f–j) are showing moderate antitubercular activity except the compound 3h, having bromine substituent on both the moieties. Replacement of the halogen groups on phenyl ring of cinnamyl moiety of compounds (3f–h) with nitro group (3j–l) enhanced the antitubercular activity (1.56 µg/mL), which is close to that of the standard Isoniazid drug (0.36 µg/mL).

Cytotoxic evaluation

The in vitro results of antitubercular activity encouraged us to evaluate their anticancer effects against a panel of four cell lines. The preliminary cytotoxicity for a set of compounds (3a–l) was screened in four cell lines comprising non-cancerous Chinese hamster ovary cells (CHO) and cancerous Colo 205 (human colon cancer), Sup-T1 (human lymphoma) and C6 glioma (rat glioma) cell lines were determined by MTT assay method. The resulting data show specific cytotoxicity of compound 3l on cancerous cell lines. Further, compound 3l is found to be more toxic (<20 % cell viability, Fig. 1b, c) towards C6 glioma and Sup-T1 cells. However, compounds 3a–k are found to be equally toxic to both cancerous and non-cancerous cells. Interestingly, compounds 3d–f showed higher cytotoxicity in colon cells when compared to C6 glioma and Sup-T1 cells (Fig. 1b–d). The results suggest that compound 3l in the series of compounds 3a–l is active on cancer cells, C6 glioma in particular. More comprehensive activity studies need to be carried out to establish the anticancer activity of the compound 3l.

Experimental

All chemicals were of research grade and were used as-obtained commercially from Aldrich. The reactions were carried out in a round-bottomed flask of 25 mL capacity at room temperature in an efficient fume hood. The progress of all the reactions was monitored by TLC, using TLC aluminium sheets precoated with silica gel 60 F254 to a thickness of 0.25 mm (Merck). Flash column chromatography was done using silica gel (Merck, 60–120 mesh). Melting points were determined on a MEL-TEMP II melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer. 1H and 13C NMR spectra were recorded on a Varian Gemini 200 MHz, Bruker Avance 300 MHz spectrometer; TMS was used as an internal standard in CDCl3/DMSO-d6. Mass spectra were recorded on VG Micro mass 7070 H (EI), QSTAR XL High resolution mass spectrometer (HRMS) and Thermofinnigan ESI ion trap Mass Spectrometer.

General experimental procedure for the preparation of N-cinnamyl-substituted isatin derivative (3a–l)

To a solution of isatin 2a (147 mg, 1.0 mmol) in acetonitrile (10 mL), K2CO3 (166 mg, 1.2 mmol) was added followed by Baylis–Hillman bromide 1a (305 mg, 1.2 mmol) at room temperature and the reaction mixture was stirred for 10–12 h. Upon completion of the reaction, the mixture was filtered and the filtrate was diluted with water (50 mL) and

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Analysis of the cytotoxic effects of various derivatives (3a–l) in non-cancerous cell line (CHO) and in cancerous cell lines (C6 glioma, Sup T1 and Colo 205) was treated with various concentrations (1 mm, 100 µm, 100 nm and 100 pm). A viability assay was carried out. Experiments were performed in triplicates and data are expressed as means of the triplicate for determination of % of cell viability.

extracted with EtOAc (50 mL × 3). The combined organic layers were dried over Na2SO4, filtered and concentrated under reduced pressure and the obtained crude product was purified by silica gel column chromatography (60–120 mesh, eluent: EtOAc/hexane, 3:7) to afford pure cinnamyl-substituted isatin derivative 3a as solid compound.

Methyl(E)-2-[{(2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-3-phenyl-2-propionate (3a) Yield: 87 %; Reddish solid; m.p. 81–83 °C; IR (KBr) ν: 2952 (Ar, C–H str.), 1744 (ester, CO str.), 1700 (CO, C–O str.), 1612 (amide, CO str.), 1573 cm–1; 1H NMR (300 MHz, CDCl3): δ 3.78 (s, 3H, CH3), 4.80 (s, 2H, CH2), 6.53 (d, 1H, J = 7.9 Hz, ArH), 6.98 (t, 1H, J = 7.5 Hz, ArH) 7.26–7.42 (m, 7H, ArH), 7.96 (s, 1H, olefin); 13C NMR (75 MHz, CDCl3): δ 37.6, 51.9, 110.9, 117.3, 122.9, 124.1, 126.1, 128.4, 128.9, 129.1, 133.9, 137.9, 142.3, 150.7, 157.7, 166.2, 182.8; MS (m/z): 322 [M+H]+; HRMS (ESI) m/z: C19H16NO4 [M+H]+ calculated: 322.1079, found: 322.1089.

Methyl(E)-2-[{(5-fluoro-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-3-phenyl-2-propionate (3b) Yield: 89 %; Reddish solid; m.p. 149–151 °C; IR (KBr) ν: 2953 (Ar, C–H str.), 1746 (ester, CO str.), 1697 (CO str.), 1608 (amide, CO str.), 1469, 1260 cm–1; 1H NMR (300 MHz, CDCl3): δ 3.78 (s, 3H, CH3), 4.80 (s, 2H, CH2), 6.54 (d, 1H, J = 8.0 Hz, ArH), 7.28–7.44 (m, 7H, ArH), 7.96 (s, 1H, olefin); 13C NMR (75 MHz, CDCl3): δ 37.5, 52.4, 112.3, 118.3, 124.8, 125.2, 128.7, 129.2, 129.3, 133.8, 137.3, 144.4, 148.8, 157.2, 166.6, 181.8; MS (m/z): 356 [M+H]+; HRMS (ESI) m/z: C19H16NO4F [M+H]+ calculated: 356.0689, found: 356.0680.

Methyl(E)-2-[{(5-chloro-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-3-phenyl-2-propionate (3c) Yield: 85 %; Reddish solid; m.p. 114–116 °C; IR (KBr) ν: 2956 (Ar, C–H str.), 1746 (ester, CO str.), 1694 (CO, str.), 1621 (amide, CO str.), 1482, 1264 cm–1; 1H NMR (300 MHz, CDCl3): δ 3.80 (s, 3H, CH3), 4.82 (s, 2H, CH2), 6.53 (dd, 1H, J = 8.8, 3.7 Hz, ArH), 7.09–7.38 (m, 7H, ArH), 7.97 (s, 1H, olefin); 13C NMR (75 MHz, CDCl3, C–F coupling observed): δ 37.4, 52.4, 112.0, 112.1, 118.0, 118.1, 124.1, 124.4, 125.2, 128.6, 129.3, 133.8, 144.3, 146.5, 157.3, 157.6, 160.6, 166.6, 182.2; MS (m/z): 340 [M+H]+; HRMS (ESI) m/z: C19H16NO4Cl [M+H]+ calculated: 340.0985, found: 340.0973.

Methyl(E)-2-[{(5-bromo-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-3-phenyl-2-propionate (3d) Yield: 85 %; Reddish solid; m.p. 144–146 °C; IR (KBr) ν: 2926 (Ar, C–H str.),
Methyl(E)-3-(4-bromophenyl)-2-[(2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (3e) Yield: 92%; Reddish solid; m.p. 146–148 °C; IR (KBr) v: 2924 (Ar, C–H str.), 1744 (ester, CO str.), 1715 (CO str.), 1604 (amide, CO str.), 1523, 1464, 1344, 1252 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): 3.81 (s, 3H, CH₃), 4.69 (s, 2H, CH₂), 6.81 (d, 1H, J = 8.3 Hz, ArH), 7.07 (t, 1H, J = 7.5 Hz, ArH), 7.48–7.57 (m, 4H, ArH), 7.94 (s, 1H, olefin), 8.21 (d, 2H, J = 8.3 Hz, ArH); ¹³C NMR (75 MHz, CDCl₃): 37.6, 52.1, 111.1, 117.3, 123.0, 123.3, 124.1, 126.9, 131.2, 132.3, 132.4, 132.6, 132.8, 136.9, 139.9, 140.7, 147.0, 150.5, 157.9, 165.8, 181.7; MS (m/z): 389 [M + Na]⁺; HRMS (ESI) m/z: C₁₉H₁₄NO₄Na [M+Na]⁺ calculated: 389.0749, found: 389.0759.

Methyl(E)-3-(4-nitrophenyl)-2-[(2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (3i) Yield: 92%; Reddish solid; m.p. 146–148 °C; IR (KBr) v: 2924 (Ar, C–H str.), 1744 (ester, CO str.), 1715 (CO str.), 1604 (amide, CO str.), 1523, 1464, 1344, 1252 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): 3.81 (s, 3H, CH₃), 4.69 (s, 2H, CH₂), 6.81 (d, 1H, J = 8.3 Hz, ArH), 7.07 (t, 1H, J = 7.5 Hz, ArH), 7.48–7.57 (m, 4H, ArH), 7.94 (s, 1H, olefin), 8.21 (d, 2H, J = 8.3 Hz, ArH); ¹³C NMR (75 MHz, CDCl₃): 35.9, 50.4, 111.2, 116.7, 121.3, 125.7, 128.1, 128.5, 130.3, 135.1, 138.4, 139.1, 145.3, 147.3, 153.9, 163.9, 179.8; MS (m/z): 401 [M + H]⁺; HRMS (ESI) m/z: C₁₉H₂₂N₂O₆Cl [M+H]⁺ calculated: 401.0540, found: 401.0544.
6.81 (dd, 1H, J = 8.3, 3.4 Hz, ArH), 7.23-7.29 (m, 2H, ArH), 7.56 (d, 2H, J = 8.5 Hz, ArH), 7.99 (s, 1H, olefin), 8.24 (d, 2H, J = 8.5 Hz, ArH); 13C NMR (75 MHz, CDCl3): δ 37.9, 52.7, 112.2, 112.4, 118.1, 123.3, 123.7, 124.3, 124.6, 128.3, 129.6, 140.5, 141.5, 146.5, 147.7, 157. 9, 160.7, 166.0, 181.8; MS (m/z): 407 [M+Na]+; HRMS (ESI) m/z: C19H14N2O6Br [M+Na]⁺ calculated: 407.0655, found: 407.0661.

Methyl(E)-3-(4-nitrophenyl)-2-[(5-bromo-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (3I) Yield: 85 %; Reddish solid; m.p. 159–161 °C; IR (KBr) ν: 2955 (Ar, C–H str.), 1742 (ester, CO str.), 1713 (CO str.), 1599 (amide, CO str.), 1514, 1471, 1343, 1260 cm⁻¹; ¹H NMR (300 MHz, CDCl3): δ 3.81 (s, 3H, CH3), 4.67 (s, 2H, CH2), 6.85 (dd, 1H, J = 9.4, 3.8 Hz, ArH), 7.22-7.28 (m, 2H, ArH), 7.58 (d, 2H, J = 8.5 Hz, ArH), 7.94 (s, 1H, olefin), 8.24 (d, 2H, J = 8.5 Hz, ArH); ¹³C NMR (75 MHz, CDCl3): δ 37.8, 52.7, 112.8, 116.6, 123.3, 123.7, 127.8, 129.1, 129.6, 136.6, 140.5, 141.7, 147.7, 149.2, 157.4, 166.0, 181.2; MS (m/z): 445 [M+H]⁺; HRMS (ESI) m/z: C19H13N2O6FNa [M+Na]⁺ calculated: 445.0035, found: 445.0046.

Antitubercular studies procedure

Twelve compounds (3a–l) were tested in vitro against M. tuberculosis H37Rv strain ATCC 27294 which is susceptible to control drugs (Rifampicin, Isoniazid, Ethambutol and Pyrazinamide). Mic was determined using agar dilution method in Middlebrook 7H11 medium with oleic acid–albumin–dextrose (OADC) growth supplement. The compounds and control drugs were dissolved in DMSO and diluted twofold to obtain ten serial dilutions of each compound. Appropriate volumes of compounds were incorporated into duplicate plates of Middlebrook 7H11 agar medium supplemented with 10 % Middlebrook supplement OADC. Inoculums of M. tuberculosis H37Rv (Mycobacterium strains) were grown in Middlebrook 7H11 agar slants with OADC growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05 %) saline diluted to 10⁻² to give a concentration of approximately 10⁷ cfu/mL. A 5 μL amount of bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of compounds and control drugs per mL. MIC values were determined after incubation at 37 °C and final readings were recorded after 28 days. The MIC (in μg/mL) was recorded as the lowest concentration/highest dilution of the compounds/control drugs that completely inhibited the growth of Mycobacterium cultures. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for the determination of MIC in duplicate.

Cytotoxicity assay procedure

The potential effects on cell viability were investigated by using the MTT assay (Sigma, USA) [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] as an indicator of metabolically active cells (Van de Loosdrecht et al., 1994; Alley et al., 1988). The four different cell lines used are CHO, Colo 205, Sup-T1 and C6 glioma were seeded into 96-well plates in a volume of 200 mL of culture medium and incubated overnight at 37 °C in a CO² incubator before addition of test compound. Cells were then exposed to known concentrations of the compound to be tested (1 mM, 100 μM, 100 nM and 100 pM expressed as final concentration) for 16 h at 37 °C in a CO² incubator with 5 % CO₂. After drug exposure, the culture medium was removed and 20 μL of MTT reagent (diluted in culture medium, 1 mg/mL) was added. After incubating for 5 h in a humidified atmosphere, the MTT/medium was removed and DMSO (200 μL) was added to dissolve the formazan crystals. Absorbance of the coloured solution was measured by an ELISA using a NJ-2300 spectrophotometer with a test wavelength of 570 nm. Results were evaluated by comparing the absorbance of the wells containing compound-treated cells with the absorbance of wells containing 0.1 % DMSO alone (solvent control). Conventionally, cell viability was estimated to be 100 % in the solvent control. All assays were performed in triplicate and mean standard deviation values were used to estimate cell viability.

Conclusion

In conclusion, a series of new Baylis–Hillman adduct-derived N-cinnamyl-substituted isatin derivatives was synthesized in a simple and efficient manner. All the synthesized compounds were evaluated for their antitubercular and anticancer activities. These compounds have inhibited Mycobacterium strains, especially compounds 3j–l have shown overall good activity on M. tuberculosis H37Rv strain ATCC 27294. The compound 3l showed a specific cytotoxicity on cancerous cell lines Colo 205, Sup-T1 and C6-glioma, whereas it is non-cytotoxic to non-cancerous cell line (CHO).

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