European Journal of Medicinal Chemistry 79 (2014) 194–202

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, crystal studies, anti-tuberculosis and cytotoxic studies of 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole derivatives

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ARTICLE INFO

Article history:
Received 15 July 2013
Received in revised form
1 April 2014
Accepted 4 April 2014
Available online 5 April 2014

Keywords:
1*H*-benzimidazoles
Anti-tuberculosis
Cytotoxic activity
Crystal structure

ABSTRACT

Series of 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole derivatives were synthesized and characterized by spectral methods. Among 21 derivatives, single crystals of **3a** and **3l** were grown and their structural parameters were evaluated. Newly synthesized compounds were screened for anti-tubercular activity and the MIC was determined against *Mycobacterium tuberculosis* H37Rv by Microplate Alamar Blue Assay (MABA) method. Majority of the compounds exhibited a promising inhibition of *M. tuberculosis* and the molecules functionalized with electron-donating groups at C-2 carbon of benzimidazole moiety were found to be more active in inhibiting *M. tuberculosis*. Further, more promising compounds *viz*., **3b**, **3i** and **3l** were tested for their cytotoxic activity. Compound **3l** was found to display excellent activity ($IC_{50} < 10$ μg mL^{-1}) with 100% cell lysis at 30 μg mL^{-1} concentration against A549 (Human lung carcinoma) and 8E5 (Human; Acute Lymphoblastic Leukemia) cell lines.

1. Introduction

Tuberculosis (TB) is an aerial contagious disease caused by species found in *Mycobacterium tuberculosis* complex that includes *M. tuberculosis* (*Mtb*). The number of cases of MDR-TB have been increased in 27 high burden countries in 2011 and 20% of previously treated cases were estimated to have MDR-TB [1]. About 9 million new cases are estimated each year with almost two million death tolls [2,3]. Thus, tuberculosis becomes a significant threat to global health. So, the novel therapeutics are necessary to treat both drug-susceptible TB and progressively common drug resistant strains since, no new chemical entities are emerged in the past 4 decades for the treatment of TB [4–8].

It is known that most of the currently existing tubercular medications are constituted by the group of nitrogen heterocyclic compounds such as isoniazid, pyrazinamide etc. Further, most of them are derived from pyridine and pyrazines [9]. In an attempt to look for better bioactive heterocyclic compounds containing nitrogen hetero atom (since most of antituberculosis compounds are based on either pyridine or pyrazines), our consideration curved in the direction of benzimidazole derivatives, as these compounds exhibit a wide spectrum of biological activities including antitubercular activity [10]. Specifically, this nucleus is a constituent of vitamin-B12 and many currently existing medications [11]. Almost all benzimidazoles with different heterocyclic substituents led to essential modification in their physico-chemical, metabolic and pharmacokinetic properties [12].

Only a few reports are available in the literature on antitubercular activity of benzimidazoles [13,14]. Further, literature survey revealed that most of the first-line anti-tuberculous drugs were constituted by amide linker (shown in Fig. 1.). However, remarkable antitumor/antiproliferative/anticancer [15–18] activity of 1-substituted benzimidazole derivatives prompted us to carry out the cytotoxic activity. Taking into account of the functional group similarity of the amide linkage [19,20], the structural similarity of the pyridopyrazine moiety, albendazole and thiabendazole [21,22] and with the aim of obtaining pharmacologically active compounds, we have envisioned that the benzimidazole scaffold could be a good starting point for the development of good *Mtb* inhibitors. In the present work, we report the synthesis of a new class of 1*H*-benzimidazole derivatives. Single crystals of some of the synthesized compounds were developed to elucidate the structural properties. Finally, the synthesized compounds were tested for their antitubercular activity against *Mtb* H37Rv strains. Further, the cytotoxicity of potential 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-

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http://dx.doi.org/10.1016/j.ejmech.2014.04.017
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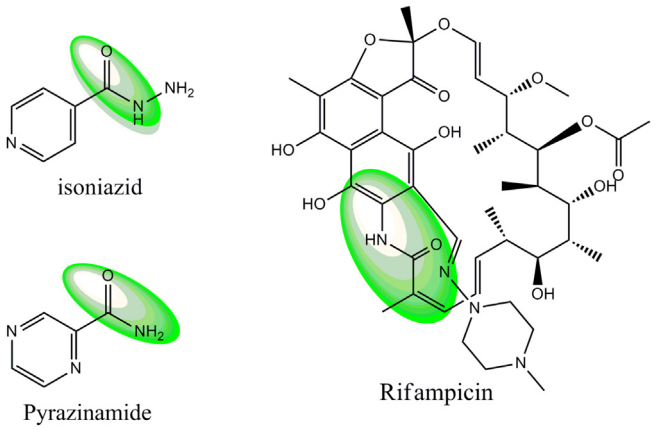


Fig. 1. Currently available anti-tubercular drugs containing amide linkage.

benzimidazole derivatives against two cell lines namely, A549 (Human lung carcinoma) and 8E5 (Human; Acute Lymphoblastic Leukemia) was also investigated.

2. Results and discussion

2.1. Chemistry

The key starting compounds, 2-aryl substituted benzimidazoles were prepared by the condensation of aryl aldehyde with *o*-phenylenediamine in the presence of *p*-TsOH [23]. Then, the 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole derivatives, portrayed in Scheme 1, were prepared by slow addition of cinnamoyl chloride to substituted benzimidazoles in dry THF in the presence of sodium hydride at 0–5 °C.

Nemoto et al. have [24] reported the synthesis of (E)-1-(1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3a**) by the reaction of cinnamic acid and benzimidazole in the presence of equimolar ratio of 1-ethyl-3-(dimethylaminopropyl)carbodimide hydrochloride for 12 h at room temperature. However, this reported method [24] suffered from disadvantages like high reaction time, use of hazardous reagents and tedious product isolation method. In view of this, we have developed a new practicable route for the synthesis of (E)-1-(1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one and its derivatives using sodium hydride as a base at 0–5 °C. In addition, the present route results in good yield in about only 2 h. The optimized experimental conditions are given in Table 1 for the synthesis of 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole derivatives.

The newly synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis data. The physical and elemental analyses of all compounds are given in experimental section. The spectral data of newly synthesized compounds are in good agreement with proposed structures and are given in experimental section.

IR spectra of newly synthesized compounds showed sharp bands ~1650–1697 cm^{-1} and 1619–1649 cm^{-1} are due to the amide carbonyl and alkenyl C=C stretching frequency, respectively. The bands at ~1340–1344 cm^{-1} and 1511–1515 cm^{-1} indicated the presence of NO_2 group in nitro substituted compounds (**3d**, **3e** and **3n**). Further, alkyl C–H stretching frequencies were observed at 2885–2928 cm^{-1} in the cases of methyl substituents. The O–H stretching frequencies were observed at 3292 and 3368 cm^{-1} in phenol derivatives (**3k**, **3m**).

In ^{1}H NMR spectrum of **3a**, the presence of a singlet at δ 8.56 ppm and absence of amine proton of the title compound confirmed that, the condensation of two moieties occurred at 1 position of benzimidazole. The ^{1}H NMR spectra of all compounds showed two doublets each around δ 6.12–7.49 ppm and δ 7.57–8.09 ppm due to vinylic protons. The coupling constants of vinylic protons ($^3J_{HH}$) at *ca* 16 Hz proved the E configuration of the double bond in all compounds.

The ^{13}C NMR of all the compounds exhibited signals around δ 128.9–133.4 ppm and δ 145.5–149.2 ppm due to vinylic carbon atoms. The carbonyl carbon appeared around δ 164.4–168.1 ppm and the peaks appeared between δ 111.1–160.2 ppm were assigned to aromatic carbon atoms. The mass spectral analyses of all the compounds have shown the *m/z* values which corresponds to their respective molecular mass. Fragmentation peaks suggests that all the molecules get fragmented as respective substituted benzimidazole and cinnamoyl group with *m/z* 131 in all the compounds. HPLC was run on gradient mode to determine the purity of the compounds. The compounds were found to be 97–99.8% pure and eluted with retention time (t_R) ranging from 13 to 19 min. The purity of the compounds is reflected in NMR and IR spectra with good and sharp peaks.

2.2. X-ray diffraction analysis

The single crystals of compounds of **3a** and **3l** were developed by slow evaporation of chloroform and alcohol respectively. Crystal data were refined [25a,b] and their properties were studied. Compounds, **3a** and **3l** were crystallized under monoclinic system with space groups $P2_1/n$ and P_12_1/c_1 respectively. The unit cell parameters of the compound, **3a** were as follows: a = 13.466 (4) Å, b = 4.9191 (13) Å, c = 19.168 (7) Å, α = 90, β = 96.38(4), γ = 90°, Z = 4 while the unit cell parameters of compound **3l** were found to be: a = 10.9768 (5) Å, b = 13.3148 (6) (Å), c = 12.5164 (7) Å, α = 90, β = 96.591 (4)°, γ = 90°, Z = 4.

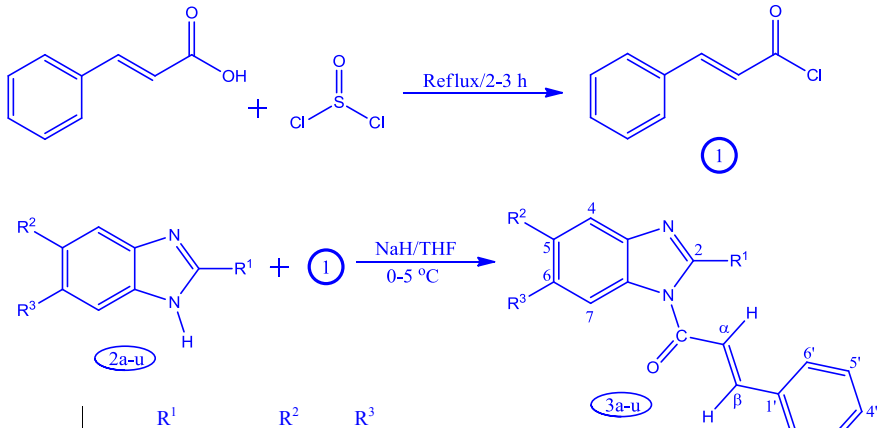
The molecules of **3a** were stabilized by weak intermolecular C–H … N interactions in the solid state, while the molecules of **3l** were stabilized by weak intermolecular C–H … H. The crystal data and structure refinement data of both **3a** and **3l** are given in supplementary material. Fig. 2 represented the ORTEP diagrams of **3a** and **3l**.

The compound **3a** was almost planar and the cinnamoyl group did not make any significant dihedral angle with benzimidazole moiety. But, when compared to crystal structure of **3a**, compound **3l** has a dihedral angle of 17.8 (3)° with cinnamoyl group due to steric hindrance of bulkier 4-CH_3-phenyl ring C16–C17 at C2 position of benzimidazole. Further, 4-CH_3-phenyl ring C16–C17 formed a dihedral angle of 115.7 (2)° with the mean plane of the nine membered benzimidazole ring system. Bond lengths and angles of both **3a** and **3l** were observed to be in normal ranges. Crystal information files (CIF) have been deposited at Cambridge Crystallographic Data Centre. The CCDC numbers for **3a** and **3l** are CCDC 946546 and CCDC 946547, respectively.

2.3. Pharmacological evaluation

2.3.1. Anti-tubercular activity assay

In vitro antituberculosis activity of all the newly synthesized compounds was investigated against *Mycobacterium* tuberculosis H37Rv strain by microplate Alamar Blue assay (MABA) method and the corresponding results are shown in Table 2 and Fig. 3. As evident from the table, all the newly synthesized compounds exhibited anti-tubercular activity, ranging from moderate to excellent values, with a minimum inhibitory concentration (MIC) range of 1.6–100.0 μg mL^{-1}. The MIC is defined as the lowest concentration (μg mL^{-1}) of the compound required to inhibit the bacterial growth, completely. Compounds **3b**, **3h**, **3i** and **3l** showed



	R^1	R^2	R^3
a	H	H	H
b	CH_3	H	H
c	H	CH_3	CH_3
d	CH_3	H	NO_2
e	H	H	NO_2
f	CH_2Cl	H	H
g	H	H	CH_3
h	C_6H_5	H	H
i	4-OCH_3-C_6H_4	H	H
j	4-Cl-C_6H_4	H	H
k	2-OH-C_6H_4	H	H
l	4-CH_3-C_6H_4	H	H
m	4-OH-C_6H_4	H	H
n	2-NO_2-C_6H_4	H	H
o	4-NO_2-C_6H_4	H	H
p	4-F-C_6H_4	H	H
q	3-Br-C_6H_4	H	H
r	3-Cl-C_6H_4	H	H
s	3-NO_2-C_6H_4	H	H
t	2-Cl-C_6H_4	H	H
u	3,4-di-Cl-C_6H_4	H	H

Scheme 1. Synthetic route for the substituted 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole.

excellent *in vitro* activity against H37Rv strain as compared to pyrazinamide and streptomycin (MIC = 3.12 and 6.25 μg mL^{-1}, respectively), although compound **3j** revealed the poorest activity (MIC > 100 μg mL^{-1}). From Table 2, no clear relation was observed between MIC and the lipophilicity (calculated log *P*). However, the physico-chemical properties seem to be one of the factors influencing the antimycobacterial activity [26]. Further, the most active compounds carry alkyl and alkoxy groups at the benzimidazole 2-position or in the para-position of phenyl substituent. The 4-methoxy derivative was observed to be less active compared to that of 4-methyl derivative indicating that the volume of the substituent might play a significant role. Further, it was evident that the methyl substituted compounds showed better activity compared to that of the 2-chloromethyl and chloro-substituted compounds. Thus, electron-donating substituents reinforced the antitubercular activity of 1-substituted benzimidazole derivatives [27,28]. De et al. [29a] have observed that the replacement of double bond of the enoyl-acyl backbone by other group such as isosteric cyclopropyl resulted in decreased activity. Lee et al. [29b] have shown from SAR studies that the presence of amide linker results in better antitubercular activity. Thus, the importance of the amide and cinnamic double bond part was evident from these biological results. However, the mechanism of action was still unknown, but the presence of enoyl-acyl backbone to the benzimidazole moiety contributes more to become a potent antitubercular agent along with the selective alkyl groups [30]. Further, extensive SAR experiments on both the enzymatic target would be required to establish unique modes of action. Furthermore, the compounds *viz.*, **3b**, **3i**, and **3l**, exhibited potent cytotoxic effect against lung carcinoma (A549 cells) and Leukemic cells (8E5 lymphocyte T-human).

2.3.2. MTT assay for cell proliferation

The potent antitubercular compounds *viz.*, **3b**, **3i** and **3l** were examined against two cancer cell lines (**A549** and **8E5**) for their cell proliferation. The compounds showed IC_{50} ranging between <10 and >30 μg mL^{-1}. It was evident from Table 3 that the 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole derivatives exhibited good to moderate activity towards both cell lines. Among the tested compounds, compound **3l** displayed excellent cytotoxicity

Table 1
Optimized conditions for production of 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole.

Entry	Base (equivalents)	Solvent	Time	Yield (%)
3a	NaOH (1.2)	Ethanol	8 h (Reflux)	10
3a	KOH (1.2)	Ethanol	8 h (Reflux)	15
3a	$NaOCH_3$ (1.2)	Methanol	8 h (Reflux)	18
3a	Piperidine (1.2)	Methanol	8 h (Reflux)	24
3a	Pyridine (1.2)	Methanol	8 h (Reflux)	21
3a	NaH (1.2)	THF	2 h (RT)	82

inhibit thioredoxin reductase through nucleophilic addition of glutathione cystine-SH residues and can potentially be exploited for use in different concentrations in chemotherapeutic and chemopreventive strategies. From a chemical point of view, the compounds having an electron-donating group in the para-position of the phenyl ring at C2 position of the benzimidazole can enhance the activity. These results illustrated that the presence of amide and olefin to the benzimidazole moiety exhibited better activity against both cancerous cell lines.

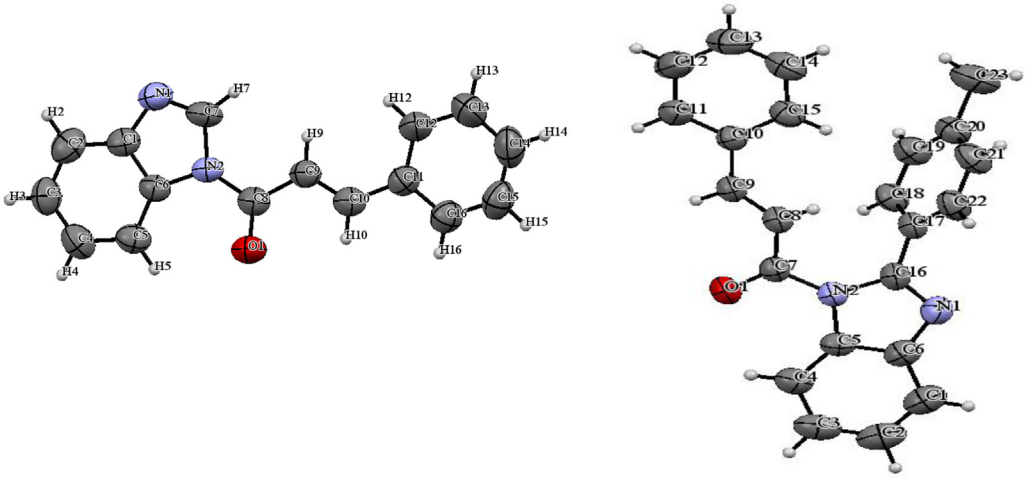


Fig. 2. ORTEP diagrams of compounds **3a** and **3l**.

($IC_{50} < 10$ μg mL^{-1}) with 100% cell lysis at 30 μg mL^{-1} concentration. E. H. Chew et al. [31] have recently showed that α, β-unsaturated carbonyl moiety, also known as the Michael acceptor can

Table 2
Anti-tubercular activities and log *P* measurements of 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole derivatives.

Compound	R^1	R^2	R^3	MIC μg mL^{-1}	log *P*[a]
3a	H	H	H	50	3.73
3b	CH_3	H	H	**3.12**	**3.81**
3c	H	CH_3	CH_3	50	4.53
3d	CH_3	H	NO_2	25	2.92
3e	H	H	NO_2	100	2.83
3f	CH_2Cl	H	H	25	4.41
3g	H	H	CH_3	50	4.15
3h	C_6H_5	H	H	**6.25**	**5.84**
3i	4-OCH_3-C_6H_4	H	H	**3.12**	**5.90**
3j	4-Cl-C_6H_4	H	H	>100	6.52
3k	2-OH-C_6H_4	H	H	25	5.58
3l	4-CH_3-C_6H_4	H	H	**1.6**	**6.29**
3m	4-OH-C_6H_4	H	H	12.5	5.36
3n	2-NO_2-C_6H_4	H	H	12.5	4.92
3o	4-NO_2-C_6H_4	H	H	50	5.80
3p	4-F-C_6H_4	H	H	25	6.01
3q	3-Br-C_6H_4	H	H	50	6.63
3r	3-Cl-C_6H_4	H	H	12.5	6.50
3s	3-NO_2-C_6H_4	H	H	50	5.78
3t	2-Cl-C_6H_4	H	H	50	6.47
3u	3,4-di-Cl-C_6H_4	H	H	25	7.13
Pyrazinamide				**3.12**	**−0.71**
Sreptomycin				**6.25**	**−5.35**
Rifampicin				**0.12**	**2.62**

Results shown in bold letters indicate more antituberculosis activity of compounds compared to others.
[a] Calculated by http://www.molinspiration.com/.

3. Conclusions

An efficient, simple and viable method for the synthesis of 1-[(2*E*)-3-phenylprop-2-enoyl]-1*H*-benzimidazole derivatives with yields ranging 65–85% was reported. All the derivatives were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopic data. Single crystals of molecules **3a** and **3l** were developed and their crystal parameters were evaluated. All the newly synthesized compounds were screened for their anti-tubercular activity. Cytotoxicity activity of the more promising tuberculosis inhibitor was investigated against 2 cancerous cell lines, A549 and 8E5.

4. Experimental protocol

4.1. Chemistry

All chemicals and solvents were purchased from Spectrochem, sd-fine Chemicals and HiMedia (India) and used without further purification. Melting points were determined in open capillary tubes on a melting point apparatus of Concord Instruments (P) Ltd., Bangalore, and are reported as uncorrected values. Carbon, hydrogen and nitrogen were estimated by using CHN analyzer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-500 MHz/Bruker Avance DPX-300 MHz/Bruker Avance FT 400 MHz NMR spectrometer in DMSO-d6/$CDCl_3$. Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (J) are expressed in Hz. Mass spectra were recorded on a Shimadzu spectrometer model QP2010 S and IR spectra were recorded on a Bruker Alpha-T FT-IR spectrophotometer using KBr optics. High resolution X-ray diffraction data were collected on Xcalibur, Eos, Gemini diffractometer and Bruker SMART APEX II diffractometer. The purity of the compounds was determined on Agilent 1200 Series HPLC

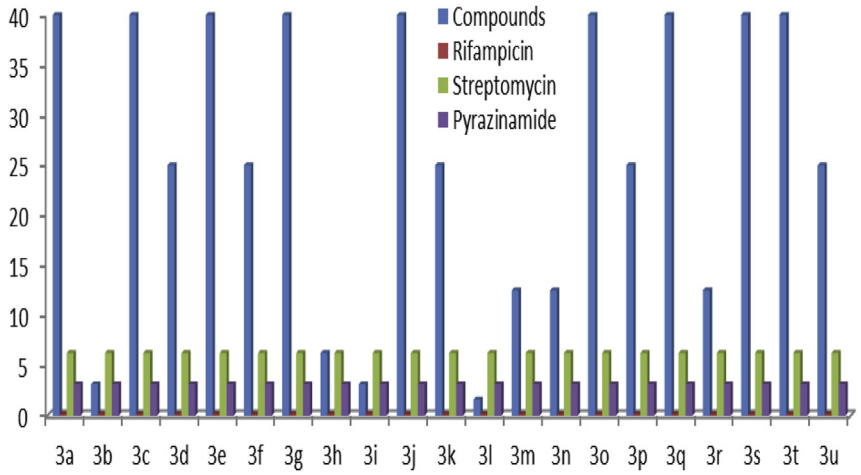


Fig. 3. Antitubercular activity of synthesized compounds **3a**–**u** against *Mtb* H37Rv strains.

instrument. The anti-tubercular and cytotoxicity activity evaluation was carried out at the Department of Microbiology and Immunology, NGH College of Dental sciences, Belagavi, Karnataka, India.

*4.1.1. General procedure for the synthesis of 2-aryl substituted benzimidazoles (**2h**–**u**)*

The series of these derivatives was synthesized using the procedure reported in the literature [23] and characterized by ^{1}H NMR. The melting points of these compounds were also compared with the reported values [32].

*4.1.2. General method for the synthesis of 1-[(2E)-3-phenylprop-2-enoyl]-1H-benzimidazole derivatives (**3a**–**u**)*

Substituted benzimidazole (1 eq.) was added slowly at reduced temperature to a solution of sodium hydride (1.1 eq.) in anhydrous THF and the contents were stirred for 15–20 min. To this, a solution of freshly prepared cinnamoyl chloride (1 eq.) in THF was added dropwise at 0–5 °C for about 30 min. The reaction was carried out in an inert atmosphere. Then, the reaction mixture was allowed to attain ambient temperature and the stirring was continued for 60–90 min (monitored by TLC). After the completion of the reaction, the volume of the THF was reduced to half *in vacuo* and poured to crushed ice and the pH was adjusted to neutral. The separated solid was filtered, washed with water, dried and finally purification was achieved on silica gel (60–120 mesh) column chromatography using ethyl acetate/hexane as the eluent.

4.1.3. General method for the determination of purity of the compounds by HPLC

The HPLC method was employed to determine the purity of compounds on Eclipse XDB-C_{18} column (50 × 4.6 mm, 5 μM) using the binary mobile phase consisting of 20 mM monopotassium phosphate at pH 4.5 (eluent A) and MeCN (eluent B). The gradient elution program was: 70% A and 30% B (0–15 min), 30% A and 70% B (15–25 min), and 70% A and 30% B (25–30 min). The sample solution was prepared by dissolving the sample (mg mL^{-1}) in methanol; MeCN (1:1 v/v) diluent. The injection volume was 5 μL and the column temperature was maintained at 25 °C. The flow rate of 1.0 mL min^{-1} was maintained throughout the run. The run time was typically 30 min. The UV detector was set at a wavelength range of 280–320 nm.

Table 3
IC_{50} values with reference to control and compound concentration.

Entry	IC_{50} (μg/mL)	
	A549	8E5
3b	>30	>30
3i	30	20
3l	<10	<10

*4.1.3.1. (E)-1-(1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3a**).* Yield: 86%; m.p: 232–234 °C; IR (KBr): 1650 (C=O), 1620 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.22 (d, 1H, *J* = 15.2 Hz, H-α), 7.41 (t, 2H, *J* = 7.2 Hz, C5, C6–H), 7.65 (t, 1H, *J* = 7.4 Hz, C4′-H), 7.81 (t, 2H, *J* = 7.4 Hz, C3′,C5′-H), 7.83–7.94 (m, 4H, C4, C7,C2′C6′-H), 8.09 (d, 1H, *J* = 15.2 Hz, H-β), 8.56 (s, 1H, C2-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 114.12, 121.06, 123.86, 128.22, 128.94, 129.15, 130.63, 130.94, 134.13, 140.21, 144.22, 147.94, 165.16; MS (*m/z*): 249 [(M + H)$^+$, a], 248 (M$^+$), 145, 131, 118, 103, 90, 77; Anal. Calcd. for $C_{16}H_{12}N_2O$ (248.09): C, 77.40%; H, 4.87%; N, 11.28%; Found: C, 77.35%; H, 4.85%; N, 11.30%; HPLC purity: 97.05%, t_R-13.7 min.

*4.1.3.2. (E)-1-(2-methyl-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3b**).* Yield: 80%; m.p: 96–98 °C; IR (KBr): 2928 (CH_3), 1677 (C=O), 1632 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d6, 300 MHz, δ ppm): 2.64 (s, 3H, C2-CH_3), 6.50 (d, 1H, *J* = 15.9 Hz, H-α), 6.99 (t, 2H, *J* = 7.2 Hz C5, C6–H), 7.29 (t, 1H, *J* = 7.4 Hz, C4′-H), 7.38 (t, 2H, *J* = 7.4 Hz, C3′,C5′-H), 7.41–7.49 (m, 4H, C4, C7, C2′, C6′-H), 7.57 (d, 1H, *J* = 15.9 Hz, H-β); ^{13}C NMR (DMSO-d6, 75 MHz, δ ppm): 14.94, 114.69, 118.93, 124.50, 127.07, 128.63, 129.18, 130.70, 131.79, 134.69, 139.15, 144.39, 147.55, 168.14; MS (*m/z*): 263 [(M + H)$^+$, a], 262 (M$^+$), 147, 131, 117, 103, 90, 77; Anal. Calcd. for $C_{17}H_{14}N_2O$ (262.11): C, 77.84%; H, 5.38%; N, 10.68%; Found: C, 77.79%; H, 5.35%; N, 10.62%; HPLC purity: 98.67%, t_R-14.2 min.

*4.1.3.3. (E)-1-(5,6-dimethyl-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3c**).* Yield: 85%; m.p: 218–220 °C; IR (KBr): 2911 and 2885 (CH_3), 1655 (C=O), 1623 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d6, 300 MHz, δ ppm): 2.48 (s, 6H, C5, C6-CH_3), 6.90 (d, 1H, *J* = 15 Hz, H-α), 7.39–7.47 (m, 3H, C4, C7,C4′-H), 7.53 (t, 2H, *J* = 7.8 Hz, C3′,C5′-H), 7.59 (t, 2H, *J* = 7.8 Hz, C2′, C6′-H), 7.79 (d, 1H, *J* = 15 Hz, H-β), 8.07 (s, 1H, C2-H); ^{13}C NMR (DMSO-d6, 75 MHz, δ ppm): 19.59, 116.29, 128.24, 128.60, 129.48, 129.64, 130.22, 130.46, 134.63, 135.26, 142.97, 147. 61, 164.39; MS (*m/z*): 277 [(M + H)$^+$, a], 276 (M$^+$), 146, 131, 116, 103, 77; Anal. Calcd. for $C_{18}H_{16}N_2O$ (276.33): C, 78.24%; H, 5.84%; N, 10.14%; Found: C, 77.85%; H, 5.85%; N, 10.25%; HPLC purity: 99.26%, t_R-14.8 min.

*4.1.3.4. (E)-1-(2-methyl-6-nitro-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3d**).* Yield: 59%; m.p: 204–206 °C; IR (KBr): 2920 (CH_3), 1696 (C=O), 1608 (C=C), 1514 and 1344 (NO_2) cm^{-1}; ^1H NMR (DMSO-d6, 500 MHz, δ ppm): 2.86 (s, 3H, C2-CH_3), 7.49 (d, 1H, J = 16 Hz, H-α), 7.69 (t, 1H, J = 7.4 Hz, C4′-H), 7.76 (t, 2H, J = 7.4 Hz, C3′,C5′-H), 7.82–7.92 (m, 3H, C4, C2′, C6′-H) 8.01 (d, 1H, J = 16 Hz, H-β), 8.16 (d, 1H, J = 7.6 Hz, C5-H), 8.23 (s, 1H, C7-H); ^{13}C NMR (DMSO-d6, 125 MHz, δ ppm): 14.80, 112.02, 116.82, 119.08, 128.53, 129.15, 129.32, 131.12, 132.56, 135.86, 142.16, 144.36, 145.67, 147.14, 166.06; MS (*m/z*): 307 (M^+), 147, 131, 120, 103, 91, 77; Anal. Calcd. for $C_{17}H_{13}N_3O_3$ (307.30): C, 66.44%; H, 4.26%; N, 13.67%; Found: C, 66.36%; H, 4.25%; N, 13.67%; HPLC purity: 98.97%, t_R-14.1 min.

*4.1.3.5. (E)-1-(6-nitro-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3e**).* Yield: 65%; m.p: 110–112 °C; IR (KBr): 1682 (C=O), 1619 (C=C), 1515 and 1342 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.38 (d, 1H, J = 15.2 Hz, H-α), 7.46 (t, 1H, J = 7.4 Hz, C4′-H), 7.52 (t, 2H, J = 7.4 Hz, C3′,C5′-H), 7.66–7.78 (m, 3H, C4, C2′, C6′-H), 7.98 (d, 1H, J = 15.2 Hz, H-β), 8.62 (s, 1H, 2-H), 8.71 (d, 1H, J = 7.2 Hz, C5-H), 8.79 (s, 1H, C7-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 111.34, 116.82, 119.24, 127.46, 128.84, 130.64, 131.33, 135.21, 141.88, 144.13, 147.56, 149.49, 166.02; MS (*m/z*): 294 [$(M + H)^+$, a], 165, 147, 131, 125, 103, 91, 77, 63; Anal. Calcd. for $C_{16}H_{11}N_3O_3$ (293.28): C, 65.53%; H, 3.78%; N, 14.33%; Found: C, 65.46%; H, 3.77%; N, 14.33%; HPLC purity: 98.47% t_R-14.5 min.

*4.1.3.6. (E)-1-(2-(chloromethyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3f**).* Yield: 63%; m.p: 150–152 °C; IR (KBr): 1678 (C=O), 1623 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 3.72 (s, 2H, C2-CH_2), 7.16 (d, 1H, J = 15 Hz, H-α), 7.24 (t, 2H, J = 7.2 Hz, C5, C6-H), 7.45 (t, 1H, J = 7.4 Hz, C4′-H), 7.56 (t, 2H, J = 7.4 Hz, C3′,C5′-H), 7.69–8.78 (m, 4H, C4, C7, C2′, C6′-H), 7.86 (d, 1H, J = 15 Hz, H-β); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 35.80, 113.97, 115.06, 123.15, 127.29, 128.34, 128.47, 130.54, 130.89, 135.94, 139.23, 141.92, 147.63, 165.82; MS (*m/z*): 296/298 (M^+, a), 165, 147, 131, 120, 103, 91, 77; Anal. Calcd. for $C_{17}H_{13}ClN_2O$ (296.75): C, 68.81%; H, 4.42%; N, 9.44%; Found: C, 68.71%; H, 4.38%; N, 9.41%; HPLC purity: 97.98%, t_R-15.2 min.

*4.1.3.7. (E)-1-(6-methyl-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3g**).* Yield: 82%; m.p: 218–220 °C (Dec); IR (KBr): 2920 (CH_3), 1695 (C=O), 1622 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz, δ ppm): 2.50 (s, 3H, C6-CH_3), 7.20 (d, 1H, J = 15.5 Hz, H-α), 7.25–7.32 (m, 2H, C4,C4′-H), 7.46–7.52 (m, 3H,C7,C3′,C5′-H), 8.05 (d, 1H, J = 15.5 Hz, H-β), 8.14–8.18 (m, 3H, C5, C2′C6′-H), 8.50 (s, 1H, C2-H); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): 21.56, 115.82, 115.94, 126.49, 128.60, 129.15, 129.87, 131.35, 133.90, 134.95, 140.01, 140.54, 148.51, 162.52; MS (*m/z*): 262/263 (M^+, a), 146, 131, 103, 77; Anal. Calcd. for $C_{17}H_{14}N_2O$ (262.31): C, 77.84%; H, 5.38%; N, 10.68%; Found: C, 77.80%; H, 5.31%; N, 10.65%; HPLC purity: 98.17% t_R-15.5 min.

*4.1.3.8. (E)-3-phenyl-1-(2-phenyl-1H-benzo[d]imidazol-1-yl)prop-2-en-1-one (**3h**).* Yield: 72%; m.p: 130–132 °C; IR (KBr): 1697 (C=O), 1630 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz, δ ppm): 6.85 (d, 1H, J = 16 Hz, H-α), 7.28 (t, 2H, J = 7.4 Hz, C5, C6-H of Benzimidazole), 7.39 (t, 1H, J = 7.2 Hz, C4′-H)7.46–7.51 (m, 3H, C3′,C5′,C4 of Ph), 7.56–7.72 (m, 6H, Ar–H), 7.93 (d, 1H, J = 16 Hz, H-β), 8.36 (d, 2H, J = 8.4 Hz, C2,C6-H of Ph); ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): 114.23, 115.46, 124.41, 127.68, 127.98, 128.63, 128.82, 129.45, 130.43, 130.82, 130.96, 131.29, 135.43, 139.43, 142.01, 147.85, 167.94; MS (*m/z*): 324/325 (M^+, a), 193, 147, 131, 103, 77; Anal. Calcd. for $C_{22}H_{16}N_2O$ (324.38): C, 81.46%; H, 4.97%; N, 8.64%; Found: C, 81.41%; H, 4.99%; N, 8.61%; HPLC purity: 98.88%, t_R-18.2 min.

*4.1.3.9. (E)-1-(2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3i**).* Yield: 78%; m.p: 98–100 °C; IR (KBr): 2918 (CH_3), 1681 (C=O), 1621 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 3.60 (s, 3H, O-CH_3 of Ph), 6.12 (d, 1H, J = 15.2 Hz, H-α), 6.83 (d, 2H, J = 8.8 Hz, C3,C5-H of Ph), 7.09 (t, 2H, J = 7.4 Hz, C5, C6-H of Benzimidazole), 7.16 (t, 1H, J = 7.2 Hz, C4′-H), 7.43 (t, 2H, J = 7.2 Hz, C3′,C5′-H), 7.44–7.52 (m, 4H, Ar–H), 7.75 (d, 1H, J = 15.2 Hz, H-β), 8.52 (d, 2H J = 8.0 Hz, C2,C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 54.86, 114.11, 114.92, 115.31, 122.96, 123.12, 128.23, 128.64, 128.88, 130.46, 130.52, 130.86, 135.34, 138.93, 141.87, 147.63, 160.82, 165.63; MS (*m/z*): 357 (M^{+3}), 227, 147, 131, 121, 103, 91, 78; Anal. Calcd. for $C_{23}H_{18}N_2O_2$ (354.40): C, 77.95%; H, 5.12%; N, 7.90%; Found: C, 77.89%; H, 5.06%; N, 7.88%; HPLC purity: 98.76%, t_R-18.8 min.

*4.1.3.10. (E)-1-(2-(4-chlorophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3j**).* Yield: 84%; m.p: 116–118 °C; IR (KBr): 1686 (C=O), 1614 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.41 (d, 1H, J = 16 Hz, H-α), 7.16 (t, 2H, J = 7.4 Hz, C5, C6-H of Benzimidazole), 7.37 (t, 1H, J = 7.2 Hz, C4′H), 7.44 (t, 2H, J = 7.2 Hz, C3′,C5′-H), 7.60–7.70 (m, 6H, Ar–H), 7.83 (d, 1H, J = 16 Hz, H-β), 8.11 (d, 2H, J = 8.1 Hz, C2,C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 114.53, 116.75, 125.02, 128.43, 128.60, 129.09, 129.12, 129.81, 130.99, 133.73, 134.29, 136.86, 142.68, 147.11, 165.32; MS (*m/z*): 358/360/359 (M^+, a), 228, 147, 131, 103, 90, 77; Anal. Calcd. for $C_{22}H_{15}ClN_2O$ (358.82): C, 73.64%; H, 4.21%; N, 7.81%; Found: C, 73.55%; H, 4.20%; N, 7.77%; HPLC purity: 99.41%, t_R-19.2 min.

*4.1.3.11. (E)-1-(2-(2-hydroxyphenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3k**).* Yield: 56%; m.p: 198–200 °C; IR (KBr): 3315 (OH), 1681 (C=O), 1633 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.73 (d, 1H, J = 16 Hz, H-α), 6.91 (d, 1H, J = 6.8 Hz, C3-H of Ph), 7.04 (t, 1H, J = 7.0 Hz, C5-H of Ph), 7.31 (m, 3H, C4-H of Ph, C5, C6-H of Benzimidazole), 7.38 (t, 1H, J = 7.4 Hz, C4′-H) 7.48 (t, 2H, J = 7.4 Hz, C3′,C5′-H), 7.61–7.69 (m, 5H, Ar–H), 7.78 (d, 1H, J = 16 Hz, H-β), 8.03 (s, 1H, –OH D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 13.50, 46.92, 114.51, 115.36, 123.10, 128.06, 128.61, 128.83, 128.92, 130.43, 130.58, 130.76, 135.36, 139.13, 141.93, 147.33, 165.88; MS (*m/z*): 340/341 (M^+, a), 209, 131, 103, 90, 77; Anal. Calcd. for $C_{22}H_{16}N_2O_2$ (340.37): C, 77.63%; H, 4.74%; N, 8.23%; Found: C, 77.56%; H, 4.76%; N, 8.21%; HPLC purity: 98.74%, t_R-17.7 min.

*4.1.3.12. (E)-3-phenyl-1-(2-(p-tolyl)-1H-benzo[d]imidazol-1-yl) prop-2-en-1-one (**3l**).* Yield: 82%; m.p: 103–105 °C; IR (KBr): 2921 (CH_3), 1683 (C=O), 1621 (C=C) cm^{-1}; ^1H NMR (DMSO-d6, 500 MHz, δ ppm): 2.37 (s, 3H, C2-CH_3 of Ph), 6.54 (d, 1H, J = 16 Hz, H-α), 7.26–7.28 (m, 4H, Ar–H), 7.36 (t, 1H, J = 7.6 Hz, C4′-H), 7.42 (t, 2H, J = 7.6 Hz, C3′,C5′-H), 7.64–7.69 (m, 4H, Ar–H), 7.76 (d, 1H, J = 16 Hz, H-β), 8.04 (d, 2H, J = 7.2 Hz, C2,C6-H of Ph); ^{13}C NMR (DMSO-d6, 125 MHz, δ ppm): 20.88, 114.08, 119.63, 121.03, 124.57, 124.87, 128.19, 128.35, 128.91, 129.19, 129.59, 131.15, 133.75, 133.83, 140.30, 142.30, 145.48, 165.49; MS (*m/z*): 338/339 (M^+, a), 207, 147, 131, 103, 77; Anal. Calcd. for $C_{23}H_{18}N_2O$ (338.40): C, 81.63%; H, 5.36%; N, 8.28%; Found: C, 81.55%; H, 5.32%; N, 8.25%; HPLC purity: 99.87%, t_R-18.9 min.

*4.1.3.13. (E)-1-(2-(4-hydroxyphenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3m**).* Yield: 71%; m.p: 120–122 °C; IR (KBr): 3280 (OH), 1680 (C=O), 1649 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.62 (d, 1H, J = 16 Hz, H-α), 6.84 (d, 2H, J = 8.4 Hz, C3,C5-H of Ph), 7.26 (t, 2H, J = 7.4 Hz, C5, C6-H of Benzimidazole), 7.39 (t, 1H, J = 7.6 Hz, C4′-H), 7.51 (t, 2H, J = 7.6, C3′,C5′-H), 7.60–7.66 (m, 4H, Ar–H), 7.86 (d, 1H, J = 16 Hz, H-β), 7.92 (d, 2H, J = 7.8 Hz, C2,C6-H of Ph), 8.12 (s, 1H, –OH D_2O exchangeable); ^{13}C

NMR ($CDCl_3$, 100 MHz, δ ppm): 114.15, 115.82, 116.93, 123.12, 123.23, 128.03, 128.42, 128.56, 130.61, 130.83, 135.23, 138.93, 142.62, 147.45, 158.82, 165.33; MS (*m/z*): 340/341 (M^+, a), 209, 131, 103, 90, 77; Anal. Calcd. for $C_{22}H_{16}N_2O_2$ (340.37): C, 77.63%; H, 4.74%; N, 8.23%; Found: C, 77.55%; H, 4.72%; N, 8.21%; HPLC purity: 98.55%, t_R-18.3 min.

*4.1.3.14. (E)-1-(2-(2-nitrophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3n**).* Yield: 59%; m.p: 168–170 °C; IR (KBr): 1680 (C=O), 1625 (C=C), 1515 and 1340 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.72 (d, 1H, *J* = 16 Hz, H-α), 7.14 (t, 2H, *J* = 7.3 Hz, C5, C6-H of Benzimidazole), 7.28 (t, 1H, *J* = 7.6 Hz, C4′-H), 7.40 (t, 2H, *J* = 7.6, C3′,C5′-H), 7.59–7.67 (m, 5H, Ar–H), 7.94 (d, 1H, *J* = 16 Hz, H-β), 8.01 (t, 1H, *J* = 6.4 Hz, C5-H of Ph), 8.12 (d, 1H, *J* = 6.7 Hz, C3-H of Ph), 8.20 (d, 1H, *J* = 6.4 Hz, C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 114.23, 115.63, 123.08, 124.61, 126.43, 128.03, 128.46, 128.73, 129.74, 130.43, 130.86, 133.45, 135.88, 139.14, 142.63, 147.27, 150.23, 165.85; MS (*m/z*): 369/370 (M^+, a), 238, 131, 121, 103, 91, 77, 63; Anal. Calcd. for $C_{22}H_{15}N_3O_3$ (369.37): C, 71.54%; H, 4.09%; N, 11.38%; Found: C, 71.45%; H, 4.01%; N, 11.37%; HPLC purity: 98.92%, t_R-19.2 min.

*4.1.3.15. (E)-1-(2-(4-nitrophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3o**).* Yield: 85%; m.p: 215–216 °C; IR (KBr): 1684 (C=O), 1628 (C=C), 1522 and 1334 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.63 (d, 1H, *J* = 16 Hz, H-α), 7.31 (t, 2H, *J* = 7.2 Hz, C5, C6-H of Benzimidazole), 7.38 (t, 1H, *J* = 7.6 Hz, C4′-H), 7.48 (t, 2H, 7.6 Hz, C3′,C5′-H), 7.49–7.51 (m, 4H, Ar–H), 7.96 (d, 1H, *J* = 16 Hz, H-β), 8.03 (d, 2H, *J* = 6.8 Hz, C2,C6-H of Ph), 8.36 (d, 2H, *J* = 8.8 Hz, C3,C5-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 114.09, 119.69, 120.82, 123.82, 125.22, 126.07, 128.57, 129.28, 130.63, 131.91, 133.41, 134.23, 137.31, 142.88, 148.60, 150.54, 165.06; MS (*m/z*): 369/370 (M^+, a), 238, 131, 121, 103, 90, 79, 62; Anal. Calcd. for $C_{22}H_{15}N_3O_3$ (369.37): C, 71.54%; H, 4.09%; N, 11.38%; Found: C, 71.46%; H, 4.03%; N, 11.35%; HPLC purity: 98.12%, t_R-17.0 min.

*4.1.3.16. (E)-1-(2-(4-fluorophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3p**).* Yield: 85%; m.p: 215–216 °C; IR (KBr):1682 (C=O), 1619 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.73 (d, 1H, *J* = 16 Hz, H-α), 7.28 (t, 2H, *J* = 7.3 Hz, C5, C6-H of Benzimidazole), 7.33–7.38 (m, 3H, Ar–H), 7.46 (t, 2H, *J* = 7.4 Hz, C3′,C5′-H), 7.62–7.68 (m, 4H, Ar–H) 7.82 (d, 1H, *J* = 16 Hz, H-β), 7.98 (d, 2H, *J* = 8.2 Hz, C2,C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 114.19, 115.69, 117.82, 123.65, 125.22, 128.11, 128.94, 129.35, 130.63, 131.91, 133.41, 136.23, 139.31, 142.88, 147.60, 163.54, 165.43; MS (*m/z*): 342 (M^+), 212, 131, 121, 103, 90, 77, 63; Anal. Calcd. for $C_{22}H_{15}FN_2O$ (342.37): C, 77.18%; H, 4.42%; N, 8.18%; Found: C, 77.22%; H, 4.38%; N, 8.16%; HPLC purity: 99.26%, t_R-17.6 min.

*4.1.3.17. (E)-1-(2-(3-bromophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3q**).* Yield: 82%; m.p: 210–212 °C; IR (KBr): 1676 (C=O), 1612 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.69 (d, 1H, *J* = 16 Hz, H-α), 7.18 (t, 2H, *J* = 7.2 Hz, C5, C6-H of Benzimidazole), 7.29 (t, 1H, *J* = 7.4 Hz, C4′-H), 7.42–7.50 (m, 4H, Ar–H), 7.62–7.68 (m, 5H, Ar–H), 7.76 (d, 1H, *J* = 16 Hz, H-β), 8.35 (d, 1H, *J* = 6.8 Hz, C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 114.09, 115.29, 122.32, 123.11, 126.67, 127.87, 128.06, 128.63, 129.04,130.45, 130.78, 131.42, 131.68, 132.93, 135.34, 139.02, 141.50, 147.20, 165.00; MS (*m/z*): 404 [$(M + H)^+$, a], 272, 147, 131, 120, 103, 77, 63; Anal. Calcd. for $C_{22}H_{15}BrN_2O$ (403.27): C, 65.52%; H, 3.75%; N, 6.95%; Found: C, 65.58%; H, 3.78%; N, 6.91%; HPLC purity: 99.31%, t_R-18.4 min.

*4.1.3.18. (E)-1-(2-(3-chlorophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3r**).* Yield: 88%; m.p: 133–134 °C; IR (KBr): 1687 (C=O), 1619 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.73 (d, 1H, *J* = 16 Hz, H-α), 7.28 (t, 2H, *J* = 7.2 Hz, C5, C6-H of Benzimidazole), 7.39 (t, 1H, *J* = 7.6 Hz, C4′-H), 7.48–7.54 (m, 4H, Ar–H), 7.62–7.71 (m, 4H, Ar–H), 7.88 (d, 1H, *J* = 16 Hz, H-β), 8.06 (s, 1H, C2-H of Ph), 8.15 (d, 1H, *J* = 6.8 Hz, C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 114.15, 115.34, 123.55, 124.86, 126.48, 127.69, 128.59, 128.86, 129.08, 129.65, 130.65, 131.27, 132.67, 135.28, 136.12, 139.19, 141.58, 148.00, 165.31; MS (*m/z*): 358 (M^+), 228, 147, 131, 103, 63; Anal. Calcd. for $C_{22}H_{15}ClN_2O$ (358.82): C, 73.64%; H, 4.21%; N, 7.81%; Found: C, 73.60%; H, 4.10%; N, 7.72%; HPLC purity: 98.89%, t_R-19.5 min.

*4.1.3.19. (E)-1-(2-(3-nitrophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3s**).* Yield: 91%; m.p: 100–102 °C; IR (KBr): 1671 (C=O), 1629 (C=C), 1511 and 1337 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.62 (d, 1H, *J* = 16 Hz, H-α), 7.35 (t, 2H, *J* = 7.2 Hz, C5, C6-H of Benzimidazole), 7.39 (t, 1H, *J* = 7.4 Hz, C4′-H) 7.46 (t, 2H, *J* = 7.4, C3′,C5′-H), 7.52–7.66 (m, 5H, Ar–H), 7.71 (d, 1H, *J* = 16 Hz, H-β), 8.31 (d, 1H, *J* = 6.8 Hz, C4-H of Ph), 8.69 (s, 1H, C2-H of Ph), 8.73 (d, 1H, *J* = 6.9 Hz, C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm):114.18, 115.23, 122.78, 123.23, 124.12, 127.95, 128.65, 128.76, 130.21, 130.87, 131.04, 131.69, 133.88, 135.20, 138.86, 141.55, 147.20, 148.45, 165.12; MS (*m/z*): 369/370 (M^+, a), 238, 131, 121, 103, 90, 79, 62; Anal. Calcd. for $C_{22}H_{15}N_3O_3$ (369.37): C, 71.54%; H, 4.09%; N, 11.38%; Found: C, 71.42%; H, 4.01%; N, 11.41%; HPLC purity: 98.47%, t_R-19.8 min.

*4.1.3.20. (E)-1-(2-(2-chlorophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3t**).* Yield: 76%; m.p: 186–188 °C; IR (KBr): 1680 (C=O), 1625 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.68 (d, 1H, *J* = 16 Hz, H-α), 7.25 (t, 2H, *J* = 7.2 Hz, C5, C6-H of Benzimidazole), 7.36–7.40 (m, 3H, Ar–H), 7.46 (t, 2H, *J* = 7.5 Hz, C3′,C5′-H), 7.60–7.74 (m, 6H, Ar–H), 7.82 (d, 1H, *J* = 16 Hz, H-β); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm):114.16, 115.33, 123.34, 127.32, 128.01, 128.54, 128.72, 129.04, 129.54, 130.19, 130.66, 130.85, 131.94, 135.45, 137.81, 138.61, 142.00, 147.50, 165.22; MS (*m/z*): 358 (M^+), 228, 147, 131, 103, 63; Anal. Calcd. for $C_{22}H_{15}ClN_2O$ (358.82): C, 73.64%; H, 4.21%; N, 7.81%; Found: C, 73.67%; H, 4.20%; N, 7.78%; HPLC purity: 99.14%, t_R-19.5 min.

*4.1.3.21. (E)-1-(2-(3,4-dichlorophenyl)-1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one (**3u**).* Yield: 82%; m.p: 166–168 °C; IR (KBr): 1675 (C=O), 1613 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 6.58 (d, 1H, *J* = 16 Hz, H-α), 7.18 (t, 2H, *J* = 7.2 Hz, C5, C6-H of Benzimidazole), 7.29 (t, 1H, *J* = 7.6 Hz, C4′-H), 7.38–7.46 (m, 3H, Ar–H), 7.57–7.63 (m, 4H, Ar–H), 7.68 (d, 1H, *J* = 16 Hz, H-β), 8.01 (s, 1H, C2-H of Ph), 8.12 (d, 1H, *J* = 6.8 Hz, C6-H of Ph); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm):114.26, 115.22, 123.14, 127.32, 127.96, 128.46, 128.68, 129.02, 130.14, 130.68 139.90, 133.01, 133.80 135.25, 139.15, 141.50, 147.46, 165.08; MS (*m/z*): 392 (M^{-1}), 394 [$(M + H)^+$, a], 261, 131, 103, 63; Anal. Calcd. for $C_{22}H_{14}Cl_2N_2O$ (393.27): C, 67.19%; H, 3.59%; N, 7.12%; Found: C, 67.24%; H, 3.63%; N, 7.08%; HPLC purity: 99.52%, t_R-19.2 min.

4.2. Methodology for the evaluation of anti-tubercular activity assay

All the synthesized molecules were screened for their potential to inhibit the growth of *M. tuberculosis* strain H37Rv using the microplate Alamar Blue assay (MABA) [33] method. The experiment was carried out in triplicate using a 96-welled plate under the standard conditions. Initially, 200 μL sterile deionized water was added to each well of the plate to minimize evaporation of medium in the test wells during incubation. Subsequently 100 μL of Middlebrook 7H9 broth was added to each well. Further, the test

compounds were serially diluted by two fold serial dilution method. Consequently, the concentrations of tested compounds were in the range of 100–0.2 μg/mL. The plates were covered and sealed with parafilm and incubated at 37 °C for 5 days. Further, 25 μL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween-80 was added to the plate and incubated for 24 h. The blue color in the well indicated the inhibition of bacterial growth while the pink color was scored as growth. Further, the minimum concentration of the compound required to inhibit the bacterial growth was determined.

4.3. Methodology for cytotoxicity assay

4.3.1. Cell culture

Human lung (A549) and lymphocyte T (8E5) cancer cell lines were used to evaluate the impact on cell viability of the tested compounds (**3b**, **3i** and **3l**) by MTT assay method [34]. A549 and 8E5 cells were cultured in RPMI 1640 and Ham's F12k medium respectively; all media were supplemented with 10% fetal calf serum (FCS). The compounds were dissolved in 0.1% DMSO and then diluted with the medium.

4.3.2. Cytotoxicity assay

In brief, exponentially growing cells (10^5 cells/well) were plated in 96-well plates. After treatment of cells with the xenobiotics, the cell monolayers were gently washed with warm phosphate-buffered saline (PBS) using a multichannel pipette. The cells were then exposed to different concentrations of drug (10–30 μg mL^{-1}) and kept for incubation for 48 h. After the incubation, 100 μL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) solution (5 mg mL^{-1}) was added to each well and cells were incubated for additional 2–3 h. After careful removal of incubation medium from the incubator, 100 μL of dimethyl sulphoxide was added and the plates were gently shaken to resuspend formed formazan and waited for few mins to form a homogenized color. The suspension was placed on a microvibrator for 5 min, and absorbances of wells containing cells and blanks were recorded at 490 nm. The experiment was executed in triplicate. The mean of the absorbance of wells was calculated with the same treatment after subtracting of blank absorbance. The results were normalized by considering control wells as 100% (maximum absorbance obtained), expressing then the results as percentage of controls.

Acknowledgments

The authors acknowledge the University Scientific Instrumentation Centre (USIC), Karnatak University, Dharwad for IR, NMR, Mass, SC-XRD and analytical data. We are also grateful to NMR Research Centre, Indian Institute of Science (IISc), Bengaluru, India for carrying out the spectral analyses.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.017.

References

[1] World Health Organization, Global Tuberculosis Report, 2012,. http://www.who.int/tb/publications/global_report/gtbr12_executivesummary.pdf (last accessed on 21.10.13).
[2] C. Dye, Global epidemiology of tuberculosis, The Lancet 367 (2006) 938–940.
[3] C. Dye, K. Lonnroth, E. Jaramillo, B.G. Williams, M. Raviglione, Trends in tuberculosis incidence and their determinants in 134 countries, Bulletin of the World Health Organization 87 (2009) 683–691.
[4] H. Tomioka, Current status of some antituberculosis drugs and the development of new antituberculous agents with special reference to their in vitro and in vivo antimicrobial activities, Current Pharmaceutical Design 12 (2006) 4047–4070.
[5] M.K. Spigelman, New tuberculosis therapeutics: a growing pipeline, Journal of Infectious Diseases 196 (2007) 28–34.
[6] A.M. Ginsberg, M. Spigelman, Challenges in tuberculosis drug research and development, Natural Medicines 13 (2007) 290–294.
[7] Z. Ma, C. Lienhardt, H. McIlleron, A.J. Nunn, X. Wang, Global tuberculosis drug development pipeline: the need and the reality, Lancet 375 (2010) 2100–2109.
[8] C. Lienhardt, A. Vernon, M.C. Raviglione, New drugs and new regimens for the treatment of tuberculosis: review of the drug development pipeline and implications for national programmes, Current Opinion in Pulmonary Medicine 16 (2010) 186–193.
[9] Beena, D.S. Rawat, Antituberculosis drug research: a critical overview, Medicinal Research Reviews 33 (2013) 693–764.
[10] K. Kumar, D. Awasthi, S.Y. Lee, I. Zanardi, B. Ruzsicska, S. Knudson, P.J. Tonge, R.A. Slayden, I. Ojima, Novel trisubstituted benzimidazoles, targetting *Mtb* FtsZ, as a new class of antitubercular agents, Journal of Medicinal Chemistry 54 (2011) 374–381.
[11] M.J. O'Niel, M. Smith, P.E. Heckelman, The Merck Index, thirteenth ed., Merck & Co. Inc., New Jersey, 2001, p. 1785.
[12] V.M. Reddy, K.R. Reddy, Synthesis and antimicrobial activity of some novel 4-(1H-benz[d]imidazol-2yl)-1,3-thiazol-2-amines, Chemical and Pharmaceutical Bulletin 58 (2010) 953–956.
[13] V. Klimešova, J. Koci, K. Waisser, J. Kaustova, New benzimidazole derivatives as antimycobacterial agents, Il Farmaco 57 (2002) 259–265.
[14] M. Pieroni, S.K. Tipparaju, S. Lun, Y. Song, A.W. Sturm, W.R. Bishai, A.P. Kozikowski, Pyrido[1,2-a]benzimidazole-based agents active against tuberculosis (TB), multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB, ChemMedChem 6 (2011) 334–342. Special Issue: Rare & Neglected Diseases.
[15] M.M. Ramla, M.A. Omar, A.M.M. El-Khamry, H.I. El-Diwani, Synthesis and antitumor activity of 1-substituted-2-methyl-5-nitrobenzimidazoles, Bioorganic & Medicinal Chemistry 14 (2006) 7324–7332.
[16] M.M. Ramla, M.A. Omar, H. Tokuda, H.I. El-Diwani, Synthesis and inhibitory activity of new benzimidazole derivatives against Burkitt's lymphoma promotion, Bioorganic & Medicinal Chemistry 15 (2007) 6489–6496.
[17] S. Demirayak, U.A. Mohsen, A.C. Karaburun, Synthesis and anticancer and anti-HIV testing of some pyrazino[1,2-a]benzimidazole derivatives, European Journal of Medicinal Chemistry 37 (2002) 255–260.
[18] A.A.E. Rashedy, H.Y.A. Enein, Benzimidazoles derivatives as potential anticancer agents, Mini-Reviews in Medicinal Chemistry 13 (2013) 399–407.
[19] K.D. Thomas, A.V. Adhikari, I.H. Chowdhury, E. Sumesh, N.K. Pal, New quinolin-4-yl-1,2,3-triazoles carrying amides, sulphonamides and amidopiperazines as potential antitubercular agents, European Journal of Medicinal Chemistry 46 (2011) 2503–2512.
[20] S. Sharma, P.K. Sharma, N. Kumar, R. Dudhe, A review on various heterocyclic moieties and their antitubercular activity, Biomedicine & Pharmacotherapy 65 (2011) 244–251.
[21] E.L. White, L.J. Ross, R.C. Reynolds, L.E. Seitz, G.D. Moore, D.W. Borhani, Slow polymerization of *Mycobacterium tuberculosis* FtsZ, Journal of Bacteriology 182 (2000) 4028–4034.
[22] A.D. Khoje, C. Charnock, B. Wan, S. Franzblau, L.L. Gundersen, Synthesis and antimycobacterial activities of non-purine analogs of 6-aryl-9-benzylpurines: imidazopyridines, pyrrolopyridines, benzimidazoles, and indoles, Bioorganic & Medicinal Chemistry 19 (2011) 3483–3491.
[23] H. Xiangming, M. Huiqiang, W. Yulu, p-TsOH catalyzed synthesis of 2-arylsubstituted benzimidazoles, Arkivoc (Archive for Organic Chemistry) (2007) 150–154 xiii.
[24] T. Nemoto, T. Ohshima, M. Shibasaki, Catalytic asymmetric synthesis of α,β-epoxy esters, aldehydes, amides, and γ,δ-epoxy β-keto esters: unique reactivity of α, β-unsaturated carboxylic acid imidazolides, Journal of the American Chemical Society 123 (2001) 9474–9475.
[25] (a) , Oxfordshire, EnglandOxford Diffraction Ltd., Xcalibur CCD System, CrysAlis Software System Version 1.171.34.40 Abingdon, 2008;
(b) G.M. Sheldrick, A short history of SHELX, Acta Crystallographica Section A 64 (2008) 112.
[26] M. Kratky, J. Vinsova, E. Novotna, J. Mandikova, V. Wsol, F. Trejtnar, V. Ulmann, J. Stolarikova, S. Fernandes, S. Bhat, J.O. Liu, Salicylanilide derivatives block Mycobacterium tuberculosis through inhibition of isocitrate lyase and methionine aminopeptidase, Tuberculosis 92 (2012) 434–439.
[27] R.B. Pathak, P.T. Chovatia, H.H. Parekh, Synthesis antitubercular and antimicrobial evaluation of 3-(4-chlorophenyl)-4-substituted pyrazole derivatives, Bioorganic & Medicinal Chemistry Letters 22 (2012) 5129–5133.
[28] A. Garcia, V. Bocanegra-Garcia, J.P. Palma-Nicolas, G. Rivera, Recent advances in antitubercular natural products, European Journal of Medicinal Chemistry 49 (2012) 1–23.
[29] (a) P. de, G.K. Yoya, P. Constant, F. Bedos-Belval, H. Duran, N. Saffon, M. Daffe, M. Baltas, Design, synthesis and biological evaluation of new cinnamic

derivatives as antituberculosis agents, Journal of Medicinal Chemistry 54 (2011) 1449–1461;
(b) I.Y. Lee, T.D. Gruber, A. Samuels, M. Yun, B. Nam, M. Kang, K. Crowley, B. Winterroth, H.I. Boshoff, C. Berry, Structure-activity relationship of antitubercular salicylanilides consistent with disruption of the proton gradient via proton shuttling, Bioorganic & Medicinal Chemistry 21 (2013) 114–126.

[30] R. Villar, E. Vicente, B. Solano, S. Perez-Silanes, I. Aldana, J.A. Maddry, A.J. Lenaerts, S.G. Franzblau, S.-H. Cho, A. Monge, R.C. Goldman, Invitro and invivo antimycobacterial activities of ketone and amide derivatives of quinoxaline 1,4-di-N-oxide, Journal of Antimicrobial Chemotherapy 62 (2008) 547–554.

[31] E. Chew, A.A. Nagle, Y. Zhang, S. Scarmagnani, P. Palaniappan, T.D. Bradshaw, A. Holmgren, A.D. Westwell, Cinnamaldehydes inhibits thioredoxin reductase and induce Nrf2: potential candidates for cancer therapy and chemoprevention, Free Radical Biology & Medicine 48 (2010) 98–111.

[32] A.B. Alloum, K. Bougrin, M. Soufiaoui, Synthèse chimio sélective des benzimidazoles sur silice traitée par le chlorure du thionyle, Tetrahedron Letters 44 (2003) 5935–5937.

[33] S.G. Franzblau, R.S. Witzig, J.C. McLaughlin, P. Torres, G. Madico, A. Hernandez, M.T. Degnan, M.B. Cook, V.K. Quenzer, R.M. Ferguson, R.H. Gilman, Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar blue assay, Journal of Clinical Microbiology 36 (1998) 362–366.

[34] A. Doyle, J.B. Griffiths, Cell and Tissue Culture for Medical Research, first ed., John Wiley and Sons Ltd, 2000, p. 409.

Glossary

TB: tuberculosis
Mtb: Mycobacterium tuberculosis
MDR-TB: multi-drug-resistant tuberculosis
A549: human lung carcinoma
8E5: human; acute lymphoblastic leukemia
p-TsOH: p-toluenesulfonic acid
THF: tetrahydrofuran
FTIR: Fourier transform infrared spectroscopy
^{1}H *NMR:* proton nuclear magnetic resonance
^{13}C *NMR:* carbon-13 nuclear magnetic resonance
MIC: minimum inhibitory concentration
IC_{50}*:* half maximal inhibitory concentration
MABA: microplate Alamar blue assay
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
FCS: fetal calf serum
PBS: phosphate-buffered saline
SC-XRD: single crystal X-ray diffraction.
Ph: phenyl ring
Ar: aromatic
HPLC: high performance liquid chromatography
MeCN: acetonitrile
t_R*:* retention time
SAR: structure activity relationship