Studies on imidazo[2,1-*b*][1,3]benzothiazole derivatives as new radiosensitizers

Krishnakishore Majalakere¹ · Sarojini Balladka Kunhana¹ · Shama Rao² · Bhuvanesh Sukhlal Kalal³ · Narayana Badiadka⁴ · Ganesh Sanjeev⁵ · Bantwal Shivaram Holla⁶

Received: 1 July 2020 / Accepted: 15 October 2020 / Published online: 27 October 2020
© Springer Nature Switzerland AG 2020

Abstract

The synthesis and characterization of potent 7-substituted-2-(4-substitutedphenyl)imidazo[2,1-*b*][1,3]benzothiazoles as effective radiosensitizers and anticarcinogenic compound is reported. The activity is determined against human liver cancer Hep G2 cell line, two parental melanoma cell lines (human melanoma cell line, A375C and mouse melanoma cell line, A375C). The compounds 7-sulfonamide-2-(4-fluorophenyl)imidazo[2,1-*b*][1,3]benzothiazole (**3f**, IC₅₀ = 0.097 μM) and 7-sulfonamide-2-(4-methylphenyl)imidazo[2,1-*b*][1,3]benzothiazole (**3g**, IC₅₀ = 0.114 μM) exhibited considerable *in vitro* anticancer activity against Hep G2 cell line while 7-bromo-2-(4-fluorophenyl)imidazo[2,1-*b*][1,3]benzothiazole showed effectiveness against both parental melanoma cell lines (**3c**, IC₅₀ = 0.04 and 0.052 μM). Further the active molecules **3f**, **3g** and **3h** are tested for radiosensitizing activity over Hep G2 cell line at 4 Gy, with the comet formed at 0.054 μM. Similarly, **3c** tested against two parental melanoma cell lines, it has proven to be more potent when combined with 2 Gy γ-radiation. The present study reveals that presence of sulfonamide in combination with methoxy substitution enhanced DNA fragmentation and there by acting as effective derivative for Hepatocellular carcinoma.

Keywords 7-Substituted-2-(4-substitutedphenyl)imidazo[2,1-*b*][1,3]benzothiazole · Anticancer · Hep G2 · Melanoma cell lines · Radiosensitizing

1 Introduction

Of late many drugs put in clinical practice have proven ineffective in combating the target cancer due to various factors that include multidrug resistance and adverse drug reactions. Hence, there always exists quite a large demand for novel anticancer drugs which instigates an interest among scientists for the development of new natural

products or synthetic compounds as potent candidates for drug development.

Radiotherapy is one of the most advanced and highly evolved areas in anticancer therapy for most of the cancers. This therapy works by directing high energy ionizing radiation towards cancer cells, thereby causing genetic changes triggering cell death [1]. In most of the cases radiation therapy loses its effectiveness due to hypoxic nature of cancer cells [2]. To overcome this, radiosensitization of

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s42452-020-03726-7>) contains supplementary material, which is available to authorized users.

✉ Sarojini Balladka Kunhana, bksaroj35@gmail.com | ¹Department of Industrial Chemistry, Mangalore University, Mangalagangothri, Karnataka 574199, India. ²Centre for Stem Cell Research and Regenerative Medicine, Justice K.S. Hegde Medical Academy, Deralakatte, Mangaluru, Karnataka 575017, India. ³Department of Biochemistry, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangaluru, Karnataka 575018, India. ⁴Department of Chemistry, Mangalore University, Mangalagangothri, Karnataka 574199, India. ⁵Microtron Centre, Department of Physics, Mangalore University, Mangalagangothri, Karnataka 574199, India. ⁶Department of Postgraduate Studies in Chemistry, Sri Dharmasthala Manjunatheshwara College, Ujire, Karnataka 574240, India.

SN Applied Sciences (2020) 2:1902 | <https://doi.org/10.1007/s42452-020-03726-7>

tumor cells has done by using certain agents known as 'radiosensitizers'. These compounds help to sensitize the tumor cells towards radiation by inducing free radical generation, denaturing macromolecules and inhibiting DNA repair mechanisms thus leading to cell death [2].

A broad spectrum of anticancer activities exhibited by sulfonamide hybrids with minimum side effect and better drug resistance-reversal ability had made them as an attractive pharmacophores. The metallo enzymes which were found in eukaryotic and many prokaryotic cells were known as carbonic anhydrases (CAs, EC 4.2.1.1) play a role in catalyzing reversible hydration/dehydration of CO_2/HCO_3 . Sulfonamide derivatives of heterocycles had exhibited strong CA inhibiting activity than other derivatives were documented. Previous reports had shown that compounds with thiophene-2-sulfonamide cores were found to be 40 times more active than benzene sulfonamide derivatives to inhibit carbonic anhydrases [3, 4]. A radio/chemosensitizing sulfonamide/sulfamide/sulfamate derivatives of nitroimidazoles act as CA IX inhibitors targeting the tumor-associated carbonic anhydrase (CA) isoforms IX and XII. They exhibited potency on hypoxia-induced extracellular acidosis in HT-29 and HeLa cell lines. The sulphonamide derivatives of these series in combination with dioxorubicin inhibited hypoxic tumors over expressing extracellular CA isozymes. The LEAD compounds for anticancer activity such as SLC-0111 [5], Indisulam [6] were being tested under the anticancer clinical trials. Fusion of thiazole and imidazole pharmacophores into a single molecular frame work might enhance the therapeutic potential of the individual scaffolds. The Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazole derivatives were proven to be potential killers of cancer cells with IC_{50} ranging from 2.8 to 8 mM [7]. As a consequence it was aspired to synthesize a series of thiazole incorporating imidazole moieties which were previously reported as antimicrobial agents [8].

Herein, it is attempted to analyze these scaffolds for their *in vitro* antiproliferative and radiosensitizing activities. A synergistic effect of anticancer and radiosensitization properties, probably would prove to be more beneficial based on the advantage of pharmaco-economic point of view.

2 Experimental

The analytical grade chemicals were purchased from Spectrochem Pvt. Ltd. Mumbai (India) and were used for reaction. The Merck silica gel 60 F254 aluminum sheets (Merck, Germany) were used for thin-layer chromatography to monitor reaction progress with ethyl acetate:hexane (3:7)

as eluent. Digital melting point/boiling point apparatus (EQ 730) was used to determine uncorrected melting point in a capillary tube. Bruker Avance FT-NMR spectrophotometer was used for recording ^1H -NMR and ^{13}C -NMR at room temperature with TMS as internal standard.

2.1 Synthesis of 6-substituted-1,3-benzothiazol-2-amine

The synthesis of the compound 6-substituted-1,3-benzothiazol-2-amine was carried out according to the procedure reported in the literature (Scheme 1) [9].

2.2 Synthesis of 7-substituted-2-(4-substitutedphenyl)imidazo[2,1-*b*][1,3]benzothiazoles

To a stirring solution of 6-substituted-1,3-benzothiazol-2-amine [0.00256 mol] in 10 ml methanol was added of 4-substituted phenacyl bromides [0.00282 mol] and subjected to reflux for 6–8 h. The precipitated solid was filtered, washed with cold methanol and dried. The products were checked for their purity with TLC and found to be pure (Scheme 2).

2.3 *In vitro* anticancer activity

2.3.1 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The anticancer activity screening of compounds **3a–h** was performed in HepG2 cell line, two parental melanoma cell line (human melanoma cell line, A375C and mouse melanoma cell line, A375C) and two drug resistant sublines (B16F10R and A375R) [10]. Cells cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/l glutamine, 1% antibiotics solution (100 U/ml penicillin G and 100 mg/ml streptomycin). Cells were grown in 25 cm^2 tissue culture flasks and maintained at 37 °C in a humidified incubator with 5% CO_2 . The Hep G2 cells were seeded in 96 well plates with density of 10,000 cells/well. The proper adhesion of the cells where obtained by incubating for 24 h in 100 μl DMEM complete medium. Varied concentrations of studied compounds were dissolved in 10% DMSO

Scheme 1 Synthesis of benzothiazole amine

Scheme 2 Synthesis of target compounds (**3a–h**)

and were put onto cells. Further incubated for 24 h and 0.5 mg/ml MTT solution was added. On incubation for 4 h the purple colored formazane crystals formed were dissolved in DMSO and OD recorded at 570 nm.

2.3.2 Radiation sensitization experiment

First, cell lines were irradiated with different doses of radiation and among them; a dose 2 Gy radiation was considered optimum for radiation sensitization experiments. Cells were seeded in tissue culture flask (Tarsons) and allowed to stand overnight for adherence. Next day, these cell lines were treated with drugs (IC_{25} dose) in media and further incubated for 24 h. After incubation, cell culture was irradiated with gamma radiation at the Centre for Application of Radioisotopes and Radiation Technology (CARRT), Mangalore University, India. Upon irradiation, the cells were seeded in triplicates into 96-well plate and incubated for 24 h. The MTT assay was performed for calculation of cell viability percentage. The unirradiated cultured cells were used as controls.

2.3.3 Comet assay

The standard radiation dosage was determined by performing comet assay for Hep G2 cell lines. The cells were exposed from 2 to 10 Gy radiation dosage and the formed comet was observed under fluorescent microscope. It was found that a dose of 4 Gy was effective to rupture the cells, hence the comet assay was performed with 4 Gy radiation dosage. About 10,000 cells/vial were placed in a vial and treated with 20 μl drug (1 mg/ml) solution which was exposed to 4 Gy γ -radiation. After 24 h of incubation Trypan blue staining was used to check the viability of cells. Then glass slides pre-coated with agarose were spread with the cells and used for alkaline comet assay. The lysis solution [10 mM Tris, 100 mM EDTA, 2.5 M NaCl, 10% DMSO, 1% Triton X-100 (pH 10)] was used for lysing the cells for 3 h. The solution was further kept for 20 min at 4 °C

in alkaline conditions (300 mM NaOH, 1 mM EDTA, pH $\geq$ 13) for DNA unwinding. The electrophoresis was performed at 4 °C for 20 min under an electrical current of 400 mA (25 V). On neutralization with TrisHCl (0.4 M), dehydration using 70% ethanol and staining with ethidium bromide (10 $\mu\text{g}/\text{ml}$) the slides were analyzed on fluorescence microscope (400 $\times$ magnification, Eclipse 50i, Nikon, Kanagawa, Tokyo).

3 Results and discussion

3.1 Chemistry and spectral characterization

The 7-substituted-2-(4-substitutedphenyl)imidazo[2,1-*b*] [1,3]benzothiazole derivatives (**3a–h**) were synthesized from 6-substituted-1,3-benzothiazol-2-amine was confirmed by their spectroscopic characterizations. The FTIR spectral details indicated the formation of the target molecules. One of the representative compound **3a** (7-bromo-2-(4-methoxyphenyl)imidazo[2,1-*b*]benzothiazole) absorbed characteristic frequencies 1683 cm^{-1} ($-\text{C}=\text{N}$), 1246 cm^{-1} ($-\text{C}-\text{O}$), 694 cm^{-1} ($-\text{C}-\text{S}$), 518 cm^{-1} ($-\text{C}-\text{Br}$) the IR active groups present in the molecule. The bending and stretching vibrational frequencies due to amine group present in the parent molecule at 3246 cm^{-1} were absent. The $^1\text{H-NMR}$ of **3a** showed singlet peak at δ 3.79 ppm corresponded to three methoxy protons substituted on phenyl ring. A doublet centered at δ 7.01 ppm integrating for two protons with coupling constant $J=8.0$ Hz could be assigned for the meta protons of phenyl ring and another doublet seen at δ 7.78 ppm ($J=8.0$ Hz) could be assigned for ortho protons of phenyl ring. Further, the signals appeared for thiazole fused phenyl ring protons as two singlets and one doublet. The ortho and one of the meta protons of bromophenyl ring appeared as singlets at δ 7.91 ppm and a doublet at δ 7.96 ppm ($J=8$ Hz) integrating for one proton each respectively. Another singlet appeared at δ 8.34 ppm could be assigned for the other meta proton of the bromophenyl ring. The signal

resonated at δ 8.65 ppm due to imidazole ring proton. Formation of the final product further confirmed by the absence of signals at δ 8.14 ppm for $-\text{NH}_2$ proton of precursor. In the ^{13}C NMR spectrum, signal obtained at δ 55.6 ppm could be assigned to methoxy group carbon and a downfield signal appeared at δ 193.4 ppm could be assigned to bridging carbon atom of imidazothiazole ring. Other signals appeared in aromatic region accounting for the carbon atoms of both phenyl rings including equivalent ones. Further, formation of the compound **3a** was strongly appended by mass spectrum LCMS with ion peak 358.2 ($\text{M}^+ - 1$) corresponding to its mass of 359.2. The physico-chemical characterization, elemental analysis and spectral characterization of these new derivatives are given in Tables 1 and 2 respectively.

In this series, para position of the benzothiazole amine was substituted with bromo and sulphonamide groups respectively. Further, these scaffolds were derivatized with variably substituted phenacyl bromides. The selection of bromo substitution was based on the pharmacological effect of halogen substitution in cancer chemotherapy. The role of halogen atoms in the drug development process mainly depended on the binding efficiency to form drug-target complexes due to steric effects. The bulky bromo group can adequately sit on binding site through halogen bonding. Of late it was understood that not only the steric effect but also other related complex interactions were the basis for the stability of protein-ligand complexes [11]. In an earlier study the poly brominated 4,5,6,7-tetrabromo-1*H*-benzotriazole was found to be potent and selective CK2 inhibitor and thus considered as reference compound for the bromo derivatives. Hence in this experiment the bromo substitution was selected to achieve better anticancer activity [12]. However, as the sulphonamide group was a proven pharmacophore, so it was selected for better radiosensitization.

3.2 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) assay

The formation of purple colored formazan in MTT assay was catalyzed by mitochondrial succinate dehydrogenase. The proliferating cells reduce MTT to MTT-formazan complex due to mitochondrial respiration. The formation of this dye indicated the cell viability which was directly proportional to the optical density of complex. The IC_{50} value was calculated by probit analysis (Fig. 1 and Table 3).

The cell viability (MTT) assay for compounds **3a–h** was been performed against Hep G2 cell line and the results are reported in Fig. 1. The newly synthesized compounds exhibited moderate IC_{50} values ranged between 0.097 to 0.162 μM . The compound **3f** emerged as the most active with IC_{50} value of 0.097 μM . As per the result, the compounds derived from bromo thiazole amine were less active compared to compounds derived from sulphonamide precursor. This can be visualized from Table 3 in which compounds **3a**, **3b** and **3c** showed higher IC_{50} values compared to compounds **3h**, **3g** and **3f** (sulfonamide substituted derivatives). Among the analyzed compounds two derivatives **3f** (sulfonamide along with fluorine) and **3g** (sulfonamide and methyl groups) at para positions on either side of the phenyl rings turned to be more potent ones. The presence of fluorine in a compound influences its cell permeability by acting as more lipophilic than hydrogen substituted compounds. The presence of fluorine makes the molecule an effective ligand to bind to the cellular targets. Likewise, the methyl group as substituent also plays crucial role in deciding the ADME property of a molecule, the methyl group can substantially boost the biological activity [13].

3.3 Comet assay

The compounds **3f**, **3g** and **3h** had low IC_{50} values and appeared to be effective, hence further taken for

Table 1 Results of physical characterization and elemental analysis

Compounds	Formula	Calculated			Melting point ($^{\circ}\text{C}$)	Yield (%)
		C	H	N		
3a	$\text{C}_{16}\text{H}_{11}\text{BrN}_2\text{OS}$	53.35	3.02	7.75	214–216	75
3b	$\text{C}_{16}\text{H}_{11}\text{BrN}_2\text{S}$	55.89	3.20	8.10	204–207	77
3c	$\text{C}_{15}\text{H}_8\text{BrFN}_2\text{S}$	51.80	2.28	8.01	221–224	75
3d	$\text{C}_{15}\text{H}_8\text{BrN}_3\text{O}_2\text{S}$	48.21	2.11	11.18	230–232	74
3e	$\text{C}_{15}\text{H}_{10}\text{N}_4\text{O}_4\text{S}_2$	48.12	2.58	14.92	>330	72
3f	$\text{C}_{15}\text{H}_{10}\text{FN}_3\text{O}_2\text{S}_2$	51.82	2.85	12.05	256–258	74
3g	$\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_2\text{S}_2$	55.92	3.78	12.20	300–302	75
3h	$\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_3\text{S}_2$	53.70	3.61	11.62	266–268	71

Table 2 Results of spectral characterization

Compound	Spectral data
3a. 7-bromo-2-(4-methoxyphenyl)imidazo[2,1-b][1,3]benzothiazole	FT-IR, ν cm^{-1} : 1683 (–C=N), 1246 (–C–O), 694 (–C–S), 518 (–C–Br). ^1H NMR (DMSO- d_6 , 400 MHz), δ ppm: 3.79 (s, 3H, –OCH ₃), 7.01 (d, 2H, $J=8$ Hz, Ar-H), 7.78 (d, 2H, $J=8$ Hz, Ar-H), 7.91 (d, 1H, $J=8$ Hz, Ar-H), 7.96 (d, 1H, $J=8$ Hz, Ar-H), 8.34 (s, 1H, Ar-H), 8.65 (s, 1H, Imidazole-H). ^{13}C (DMSO- d_6 , 100 MHz): δ ppm: 56.0, 75.4, 113.6, 114.8, 115.7, 123.0, 123.3, 125.6, 127.9, 128.0, 129.4, 137.9, 143.2, 159.7, 161.0, 193.4. LCMS (ESI-MS): m/z 358.2 ($M^+ - 1$).
3b. 7-bromo-2-(4-methylphenyl)imidazo[2,1-b][1,3]benzothiazole	FT-IR, ν cm^{-1} : 1635 (–C=N), 1201 (–C–C), 692 (–C–S), 501 (–C–Br). ^1H NMR (DMSO- d_6 , 400 MHz), δ ppm: 2.33 (s, 3H, –CH ₃), 7.23 (d, 2H, $J=7.6$ Hz, Ar-H), 7.76 (d, 2H, $J=6.8$ Hz, Ar-H), 7.73 (br s, 1H, Ar-H), 7.92 (d, 1H, $J=8.4$ Hz, Ar-H), 8.35 (s, 1H, Ar-H), 8.73 (s, 1H, Imidazole-H). ^{13}C (DMSO- d_6 , 100 MHz) δ ppm: 21.1, 113.6, 115.2, 123.0, 123.3, 127.9, 128.0, 129.4, 132.4, 137.2, 137.9, 143.2, 159.7. LCMS (ESI-MS): m/z 342.5 ($M^+ - 1$).
3c. 7-bromo-2-(4-fluorophenyl)imidazo[2,1-b][1,3]benzothiazole	FT-IR, ν cm^{-1} : 1646 (–C=N), 1251 (–C–F), 698 (–C–Br). ^1H NMR (DMSO- d_6 , 400 MHz), δ ppm: 7.26 (d, 2H, $J=8.4$ Hz, Ar-H), 7.75 (d, 1H, $J=8.4$ Hz, Ar-H), 7.86 (br s, 3H, Ar-H), 8.35 (s, 1H, Ar-H), 8.76 (s, 1H, Imidazole-H). ^{13}C (DMSO- d_6 , 100 MHz) δ ppm: 115.2, 116.2, 116.4, 117.8 (d, $^1J_{\text{CF}}=160$ Hz), 122.9 (d, $^1J_{\text{CF}}=264$ Hz), 128.94 (d, $^1J_{\text{CF}}=12$ Hz), 129.8, 138.0, 143.2, 147.3, 159.7, 162.0, 164.1. LCMS (ESI-MS): m/z 346.3 ($M^+ + 1$).
3d. 7-bromo-2-(4-nitrophenyl)imidazo[2,1-b][1,3]benzothiazole	FT-IR, ν cm^{-1} : 1639 (–C=N), 1517 (–NO ₂ asymmetric), 1336 (–NO ₂ symmetric), 624 (–C–Br). ^1H NMR (DMSO- d_6 , 400 MHz), δ ppm: 7.75 (d, 1H, $J=8.4$ Hz, Ar-H), 7.94 (d, 1H, $J=8.4$ Hz, Ar-H), 8.07 (d, 2H, $J=8.4$ Hz, Ar-H), 8.29 (d, 2H, $J=8.4$ Hz, Ar-H), 8.36 (s, 1H, Ar-H), 9.06 (s, 1H, Imidazole-H). ^{13}C (DMSO- d_6 , 100 MHz) δ ppm: 113.6, 115.2, 123.0, 123.3, 125.0, 126.8, 127.9, 129.4, 137.1, 137.9, 143.2, 145.8, 159.7. LCMS (ESI-MS): m/z 375.2 ($M^+ + 1$).
3e. 2-(4-nitrophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide	FT-IR, ν cm^{-1} : 3259 (–NH ₂), 1639 (–C=N), 1518 (–NO ₂ asymmetric), 1317 (–NO ₂ symmetric), 1159 (–S=O). ^1H NMR (DMSO- d_6 , 500 MHz): δ ppm: 7.13 (s, 1H, imidazole-H), 7.47 (s, 2H, SO ₂ NH ₂), 7.90 (d, 2H, $J=7.5$, Ar-H), 8.14 (d, 1H, $J=7.5$ Hz, Ar-H), 8.30 (d, 2H, $J=7.5$ Hz, Ar-H), 8.43 (d, 1H, $J=7.5$ Hz, Ar-H), 8.66 (s, 1H, Ar-H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ ppm: 115.2, 117.6, 118.0, 122.6, 123.2, 125.0, 126.8, 137.1, 138.0, 143.2, 145.8, 147.3, 159.7; LCMS (ESI-MS): m/z 375.06 ($M^+ + 1$).
3f. 2-(4-fluorophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide	FT-IR, ν cm^{-1} : 3288 (–NH ₂), 1631 (–C=N), 1325 (–C–F), 1159 (–S=O). ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: δ 7.15 (dd, 3H, $J=8$ Hz, $J=10$ Hz, Ar-H), 7.63 (dd, 2H, $J=6$ Hz, $J=7.5$ Hz, Ar-H), 7.84 (s, 2H, SO ₂ NH ₂), 8.14 (dd, 1H, $J=7.5$ Hz, $J=7.5$ Hz, Ar-H), 8.43 (d, 1H, $J=7.5$ Hz, Ar-H), 8.67 (s, 1H, imidazole-H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ ppm: 113.6, 115.2, 116.3 (d, $^1J_{\text{CF}}=88$ Hz), 123.0, 123.3, 127.93, 129.6 (d, $^1J_{\text{CF}}=124$ Hz), 129.8, 137.9, 143.25, 159.78, 162.0, 164.1; LCMS (ESI-MS): m/z 348.06 ($M^+ + 1$).
3g. 2-(4-methylphenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide	FT-IR, ν cm^{-1} : 3246 (–NH ₂), 1652 (–C=N), 1151 (–S=O). ^1H NMR (DMSO- d_6 , 500 MHz) δ ppm: δ 2.34 (s, 3H, –CH ₃), 7.13 (s, 1H, imidazole-H), 7.29 (d, 2H, $J=7.5$ Hz, Ar-H), 7.59 (d, 2H, $J=7$ Hz, Ar), 7.69 (s, 2H, SO ₂ NH ₂), 8.13 (d, 1H, $J=7.5$ Hz, Ar-H), 8.43 (d, 1H, $J=7.5$ Hz, Ar-H), 8.67 (s, 1H, Ar-H). ^{13}C NMR (75.5 MHz, DMSO- d_6) δ ppm: 21.1, 115.2, 117.6, 118.0, 112.6, 123.2, 128.0, 129.4, 132.4, 137.2, 138.0, 143.2, 147.3, 159.2; LCMS (ESI-MS): m/z 407.99

Table 2 (continued)

Compound	Spectral data
3h . 2-(4-methoxyphenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide	FT-IR, ν cm^{-1} : 3392 ($-\text{NH}_2$), 1641 ($-\text{C}=\text{N}$), 1153 ($-\text{SO}$), 623 ($-\text{C}-\text{S}$) ^1H NMR ($\text{DMSO}-d_6$, 500 MHz) δ ppm: 3.81 (s, 3H, $-\text{OCH}_3$), 7.03 (d, 2H, $J=7.5$ Hz, Ar-H), 7.13 (s, 1H, imidazole-H), 7.61 (d, 2H, $J=7.5$ Hz, Ar-H), 7.94 (s, 2H, SO_2NH_2), 8.13 (d, 1H, $J=7.5$ Hz, Ar-H), 8.43 (d, 1H, $J=7.5$ Hz, Ar-H), 8.67 (s, 1H, Ar-H). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ ppm: 56.0, 114.8, 115.2, 117.6, 118.0, 122.6, 123.2, 125.6, 128.0, 138.0, 143.2, 147.3, 159.7, 161.0; LCMS (ESI-MS): m/z 344.08 (M^++H).

Fig. 1 Cell viability of Hep G2 cells as determined by MTT assay of **3a–h** **Table 3** IC_{50} value of compounds **3a–h** by MTT assay in 1 $\mu\text{g}/\mu\text{l}$ concentration

Compound	R	R ^I	IC_{50} in μM
3a	–Br	– OCH_3	0.122 ± 0.28
3b	–Br	– CH_3	0.162 ± 0.15
3c	–Br	–F	0.154 ± 0.22
3d	–Br	– NO_2	0.117 ± 0.19
3e	– SO_2NH_2	– NO_2	0.148 ± 0.24
3f	– SO_2NH_2	–F	0.097 ± 0.29
3g	– SO_2NH_2	– CH_3	0.114 ± 0.35
3h	– SO_2NH_2	– OCH_3	0.115 ± 0.32

Fig. 2 Comet assay of **3f**

radiosensitization study against Hep G2 cell line. The comet assay for all the three compounds were performed at 0.054 μM (half of IC_{50}) drug concentration irradiating with 4 Gy γ -ray (Figs. 2, 3 and 4). The study showed that comet formed from **3h** had long tail formation (27.34 ± 0.91) compared to **3f** (24.58 ± 0.48) and **3g**

(16.86 ± 1.08) (Table 4), shown to be effective among tested compounds as DNA damaging agent.

Fig. 3 Comet assay of **3g** Fig. 4 Comet assay of **3h****Table 4** Determination of apoptosis by comet assay

Compound	Percentage DNA damage
3f	24.58 ± 0.48
3g	16.86 ± 1.08
3h	27.34 ± 0.91

Table 5 Cell cytotoxic effect of compounds

Compounds	A375		B16F10	
	IC ₅₀ (μM)			
	Parental	Resistant	Parental	Resistant
3a	0.123	0.105 ± 0.09	0.071	0.126 ± 0.17
3b	0.163	0.296 ± 0.15	0.035	0.154 ± 0.1
3c	0.046	0.047 ± 0.13	0.033	0.102 ± 0.21
3d	0.236	0.216	0.231	0.244
3e	0.504	0.563	0.533	0.353
3f	0.917	0.183	0.168	0.217
3g	0.927	0.185	0.170	0.219
3h	0.885	0.177	0.163	0.209

Fig. 5 MTT cell viability assay of melanoma cells treated with **3c** along with irradiation

3.4 Radiosensitizing study on melanoma cells

Using dose-response curves for two parental melanoma cell lines, IC_{50} values of all the compounds were calculated and results are presented in Table 5. Among the seven tested compounds, **3c** exhibited the best cytotoxic activity against all parental (A375C and B16F10C) and drug resistant (A375R and B16F10R) melanoma cell lines. All the other compounds afforded moderate anticancer activity against cancer cell lines.

The parental and drug-resistant cells of A375 and B16F10 cells were pretreated with an IC_{50} concentration of **3c** for 24 h and irradiated with 2.0 Gy γ -radiation. The cell viability in melanoma cells with irradiation at 2.0 Gy and **3c**, was 20–38% respectively (Fig. 2). Treatment with both **3c** and irradiation showed synergistic effects, i.e., cell viability was less than cell treated individually or untreated. The cell viability was significantly reduced ($p < 0.001$) in both cell lines but A375 cells were found to be more sensitive. The concentration of melanin was estimated by the differences in the absorbance at 470 nm. The pretreatment with **3c** had shown to decrease in absorbance at 470 nm for both cell lines compared to untreated cells (Fig. 5).

4 Conclusion

A series of bromo and sulphonamide substituted benzothiazole derivatives (**3a–h**) were synthesized and characterized by spectroscopic techniques. The new molecules were subjected to antiproliferative activity on Hep G2 and two parental melanoma cell lines. Among the synthesized compounds **3f** (sulfonamide along with fluorine) and **3g** (sulfonamide and methyl groups) at para positions on either side of the phenyl rings appear to be cytotoxic agents with IC_{50} 0.097 and 0.114 μ M respectively for Hep G2 cell line. The radiosensitizing effect of these two compounds at 0.054 μ M (half of IC_{50}) on hypoxic HepG2 cells was evident by comet assay. The concomitant effect of these two compounds on cytotoxic activity and radiosensitization on HepG2 cells seem to be promising. While **3c** (bromo substitution along with fluorine) was potent against two parental melanoma cell lines, with IC_{50} 0.046 and 0.052 μ M respectively. When combined with 2 Gy γ -radiation, **3c** seemed to be more potent on melanoma cells with 30–40% viability. Hence, these molecules which were synthesized by adopting simple procedures could be potential drug candidates to be exploited for radiosensitization during radiation associated chemotherapy.

Compliance with ethical standards

Conflict of interest The corresponding author states that there is no conflict of interest.

References

1. Jayam R, Vijay KB, Venkata SK, Uday SR, Venkata S (2014) Radiosensitizers, radioprotectors, and radiation mitigators. *Indian J Dent Res* 25:83–90. <https://doi.org/10.4103/0970-9290.131142>
2. David RR, Daphne A (2013) Molecular targets and mechanisms of radiosensitization using DNA damage response pathways. *Future Oncol* 9(2):219–233. <https://doi.org/10.2217/fon.12.185>
3. Ozensoy GO, Capasso C, Supuran CT (2016) A magnificent enzyme superfamily: Carbonic anhydrases, their purification and characterization. *J Enzyme Inhib Med Chem* 31:689–694. <https://doi.org/10.3109/14756366.2015.1059333>
4. Elena A, Jessica R, Silvia P, Francesca B, Anna L, Fabrizio C, Claudio TS, Lido C (2019) The carbonic anhydrase IX inhibitor SLC-0111 sensitises cancer cells to conventional chemotherapy. *J Enzyme Inhib Med Chem* 34(1):117–123. <https://doi.org/10.1080/14756366.2018.1532419>
5. Cecilia AH, Pierre JM, Stuart RM, Harvey S, John MS, Robert LS, Michael FS, Kenneth LS (1994) 3-Substituted thieno[2,3-b][1,4]thiazine-6-sulfonamides. A novel class of topically active carbonic anhydrase inhibitors. *Med Chem* 37:240–247. <https://doi.org/10.1021/jm00028a006>
6. Supuran CT (2003) Indisulam: an anticancer sulfonamide in clinical development. *Expert Opin Investig Drugs* 12(2):283–287. <https://doi.org/10.1517/13543784.12.2.283>
7. Kumbhare RM, Vijay Kumar K, Janaki RM, Dadmal T, Pushpavalli SN, Mukhopadhyay D, Divya B, Anjana DT, Kosurkar U, Pal-Bhadra M (2011) Synthesis and biological evaluation of novel Mannich bases of 2-arylimidazo[2,1-b]benzothiazoles as potential anti-cancer agents. *Eur J Med Chem* 46:4258–4266. <https://doi.org/10.1016/j.ejmech.2011.06.031>
8. Swetha KM, Zhen-Zhen L, Vijaya KK, Rammohan RYB, Balaraju T, Vijai KRT, Cheng-He Z (2018) Azoalkyl ether imidazo[2,1-b]benzothiazoles as potentially antimicrobial agents with novel structural skeleton. *Bioorg Med Chem Lett* 28:2426–2431. <https://doi.org/10.1016/j.bmcl.2018.06.016>
9. Chandak N, Jitender KB, Rajnesh KS (2012) Inhibitors of apoptosis in testicular germ cells: Synthesis and biological evaluation of some novel IBTs bearing sulfonamide moiety. *Eur J Med* 59:203–208. <https://doi.org/10.1016/j.ejmech.2012.11.015>
10. Bhuvanesh SK, Vinitha RP, Santosh KB, Hiriyur MS (2019) HDAC2 inhibitor valproic acid increases radiation sensitivity of drug-resistant melanoma cells. *Med Sci (Basel)* 7(3):51. <https://doi.org/10.3390/medsci7030051>
11. Hernandez MZ, Cavalcanti SMT, Moreira DRM, de Azevedo Junior WF, Leite ACL (2010) Halogen atoms in the modern medicinal chemistry: hints for the drug design. *Curr Drug Targets* 11(3):303–314. <https://doi.org/10.2174/138945010790711996>
12. Baldrighi M, Metrangolo P, Pilati T, Resnati G, Terraneo G (2017) Halogen and hydrogen bonding in multicomponent crystals of tetrabromo-1H-benzotriazole. *Crystals* 7(11):332. <https://doi.org/10.3390/cryst7110332>
13. Uwabagira N, Sarojini B, Poojary B (2018) N-(3-Chloro-2-methylphenyl)-4-(4-fluorophenyl)-1,3-thiazol-2-amine. *Molbank* 2018(1):M975. <https://doi.org/10.3390/m975>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.