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Original article

Synthesis and biological evaluation of novel Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazoles as potential anti-cancer agents

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ABSTRACT

A new series of Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazoles were synthesized and evaluated for their anti-cancer activity. These compounds showed better cytotoxicity activity with IC_{50} values ranging from 2.8 to 8.0 μM in HepG2, MCF-7 and HeLa cell lines. Further mechanism aspects responsible for the anti-cancer activity of two promising compounds **3c** and **3f** in HepG2 cell line were studied. Interestingly, **3c**, **3f** induced G2/M cell cycle arrest with down regulation of cyclin B and up regulation of Chk2 protein. Moreover, compounds **3c**, **3f** also showed the characteristic features of apoptosis such as enhancement in the levels of caspase-3. Treatments with compounds led to a decrease in levels of vital cell proliferation proteins such as Jun (C-Jun, JunB), p38 MAPK, p-JNK and PKCα. The compound **3f** of the series could be considered as the potential lead for its development as a novel anti-cancer agent.

1. Introduction

Cancer chemotherapy represents one of the most relevant challenges of the chemists and oncologist. In order to gain new insights into the complexity of the disease, robust screening methods for evaluating different natural or synthetic drugs have been carried out from the scientific community. Numerous studies have been carried out to screen compounds with antitumour activity against different cancer cell lines including hepatocellular carcinoma, breast cancer, acute myelogenous leukaemia, non-small cell lung carcinoma etc.

World statistics on cancers have revealed that hepatocarcinoma is the sixth most common malignancy which is highly resistant to chemotherapeutic treatment resulting in increased mortality rates [1–3]. Therefore, in this respect, the benzothiazoles constitutes an important scaffold of drugs, possessing several pharmacological functions, rendering this molecule and its derivatives as powerful antitumour agents particularly for the treatment of hepatocarcinoma as observed in our study. Also, in this context the study of signalling pathways and key molecules that regulate cell proliferation, oncogenesis, invasion and metastasis of liver cancer such as AP-1 (C-Jun, JunB), NF-kB, p38 MAPK would drive the way to the development of selective targeted therapies for hepatocarcinoma [4].

Earlier studies on benzothiazoles demonstrated interesting pharmacological activities including antitubercular [5], antimalarial [6], anticonvulsant [7,8], antihelmintic [9,10], analgesic [11], anti-inflammtory [12], antidiabetic [13] and antitumor activities [14–17]. In recent years, several attempts were made for modifying the benzothiazole nucleus to improve their antitumor activities. Modifications on the benzothiazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. Among them, imidazo benzothiazoles, as well as, polymerized benzothiazoles and other substituted benzothiazoles such as 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610) (Fig. 1) has been shown to exhibit exquisitely potent (GI50 < 0.1 nM) and selective *in vitro* antitumor properties in human cancer cell lines (e.g., colon, nonsmall- cell lung and breast subpanels) of the National Cancer Institute (NCI) 60 human cancer cell line screen [18] and also exhibited remarkable antitumor activity against malignant cell lines [19]. Among the imidazo benzothiazole class

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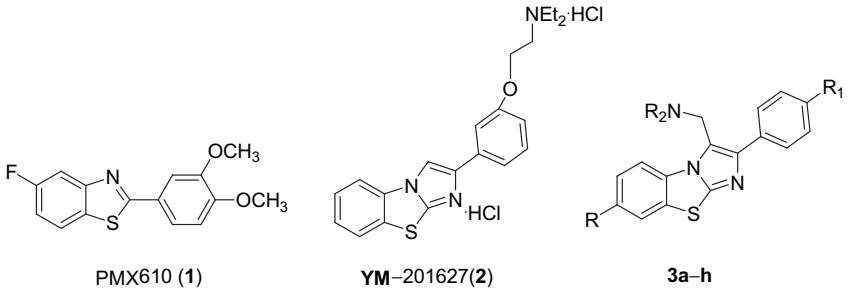
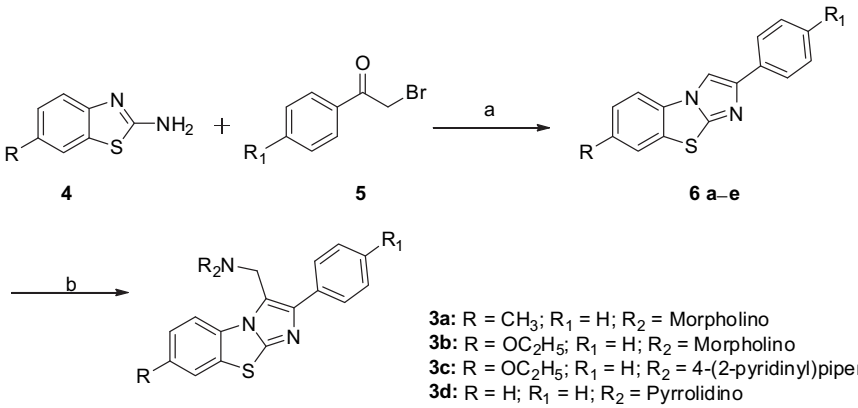


Fig. 1. Structure of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (**1**), *N,N*-Diethyl-2-(3-imidazo [2,1-b] [1,3]benzothiazole-2-ylphenoxy)ethnamine dihydrochloride (**2**), Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazoles derivatives (**3**).



3a: R = CH_3; R_1 = H; R_2 = Morpholino
3b: R = OC_2H_5; R_1 = H; R_2 = Morpholino
3c: R = OC_2H_5; R_1 = H; R_2 = 4-(2-pyridinyl)piperazino
3d: R = H; R_1 = H; R_2 = Pyrrolidino
3e: R = CH_3; R_1 = H; R_2 = Pyrrolidino
3f: R = F; R_1 = H; R_2 = Pyrrolidino
3g: R = OC_2H_5; R_1 = H; R_2 = Pyrrolidino
3h: R = H; R_1 = Cl; R_2 = Pyrrolidino

Scheme 1. Reagents and conditions: (a) Ethanol, MW, 180 W, 4–5 Min, 77–82%; (b) $ZnCl_2$, secondary amine, HCHO, Ethanol, 4–5 h, rt, 84–91%.

of compounds, 2-arylimidazo [2,1-b] [1,3]benzothiazole derivative (YM-201627) (Fig. 1) was found to be a potent and orally active antitumor agent and useful for treatment of solid tumors [20]. Aminoalkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [21,22]. Further, the presence of nitrogen atom may impart interesting biological activities to the parent compound [23–25]. Hence in continuation of our efforts on the design of novel anti-cancer agents [26–28] and keeping in mind the medicinal importance of imidazo benzothiazole moiety, it was thought worthwhile to synthesize certain novel derivatives of benzothiazoles like Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazoles and screen them for their biological activities particularly for their anti-cancer activity. Representatives of some biologically important benzothiazole compounds have been illustrated in Fig. 1.

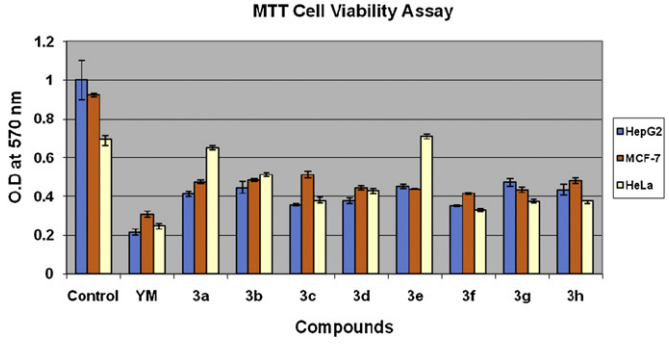


Fig. 2. Cell viability observed after the treatment with compounds at 4 μM concentration by MTT assay. Here HepG2, MCF-7 and HeLa cells were treated with various compounds (**3a**–**h**) at 4 μM concentration as indicated for 24 h in 96 well plates at 10,000 cells per well. Compound YM-201627 (YM) was used as standard control. Control indicates the untreated cells.

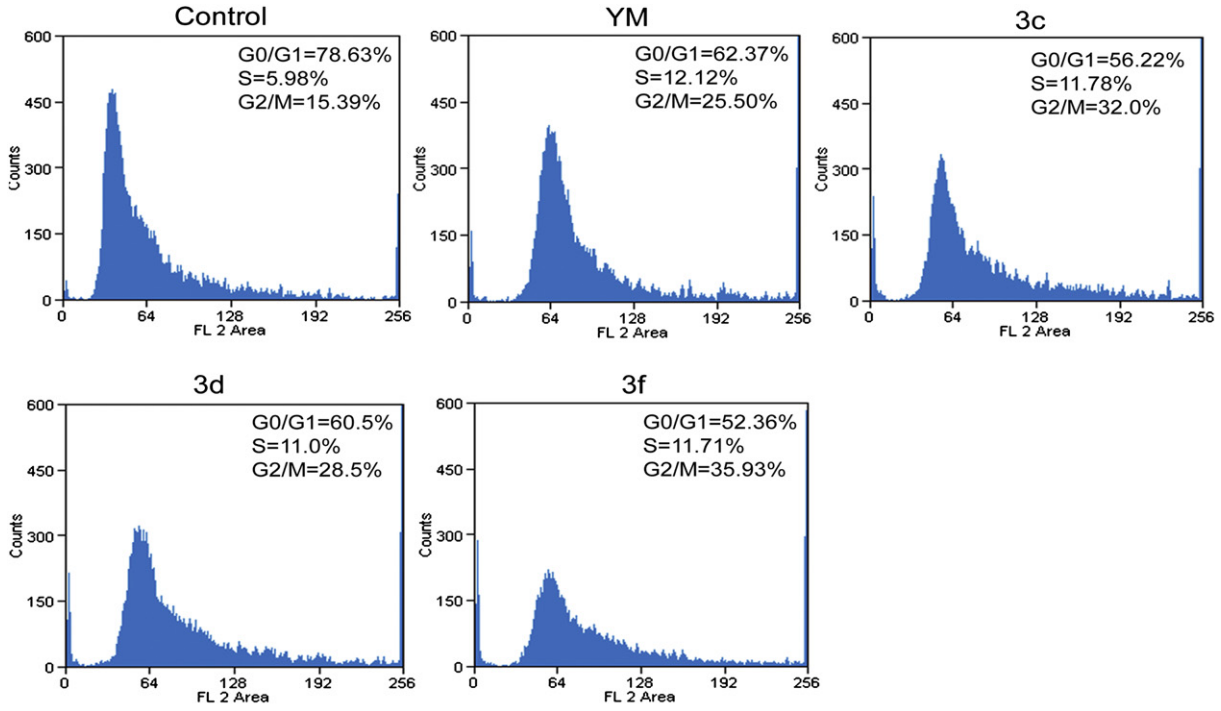
2. Chemistry

The 2-aryl-imidazo(2,1-b) benzothiazole derivatives (**6a**–**e**) were prepared by the condensation of substituted 2-aminobenzothiazoles **4** with the appropriate 2-bromoacetophenone **5** under

Table 1
IC_{50} values for compounds **3a**–**h** in selected cancer cell lines as well as normal cells (HEK).

	IC_{50}(μM)	IC_{50} (μM)	IC_{50}(μM)	IC_{50}(μM)
	HEK[a]	HepG2[b]	MCF-7[c]	HeLa[d]
YM	17.5 ± 0.2	2.02 ± 0.136	3.59 ± 0.13	4.3 ± 0.08
3a	19 ± 0.5	3.288 ± 0.112	3.8 ± 0.064	7.59 ± 0.11
3b	21 ± 0.25	3.52 ± 0.232	4.2 ± 0.052	5.89 ± 0.11
3c	16 ± 0.5	2.84 ± 0.039	4.45 ± 0.139	4.36 ± 0.13
3d	18.5 ± 0.75	3.01 ± 0.12	3.869 ± 0.004	4.65 ± 0.196
3e	17.5 ± 0.53	3.6 ± 0.072	3.8 ± 0.13	8.09 ± 0.138
3f	15 ± 0.22	2.8 ± 0.031	3.60 ± 0.043	3.82 ± 0.1
3g	18.80 ± 0.42	3.76 ± 0.16	3.76 ± 0.13	4.3 ± 0.08
3h	21.2 ± 0.75	3.464 ± 0.224	4.07 ± 0.121	4.33 ± 0.15

HEK[a] = Human embryonic kidney cells; HepG2[b]= Hepatoma; MCF-7[c] = breast cancer cells; HeLa[d] = Human cervical cancer cells. The data obtained here is from three independent experiments and Standard deviation (S.D) values were derived.



Cell cycle distribution of HepG2 cell line using **3a- h** at 4 μM concentration

Compounds	Cell cycle distribution (%)		
	GO/G1	S	G2/M
Control	80.37 ± 0.58	5.36 ± 0.37	14.26 ± 1.62
YM	64.19 ± 1.68	13.37 ± 1.08	22.43 ± 2.72
3a	70.89 ± 0.97	8.20 ± 0.26	20.90 ± 1.21
3b	73.54 ± 0.414	5.99 ± 0.011	20.46 ± 0.41
3c	55.24 ± 1.13	13.42 ± 1.44	31.33 ± 1.15
3d	60.20 ± 0.97	10.52 ± 0.5	29.27 ± 0.93
3e	73.97 ± 0.15	7.13 ± 0.15	18.95 ± 0.05
3f	55.65 ± 3.09	9.37 ± 2.14	34.97 ± 0.96
3g	60.12 ± 0.97	12.64 ± 0.55	27.23 ± 1.07
3h	81.75 ± 1.08	4.58 ± 0.53	13.66 ± 0.80

Fig. 3. FACS analysis of cell cycle distribution of HepG2 cells after treatment with compounds **3c**, **3d** and **3f** at 4 μM concentration for 24 h. Compound **YM-201627 (YM)** was used as standard control. Control = untreated cells.

microwave irradiation conditions at 180 W for 4 min. Alkylation of compounds (**6a**–**e**) by reacting with cyclic secondary amines and formic aldehyde in the presence of $ZnCl_2$ as catalyst provided the final compounds **3a**–**h** as shown in Scheme 1.

3. Result and discussion

3.1. Biological evaluation

3.1.1. Anti-proliferative activity

The representative compounds **3a**–**h** were evaluated for their *in vitro* antiproliferative activity against three cancer cell lines such as Hepato carcinoma cell line (HepG2), Breast carcinoma cell line (MCF-7) and Human cervical carcinoma cell line (HeLa) by MTT assay. MTT assay, is a mitochondrial function assay that is based on the ability of viable cells to reduce the MTT to insoluble formazan crystals by mitochondrial dehydrogenase [29]. Treatment of these three cancer cell lines with compounds (**3a**–**h**) at a concentration of 4 μM for 24 h inhibited cell proliferation and particularly the effect is more pronounced in HepG2 cells. (Fig. 2). IC_{50} values were shown in Table 1. From these IC_{50} values, the selectivity index (SI) was calculated for each of the cell line tested. Selectivity Index (SI) for a compound is calculated as the ratio of IC_{50} for the normal cell line to the IC_{50} for the cancer cell line. The selectivity index of this series of compounds are in the range of 4.86–6.14, 3.59–5.2 and 2.163–4.89 in HepG2, MCF-7 and HeLa cell lines respectively, Where as standard positive control YM-201627 has shown SI values of 8.663, 4.87 and 4.069 in HepG2, MCF-7 and HeLa cell lines respectively. Among all the compounds synthesized, compounds **3c**, **3d** and **3f** were found to be more toxic than others of this series. The structure - activity relationship studies revealed that the introduction of a fluorine atom in the 7th position of the target compounds (**3f**) enhanced the cytotoxic activity. Similarly, the Mannich base of imidazobenzothiazole at the 3rd position derived from various secondary cyclic amines also lead to an increase in cytotoxic activity. Further, Trypan blue exclusion assay was conducted and is based on the principle that live cells posseses intact cell membranes that excludes the trypan blue dye where as dead cells do not. Our results revealed the superior cytotoxic activity of **3c**, **3d** and **3f** compared to other compounds of the same series (data not shown).

3.1.2. Cell cycle effects

To further investigate the differential growth inhibition mechanism mediated by benzothiazole compounds on cell cycle dynamics. HepG2 cells were synchronized with Nocodazole treatment and compounds **3c**, **3d** and **3f** were tested. The cell cycle distribution was analyzed by treating HepG2 cells at 4 μM

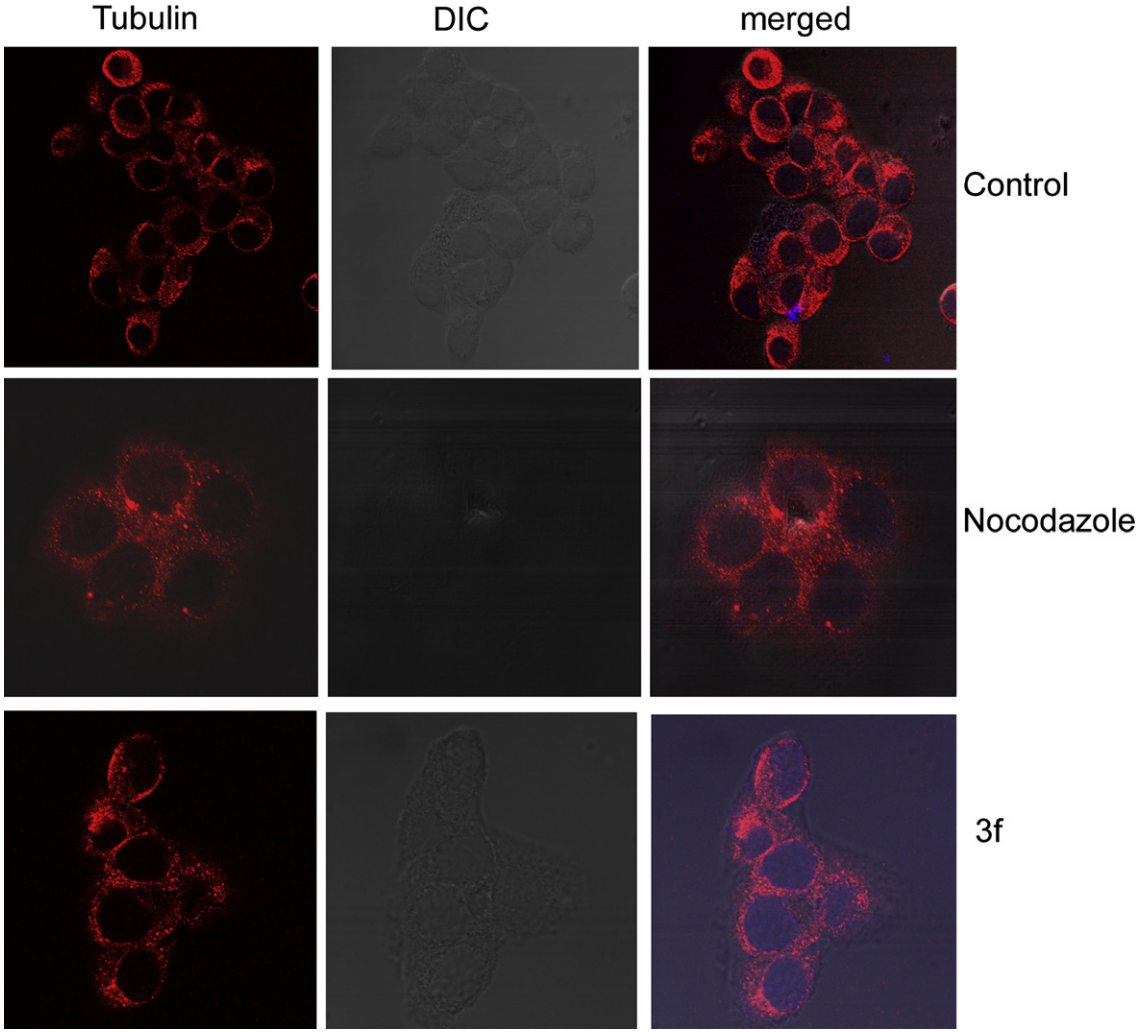


Fig. 4. Effect of compounds on the microtubule network in HepG2 cells. Cells were treated with compounds Nocodazole, **3f** at 4 μM for 24 h. The cells were stained with α-tubulin antibody. The tubulin pattern was observed as red coloured because secondary antibody used here was Cy3 labelled.

concentration for 24 h by flow cytometry. Results indicated 32% (**3c**), 35% (**3f**), 28% (**3d**) of cells in G2/M phase respectively, where as, the positive control YM-201627(**YM**) and control i.e untreated cells showed 25% and 15% of G2/M phase cells respectively as shown in Fig. 3a and b. Among all the compounds tested compounds **3c** and **3f** could be considered as the most effective derivatives to produce cell cycle arrest Fig. 3.

3.1.3. Effect on tubulin polymerization

Previous studies [30] showed that the inhibition of tubulin polymerization is associated with G2-M phase cell cycle arrest. As we have observed the G2/M phase arrest, it was considered of interest to understand the underlying mechanism of these compounds. Since **3f**, is the most effective compound in causing G2/M arrest among all the compounds tested the effect of this compound on tubulin polymerization was studied by immunofluoroscence microscopy studies using α-tubulin antibody. These results showed that **3f** functions similar to Nocodazole (the standard tubulin polymerization inhibitor), and effected tubulin cytoskeleton as shown in Fig. 4.

3.1.4. Effect on CyclinB1and check point 2 (Chk2) proteins

It is known from the previous studies that the cell cycle progression is regulated by the expression of some cell cycle specific cyclins. Hence, to understand the mechanism underlying the G2/M cell cycle arrest in these compounds, we examined the effect of compounds (**3c**, **3f**) on the expression of Cyclin B1, which controls cell cycle progression from G2 to M phases [31]. It is observed from the results that there is down regulation of Cyclin B1, particularly in case of compound **3f** treated HepG2 cells as shown in Fig. 5. G2/M phase arrest provides sufficient time for cells to repair its damaged DNA before proceeding to the next phase of cell cycle. Thus it was considered of interest to study the expression levels of important check point protein such as Check point 2 (Chk2) that play a vital role in the G2/M phase. HepG2 cells were treated with compounds (**3c** and **3f**) at 4 μM concentration for 24 h. It was observed that there is an up-regulation of Chk2 protein in case of compounds **3c** and **3f**, especially in **3f** compound treated cells as shown in Fig. 5.

3.1.5. Effect on proteins that regulate cell cycle in HepG2 cells

Previous studies [32] revealed that MAPK family of proteins such as JNK, ERK and p38 are involved in processes such as DNA damage. Moreover, MAPK family proteins such as MAPK and Phosphorylated forms of c-Jun terminal Kinase (p-JNK) regulate the proteins that are tightly associated with G2/M arrest [33,34]. Hence to understand the role and possible involvement of MAPK and JNK in this event, HepG2 cells were treated with compounds **3c** and **3f** at 4 μM concentration for 24 h. The cell lysates were subjected to Western analysis and a down regulation of these two proteins (MAPK and p-JNK) suggested a G2/M arrest as shown in Fig. 6.

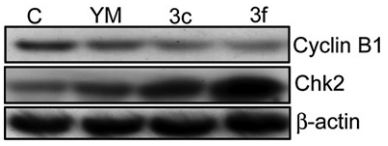


Fig. 5. Effect of compounds on Cyclin B1 and Chk2. HepG2 Cells were treated with 4 μM concentration of compounds (**3c**, **3f**) for 24 h. The cell lysates have been collected and observed the levels of Cyclin B and Chk2 proteins using specific antibody. β-actin was used as loading control. C: Control (untreated). Compound YM was used as standard control.

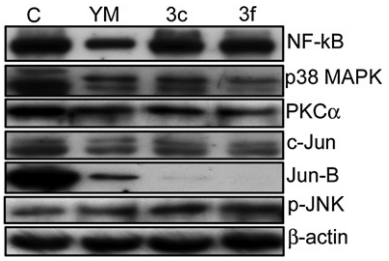


Fig. 6. Effect of compounds on cell cycle and cell proliferation related proteins. HepG2 Cells were treated with 4 μM concentration of compounds (**3c**, **3f**) for 24 h. YM was used as standards control. The cell lysates have been collected and observed the levels of NF-kB (p65), PKC α, p38 MAPK, C-Jun, JunB, p-JNK protiens using specific antibody. β-actin was used as loading control. C: control (untreated). Compound YM were used as standard control.

3.1.6. Effect on proteins that regulate cell proliferation in HepG2 cells

Further interested to study the effect of these compounds on other protein factors (c-Jun, JunB and NF-kB) that play vital role in active cell proliferation and drug resistance [35,36]. Moreover, NF-kB (p65) is a key molecule that is constitutively activated and caused the hepatocarcinoma the suppression of which leads to abrogation of cell proliferation [37,38]. c-Jun/JunB inactivation induces apoptosis in cancer cells. In the absence of c-Jun, JunB can act as a tumor promoter and inactivation of both, c-Jun and JunB, could provide a valuable strategy for antitumor intervention [39]. Hence, the HepG2 cells were treated with these compounds (**3c** and **3f)** at 4 μM concentration for 24 h and western analysis was carried out. Surprisingly from the results it is observed that there is down regulation of c-Jun and its other isoform B-Jun and NF-kB proteins, particularly in compound **3f** treated cells as shown in Fig. 6. Thus, this reveals the antiproliferative action of these compounds on HepG2 cells. Further it is considered of interest to evaluate the levels of up-stream activators of JNK pathway such as PKCα control the processes such as cell proliferation and apoptosis [40]. Here, the

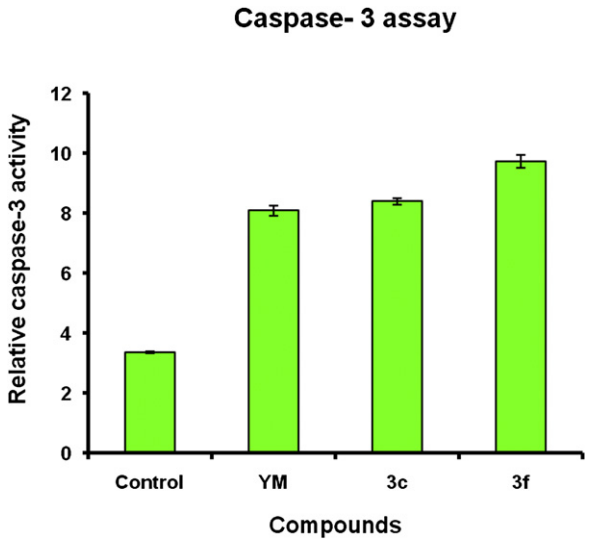


Fig. 7. Effect of compound on caspase-3 activation in HepG2 cells. The increased enzymatic activity of caspase-3, in apoptosis after the treatment of compounds (**3c**, and **3f**) was determined by flourimetry. The cleavage of peptide by caspase-3 releases the fluorophore AFC that was quantified at their excitation wavelength of 400 nm and emission wave length of 505 nm. *Y*-axis represents relative caspase-3 activity and *X*-axis represents compound treatments (**YM**, **3c** and **3f**). Compound YM-201627 (YM) was used as standard. Control indicates theuntreated cells.

HepG2 cells were treated with compounds (**3c** and **3f**) at 4 μM concentration for 24 h and observed for the level of PKCα protein. The down regulation of PKCα protein was observed in compound treated cells as shown in Fig. 6. This revealed that these compounds affect the PKCα dependent pathway in HepG2 cells.

3.1.7. Effect on caspases

Previous studies [41] reported that caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. Programmed cell death or apoptosis was mediated by death proteases known as caspases. Out of all caspases the Caspase-3 is a frequently activated death protease and is indispensible for DNA fragmentation, chromatin condensation and the formation of apoptotic bodies in all the cell types tested [42]. To understand the apoptosis inducing nature of these compounds the caspase-3 activity was carried out by Fluorescence based method. In compound treated cells particularly **3c** and **3f** the caspase-3 activation was up-regulated more than two-fold in comparison to control. The order of caspase-3 activation was **3f** > **3c** (Fig. 7). Here **YM** (YM-201627) was used as standard.

4. Conclusion

A new class of Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazoles compounds were synthesised and evaluated for their antiproliferative activities in three cancer cell lines. From the MTT proliferation assay, it was observed that compound **3f** isthe most effective antiproliferative agent than the other compounds of the series against all three cell lines (HepG2, MCF-7 and HeLa) when tested at 4 μM concentration. The FACS analysis also showed more population in G2/M phase in compounds **3c**, **3d** and **3f** indicating that these compounds caused G2/M cell cycle arrest with **3f** being the most effective. From the results of the detailed biological assays, drastic down regulation of cell cycle regulatory protein such as Cyclin B as well up-regulation DNA damage specific check point protein (Chk2) protein was observed, suggesting that these proteins are responsible for cell cycle blockade at G2/M phase in compound **3f**. Moreover, caspase-3 protein level was increased that indicate apoptotic inducing nature of the compounds **3c** and **3f**. Further downstream proteins which have a potential role in cell cycle as well as proliferation such as MAPK family (p38 and p-JNK) and Jun family proteins were down regulated. These findings concretely support the important cytotoxic as well as cell cycle regulatory role of these Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazoles compounds. In this study an insight into the cell cycle regulatory role as well as apoptotic inducing ability of the compound **3f** was elucidated. These studies suggest that compound **3f** has the potential to be taken up for further, particularly against hepatocaricinoma.

5. Experimental Protocols

All chemicals and reagents were purchased from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C spectra were recorded Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI + software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

5.1. Synthesis of 2-phenyl imidazo [2,1-b]benzothiazole under microwave heating

*5.1.1. 2-Phenyl imidazo [2,1-b]benzothiazole (**6a**)*

A mixture of 2-aminobenzothiazole **4a** (1.00 g, 6.67 mmol), 2-bromo acetophenone **5a** (1.32 g, 6.67 mmol), and ethanol (8 mL) were taken in a 20 mL pressure tube and were subjected to microwave irradiation (CEM Discover, 180 W, 250 psi, 100 °C) for 4 min the reaction mixture was cooled to room temperature and the resulting white precipitate was filtered, and air-dried to give 3.29 g of 2-phenylimidazo [2,1-b]benzothiazolehydrobromide. The hydrobromide was suspended in ethanol (20 mL) in a 100 ml of two necked flask equipped with a reflux condenser and dropping funnel. To this, triethylamine (5 mL) was added drop wise and refluxed for 5 min. The solution was cooled to room temperature and poured into ice water (50 mL). The resulting white precipitate was filtered and air-dried to obtained **6a**. Yield 2.05 g, 82%; mp 98–100 °C. IR (KBr, ν_{max} cm^{-1}): 3041 (Ar–H), 1667 (C=N), 678 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.26 (s, 1H, imidazole-**H**), 7.67–7.88 (m, 3H, Ar**H**), 7.47 (dt, 1H, J = 8.30 Hz and J = 0.75 Hz, Ar**H**), 7.32–7.42 (m, 4H, Ar**H**), 7.24 (t, 1H, J = 7.36 Hz, Ar**H**); MS (ESI): *m/z* 251 (M + 1)$^+$.

*5.1.2. 7-Methyl-2-phenyl imidazo [2,1-b] benzothiazole (**6b**)*

This compound was prepared according to the method described for compound **6a**, employing Compound **5a** (1.32 g, 6.67 mmol) and compound **4b** (1.09 g, 6.67 mmol) to obtain the pure product **6b** as a white solid, yield 2.11 g, 80%; mp 160–162 °C. IR (KBr, ν_{max} cm^{-1}):3072 (Ar–H), 1678 (C=N), 697 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.28 (s, 1H, imidazole-**H**), 7.94 (d, 1H, J = 7.95 Hz, Ar**H**), 7.64 (d, 2H, J = 7.16 Hz, Ar**H**), 7.49 (s, 1H, Ar**H**), 7.41 (t, 2H, J = 7.95 Hz, Ar**H**), 7.32 (t, 1H, J = 7.16 Hz, Ar**H**), 7.19 (dd, 1H, J = 7.95 Hz and J = 0.79 Hz, Ar**H**), 2.48 (s, 3H, -C**H$_3$**); MS (ESI): *m/z* 269 (M + 1)$^+$.

*5.1.3. 7-Fluoro-2-phenyl imidazo [2,1-b] benzothiazole (**6c**)*

This compound was prepared according to the method described for compound **6a**, employing Compound **5a** (1.32 g, 6.67 mmol) and compound **4c** (1.12 g, 6.67 mmol) to obtain the pure product **6b** as a white solid, yield 2.06 g, 77%; mp 156–159 °C. IR (KBr, ν_{max} cm^{-1}): 3088 (Ar–H), 1643 (C=N), 1340 (C–F), 654 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.27 (s, 1H, imidazole-**H**), 8.08 (q, 1H, J = 4.53 Hz, Ar**H**), 7.63 (d, 2H, J = 7.55 Hz, Ar**H**), 7.36–7.45 (m, 3H, Ar**H**), 7.27–7.34 (m, 1H, Ar**H**), 7.11 (dt, 1H, J = 8.30 Hz and J = 2.26 Hz, Ar**H**); MS (ESI): *m/z* 269 (M + 1)$^+$.

*5.1.4. 7-Ethoxy-2-phenyl imidazo [2,1-b] benzothiazole (**6d**)*

This compound was prepared according to the method described for compound **6a**, employing Compound **5a** (1.32 g, 6.67 mmol) and compound **4d** (1.29 g, 6.67 mmol) to obtain the pure product **6b** as a white solid, yield 2.38 g, 81%; mp 179–181 °C. IR (KBr, ν_{max} cm^{-1}): 3097 (Ar–H), 1658 (C=N), 1080 (C–O–C), 647 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.24 (s, 1H, imidazole-**H**), 7.96 (d, 1H, J = 8.87 Hz, Ar**H**), 7.64 (d, 2H, J = 7.36 Hz, Ar**H**), 7.40 (t, 2H, J = 7.17 Hz, Ar**H**), 7.30 (t, 1H, J = 7.17 Hz, Ar**H**), 7.16 (d, 1H, J = 2.455 Hz, Ar**H**), 6.91 (dd, 1H, J = 9.06 Hz and J = 2.45 Hz, Ar**H**), 4.08 (q, 2H, J = 6.98, –O**CH$_2$**), 1.48 (t, 3H, J = 6.98 Hz, -C**H$_3$**); MS (ESI); *m/z* 295 (M + 1)$^+$.

*5.1.5. 2-(4-Chlorophenyl) imidazo [2,1-b]benzothiazole (**6e**)*

This compound was prepared according to the method described for compound **6a**, employing Compound **5b** (1.89 g, 6.67 mmol) and compound **4a** (1.0 g, 6.67 mmol) to obtain the pure

product **6b** as a white solid, yield 2.21 g, 78%; mp 165–168 °C. IR (KBr, ν_{max} cm^{-1}): 3057 (Ar–H), 1677 (C=N), 688 (C–S–C), 647 (C–Cl); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.26 (s, 1H, imidazole-**H**), 8.07 (d, 1H, J = 9.06 Hz, Ar**H**), 7.69 (d, 1H, J = 8.34 Hz, Ar**H**), 7.63 (d, 2H, J = 8.78 Hz, Ar**H**), 7.39 (t, 3H, J = 8.34 Hz, Ar**H**), 7.31 (t, 1H, J = 8.34 Hz, Ar**H**); (ESI): m/z 285.

5.1.6. Synthesis of 3-(morpholinomethyl)-2-phenyl-7-methyl-imidazo [2,1-b]benzothiazole (**3a**)

A mixture of 7-methyl-2-phenyl imidazo [2,1-b] benzothiazole **6b** (264 mg, 1 mmol), morpholine (241 mg, 3 mmol), formalin (1 mL) and catalytic amount of anhydrous $ZnCl_2$ (0.135 mg, 0.1 mmol) in ethanol was stirred at room temperature for 4 h. The progress of the reaction was monitored with TLC. After completion of the reaction, ethanol was removed under reduced pressure and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined extract was washed with water (1 × 5 mL) and brine (1 × 5 mL) and was dried over anhyd. $MgSO_4$. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography silica gel (60–120 mesh) using ethyl acetate-hexane(1:9) as eluent to afford compound **3a** as a white solid, yield 330 mg, 91%; mp 132–134 °C; IR (KBr, ν_{max} cm^{-1}): 3064 (Ar–H), 2885 & 2811 (C–H), 1651 (C=N), 1067 (C–O–C), 729 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.94 (d, 1H, J = 7.87 Hz, Ar**H**), 7.64 (d, 2H, J = 7.11 Hz, Ar**H**), 7.49 (s, 1H, Ar**H**), 7.41 (t, 2H, J = 7.87 Hz, Ar**H**), 7.32 (t, 1H, J = 7.11 Hz, Ar**H**), 7.19 (dd, 1H, J = 7.87 Hz and J = 0.78 Hz, Ar**H**), 4.0 (s, 2H, methelene-**H**), 3.64 (t, 4H, J = 3.96 Hz, morpholine-**H**), 2.56 (t, 4H, J = 3.96 Hz, morpholine-**H**), 2.48 (s, 3H, –C**H$_3$**); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 148.5, 145.7, 137.9, 134.7, 133.5, 130.5, 129.7, 128.5, 124.8, 122.5, 115.6, 60.4, 51.8, 45.3, 28.7; ESI-MS: 364 (M + 1)$^+$; HRMS (ESI m/z) for $C_{21}H_{21}N_3OS$, calcd 364.1545, found 364.1561.

5.1.7. 3-(morpholinomethyl)-2-phenyl-7-ethoxy-imidazo [2,1-b] benzothiazole (**3b**)

This compound was prepared according to the method described for compound **3a**, employing compound 7-Ethoxy -2-phenyl imidazo [2,1-b]benzothiazole **6d** (294 mg, 1 mmol) and compound morpholine (241 mg, 3 mmol) to obtain the pure product **3b** as a white solid, yield 334 mg, 85%; mp 169 °C. IR (KBr, ν_{max} cm^{-1}): 3062 (Ar–H), 2892 & 2813 (C–H), 1677 (C=N), 1087 &1073 (C–O–C), 678 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.94 (d, 1H, J = 8.79 Hz, Ar**H**), 7.62 (d, 2H, J = 7.24 Hz, Ar**H**), 7.41 (t, 2H, J = 7.08 Hz, Ar**H**), 7.32 (t, 1H, J = 7.08 Hz, Ar**H**), 7.15 (d, 1H, J = 2.39 Hz, Ar**H**), 6.89 (dd, 1H, J = 8.99 Hz and J = 2.39 Hz, Ar**H**), 4.06 (q, 2H, J = 6.88 Hz, Ar**H**), 3.98 (s, 2H, methelene-**H**), 3.62 (t, 4H, J = 3.94 Hz, morpholine-**H**), 2.61 (t, 4H, J = 3.94 Hz, morpholine-**H**), 1.48 (t, J = 6.88 Hz, 3H, –C**H$_3$**); ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.9, 145.9, 138.9, 134.7, 133.8, 130.7, 129.9, 128.7, 125.1, 122.9, 115.9, 62.4, 51.8, 42.1, 34.7, 21.5; ESI-MS: 394 (M + 1)$^+$; HRMS (ESI m/z) for $C_{22}H_{23}N_3O_2S$, calcd 394.1527, found 394.1518.

5.1.8. 3-(4-(2-pyridinyl)piperazinomethyl)-2-phenyl-7-ethoxy-imidazo [2,1-b]benzothiazole (**3c**)

This compound was prepared according to the method described for compound **3a**, employing compound 7-Ethoxy-2-phenyl imidazo [2,1-b] benzothiazole **6d** (294 mg, 1 mmol) and compound pyridinyl piperzine (326 mg, 2 mmol) to obtain the pure product **3c** as a white solid, yield 408 mg, 87%; mp 160 °C. IR (KBr, ν_{max} cm^{-1}): 3057 (Ar–H), 2857 & 2799 (C–H), 1681 (C=N), 1063 (C–O–C), 737 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.11 (dd, 1H, J = 5.28 Hz, and J = 1.51 Hz, pyridinyl-**H**), 8.04 (d, 1H, J = 9.06 Hz, pyridinyl-**H**), 7.59–7.69 (m, 2H, pyridinyl-**H**), 7.39 (t, 3H, J = 7.55 Hz, Ar**H**), 7.30 (d, 1H, J = 6.79 Hz, Ar**H**), 7.14–7.19 (m, 1H, Ar**H**), 6.90 (dd, 1H, J = 9.06 Hz and J = 3.022 Hz, Ar**H**), 6.51–6.59 (m, 2H, Ar**H**), 4.08 (q, 2H, J = 6.79 Hz, –OC**H$_2$**), 4.02 (s, 2H, methelene-**H**), 3.56 (t, 4H, J = 4.53 Hz, pyridinylpiperzine-**H**), 2.70 (t, 4H, J = 4.53 Hz, pyridinylpiperzine-**H**), 1.46 (t, 3H, J = 6.79 Hz, –C**H$_3$**); ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.9, 138.4, 133.9, 133.1, 130.1, 129.7, 125.8, 124.9, 123.8, 115.9, 113.8, 107.6, 77.9, 77.4, 76.9, 52.4, 51.2, 45.3, 33.7, 20.3; ESI-MS: 470 (M + 1)$^+$; HRMS (ESI m/z) for $C_{27}H_{27}N_5OS$, calcd 470.1911, found 470.1897.

5.1.9. 3-(pyrrolidinomethyl)-2-phenyl-imidazo [2,1-b] benzothiazole (**3d**)

This compound was prepared according to the method described for compound **3a**, employing compound 2-phenyl imidazo [2,1-b]benzothiazole **6a** (250 mg, 1 mmol) and compound pyrolidine (213 mg, 3 mmol) to obtain the pure product **3d** as a white solid, yield 286 mg, 86%; mp 127 °C. IR (KBr, ν_{max} cm^{-1}): 3067 (Ar–H), 2887 & 2763 (C–H), 1678 (C=N), 684 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.71–7.89 (m, 3H, Ar**H**), 7.48 (dt, 1H, J = 8.43 Hz and J = 1.12 Hz, Ar**H**), 7.32–7.42 (m, 4H, Ar**H**), 7.24 (t, 1H, J = 7.43 Hz, Ar**H**), 4.07 (s, 2H, methelene-**H**), 2.62 (t, 4H, J = 8.43 Hz, pyrolidine-**H**), 1.81 (t, 4H, J = 8.43 Hz, pyrolidine-**H**); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.1, 145.9, 137.8, 135.1, 133.8, 130.5, 129.7, 128.4, 123.1, 121.4, 115.8, 60.9, 51.7, 29.7; ESI-MS: 334 (M + 1)$^+$; HRMS (ESI m/z) for $C_{20}H_{19}N_3S$, calcd 334.1353, found 334.1364.

5.1.10. 3-(pyrrolidinomethyl)-2-phenyl-7-methyl-imidazo [2,1-b] benzothiazole (**3e**)

This compound was prepared according to the method described for compound **3a**, employing compound 7-methyl-2-phenyl imidazo [2,1-b]benzothiazole **6b** (264 mg, 1 mmol) and compound pyrolidine (213 mg, 3 mmol) to obtain the pure product **3e** as a white solid, yield 305 mg, 88%; mp 129 °C. IR (KBr, ν_{max} cm^{-1}): 3067 (Ar–H), 2958 & 2867 (C–H), 1674 (C=N), 701 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, 1H, J = 7.86 Hz, Ar**H**), 7.68 (d, 2H, J = 7.14 Hz, Ar**H**), 7.52 (s, 1H, Ar**H**), 7.44 (t, 2H, J = 7.86 Hz, Ar**H**), 7.34 (t, 1H, J = 7.14 Hz, Ar**H**), 7.19 (dd, 1H, J = 7.86 Hz and J = 1.24 Hz, Ar**H**), 4.06 (s, 2H, methelene-**H**), 2.56 (t, 4H, J = 4.04 Hz, pyrolidine-**H**), 2.42 (s, 3H, -C**H$_3$**), 1.80 (t, 4H, J = 4.04 Hz, pyrolidine-**H**); ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.9, 145.7, 136.9, 134.7, 134.1, 131.7, 129.7, 128.5, 124.9, 122.8, 121.5, 115.6, 60.6, 51.9, 45.2, 28.7; ESI-MS: 348 (M + 1)$^+$; HRMS (ESI m/z) for $C_{21}H_{21}N_3S$, calcd 348.1502, found 348.1514.

5.1.11. 3-(pyrrolidinomethyl)-2-phenyl-7-fluoro-imidazo [2,1-b] benzothiazole (**3f**)

This compound was prepared according to the method described for compound **3a**, employing compound 7-Fluoro-2-phenyl imidazo [2,1-b]benzothiazole **6c** (268 mg, 1 mmol) and compound pyrolidine (213 mg, 3 mmol) to obtain the pure product **3f** as a white solid, yield 298.35 mg, 85%; mp 136 °C. IR (KBr, ν_{max} cm^{-1}): 3054 (Ar–H), 2876 & 2799 (C–H), 1632 (C=N), 1151 (C–F), 678 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (q, 1H, J = 5.48 Hz, Ar**H**), 7.64 (d, 2H, J = 8.34 Hz, Ar**H**), 7.35–7.47 (m, 3H, Ar**H**), 7.27–7.32 (m, 1H, Ar**H**), 7.11 (dt, 1H, J = 7.96 Hz and J = 2.15 Hz, Ar**H**), 4.05 (s, 2H, methelene-**H**), 2.52 (t, 4H, J = 4.53 Hz, pyrolidine-**H**), 1.75 (t, 4H, J = 4.53 Hz, pyrolidine-**H**); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.7, 145.7, 137.6, 134.8, 133.6, 130.4, 129.7, 128.4, 121.9, 115.8, 60.9, 45.4; ESI-MS: 352 (M + 1)$^+$; HRMS (ESI m/z) for $C_{20}H_{18}FN_3S$, calcd 352.1263, found 352.1247.

5.1.12. 3-(pyrrolidinomethyl)-2-phenyl-7-ethoxy-imidazo [2,1-b] benzothiazole (**3g**)

This compound was prepared according to the method described for compound **3a**, employing compound 7-ethoxy-2-phenyl imidazo [2,1-b]benzothiazole **6d** (294 mg, 1 mmol) and compound pyrolidine (213 mg, 3 mmol) to obtain the pure product

3g as a white solid, yield 327 mg, 87%; m.p 147 °C. IR (KBr, ν_{max} cm^{-1}): 3065 (Ar–H), 2877 & 2763 (C–H), 1669 (C=N), 1079 (C–O–C), 678 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 1H, J = 9.06 Hz, Ar**H**), 7.67 (d, 2H, J = 8.75 Hz, Ar**H**), 7.44 (t, 2H, J = 7.84 Hz, Ar**H**), 7.31 (t, 1H, J = 7.84 Hz, Ar**H**), 7.16 (d, 1H, J = 3.45 Hz, Ar**H**), 6.91 (dd, 1H, J = 9.54 Hz, and J = 3.45 Hz, Ar**H**), 4.08 (q, 2H, J = 7.65 Hz, -OC**H$_2$**), 4.02 (s, 2H, methelene-**H**), 2.62 (t, 4H, J = 4.47 Hz, pyrolidine-**H**), 1.80 (t, 4H, J = 4.47 Hz, pyrolidine-**H**), 1.49 (t, 3H, J = 7.65 Hz, –C**H$_3$**), ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.3, 146.1, 135.7, 133.8, 130.5, 128.7, 124.7, 122.9, 121.9, 116.5, 60.9, 51.6, 45.2, 29.7, 19.6; ESI-MS: 378 $(M + 1)^+$; HRMS (ESI m/z) for $C_{22}H_{23}N_3OS$, calcd 378.1614, found 378.1628.

5.1.13. 3-(pyrrolidinomethyl)-2-(4-chlorophenyl) imidazo- [2,1-b] benzothiazole (**3h**)

This compound was prepared according to the method described for compound **3a**, employing compound 2-(4-chlorophenyl) imidazo [2,1-b]benzothiazole **6e** (284 mg, 1 mmol) and compound pyrolidine (213 mg, 3 mmol) to obtain the pure product **3h** as a white solid, yield 308 mg, 84%; mp 139 °C. IR (KBr, ν_{max} cm^{-1}): 3057 (Ar–H), 2887 & 2789 (C–H), 1681 (C=N), 741 (C–S–C), 674 (C–Cl); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (d, 1H, J = 8.30 Hz, Ar**H**), 7.70 (d, 1H, J = 7.55 Hz, Ar**H**), 7.63 (d, 2H, J = 8.30 Hz, Ar**H**), 7.39 (t, 3H, J = 7.55 Hz, Ar**H**), 7.31 (t, 1H, J = 7.55 Hz, Ar**H**), 4.07 (s, 2H, -C**H$_2$**), 2.61 (t, 4H, J = 4.53 Hz, pyrolidine-**H**), 1.80 (t, 4H, J = 4.53 Hz, pyrolidine-**H**); ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.4, 145.7, 137.8, 134.9, 133.6, 130.5, 129.6, 128.1, 124.5, 122.3, 115.6, 60.5, 51.8, 29.7; ESI-MS: 368 $(M + 1)^+$; HRMS (ESI m/z) for $C_{20}H_{18}ClN_3S$, calcd 368.1108, found 368.1121.

5.2. Cell culture

The HepG2 (human hepato-carcinoma), MCF-7 cells (human breast cancer) and HeLa (human cervical cancer) were incubated by using Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum, 100 μg/mL pencillin-G and 100 μg/mL streptomycin sulfate (Sigma). The cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in the incubator.

5.3. Cell viability

Cell viability was assessed by the MTT based assay HepG2, MCF-7 and HeLa cells were seeded in a 96-well plate (TPP) at a cell density of 10,000 cells/well. After overnight incubation, the cells were treated with compounds **3a**–**h** and YM-201627 **(YM)** was incubated for 24 h. The medium was then discarded and replaced with fresh 100 μL media followed by addition of 10 μL of MTT dye. Plates were incubated at 37 °C for 2 h. The absorbance (O.D) was measured at 570 nm using Multimode Varioskan Flash (Thermo Fisher Scientifics).

5.4. Cell cycle analysis

5×10^5 cells each of HepG2 cells cell line were seeded in 60 mm dish and were allowed to grow for 24 h. A concentration of 4 μM of **3a**–**h**, **YM-201627 (YM)** was added to the culture media, and the cells were incubated for an additional 24 h. Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70% ethanol at 4 °C for 30 min, washed with PBS and incubated with 1 mg/ml RNaseA solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 μL of DNA staining solution [10 mg of Propidium Iodide (PI), 0.1 mg of trisodium citrate, and 0.03 mL of Triton X-100 were dissolved in 100 mL of sterile MilliQ water at room temperature for 30 min in the dark]. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit Software.

5.5. Protein extraction and Western blot analysis

Total cell lysates from cultured HepG2cells were obtained by lysing the cells in ice-cold RIPA buffer (1XPBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) and containing 100 μg/mL PMSF, 5 μg/mL Aprotinin, 5 μg/mL leupeptin, 5 μg/mL pepstatin and 100 μg/mL NaF. After centrifugation at 12,000 rpm for 10 min, the protein in supernatant was quantified by Bradford method (BIO-RAD) using Multimode varioskan instrument (Thermo-Fischer Scientifics). Fifty micrograms of protein per lane was applied in 12% SDS-polyacrylamide gel. After electrophoresis, the protein was transferred to polyvinylidine difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in TBS + 0.1% Tween20 (TBST) containing 5% blocking powder (Santacruz). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight (O/N). Mouse monoclonal antibodies Chk2, CyclinB1, NF-kB (p65) and rabbit polyclonal antibodies MAPK, PKCα, JunB and β-actin antibodies were purchased from Imgenex, USA. Rabbit polyclonal antibodies C-Jun and p-JNK was purchased from Santacruz Company. The membrane was incubated with corresponding horseradish peroxidase-labeled secondary antibody (1:2000) (Santa Cruz) at room temperature for 1 h. Membranes were washed with TBST three times for 15 min and the blots were visualized with chemiluminescence reagent (Thermo Fischer Scientifics Ltd.). The X-ray films were developed with developer and fixed with fixer solution.

5.6. Caspase-3 assay

The caspase-3 fluorescent assay kit (Clonetech,CA) was applied to evaluate the caspase-3 activity, using the procedures provided by the manufacturer HepG2 cells were treated with compounds **YM-201627(YM)**, **3c** and **3f** at 4 μM concentration as obtained from FACS analysis. Cell lysates were added to the 2× reaction buffer containing containing DTT and caspase substrate was added and incubation was carried out at 37 °C for 1 h. Readings were taken at excitation wavelength 400 nm and emission wavelength 505 nm.

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References

[1] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, CA Can. J. Clin. 55 (2005) 74–108.
[2] S. Whittaker, R. Marais, A.X. Zhu, Oncogene 29 (2010) 4989–5005.
[3] M.F. Mulcahy, Curr. Treat. Options Oncol. 6 (2005) 423–435.
[4] C.J. Weng, C.F. Chau, Y.S. Hsieh, S.F. Yang, G.C. Yen, Carcinogenesis 29 (2008) 147–156.
[5] P.J. Palmer, R.B. Trigg, J.V. Warrington, J. Med. Chem. 14 (1971) 248–251.
[6] A. Burger, S.N. Sawhney, J. Med. Chem. 11 (1968) 270–273.
[7] R.D. Chakole, N.D. Amnerkar, P.B. Khedekar, K.P. Bhusari, Indian J. Heterocyclic Chem. 15 (2005) 27.
[8] N. Siddiqui, S.N. Pandeya, S.A. Khau, J. Stables, A. Rana, M. Alam, F. Md. Arshad, M.A. Bhat, Bioorg. Med. Chem. 17 (2007) 255–259.
[9] E. Jayachandran, K. Bhatia, L.V.G. Naragud, A. Roy, Indian Drug. 40 (2003) 408–411.

[10] H.D. Brown, Chem. Abstr. 65 (1966) 18593 US Pat. 3,278,547, 1966.
[11] N. Siddiqui, M. Alam, A.A. Siddiqui, Asian J. Chem. 16 (2004) 1005–1008.
[12] B.M. Gurupadayya, M. Gopal, B. Padmashali, V.P. Vaidya, Indian J. Heterocyclic Chem. 15 (2005) 169–172.
[13] H.M. Diaz, R.V. Molina, R.O. Andrade, D.D. Coutino, L.M. Franco, S.P. Webster, M. Binnie, S.E. Soto, M.I. Barajas, I.L. Rivera, G.N. Vazquez, Bioorg. Med. Chem. Lett. 18 (2008) 2871–2877.
[14] A. Rana, N. Siddiqui, S.A. Khan, Indian J. Pharm. Sci. 69 (2007) 10–17.
[15] D.F. Shi, T.D. Bradshaw, S. Wrigley, C.J. McCall, P. Lelieveld, I. Fichtner, M.F.G. Stevens, J. Med. Chem. 39 (1996) 3375–3384.
[16] I. Hutchinson, M.S. Chua, H.L. Browne, V. Trapani, T.D. Bradshaw, A.D. Westwell, M.F.G. Stevens, J. Med. Chem. 44 (2001) 1446–1455.
[17] S.H.L. Kok, R. Gambari, C.H. Chui, M.C.W. Yuen, E. Lin, R.S.M. Wong, F.Y. Lau, G.Y.M. Cheng, W.S. Lam, S.H. Chan, K.H. Lam, C.H. Cheng, P.B.S. Lai, M.W.Y. Yu, F. Cheung, J.C.O. Tanga, A.S.C. Chan, Bioorg. Med. Chem. 16 (2008) 3626–3631.
[18] G.M. Catriona, W. Geoffrey, C.P. Jean, L.S. Erica, D.B. Tracey, F.G.S. Malcolm, D.W. Andrew, J. Med. Chem. 49 (2006) 179–185.
[19] Y.A. Al-Soud, Arkivoc 15 (2008) 225–238.
[20] A. Nobuaki, I. Yukitaka, Y. Mayumi, K. Sadao, T. Katsunori, S. Kiyohiro, M. Akira, K. Masafumi, S. Masayuki, Cancer Lett. 238 (2006) 119–127.
[21] M. Tramontini, Synthesis 1973 (1973) 703–775.
[22] M. Tramontini, L. Angliolini, Tetrahedron 46 (1990) 1791–1837.
[23] H.I. Gul, T. Ojanen, J. Vepsalainen, M. Gul, E. Erciyas, O. Hanninen, Arzneim-Forsch 51 (2001) 72–75.
[24] H.I. Gul, M. Gul, E. Erciyas, Arzneim-Forsch 52 (2002) 628–635.
[25] H.I. Gul, A.A. Denizci, E. Erciyas, Arzneim-Forsch 52 (2002) 773–777.
[26] A. Kamal, E.V. Bharathi, M.J. Ramaiah, D. Dastagiri, J.S. Reddy, A. Viswanath, F. Sultana, S.N.C.V.L. Pushpavalli, M.P. Bhadra, H.K. Srivastava, G.N. Sastry, A. Juvekar, S. Sen, S. Zingde, Bioorg. Med. Chem. 18 (2010) 526–542.
[27] A. Kamal, K. Sreekanth, P.P. Kumar, N. Shankaraiah, G. Balakishan, M.J. Ramaiah, S.N.C.V.L. Pushpavalli, P. Ray, M.P. Bhadra, Eur. J. Med. Chem. 45 (2010) 2173–2181.
[28] A. Kamal, J. Surendranadha Reddy, M.J. Ramaiah, D. Dastagiri, E.V. Bharathi, M.V. Prem Sagar, S.N.C.V.L. Pushpavalli, P. Ray, M.P. Bhadra, Med. Chem. Commun. 1 (2010) 355–360.
[29] A.A. Van de Loosdrecht, R.H.J. Beelen, G.J. Ossenkoppele, M.G. Broekhoven, M.M.A.C. Langenhuijsen, J. Immunol. Methods 174 (1994) 311–320.
[30] C. Kanthou, O. Greco, A. Stratford, I. Cook, R. Knight, O. Benzakour, G. Tozer, Am. J. Patho. 165 (2004) 1401–1411.
[31] P.M. O'connor, D.K. Ferris, M. Pagano, G. Draetta, J. Pines, T. Hunter, D.L. Longo, K.W. Kohn, J. Biol. Chem. 268 (1993) 8298–8308.
[32] C. Van Hoof, J. Goris, Biochem. Biophys. Acta. 1640 (2003) 97–104.
[33] M.H. Cobb, Prog. Biophys. Mol. Biol. 71 (1999) 479–500.
[34] C. Zhang, H. Zhu, X. Yang, J. Lou, D. Zhu, W. Lu, Q. He, B. Yang, J. Cancer Res. Clin. Oncol. 136 (2009) 437–445.
[35] B. Stein, A.S. Baldwin Jr., Ballard, D.W. Greene, W.C. Angel, P.P. Herrlich, EMBO. J. 12 (1993) 3879–3891.
[36] M.L. Smith, S.C. Wise, D.T. Hendricks, A.L. Sabichi, T. Bos, P. Reddy, P.H. Brown, M.J. Birrer, Oncogene 18 (1999) 6063–6070.
[37] D.I. Tai, S.L. Tsai, Y.H. Chang, S.N. Huang, T.C. Chen, K.S. Chang, Y.F. Liaw, Cancer Sci. 89 (2000) 2274–2281.
[38] A. Garg, B.B. Aggarwal, Leukemia 16 (2002) 1053–1068.
[39] E.N. Gurzov, L. Bakiri, J.M. Alfaro, E.F. Wagner, M. Izguierdo, Oncogene 27 (2008) 641–652.
[40] S. Frutos, J. Moscat, M.T. Diaz-Meco, J. Biol. Chem. 274 (1999) 10765–10770.
[41] R.U. Janicke, M.L. Sprengart, M.R. Wati, A.G. Porter, J. Biol. Chem. 273 (1998) 9357–9360.
[42] A.G. Porter, R.U. Janicke, Cell Death Differ. 6 (1999) 99–104.