Med Chem Res (2014) 23:1934–1940
DOI 10.1007/s00044-013-0787-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

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

Sriramoju Bharath Kumar · Mettu Ravinder · Golla Kishore · Vaidya Jayathirtha Rao · Perumal Yogeeswari · Darmarajan Sriram

Received: 12 June 2013 / Accepted: 10 September 2013 / Published online: 26 September 2013
© Springer Science+Business Media New York 2013

Abstract Baylis–Hillman adduct-derived *N*-cinnamyl-substituted isatin derivatives were synthesized and evaluated for their antitubercular activity on *Mycobacterium tuberculosis* $H_{37}Rv$ 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 (1*H*-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), anticancer (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

S. B. Kumar · M. Ravinder (✉) · V. Jayathirtha Rao (✉)
Crop Protection Chemicals Division, Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500607, India
e-mail: ravi_iictindia@yahoo.co.in

V. Jayathirtha Rao
e-mail: jrao@iict.res.in

G. Kishore
Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India

P. Yogeeswari · D. Sriram (✉)
Medicinal Chemistry & Antimycobacterial Research Laboratory Group, Birla Institute of Technology & Science-Pilani, Hyderabad 500078, India
e-mail: drdsriram@yahoo.com

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

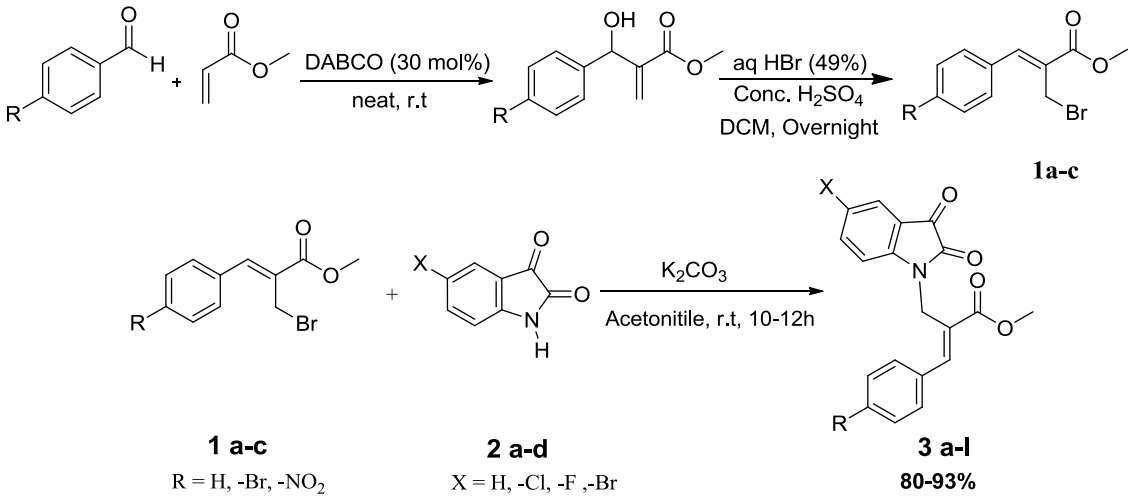
Chemistry

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. H_2SO_4/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, K_2CO_3, NEt_3, NaOMe and in various solvents such as DMF, acetonitrile and MeOH (Scheme 1). In our study, it was observed that K_2CO_3 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 (S_N2 and S_N2') 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 S_N2-type product was obtained exclusively rather than S_N2'-type product. We reasoned that bulky isatin nucleophile fails to attack at β-position of the Baylis–Hillman bromide (S_N2' path) due to steric repulsion gained by aromatic group, hence it is attacking at steric-free allyl carbon having bromide group via S_N2 path and leads to the compound **I** exclusively which is confirmed by spectral analysis. A series of compounds (**3b–l**) 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* $H_{37}Rv$ strain ATCC 27294 by agar dilution method.

Pharmacology

Antitubercular studies

All the synthesized compounds (**3a–l**) were screened for their in vitro antitubercular activity against MTB (*M. tuberculosis* $H_{37}Rv$ 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



3a, R = H, X = H; **3b**, R = H, X = Cl; **3c**, R = H, X = F; **3d**, R = H, X = Br; **3e**, R = Br, X = H; **3f**, R = Br, X = Cl
3g, R = Br, X = F; **3h**, R = Br, X = Br; **3i**, R = NO_2, X = H; **3j**, R = NO_2, X = Cl; **3k**, R = NO_2, X = F; **3l**, R = NO_2, X = Br

Scheme 1 Synthesis of *N*-cinnamyl-substituted isatin derivatives

Scheme 2 Nucleophilic substitution on Baylis–Hillman bromide

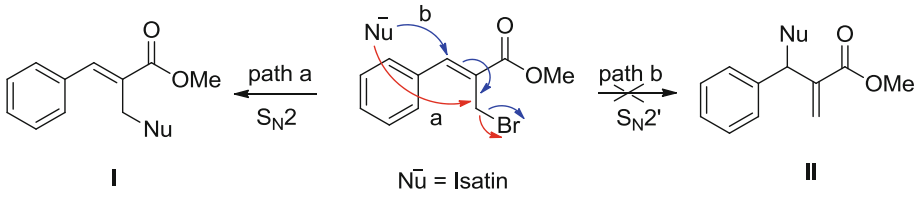


Table 1 In vitro antitubercular evaluation of **3a–l** against *M. tuberculosis* $H_{37}Rv$ ATCC 27294

Compound	MIC (μg/mL) agar dilution method
3a	25
3b	12.5
3c	12.5
3d	12.5
3e	25
3f	6.25
3g	6.25
3h	12.5
3i	6.25
3j	**1.56**
3k	**1.56**
3l	**1.56**
Rifampicin	0.1
Isoniazid	0.36
Ethambutol	7.64
Pyrazinamide	50.77

Bold values indicate the compounds which are exhibiting better activity than other compounds

MIC minimum inhibitory concentration

μg/mL. 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**, **3g**) 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-T1cells (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 F_{254} 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 ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz, Bruker Avance 300 MHz spectrometer; TMS was used as an internal standard in $CDCl_3$/DMSO-d_6. 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), K_2CO_3 (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

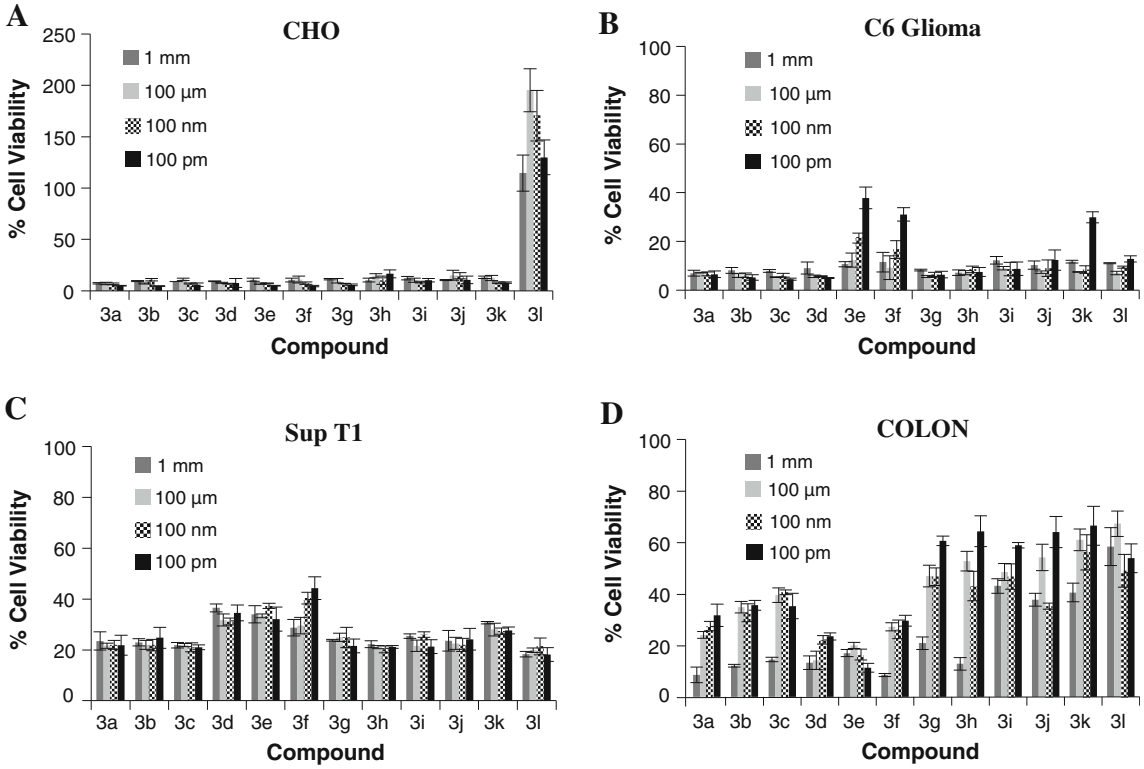


Fig. 1 Analysis of the cytotoxic effects of various derivatives (**3a–l**) in non-cancerous cell line (CHO) and in cancerous cell lines (C6 glioma, Suo T1 and Colo 205) was treated with various concentrations (1 mm, 100 µm, 100 nm and 100 pm). A viability assay was carried out. Experiments were performed 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 Na_2SO_4, 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, *str.*), 1612 (amide, CO *str.*), 1469, 1256 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.78 (s, 3H, CH_3), 4.81(s, 2H, CH_2), 6.55 (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); ^{13}C NMR (75 MHz, DMSO-d_6): 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*: $C_{19}H_{16}NO_4$ $[M+H]^+$ calculated: 322.1079, found: 322.1089.

*Methyl(E)-2-[(5-chloro-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, $CDCl_3$): δ 3.78 (s, 3H, CH_3), 4.80 (s, 2H, CH_2), 6.54 (d, 1H, $J = 8.0$ Hz, ArH), 7.28–7.44 (m, 7H, ArH), 7.96 (s, 1H, olefin); ^{13}C NMR (75 MHz, $CDCl_3$): 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*: $C_{19}H_{15}NO_4Cl$ $[M+H]^+$ calculated: 356.0689, found: 356.0680.

*Methyl(E)-2-[(5-fluoro-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-3-phenyl-2-propionate (**3c**)* Yield: 80 %; 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, $CDCl_3$): δ 3.80 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 6.53 (dd, 1H, $J = 8.8, 3.7$ Hz, ArH), 7.09–7.38 (m, 7H, ArH), 7.97 (s, 1H, olefin); ^{13}C NMR (75 MHz, $CDCl_3$, 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*: $C_{19}H_{15}NO_4F$ $[M+H]^+$ 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.*),

1742 (ester, CO *str.*), 1700 (CO, *str.*), 1605 (amide, CO *str.*), 1462, 1255 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 3H, CH_3), 4.83 (s, 2H, CH_2), 6.49 (d, 1H $J = 8.3$ Hz, ArH), 7.28 (d, 2H, $J = 7.3$ Hz, ArH) 7.35–7.40 (m, 3H, ArH), 7.52 (dd, 1H, $J = 8.3$, 2.1 Hz, ArH) 7.58 (d, 1H, $J = 2.1$ Hz, ArH), 8.01 (s, 1H, olefin); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.4, 52.4, 112.7, 116.2, 118.6, 125.1, 127.6, 128.6, 129.3, 133.7, 140.1, 144.4, 149.2, 157.0, 166.5, 181.6; MS (*m/z*): 400 $[M+H]^+$; HRMS (ESI) *m/z*: $C_{19}H_{15}NO_4Br$ $[M+H]^+$calculated: 400.0184, found: 400.0182.

*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. 104–106 °C; IR (KBr) ν: 2925 (Ar, C–H *str.*), 1739 (ester, CO *str.*), 1715 (CO, *str.*), 1609 (amide, CO *str.*), 1465, 1252 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.78 (s, 3H, CH_3), 4.78 (s, 2H, CH_2), 6.64 (d, 1H, $J = 8.3$ Hz, ArH), 7.05 (t, 1H, $J = 7.3$ Hz, ArH), 7.22 (d, 2H, $J = 8.3$ Hz, ArH) 7.45 (t, 1H, $J = 7.3$ Hz, ArH), 7.51 (m, 3H, ArH), 7.86 (s, 1H, olefin); ^{13}C NMR (50 MHz, DMSO-d_6): δ 37.6, 52.0, 111.0, 117.3, 122.4, 123.0, 124.1, 126.9, 131.2, 131.3, 133.1, 137.9, 141.0, 150.7, 157.8, 166.1, 182.8; MS (*m/z*): 400 $[M+H]^+$; HRMS (ESI) *m/z*: $C_{19}H_{15}NO_4Br$ $[M+H]^+$calculated: 400.0184, found: 400.0185.

*Methyl(E)-3-(4-bromophenyl)-2-[(5-chloro-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (**3f**)* Yield: 90 %; Reddish solid; m.p. 154–156 °C; IR (KBr) ν: 2949 (Ar, C–H *str.*), 1745 (ester, CO *str.*), 1717 (CO, *str.*), 1608 (amide, CO *str.*), 1467, 1269 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.77 (s, 3H, CH_3), 4.73 (s, 2H, CH_2), 6.65 (d, 1H, $J = 8.3$ Hz, ArH), 7.24 (d, 2H, $J = 8.3$ Hz, ArH), 7.41–7.44 (dd, 1H, $J = 8.3$, 2.3 Hz, ArH) 7.48 (d, 1H, $J = 2.3$ Hz, ArH), 7.51 (d, 2H, $J = 8.3$ Hz, ArH), 7.86 (s, 1H, olefin); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.6, 52.4, 112.3, 118.3, 123.6, 124.8, 125.9, 129.3, 130.3, 131.8, 132.6, 137.3, 143.0, 148.8, 157.4, 166.3, 181.6; MS (*m/z*): 434 $[M+H]^+$; HRMS (ESI) *m/z*: $C_{19}H_{14}NO_4ClBr$ $[M+H]^+$ calculated: 433.9794, found: 433.9779.

*Methyl(E)-3-(4-bromophenyl)-2-[(5-fluoro-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (**3g**)* Yield: 93 %; Reddish solid; m.p. 89–91 °C; IR (KBr) ν: 2925 (Ar, C–H *str.*), 1740 (ester, CO *str.*), 1713 (CO, *str.*), 1622 (amide, CO *str.*), 1484, 1263 cm^{-1}; ^1H NMR (300 MHz,$CDCl_3$): δ 3.78 (s, 3H, CH_3), 4.74 (s, 2H, CH_2), 6.65 (dd, 1H, $J = 8.5$, 3.6 Hz, ArH), 7.15–7.25 (m, 4H, ArH), 7.50 (d, 2H, $J = 8.5$ Hz, ArH) 7.86 (s, 1H, olefin); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.6, 52.5, 111.8, 112.1, 118.1, 123.6, 124.2, 124.5, 126.0, 130.4, 131.9, 132.7, 142.9, 146.6, 157.4, 157.7, 160.6, 166.5, 182.0; MS (*m/z*): 418 $[M+H]^+$; HRMS (ESI) *m/z*: $C_{19}H_{14}NO_4FBr$ $[M+H]^+$ calculated: 418.0090, found: 418.0084.

*Methyl(E)-3-(4-bromophenyl)-2-[(5-bromo-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (**3h**)* Yield: 89 %; Reddish solid; m.p. 169–171 °C; IR (KBr) ν: 2923 (Ar, C–H *str.*), 1744 (ester, CO *str.*), 1715 (CO, *str.*), 1604 (amide, CO *str.*), 1464, 1269 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.78 (s, 3H, CH_3), 4.73 (s, 2H, CH_2), 6.60 (d, 1H, $J = 8.3$ Hz, ArH), 7.24 (d, 2H, $J = 8.3$ Hz, ArH), 7.52 (d, 2H, $J = 8.3$ Hz, ArH), 7.56–7.60 (dd, 1H, $J = 8.3$, 2.3 Hz, ArH), 7.63 (d, 1H, $J = 8.3$ Hz, ArH), 7.87 (s, 1H, olefin); ^{13}C NMR(75 MHz, $CDCl_3$): δ 37.6, 52.3, 111.7, 118.7, 123.2, 124.7, 125.7, 129.1, 130.1, 131.7, 132.9, 136.1, 142.3, 149.9, 158.1, 167.4, 181.7; MS (*m/z*): 478 $[M+H]^+$; HRMS (ESI) *m/z*: $C_{19}H_{14}NO_4Br_2$ $[M+H]^+$ calculated: 477.9289, found: 477.9275.

*Methyl(E)-3-(4-nitrophenyl)-2-[(2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (**3i**)* Yield: 90 %; Reddish solid; m.p. 146–148 °C; IR (KBr) ν: 2924 (Ar, C–H *str.*), 1744 (ester, CO *str.*), 1715 (CO, *str.*), 1608 (amide, CO *str.*), 1523, 1464, 1344, 1252 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.81 (s, 3H, CH_3), 4.69 (s, 2H, CH_2), 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); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.6, 52.1, 111.1, 117.3, 123.0, 123.3, 124.1, 129.0, 130.2, 137.9, 139.9, 140.9, 147.0, 150.5, 157.9, 165.8, 182.6; MS (*m/z*): 389 $[M+Na]^+$; HRMS (ESI) *m/z*: $C_{19}H_{14}N_2O_6$ Na $[M+Na]^+$calculated: 389.0749, found: 389.0759.

*Methyl(E)-3-(4-nitrophenyl)-2-[(5-chloro-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (**3j**)* Yield: 92 %; Reddish solid; m.p. 164–166 °C; IR (KBr) ν: 2924 (Ar, C–H *str.*), 1744 (ester, CO *str.*), 1715 (CO, *str.*), 1604 (amide, CO *str.*), 1514, 1475, 1344, 1262 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.81 (s, 3H, CH_3), 4.66 (s, 2H, CH_2), 6.86 (d, 1H, $J = 8.3$ Hz, ArH), 7.47–7.51(m, 2H), 7.59 (d, 2H, $J = 8.1$ Hz, ArH),7.95 (s, 1H, olefin), 8.25 (d, 2H, $J = 8.1$ Hz, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 35.9, 50.4, 111.2, 116.7, 121.5, 125.7, 127.1, 128.5, 130.3, 135.1, 138.4, 139.1, 145.3, 147.3, 155.9, 163.9, 179.8; MS (*m/z*): 401 $[M+H]^+$; HRMS (ESI) *m/z*: $C_{19}H_{14}N_2O_6Cl$ $[M+H]^+$ calculated: 401.0540, found: 401.0544.

*Methyl(E)-3-(4-nitrophenyl)-2-[(5-fluoro-2,3-dioxo-2,3-dihydro-1H-1-indolyl)methyl]-2-propionate (**3k**)* Yield: 82 %; Reddish solid; m.p. 169–171 °C; IR (KBr) ν: 2924 (Ar, C–H *str.*), 1739 (ester, CO *str.*), 1713 (CO, *str.*), 1616 (amide, CO *str.*), 1514, 1484, 1342, 1265 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.81 (s, 3H, CH_3), 4.72 (s, 2H, CH_2),

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); ^{13}C NMR (75 MHz, $CDCl_3$): 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*: $C_{19}H_{13}N_2O_6FNa$ $[M+Na]^+$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 (**3l**)* 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}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (s, 3H, CH_3), 4.67 (s, 2H, CH_2), 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); ^{13}C NMR (75 MHz, $CDCl_3$): 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*: $C_{19}H_{14}N_2O_6Br$ $[M+H]^+$ calculated: 445.0035, found: 445.0046.

Antitubercular studies procedure

Twelve compounds (**3a–l**) were tested in vitro against *M. tuberculosis* $H_{37}R_V$ 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* $H_{37}Rv$ (*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 10^7 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 CO_2 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 CO_2 incubator with 5 % CO_2. 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 microplate 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* $H_{37}Rv$ 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).

Acknowledgments The authors thank Director, IICT for encouragement of this Main Lab Project work. We are grateful to Prof. Anand Kumar Kondapi, Department of Biochemistry, University of Hyderabad for helping in bio-evaluation of anticancer activity. SBK and MR thank CSIR, New Delhi for fellowships.

References

Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR (1988)

Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 48:589–601

Basavaiah D, Sekhara Reddy B, Badsara SS (2010) Recent contributions from the Baylis–Hillman reaction to organic chemistry. Chem Rev 110:5447–5674

Buchholz R, Hoffmann HMR (1991) α-Methylidene- and α-alkylidene-β-lactams from nonproteinogenic amino acids. Helv Chem Acta 74:1213–1220

Chen LR, Wang YC, Lin YW, Chou SY, Chen SF, Liu T, Wu YT, Kuo CJ, Chen TS, Juang SH (2005) Synthesis and evaluation of isatin derivatives as effective SARS coronavirus 3CL protease inhibitors. Bioorg Med Chem Lett 15:3058–3062

Chu W, Zhang J, Zeng C, Rothfuss J, Tu Z, Chu Y, Reichert DE, Welch MJ, Mach RH (2005) *N*-Benzylisatin sulfonamide analogues as potent caspase-3 inhibitors: synthesis, in vitro activity, and molecular modeling studies. J Med Chem 48:7637–7647

El-Sawi EA, Mostafa TB, Mostafa BB (1998) Studies on the molluscicidal action of some isatin derivatives against *Biomphalaria alexandrina* in Egypt. J Egypt Soc Parasitol 28:481–486

Guo Y, Chen F (1986) TLC-UV-spectrophotometric and TLC-scanning determination of isatin in leaf of isatis. Zhongcaoyao 17:8–11

Imam SA, Varma RS (1975) Isatin-3-anils as excystment and cysticidal agents against *Schizopyrenus russelli*. Experiential 31:1287–1288

Ischia M, Palumbo A, Prota G (1988) Adrenalin oxidation revisited. New products beyond the adrenochrome stage. Tetrahedron 44:6441–6446

Matesic L, Locke JM, Bremner JB, Pyne SG, Skropeta D, Ranson M, Vine KL (2008) *N*-Phenyl and *N*-naphthylmethyl isatins and analogues as in vitro cytotoxic agents. Bioorg Med Chem 16:3118–3124

Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, Ginsberg MS, Kim ST, Baum CM, DePrimo SE, Li JZ, Bello CL, Theuer CP, Gearge DJ, Rini BI (2006) Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol 24:16–24

Narender P, Srinivas U, Ravinder M, Anand Rao B, Ramesh Ch, Harakishore K, Gangadasu B, Murthy USN, Jayathirtha Rao V (2006) Synthesis of multisubstituted quinolines from Baylis–Hillman adducts obtained from substituted 2-chloronicotinaldehydes and their antimicrobial activity. Bioorg Med Chem 14:4600–4609

Oblack M, Gradolnik G, Kotnik M, Jerala R, Filipic M, Solmajer T (2005) In silico fragment-based discovery of indolin-2-one analogues as potent DNA gyrase inhibitors. Bioorg Med Chem Lett 15:5207–5210

Pandeya SN, Smitha S, Jyoti M, Sridhar SK (2005) Biological activities of isatin and its derivatives. Acta Pharm 55:27–46

Prenen H, Cools J, Mentens N, Folens C, Sciot R, Schoffski P, Van Oosterom A, Marynen P, Debiec-Rychter M (2006) Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. Clin Cancer Res 8:2622–2627

Raj R, Singh P, Haberkern NT, Faucher RM, Patel N, Land KM, Kumar V (2012) Synthesis of 1H-1,2,3-triazole linked β-lactam-isatin bi-functional hybrids and preliminary analysis of in vitro activity against the protozoal parasite *Trichomonas vaginlis*. Eur J Med Chem 63:897–906

Ravinder M, Sadhu PS, Santhoshi A, Narender P, Swamy GYSK, Ravikumar K, Jayathirtha Rao V (2010) Synthesis of new aminonicotinate derivatives from acetylated Baylis–Hillman adducts and enamino esters via a consecutive [3+3]—annulation protocol. Synthesis 2010:573–578

Ravinder M, Mahendar B, Saidulu M, Venkata Hamsini K, Narendar Reddy T, Rohit Ch, Sanjay Kumar B, Jayathirtha Rao V (2012) Synthesis and evaluation of novel 2-pyridone derivatives as inhibitors of phosphodiesterase3 (PDE3): a target for heart failure and platelet aggregation. Bioorg Med Chem Lett 22:6010–6015

Silva JFM, Garden SJ, Pinto A (2001) The chemistry of isatins: a review from 1975 to 1999. J Braz Chem Soc 12:273–324

Sing V, Batra S (2008) Advances in the Baylis–Hillman reaction-assisted synthesis of cyclic frameworks. Tetrahedron 64:4511–4574

Singh P, Kaur S, Kumar V, Bedi PMS, Mahajan MP, Sehar I, Pal HC, Saxena AK (2011) Synthesis and in vitro cytotoxic evaluation of *N*-alkylbromo and *N*-alkylphthalimido-isatins. Bioorg Med Chem Lett 21:3017–3020

Sriram D, Yogeeswari P, Gopal G (2005) Synthesis, anti-HIV and antitubercular activities of lamivudine prodrugs. Eur J Med Chem 40:1373–1376

Sriram D, Yogeeswari P, Meena K (2006) Synthesis, anti-HIV and antitubercular activities of isatin derivatives. Pharmazie 61:274–277

Van de Loosdrecht AA, Beelen RHJ, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MMAC (1994) A tetrazolium-based cocl-orimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines patients with acute myeloid leukemia. J Immunol Methods 174:311–320

Varma RS, Nobles WL (1967) Synthesis and antiviral and antibacterial activity of certain *N*-dialkylaminomethylisatin β-thiosemicarbozones. J Med Chem 10:972–974

Varma RS, Nobles WL (1975) Antiviral, antibacterial, and antifungal activities of isatin *N*-mannich bases. J Pharm Sci 64:881–882

Webber SE, Tikhe J, Worland ST, Fuhrman SA, Hendrickson TF, Matthews DA, Love RA, Patick AK, Meador JW, Ferre RA, Brown EL, DeLisle DM, Ford CE, Binford SL (1996) Design, synthesis and evaluation of nonpeptidic inhibitors of human rhinovirus 3C protease. J Med Chem 39:5072–5082

Zhou L, Liu Y, Zhang W, Wei P, Huang C, Pei J, Yuan Y, Lai L (2006) Isatin compounds as noncovalent SARS coronavirus 3C-like protease inhibitors. J Med Chem 49:3440–3443