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Original article

Inhibitors of apoptosis in testicular germ cells: Synthesis and biological evaluation of some novel IBTs bearing sulfonamide moiety

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ABSTRACT

Pifithrin-α, a known p53 inactivator, inhibits p53-dependant mitochondrial cell death induced by toxins or γ-radiation. It has been found that aromatic IBT analogues of PFT-α are more cytoprotective and nonpeptide-based, isatin sulfonamides selectively inhibit caspases 3 and 7, responsible for mitochondrial mediated apoptosis. Therefore, we envisioned the synthesis of novel IBTs **4** and **5** bearing sulfonamide moiety and observed the mitigating effects of these IBTs in rescue of malathion induced apoptosis in testicular germ cells of goat. Two IBTs (**4b**; R = CH_3, **5b**; R_1 = Cl) showed very high survival rate of cells whereas IBT **4f** (R = NO_2) showed some exceptional behaviour by increasing the apoptosis. These IBTs nullify the cytotoxic effect of malathion on mitochondria, following p53-independent pathway.

1. Introduction

Apoptosis is a programmed cell death that affects the single scattered cells in the midst of a normal living tissue and plays a key role in regulating development, homeostasis, and immune defence by clearing redundant or abnormal cells in organisms. A delicate balance between pro-apoptotic and anti-apoptotic mechanisms determines whether a cell death signal can activate the execution of the apoptotic program. In humans, hypo-apoptosis as well as hyper-apoptosis, both can lead to severe pathological consequences. For example, suppression of the apoptotic machinery is responsible for severe autoimmune diseases and is a hallmark of cancer [1,2] whereas high rate of apoptosis sometimes leads to tissue as well as nerve degeneration resulting into severe neurological disorders such as Alzheimer's disease, Parkinson's disease, stroke etc [3,4]. Apoptosis sometimes enhances due to overexpression of genes, such as p53, which are involved in controlling the whole apoptotic mechanism in our body or can be induced by toxins or ionizing radiations [5]. Mitochondria plays an important role in controlling cell death and follows either p53-dependent or -independent pathway of apoptosis. During apoptosis, mitochondria releases a number of pro-apoptotic proteins such as Apoptois Inducing Factor (AIF), Smac/DIABLO, Cytochrome C etc. which are involved in apoptosome formation and its formation leads to activation of caspases, also called caspase cascade, responsible for induction of apoptosis [6–8]. Of late, p53 inactivators have gained much attention of chemists as well as oncologists due to their possible applications in neurodegenerative disorders, cancer chemotherapy as well as diseases related to signalling pathways [3,9,10]. Recently, a small molecule, Pifithrin-α, also known as PFT-α, was originally identified as a leading compound of the known p53 inactivators after broad screening of 10,000 compounds which inhibits cell death from γ-radiation as well as protecting mice from lethal genotoxic stress, focal cortical ischaemic injury, and neuronal excitotoxic damage [9,11,12]. This protection by PFT-α has been attributed to inhibition of p53 transactivation *via* diminishing p53-dependent and -independent mitochondria-mediated cell death *in vivo* and *in vitro* [9,13]. It's a matter of great debate from a long time within chemists and biologists that whether ring opened PFT-α is active or its ring closed form. NMR experiments have proved many times this fact that PFT-α as well as its ring opened analogues cyclize *in situ* in protic solution and it has recently been reported that cyclized analogues of PFT-α, known as imidazo[2,1-*b*]benzothiazoles (IBTs) are more potent p53 inactivators than reference as well as opened analogues [14,15]. Another report confirmed that the aromatic IBT analogues are more protective against dexamethasone as well as γ-radiation-

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induced apoptosis [5] but at the same time, they were found to be unable to modulate the transcriptional activity of p53, indicating the role of these aromatic IBTs towards p53-independent apoptosis [16]. It is well known in the literature that malathion exerts its cytotoxic effects by the induction of apoptosis *via* direct effect on mitochondria [17]. Thus, the chemoprotection of cells from apoptosis induced by toxins or ionizing radiation can be important for biodefense and in the treatment of acute injuries. It has been discovered that targeting only two capsases 3 and 7 alone are sufficient for blocking apoptosis and first time reported nonpeptide-based caspase inhibitors, the isatin sulfonamides are finding their selectivity towards caspases 3 and 7 by interacting primarily with the S_2 subsite, and not binding in the caspase primary aspartic acid binding pocket (S_1), suggesting a big role of sulfonamide group [18,19]. Appreciating these findings, in the present investigation, we synthesized two series of small heterocycles, imidazobenzothiazoles (IBTs, **4** and **5**) bearing sulfonamide moiety, structurally related to PFT-α (Fig. 1) and observed the mitigating effects of novel IBTs in rescue of malathion induced apoptosis in testicular germ cells of goat (*Capra hircus*). To the best of our knowledge, this is in itself the first study of IBTs towards inhibition of apoptosis in testicular germ cells, particularly on a domestic animal.

2. Results and discussion

2.1. Chemistry

The synthetic route used to synthesize the target imidazobenzothiazoles, IBTs **4** and **5** is outlined in Scheme 1. The starting material, 2-aminobenzothiazole-6-sulfonamide **1** was synthesized in two steps because the conventional method of preparing 2-aminobenzothiazoles in a single step from appropriate anilines by treatment with Br_2/KSCN unfortunately failed to give **1** [20]. Therefore, the required starting material was prepared from sulfanilamide by first converting into 4-aminosulfonylphenylthiourea using ammonium thiocyanate in acidic medium followed by its oxidative cyclization with Br_2 in chloroform [21]. Reaction of **1** with either appropriate *p*-substituted phenacyl bromide **2** or 6-substituted-3-bromoacetylcoumarin **3** in refluxing 2-methoxyethanol followed by neutralization using aqueous ammonia yielded corresponding imidazobenzothiazoles, IBTs **4** and **5** respectively [5,22]. Spectral data (^{1}H NMR, ^{13}C NMR, IR and mass) of the newly synthesized compounds were in full agreement with the proposed structures. In general, both IBTs **4** and **5** were identified by the presence of characteristic imidazo proton (C_4–H) which appeared during cyclization resonating in the range δ 8.76–9.11 as a singlet in ^{1}H NMR. All coumarin IBTs (**5a**–**c**) were showing characteristic C=O stretch of lactone nature at 1720 cm^{-1} in FT-IR.

2.2. Apoptosis inhibition studies

All the newly synthesized IBTs (**4a**–**4f** and **5a**–**5c**) were evaluated for their *in vitro* inhibition of apoptosis induced by malathion in testicular germ cells. Results obtained are reported in Table 1. Out of IBTs (**4a**–**4f**) with phenyl analogues, **4b** having a methyl substituent in the phenyl ring was found to be the most potent inhibitor exhibiting 64 ± 4.35% average cell survival when compared with controlled % average cell survival (CACV; 67 ± 4.58) and malathion tested % average cell survival (41 ± 6.24). Three compounds namely **4a** (R = H), **4d** (R = Cl) and **4e** (R = Br) showed mild activity exhibiting % average cell survival 50 ± 5.29, 56 ± 3.00 and 52 ± 4.58 respectively. Compound **4c** (R = F) showed weaker protective ability exhibiting 43 ± 4.00% average cell survival. Surprisingly, compound **4f** (R = NO_2) was found to be promoter of apoptosis exhibiting 39 ± 2.00% average cell survival. Out of IBTs (**5a**–**5c**) with coumarin analogues, **5b** (R′ = Cl) was found to be the most potent inhibitor exhibiting 61 ± 6.00% average cell survival whereas compounds **5a** (R′ = H) and **5c** (R′ = Br) showed weaker cytoprotective activity exhibiting 46 ± 4.35 and 48 ± 5.56% average cell survival, respectively. Figs. 2 and 3 are the fluorescent photographs of testicular germ cells in control group showing pinkish normal germ cells with their intact cell membranes and round nuclei, taken at × 100 and × 400 resolution respectively. Fig. 4 is the fluorescent photograph of malathion treated testicular germ cells showing apoptotic germ cells with bright green condensed nuclei, taken at × 400 resolution. Figs. 5 and 6 are the fluorescent photographs of malathion treated testicular germ cells

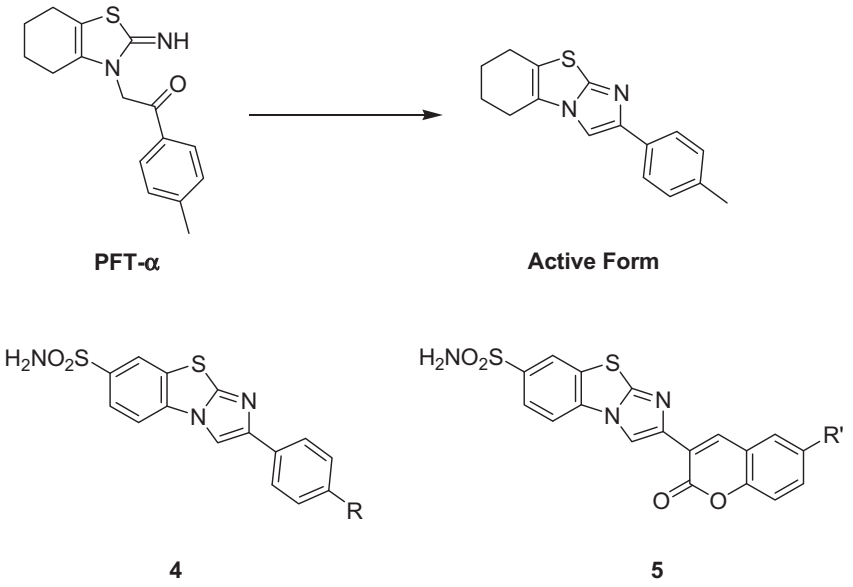
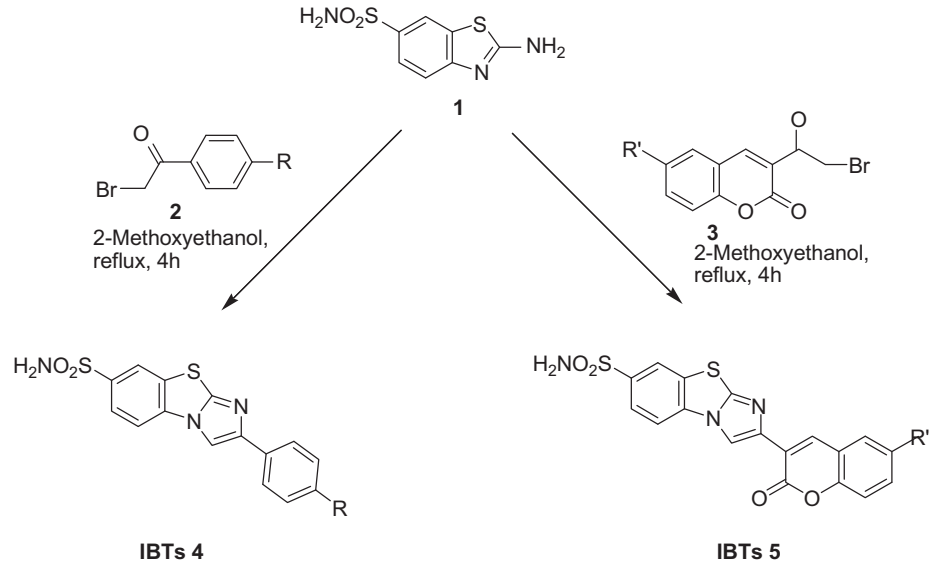


Fig. 1. Structure of Pifithrin-α (**PFT-α**) and the general structure of related analogues bearing sulfonamide moiety.



Compound **2** & **4**	**a**	**b**	**c**	**d**	**e**	**f**
R	H	CH_3	F	Cl	Br	NO_2
Compound **3** & **5**	**a**	**b**	**c**			
R'	H	Cl	Br			

Scheme 1. Synthesis of novel imidazobenzothiazoles (IBTs) **4** & **5**.

supplemented with compound **4b** and **5b** respectively showing decrease in number of apoptotic germ cells, taken at × 400 resolution. Results are summarized graphically in Fig. 7.

3. Discussion and conclusions

All the newly synthesized IBTs (**4a**–**4f** and **5a**–**5c**) were evaluated for their *in vitro* inhibition of apoptosis. It has been reported that PFT-α does not remain in uncyclized state in protic solution and get converted into ring closed form, well known as IBT, after few hours as confirmed by NMR experiments and exhibits anti-apoptotic activity [14,15]. Therefore, additional compounds were synthesized to optimize the IBT scaffold for making other isosteric analogues for anti-apoptotic activity in the search of a more potent inhibitor as well as to suppress mitochondrial mediated cell death induced by toxins. As a primary screen for biological activity, each compound was tested for its ability to prevent malathion induced cell death. Notably, all of the compounds that were tested for anti-apoptotic activity were analogous to the cyclized form of PFT-α. Critical features to make an IBT suitable for better anti-apoptotic activity were aromatization, position as well as nature of the substituent introduced whether electron releasing or withdrawing, and surface area of newly designed IBT. We shall discuss each point one by one in the same order as written above. It has already been reported in the literature that aromatic analogues of IBTs are highly active [5]. Therefore, we synthesized sulfonamide bearing aromatic

Table 1
Malathion induced inhibition of apoptosis in testicular germ cells.

Compound no.	Group **R** or **R'**	Average cell survival ± standard deviation (%)[a]
4a	H	50 ± 5.29*
4b	CH_3	64 ± 4.35*
4c	F	43 ± 4.00*
4d	Cl	56 ± 3.00*
4e	Br	52 ± 4.58*
4f	NO_2	39 ± 2.00
5a	H	46 ± 4.35*
5b	Cl	61 ± 6.00*
5c	Br	48 ± 5.56*
MACV[b]		41 ± 6.24*
CACV[c]		67 ± 4.58

*$p < 0.05$ (t test).
[a] Compound tested at 10 μM. Malathion toxic at 10 μM, tested at 5 μM.
[b] MACV: Malathion tested % average cell survival.
[c] CACV: Control % average cell survival.

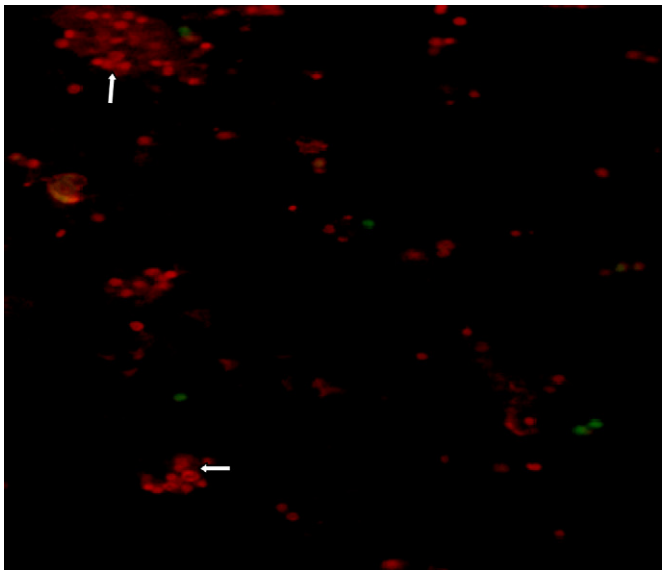


Fig. 2. Fluorescent photograph of testicular germ cells showing pinkish normal germ cells with their intact cell membranes and round nuclei in control group (arrow). × 100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

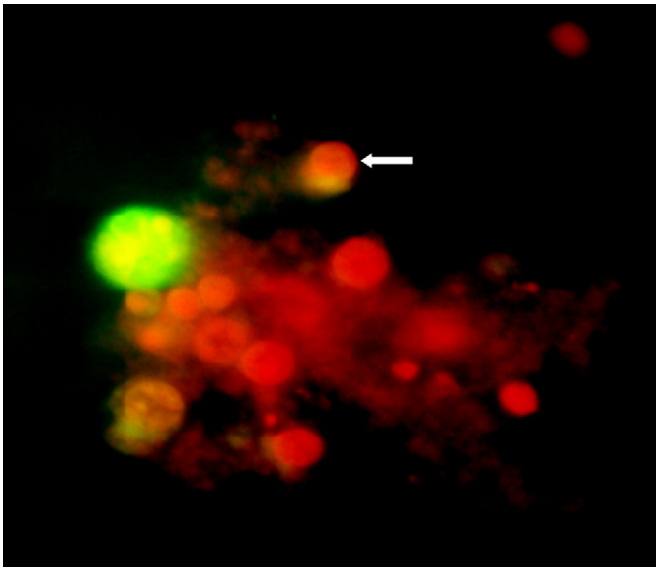


Fig. 3. Fluorescent photograph of testicular germ cells showing pinkish normal germ cell with their intact cell membranes and round nuclei in control group (arrow). × 400. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

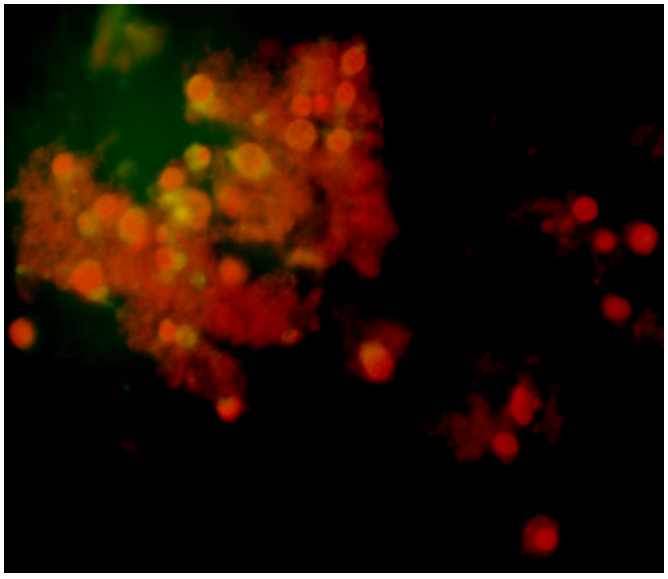


Fig. 5. Fluorescent photograph of malathion treated testicular germ cells supplemented with compound **4b** showing decrease in number of apoptotic germ cells. × 400.

analogues and found significant results. However, in our case, we noticed that nature of the substituent plays a big role in deciding the anti-apoptotic activity. In general, more the electron releasing nature of the substituent, better the anti-apoptotic activity of that IBT analogue. The trend with respect to substituent on the phenyl ring of IBTs **4** and coumarin ring of IBTs **5** was found to be $CH_3 > Cl > Br > H > F > NO_2$. In case of NO_2, this trend became so enormous that our IBT **4f** showed some unusual behaviour by increasing the apoptosis suggesting that R and R′ should not be strongly electron withdrawing in nature to make an IBT suitable for apoptotic inhibition. Literature survey shows that NO_2 substituted IBTs don't serve as better anti-apoptotic [5] but they are better p53 inactivators [14,15]. As reported in the literature that larger the surface area of an IBT, better would it show anti-apoptotic activity [5]. Therefore, we also tried to observe the role of surface area in controlling the anti-apoptotic activity of IBT by making some coumarin analogues (**5a**–**5c**) and compared the results with its phenyl analogues (**4a**, **4d** and **4e**) respectively. The results do not show a general trend as the anti-apoptotic activity of **5b** was significant while that of **5a** and **5c** was very weak. This indicates that replacement of a phenyl ring with a coumarin moiety understandably involves varying electronic effects besides increasing the surface area. After observing and comparing the anti-apoptotic results, we concluded that IBTs **4b** and **5b** exhibited significant protection against malathion induced apoptosis and may be promising for further development. It is reported in the literature

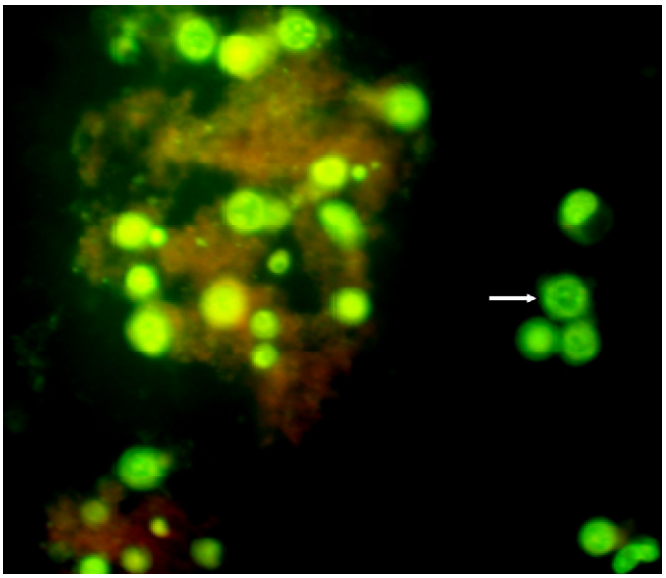


Fig. 4. Fluorescent photograph of malathion treated testicular germ cells showing apoptotic germ cells with bright green condensed nuclei (arrow). X 400. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

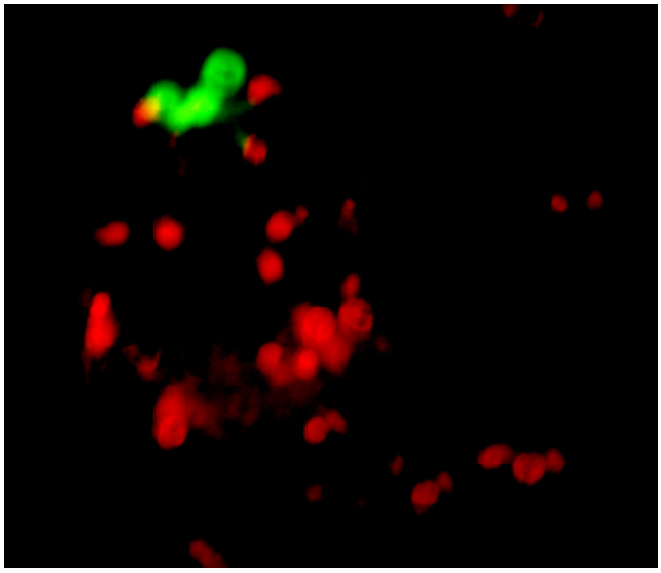


Fig. 6. Fluorescent photograph of malathion treated testicular germ cells supplemented with compound **5b** showing decrease in number of apoptotic germ cells. × 400.

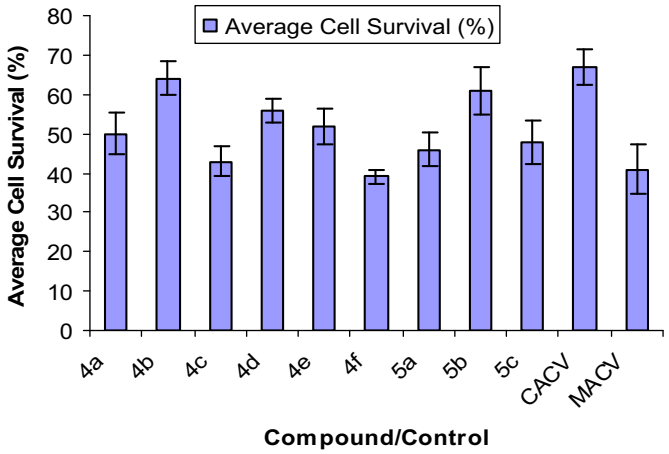


Fig. 7. Inhibition of malathion induced apoptosis.

that apoptosis induced by irradiations is p53-dependent while dexamethasone induced apoptosis is not [23]. Data suggests that these IBTs nullify the apoptotic (cytotoxic) effect of malathion on mitochondria which is not following p53-dependant pathway, confirming a recent report [16] because IBT **4f** (p-NO_2) which is expected to be better p53 inactivator, is not showing promising anti-apoptotic activity. Thus, these novel IBTs will provide deeper insight in understanding the mechanism of apoptosis *via* mitochondrial pathway in a much better way and may thus influence therapeutic strategy.

4. Experimental protocols

All reactions were carried out under atmospheric pressure. Melting points were determined on glass slide using MR-VIS LABINDIA VISUAL Melting Range Apparatus and are uncorrected. The infrared (IR) spectra were recorded on ABB MB 3000 DTGS FT-IR instrument using the KBr pellet technique. ^{1}H NMR and ^{13}C NMR spectra were recorded in pure DMSO-d_6 on Bruker NMR spectrometers at 300 MHz and 75.5 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in δ, ppm. Mass spectra (*DART-MS*) were recorded on a JEOL-AccuTOF JMS-T100LC Mass spectrometer having a DART (*Direct Analysis in Real Time*) source in ES$^+$ mode. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of chloroform and methanol as eluent. Iodine or UV lamp was used as a visualizing agent. Abbreviations 's' for singlet, 'd' for doublet, 'm' for multiplet, 'ex' for exchangeable proton are used for NMR assignments and 's' for strong, 'm' for medium for IR assignments. 'd' stands for decomposition in melting point data.

4.1. General procedure for the synthesis of imidazobenzothiazoles (IBTs), ***4a–e*** *and* ***5a–c***

A mixture of 2-aminobenzothiazole-6-sulfonamide (**1**, 4 mmol) and appropriate *p*-substituted phenacyl bromide or 6-substituted-3-bromoacetylcoumarin (**2** or **3**, 4.8 mmol) was refluxed in 2-methoxyethanol (15 mL) for 4 h. The reaction mixture was cooled to room temperature and neutralized by adding cold aqueous ammonia (10%, 40 mL) slowly with vigorous stirring till yellow or orange coloured solid precipitated out which was filtered, washed with excess of water followed by cold ethanol (20 mL) and crystallized either from chloroform–methanol (3 : 1; **4a–e**) or from DMF-ethanol (1 : 1; **4f**, **5a–c**) to afford the target IBTs.

4.1.1. 2-Phenylimidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (***4a***)

Yield: 70%; m.p. 294–296 °C (d); IR (KBr) cm^{-1}: 3302 & 3148 (m, N–H stretch), 1589 (s, C=N stretch), 1543 (m, N–H bend), 1497 (s, C=C stretch), 1335 & 1165 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.82 (s, 1H, imidazo C$_4$–H), 8.55 (s, 1H, Ar), 8.15 (d, 1H, J = 8.4 Hz, Ar), 8.00 (d, 1H, J = 8.4 Hz, Ar), 7.88 (d, 2H, J = 7.2 Hz, Ar), 7.49 (s, ex, 2H, SO_2NH_2), 7.44 (m, 2H, Ar), 7.32 (m, 1H, Ar); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 148.5, 147.3, 141.0, 134.1, 133.8, 130.3, 129.2, 128.0, 125.2, 124.9, 123.3, 114.0, 109.7; DART-MS: *m/z* 330.07 (M + H)$^+$, $C_{15}H_{11}N_3O_2S_2H^+$ calcd. 330.02.

4.1.2. 2-(4-Methylphenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (***4b***)

Yield: 62%; m.p. 300–302 °C (d); IR (KBr) cm^{-1}: 3356 & 3148 (s, N–H stretch), 1589 (s, C=N stretch), 1543 (m, N–H bend), 1497 (s, C=C stretch), 1335 & 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.76 (s, 1H, imidazo C$_4$–H), 8.54 (s, 1H, Ar), 8.13 (d, 1H, J = 8.4 Hz, Ar), 8.00 (d, 1H, J = 8.4 Hz, Ar), 7.77 (d, 2H, J = 7.8 Hz, Ar), 7.49 (s, ex, 2H, SO_2NH_2), 7.25 (d, 2H, J = 7.8 Hz, Ar), 2.33 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 148.3, 147.4, 141.0, 137.2, 134.1, 131.2, 130.2, 129.8, 125.2, 124.9, 123.4, 113.9, 109.2, 21.2 (CH_3); DART-MS: *m/z* 344.08 (M + H)$^+$, $C_{16}H_{13}N_3O_2S_2H^+$ calcd. 344.04.

4.1.3. 2-(4-Fluorophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (***4c***)

Yield: 65%; m.p. 256–258 °C (d); IR (KBr) cm^{-1}: 3325 & 3155 (m, N–H stretch), 1597 (s, C=N stretch), 1551 (m, N–H bend), 1497 (s, C=C stretch), 1327 & 1165 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.80 (s, 1H, imidazo C$_4$–H), 8.55 (s, 1H, Ar), 8.13 (d, 1H, J = 8.4 Hz, Ar), 8.00 (d, 1H, J = 8.4 Hz, Ar), 7.88–7.93 (m, 2H, Ar), 7.49 (s, ex, 2H, SO_2NH_2), 7.29 (t, 2H, J = 8.7 Hz, Ar); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 162.0 (d, $^1J_{CF}$ = 244.6 Hz), 148.5, 146.1, 140.7, 134.0, 130.2, 130.0, 127.1 (d, $^3J_{CF}$ = 8.3 Hz), 124.8, 123.1, 115.9 (d, $^2J_{CF}$ = 22.6 Hz), 113.9, 109.2; DART-MS: *m/z* 348.06 (M + H)$^+$, $C_{15}H_{10}FN_3O_2S_2H^+$ calcd. 348.01.

4.1.4. 2-(4-Chlorophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (***4d***)

Yield: 75%; m.p. 312–314 °C (d); IR (KBr) cm^{-1}: 3348 & 3256 (m, N–H stretch), 1589 (s, C=N stretch), 1543 (m, N–H bend), 1497 (s, C=C stretch), 1327 & 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.87 (s, 1H, imidazo C$_4$–H), 8.56 (s, 1H, Ar), 8.12 (d, 1H, J = 6.6 Hz, Ar), 8.01 (d, 1H, J = 6.6 Hz, Ar), 7.88 (d, 2H, J = 6.3 Hz, Ar), 7.50–7.55 (m, 4H, SO_2NH_2, Ar); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 148.7, 146.0, 141.2, 134.1, 132.9, 132.3, 130.3, 129.3, 126.9, 125.0, 123.5, 114.0, 110.2; DART-MS: *m/z* 364.03 (M + H)$^+$, $C_{15}H_{10}ClN_3O_2S_2H^+$ calcd. 363.99.

4.1.5. 2-(4-Bromophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (***4e***)

Yield: 60%; m.p. 298–300 °C (d); IR (KBr) cm^{-1}: 3333 & 3240 (m, N–H stretch), 1643 (s, C=N stretch), 1558 (m, N–H bend), 1528 (s, C=C stretch), 1311 & 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.88 (s, 1H, imidazo C$_4$–H), 8.55 (s, 1H, Ar), 8.13 (d, 1H, J = 8.1 Hz, Ar), 8.01 (d, 1H, J = 8.1 Hz, Ar), 7.83 (d, 2H, J = 7.8 Hz, Ar), 7.64 (d, 2H, J = 7.8 Hz, Ar), 7.50 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 148.7, 146.1, 141.2, 134.0, 133.2, 132.1, 130.3, 127.2, 125.0, 123.4, 120.8, 114.0, 110.3; DART MS m/z 407.99 (M + H)$^+$, $C_{15}H_{10}BrN_3O_2S_2H^+$ calcd. 407.93.

4.1.6. 2-(4-Nitrophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (***4f***)

Yield: 82%; m.p. > 330 °C; IR (KBr) cm^{-1}: 3348 & 3140 (s, N–H stretch), 1597 (s, C=N stretch), 1543 (m, N–H bend), 1504 (s, C=C

stretch), 1335 & 1165 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.11 (s, 1H, imidazo C_4–H), 8.57 (s, 1H, Ar), 8.31 (d, 2H, J = 8.1 Hz, Ar), 8.16 (d, 1H, J = 8.4 Hz, Ar), 8.11 (d, 2H, J = 8.1 Hz, Ar), 8.02 (d, 1H, J = 8.4 Hz, Ar), 7.53 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 149.5, 146.6, 145.0, 141.5, 140.4, 133.9, 130.5, 125.8, 125.0, 124.7, 123.5, 114.3, 112.6; DART-MS: *m/z* 375.06 $(M + H)^+$, $C_{15}H_{10}N_4O_4S_2H^+$ calcd. 375.01.

4.1.7. 2-(2-Oxo-2H-chromen-3-yl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (**5a**)

Yield: 85%; m.p. > 330 °C; IR (KBr) cm^{-1}: 3271 & 3140 (m, N–H stretch), 1720 (s, lactone C=O stretch), 1605 (s, C=N stretch), 1497 (s, C=C stretch), 1311 & 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.87 (s, 1H, imidazo C_4–H), 8.62 (s, 1H, Ar), 8.51 (s, 1H, coumarin C_4–H), 8.35 (d, 1H, J = 8.4 Hz, Ar), 7.94 (d, 1H, J = 8.4 Hz, Ar), 7.80 (d, 1H, J = 7.5 Hz, coumarin), 7.49–7.56 (m, 3H, SO_2NH_2, coumarin), 7.37 (d, 1H, J = 8.1 Hz, coumarin), 7.30 (t, 1H, J = 7.2 Hz, coumarin); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 158.9, 152.7, 141.4, 140.5, 137.3, 134.1, 131.9, 130.3, 129.1, 125.2, 124.9, 123.3, 120.4, 119.7, 116.3, 114.6, 114.3; DART-MS: *m/z* 398.12 $(M + H)^+$, $C_{18}H_{11}N_3O_4S_2H^+$ calcd. 398.01.

4.1.8. 2-(6-Chloro-2-oxo-2H-chromen-3-yl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (**5b**)

Yield: 80%; m.p. > 330 °C; IR (KBr) cm^{-1}: 3348 & 3163 (m, N–H stretch), 1720 (s, lactone C=O stretch), 1597 (s, C=N stretch), 1497 (s, C=C stretch), 1327 & 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.93 (s, 1H, imidazo C_4–H), 8.64 (s, 1H, Ar), 8.53 (s, 1H, coumarin C_4–H), 8.39 (d, 1H, J = 8.4 Hz, Ar), 7.94–7.98 (m, 2H, Ar, coumarin C_5–H), 7.57 (d, 1H, J = 8.7 Hz, coumarin), 7.51 (s, ex, 2H, SO_2NH_2), 7.44 (d, 1H, J = 8.4 Hz, coumarin); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 158.5, 151.2, 141.5, 140.2, 135.9, 134.0, 131.2, 130.3, 128.9, 128.0, 124.9, 123.3, 121.1, 118.3, 114.7; DART-MS: *m/z* 432.09 $(M + H)^+$, $C_{18}H_{10}ClN_3O_4S_2H^+$ calcd. 431.98.

4.1.9. 2-(6-Bromo-2-oxo-2H-chromen-3-yl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (**5c**)

Yield: 76%; m.p. > 330 °C; IR (KBr) cm^{-1}: 3340 & 3163 (m, N–H stretch), 1720 (s, lactone C=O stretch), 1597 (s, C=N stretch), 1497 (s, C=C stretch), 1327 & 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.98 (s, 1H, imidazo C_4–H), 8.68 (s, 1H, Ar), 8.56 (s, 1H, coumarin C_4–H), 8.42 (d, 1H, J = 8.4 Hz, Ar), 8.16 (s, 1H, coumarin C_5–H), 7.96 (d, 1H, J = 8.4 Hz, Ar), 7.73 (d, 1H, J = 8.7 Hz, coumarin), 7.51 (s, ex, 2H, SO_2NH_2), 7.42 (d, 1H, J = 8.7 Hz, coumarin); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 158.4, 151.6, 149.0, 141.4, 140.2, 135.7, 134.0, 130.9, 130.3, 124.9, 123.3, 121.5, 121.3, 118.4, 116.8, 114.6; DART-MS: *m/z* 476.00 $(M + H)^+$, $C_{18}H_{10}BrN_3O_4S_2H^+$ calcd. 475.92.

4.2. Biological assays

4.2.1. Collection of materials and isolation of cells

Goat testes were collected from the slaughter houses around Kurukshetra (26°6′ N, 76°5′ E), INDIA and brought to laboratory in normal saline at 4 °C. Then the testes were decapsulated and cut into smaller pieces. The testicular cells were isolated and washed three times with Dulbecco's modified Eagle's Medium (DMEM) for cell culture.

4.2.2. Reagents/chemicals

Malathion, DMSO, DMEM, phosphate buffer saline (PBS), antibiotics (penicillin, streptomycin).

4.2.3. Apoptosis assays

Testicular germ cells were harvested from mature goat (*C. hircus*) testes and cultured in DMEM medium supplemented with antibiotics (200-unit having concentration of penicillin 100 IU/mL and streptomycin 100 IU/mL) in CO_2 incubator (5% CO_2, 95% humidity, 38 °C). The cells were plated at a density of 10^5 cells/mL and pre-incubated with 10 μM of each tested compound in DMSO for 30 min before induction of apoptosis. Then the apoptosis was induced with 5 μM malathion in DMSO. The cell apoptosis was assayed by Acridine Orange staining after 6 h of culture duration under the fluorescence microscope (Olympus, Japan) using 500–525 nm filters [5,24]. Normal cells were identified by their intact cell membranes and round nucleus with scanty chromatin. Cells with bright green condensed nuclei (intact or fragmented) were interpreted as apoptotic.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.11.015.

References

[1] D. Hanhan, R.A. Weinberg, Cell 100 (2000) 57–70.
[2] C.B. Thompson, Science 267 (1995) 1456–1462.
[3] X.X. Zhu, Q.S. Yu, R.G. Cutler, C.W. Culmsee, H.W. Holloway, D.K. Lahiri, M.P. Matson, N.H. Greig, J. Med. Chem. 45 (2002) 5090–5097.
[4] J. Yuan, B.A. Yanker, Nature 407 (2000) 802–809.
[5] S.D. Barchéchath, R.I. Tawatao, M. Corr, D.A. Carson, H.B. Cottam, J. Med. Chem. 48 (2005) 6409–6422.
[6] C. Wang, R.J. Youle, Annu. Rev. Genet. 43 (2009) 95–118.
[7] S. Rolland, B. Conradt, Cell. Death Differ. 13 (2006) 1281–1286.
[8] E. Gulbins, S. Dreschers, J. Bock, Exp. Physiol. 88 (2003) 85–90.
[9] P.G. Komarov, E.A. Komarova, R.V. Kondratov, K. Christov-Tselkov, J.S. Coon, M.V. Chernov, A.V. Gudkov, Science 285 (1999) 1733–1737.
[10] E.A. Komarova, A.V. Gudkov, Biochem. (Moscow) 65 (2000) 41–48.
[11] C. Culmsee, S. Bondada, M.P. Mattson, Mol. Brain Res. 87 (2001) 257–262.
[12] C. Culmsee, X. Zhu, Q.-S. Yu, S.L. Chan, S. Camandola, Z. Guo, N.H. Greig, M.P. Mattson, J. Neurochem. 77 (2001) 220–228.
[13] E.A. Komarova, N. Neznanov, P.G. Komarov, M.V. Chernov, K. Wang, A.V. Gudkov, J. Biol. Chem. 278 (2003) 15465–15468.
[14] N. Pietrancosta, A. Moumen, R. Dono, P. Lingor, V. Planchamp, F. Lamballe, M. Bähr, J.L. Kraus, F. Maina, J. Med. Chem. 49 (2006) 3645–3652.
[15] N. Pietrancosta, F. Maina, R. Dono, A. Moumen, C. Garino, Y. Laras, S. Burlet, G. Quélvér, J.L. Kraus, Bioorg. Med. Chem. Lett. 15 (2005) 1561–1564.
[16] M.S. Christodoulou, F. Colombo, D. Passarella, G. Ieronimo, V. Zuco, M.D. Cesare, F. Zunino, Bioorg. Med. Chem. 19 (2011) 1649–1657.
[17] X.Y. Chen, J.Z. Shao, L.X. Xiang, X.M. Liu, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 142 (2006) 36–45.
[18] D. Lee, S.A. Long, J.L. Adams, G. Chan, K.S. Vaidya, T.A. Francis, K. Kikly, J.D. Winkler, C.-M. Sung, C. Debouk, S. Richardson, M.A. Levy, W.E. DeWolf Jr., P.M. Keller, T. Tomaszek, M.S. Head, M.D. Ryan, R.C. Haltiwanger, P.-H. Liang, C.A. Janson, P.J. McDevitt, K. Johanson, N.O. Concha, W. Chan, S.S. Abdel-Meguid, A.M. Badger, M.W. Lark, D.P. Nadeau, L.J. Suva, M. Gowen, M.E. Nuttall, J. Biol. Chem. 275 (2000) 16007–16014.
[19] M.E. Nuttall, D. Lee, B.A. McLaughlin, J.A. Erhardt, Drug Discov. Today 6 (2001) 85–91.
[20] H.P. Kaufmann, W. Oehring, A. Clauberg, Arch. Pharm. 266 (1928) 197.
[21] P.K. Sharma, N. Chandna, S. Kumar, P. Kumar, S. Kumar, P. Kaushik, D. Kaushik, Med. Chem. Res. 21 (2012) 3757–3766.
[22] A.N. El-Shorbagi, S.I. Sakai, M.A. El-Gendy, N. Omar, H.H. Farag, Chem. Pharm. Bull. 36 (1988) 4760–4768.
[23] A.R. Clarke, C.A. Purdie, D.J. Harrison, R.G. Morris, C.C. Bird, M.L. Hooper, A.H. Wyllie, Nature 362 (1993) 849–852.
[24] C.A. Evans, P.J. Owen, A.D. Whetton, C. Dive, Cancer Res. 53 (1993) 1735–1738.