Chapter 3

Synthesis of Some Novel Imidazobenzothiazoles (IBTs) as Inhibitors of Apoptosis
CHAPTER 3. Synthesis of Some Novel Imidazobenzothiazoles (IBTs) as Inhibitors of Apoptosis

3.1. Motivation for the current work

Apoptosis is a physiological 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 defense by clearing redundant or abnormal cells in organisms. The term ‘programmed cell death’ was introduced in 1964 by Lockshin and Williams¹ who proposed that cell death during development is not of accidental nature but follows a sequence of controlled steps leading to locally and temporally defined self-destruction. Eventually, the term ‘apoptosis’ had been coined in order to describe the morphological processes leading to controlled cellular self-destruction and was first introduced by Kerr, Wyllie and Currie in 1972.² Word ‘apoptosis’ is of Greek origin which means “falling off or dropping off”, in analogy to leaves falling off trees or petals dropping off flowers, emphasizing that the death of living matter is an integral and necessary part of the life cycle of 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.

Apoptotic cells can easily be recognized from normal healthy cells due to occurrence of various stereotypical morphological changes such as shrinkage and deformation of apoptotic cell and loss of contact from its neighbouring cells, condensation of chromatin and its margination at the nuclear membrane, blebbing or budding on the plasma membrane, and finally fragmentation of cell into compact membrane-enclosed structures, called ‘apoptotic bodies’ which contain cytosol, the condensed chromatin, and organelles. These apoptotic bodies are then engulfed by macrophages and thus removed from the tissue without causing an inflammatory response. Those morphological changes are a consequence of characteristic molecular and biochemical events occurring in an apoptotic cell, most notably the activation of proteolytic enzymes which eventually mediate the cleavage of DNA into oligonucleosomal fragments as well as the cleavage of a multitude of specific protein substrates which usually determine the integrity and shape of the cytoplasm or
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Apoptosis is in contrast to the necrotic mode of cell-death in which case the cells suffer a major insult, resulting in a loss of membrane integrity, swelling and disruption of the cells. During necrosis, the cellular contents are released uncontrolled into the cell’s environment which results in damage of surrounding cells and a strong inflammatory response in the corresponding tissue (Figure 3.1).4

Figure 3.1. Comparison between apoptosis and necrosis5

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 cancer6,7 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.8,9 Apoptosis sometimes enhances due to overexpression of genes, such as p53, which are involved in controlling the whole apoptotic mechanism in our body, or it can be induced by toxins or ionizing radiations.10 Mitochondria plays an important role in controlling cell death and follows either p53-dependent or p53-independent pathway of apoptosis. During apoptosis, mitochondria releases a number of pro-apoptotic proteins such as Apoptosis Inducing Factor (AIF), second mitochondrial-derived activator of caspase (Smac), direct IAP-binding protein with low PI (DIABLO), Cytochrome C, etc. which are involved in apoptosome formation which leads to activation of caspases, also called caspase cascade, responsible for induction of apoptosis11-13 (Figure 3.2). Caspases are aspartate-directed cysteine proteases, and failing of their activation may cause tumour development and several auto-immune diseases. At least 14 mammalian caspases (caspase-1 to 14) have been recognized that can be categorized into three functional groups such as initiators (e.g., casp-2, 8, 9 and 10), effectors (e.g., casp-3, 6 and 7) and the prototypical members of
a subclass of caspases involved in cytokine activation termed inflammatory caspases (casp-1, 4, 5, 11, 12 and 13) (Table 3.1).

**Figure 3.2.** Role of mitochondria during apoptosis

**Table 3.1.** Common known caspases involved in apoptosis (or programmed cell death)

<table>
<thead>
<tr>
<th>Cysteiny1 aspartic acid-protease (caspase)</th>
<th>Type</th>
<th>Name</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiators (or Apical)</td>
<td>Caspase-2</td>
<td>ICH1, Nedd2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspase-8</td>
<td>FLICE, MACH1, MCH5, FADD-like Ice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspase-9</td>
<td>MCH6, CELAP6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspase-10</td>
<td>FLICE2, MCH4</td>
<td></td>
</tr>
<tr>
<td>Effectors (or executioner)</td>
<td>Caspase-3</td>
<td>CPP32, YAMA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspase-6</td>
<td>MCH2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspase-7</td>
<td>MCH3, CMH, CELAP3</td>
<td></td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Inflammatory caspases</th>
<th>Caspase-1</th>
<th>ICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-4</td>
<td>ICH2, TX, ICErII</td>
<td></td>
</tr>
<tr>
<td>Caspase-5</td>
<td>ICErIII, TY</td>
<td></td>
</tr>
<tr>
<td>Caspase-11</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Caspase-12</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Caspase-13</td>
<td>ERICE</td>
<td></td>
</tr>
<tr>
<td>Caspase-14</td>
<td>MICE</td>
<td></td>
</tr>
</tbody>
</table>

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 signaling pathways.\(^8,15,16\) Recently, a small molecule, Pifithrin-\(\alpha\), also known as PFT-\(\alpha\), was originally identified as a leading compound of the known p53 inactivators after broad screening of 10,000 compounds that inhibits cell death from \(\gamma\)-radiation as well as protecting mice from lethal genotoxic stress, focal cortical ischemic injury, and neuronal excitotoxic damage.\(^16-18\) This protection by PFT-\(\alpha\) has been attributed to inhibition of p53 transactivation via diminishing p53-dependant and p53-independent mitochondria-mediated cell death \textit{in vivo} and \textit{in vitro}.\(^16,19\) It’s a matter of great debate from a long time within communities of chemists and biologists that whether ring opened PFT-\(\alpha\) is active or its ring closed form. NMR experiments have proved that PFT-\(\alpha\) as well as its ring opened analogues cyclize \textit{in situ} in protic solution and it has recently been reported that cyclized analogues of PFT-\(\alpha\), known as imidazo[2,1-\(b\)]benzothiazoles (IBTs) are more potent p53 inactivators than PFT-\(\alpha\) as well as opened analogues.\(^20,21\) Another report confirmed that the aromatic IBT analogues are more protective against dexamethasone or \(\gamma\)-radiation-induced apoptosis\(^10\) 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.\(^22\) It is known in the literature that malathion exerts its cytotoxic effects by the induction of apoptosis \textit{via} direct effect on mitochondria.\(^23\) 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 caspases 3 and 7 alone are sufficient for blocking apoptosis, and nonpeptide-based caspase inhibitors, the isatin sulfonamides 1, 2, 3 and 4 (Figure 3.3), 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.\(^{24,25}\)

![Figure 3.3. Some of the isatin sulfonamides](image)

Appreciating these findings, in the present investigation, we synthesized two series of small heterocycles, imidazo[2,1-b]benzothiazoles (IBTs, 5 and 6) bearing sulfonamide moiety, structurally related to PFT-α (Figure 3.4) and observed the

![Figure 3.4. Structure of Pifithrin-α (PFT-α) and the general structure of related analogues bearing sulfonamide moiety](image)
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.

### 3.2. Synthetic discussion for imidazo[2,1-b]benzothiazoles (IBTs 5 and 6)

The synthetic route used to synthesize the target imidazo[2,1-b]benzothiazoles, IBTs 5 and 6 is outlined in Scheme 3.1. The starting material, 2-aminobenzothiazole-6-sulfonamide 9 was synthesized in two steps because the conventional method of preparing 2-aminobenzothiazoles in a single step from appropriate anilines by treatment with Br₂/KSCN unfortunately failed to give 9. Therefore, the required

![Scheme 3.1. Synthesis of some novel imidazo[2,1-b]benzothiazoles (IBTs 5 and 6)](image-url)
starting material 9 was prepared from sulfanilamide 7 by first converting it into 4-aminosulfonylanilidheioureia 8 using ammonium thiocyanate in acidic medium followed by oxidative cyclization of 8 with Br₂ in chloroform. Reaction of 9 with either appropriate p-substituted phenacyl bromide 10 or 6-substituted-3-bromoacetylcoumarin 11 in refluxing 2-methoxynethanol followed by neutralization using aqueous ammonia yielded imidazo[2,1-b]benzothiazoles, IBTs 5 and 6 respectively. The synthetic details for each step are given in the following text.

3.2.1. Synthesis of 4-aminosulfonylanilidheioureia (8)

4-Aminosulfonylanilidheioureia 8 was prepared from sulfanilamide 7 by its reaction with ammonium thiocyanate in acidic conditions. Plausible mechanism for this reaction, depicted in Scheme 3.2, involves the initial nucleophilic attack of amino group of 7 on carbon atom of thiocyanate anion to generate an intermediate 12 which on rearrangement under acidic conditions is converted into 8.

3.2.2. Synthesis of 2-aminobenzothiazole-6-sulfonamide (9)

Having 4-aminosulfonylanilidheioureia 8 in hand, it was converted into 2-aminobenzothiazole-6-sulfonamide 9. To achieve this, 8 was suspended in chloroform followed by dropwise addition of a solution of bromine in chloroform. The resulting mixture was refuxed for 1h. After distilling off excess chloroform, orange colored semi-solid product obtained was treated with freshly prepared sulfurous acid till orange color gets discharged and the resulting mixture was refuxed for 1h. It was then cooled and neutralized with cold aqueous ammonia till solid separated out which was filtered, washed with excess of water, dried and crystallized from ethanol to afford 9 in moderate yield. Plausible mechanism of the reaction, depicted in Scheme 3.3, presumably involves the initial aromatic electrophilic substitution by bromine at position ortho to thiourea group and meta to sulfonamide group in 8. The intermediate 13 thus obtained undergoes cyclization by replacement of bromine through sulfur
attack with simultaneous carbon nitrogen double bond formation and removal of HBr to generate a new intermediate 14 which undergoes rearrangement to form final product 9.

Scheme 3.3. Plausible mechanism for 2-aminobenzothiazole-6-sulfonamide (9) synthesis

The 1H NMR spectrum of 9 in DMSO-d6 displayed two exchangeable singlets due to two protons each, one at δ 7.87 and another at δ 7.21 which were assigned to NH2 and SO2NH2 respectively. The remaining three aromatic protons were clearly assigned as one singlet due to one proton at δ 8.12 and two doublets corresponding to one proton each at δ 7.66 and δ 7.42 due to vicinal positions.

3.2.3. Synthesis of imidazo[2,1-b]benzothiazoles (IBTs 5 and 6)

After the successful synthesis of 2-aminobenzothiazole-6-sulfonamide 9, we turned our attention to its reaction with various p-substituted phenacyl bromides (10) and 6-substituted-3-bromoacetylcoumarins (11) (Scheme 3.1). Proposed mechanism for the synthesis of imidazo[2,1-b]benzothiazoles 5 and 6 is depicted in Scheme 3.4.
The first step is the formation of an acyclic intermediate 15 by the nucleophilic attack of nitrogen of benzothiazole ring (9) on the bromine-carrying carbon of 10 or 11 resulting in the formation of the N-C bond with simultaneous transfer of amino lone pair of electrons towards C-2 of the thiazole ring accompanied by the elimination of HBr molecule. In the next step, an attack by the lone pair of electrons from imino nitrogen atom of 15 at the carbonyl carbon yields a cyclic intermediate 16 which looses a water molecule leading to the formation of fused imidazobenzothiazoles 5 or 6.

Reaction of 9 with phenacyl bromide (10a) was first to be investigated. Thus a mixture of 9 and 10a was refluxed in 2-methoxyethanol till the TLC indicated the complete disappearance of the starting material. The resulting solution was neutralized with aqueous ammonia in order to convert the HBr salt of 5a to its free form affording 2-phenylimidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (5a) in 70% yield. The structure of 5a was assigned on the basis of its IR, $^1$H NMR and $^{13}$C NMR spectral data and was confirmed on the basis its mass spectrum (DART-MS). The IR spectrum of 5a showed two absorption bands at 3302 cm$^{-1}$ & 3148 cm$^{-1}$ which were assigned to the N-H stretchings of SO$_2$NH$_2$ group. The functional group region of the spectrum also exhibited absorption bands at 1589 cm$^{-1}$ (C=N stretching), 1543 cm$^{-1}$ (C=C stretching), 1497 cm$^{-1}$ (N-H bending), and 1335 cm$^{-1}$ & 1165 cm$^{-1}$ (SO$_2$ stretchings). $^1$H NMR of 5a displayed a characteristic singlet for one proton at $\delta$ 8.83 which was clearly assigned to imidazo proton (C$_4$-H) that appeared due to cyclization. An exchangeable singlet due to two protons resonating at $\delta$ 7.51 was assigned to amino group of SO$_2$NH$_2$. Three aromatic protons of the benzothiazole ring were easily identified as a singlet at $\delta$ 8.55 and two doublets having a coupling constant of 8.4 Hz at $\delta$ 8.15 & $\delta$ 8.00. The aromatic protons of the phenyl ring displayed a typical pattern showing ortho, meta & para protons in the ratio 2 : 2 : 1 with a uniform coupling constant of 7.5 Hz.

Other imidazo[2,1-b]benzothiazoles, 5b-5f and 6a-6c were also synthesized following the same procedure and showed IR spectral characteristics similar to that of 5a. Besides displaying other appropriate signals, in general, $^1$H NMR spectra of 5 and 6 displayed a characteristic singlet due to imidazo proton (C$_4$-H) in the range $\delta$ 8.76-9.11 that was taken as a direct indication of cyclization to form IBTs. An exchangeable singlet for two protons appeared in the narrow region $\delta$ 7.51-7.53,
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sometimes merging with aromatic protons that was assigned to SO$_2$NH$_2$ group. The presence of a methyl group attached to aromatic ring in 5b was ascertained on the basis of a singlet for three protons at $\delta$ 2.33 and a signal at $\delta$ 21.2 in its $^1$H NMR and $^{13}$C NMR spectrum respectively. The presence of fluorine at the para position of the aromatic ring in 5c was confirmed by the presence of three doublets at $\delta$ 162.0 (d, $^1J_{CF} = 244.6$ Hz), 127.1 (d, $^3J_{CF} = 7.5$ Hz) and 115.9 (d, $^2J_{CF} = 22.6$ Hz) in its $^{13}$C NMR spectrum. All coumarin IBTs (6a-6c) were showing characteristic C=O stretching of lactone at 1720 cm$^{-1}$ in FT-IR. A singlet due to one proton in the region $\delta$ 8.51-8.56 in $^1$H NMR spectra of coumarin IBTs (6a-6c) was assigned to coumarin C$_4$-H. Another singlet due to one proton at $\delta$ 8.16 in 6c was assigned to coumarin C$_5$-H which was merged with aromatic protons in 6b.

3.3. Apoptosis Inhibition Studies

Collection of Materials and Isolation of Cells
Goat testes were collected from the slaughter houses around Kurukshetra (26°6’ N, 76°5’ E), 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.

Reagents/Chemicals
Malathion, dimethylsulfoxide (DMSO), DMEM, phosphate buffer saline (PBS), antibiotics (penicillin, streptomycin).

3.3.1. Apoptosis Assays
Testicular germ cells were harvested from mature goat (Capra 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 μmol of each tested compound in DMSO for 30 minutes before induction of apoptosis. Then the apoptosis was induced with 5 μmol 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.$^{10,20}$ 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.

### 3.3.2. Apoptotic Inhibition Results

All the newly synthesized IBTs (5a-5f and 6a-6c) were evaluated for their *in vitro* inhibition of apoptosis induced by malathion in testicular germ cells. Results obtained are reported in Table 3.2. Out of IBTs (5a-5f) with phenyl analogues, 5b 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.

**Table 3.2. Malathion induced inhibition of apoptosis in testicular germ cells**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Group R or R’</th>
<th>Average Cell Survival ± Standard Deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>H</td>
<td>50±5.29*</td>
</tr>
<tr>
<td>5b</td>
<td>CH₃</td>
<td>64±4.35*</td>
</tr>
<tr>
<td>5c</td>
<td>F</td>
<td>43±4.00*</td>
</tr>
<tr>
<td>5d</td>
<td>Cl</td>
<td>56±3.00*</td>
</tr>
<tr>
<td>5e</td>
<td>Br</td>
<td>52±4.58*</td>
</tr>
<tr>
<td>5f</td>
<td>NO₂</td>
<td>39±2.00</td>
</tr>
<tr>
<td>6a</td>
<td>H</td>
<td>46±4.35*</td>
</tr>
<tr>
<td>6b</td>
<td>Cl</td>
<td>61±6.00*</td>
</tr>
<tr>
<td>6c</td>
<td>Br</td>
<td>48±5.56*</td>
</tr>
<tr>
<td>MACV</td>
<td></td>
<td>41±6.24*</td>
</tr>
<tr>
<td>CACV</td>
<td></td>
<td>67±4.58</td>
</tr>
</tbody>
</table>

MACV: Malathion tested % average cell survival.
CACV: Control % average cell survival.
Compound tested at 10 μm.
Malathion toxic at 10 μm, tested at 5 μm.
* p < 0.05 (*t* test)
(MACV; 41±6.24). Three compounds namely 5a (R = H), 5d (R = Cl), 5e (R = Br) showed mild activity exhibiting % average cell survival 50±5.29, 56±3.00 and 52±4.58 respectively. Compound 5c (R = F) showed weaker protective ability exhibiting 43±4.00% average cell survival. Surprisingly, compound 5f (R = NO₂) was found to be promoter of apoptosis inspite of inhibiting exhibiting 39±2.00% average cell survival. Out of IBTs 6a-6c with coumarin analogues, 6b (R’ = Cl) was found to be the most potent inhibitor exhibiting 61±6.00% average cell survival whereas compounds 6a (R’ = H) and 6c (R’ = Br) showed weaker cytoprotective activity exhibiting 46±4.35 and 48±5.56% average cell survival, respectively. Figures 3.5 and 3.6 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 X 100 and X 400 resolution respectively. Figure 3.7 is the fluorescent photograph of malathion treated testicular germ cells showing apoptotic germ cells with bright green condensed nuclei taken at X 400 resolution. Figures 3.8 and 3.9 are the fluorescent photographs, taken at X 400 resolution, of malathion treated testicular germ cells

**Figure 3.5.** Fluorescent photograph of testicular germ cells showing pinkish normal germ cells with their intact cell membranes and round nuclei in control group (arrow). X 100
Figure 3.6. Fluorescent photograph of testicular germ cells showing pinkish normal germ cell with their intact cell membranes and round nuclei in control group (arrow). X 400

Figure 3.7. Fluorescent photograph of malathion treated testicular germ cells showing apoptotic germ cells with bright green condensed nuclei (arrow). X 400
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**Figure 3.8.** Fluorescent photograph of malathion treated testicular germ cells supplemented with compound 5b showing decrease in number of apoptotic germ cells. X 400

**Figure 3.9.** Fluorescent photograph of malathion treated testicular germ cells supplemented with compound 6b showing decrease in number of apoptotic germ cells. X 400
supplemented with compound 5b and 6b respectively showing decrease in number of apoptotic germ cells. A graphical summary of the apoptosis results is presented in Figure 3.10.

![Average Cell Survival (%)](image)

**Figure 3.10.** Inhibition of malathion induced apoptosis

### 3.3.3. Discussion and Conclusions

All the newly synthesized IBTs (5a-5f and 6a-6c) 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.\(^{20,21}\) 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 the 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\textsuperscript{10} that aromatic analogues of IBTs are highly active. Therefore, we synthesized sulfonamide bearing aromatic 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 the IBT analogue. The trend with respect to substituent on the phenyl ring of IBTs 5 and coumarin ring of IBTs 6 was found to be $\text{CH}_3 > \text{Cl} > \text{Br} > \text{H} > \text{F} > \text{NO}_2$. In case of NO$_2$, this trend became so enormous that our IBT 5f showed some unusual behavior by increasing the apoptosis suggesting that 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\textsuperscript{10} but they are better p53 inactivators.\textsuperscript{20,21} As reported in the literature that larger the surface area of an IBT,\textsuperscript{10} better would it tend to show anti-apoptotic activity. Therefore, we also tried to observe the role of surface area in controlling the anti-apoptotic activity of IBT by making some coumarin analogues (6a-6c) and compared the results with its phenyl analogues (5a, 5d and 5e) respectively. The results do not show a general trend as the anti-apoptotic activity of 6b was significant while that of 6a and 6c 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 5b and 6b exhibited significant protection against malathion induced apoptosis and may be promising for further development. It is reported in the literature\textsuperscript{30} that apoptosis induced by irradiations is p53 dependent while dexamethasone induced apoptosis is not. 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\textsuperscript{22} because IBT 5f ($p$-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 \textit{via} mitochondrial pathway in a much better way and may thus influence therapeutic strategy.
3.4. Experimental section

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. IR spectra were recorded on ABB MB 3000 DTGS FT-IR instrument using the KBr pellet technique. The $^1$H NMR and $^{13}$C NMR spectra were recorded in pure DMSO-$d_6$ on Bruker NMR spectrometer at 300 MHz and 75.5 MHz, respectively. The δ values are given in ppm relative to tetramethylsilane (TMS) as internal standard. 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 (detected by disappearance of signal upon D$_2$O addition) are used for NMR assignments and ‘s’ for strong, ‘m’ for medium for IR assignments. ‘d’ stands for decomposition in melting point data.

Typical two-step procedure for the synthesis of 2-aminobenzothiazole-6-sulfonamide (9):

**Step 1: Synthesis of 4-aminosulfonylphenylthiourea (8)**

Sulfanilamide (7, 200 mmol) was dissolved in a mixture of conc. HCl (18 mL) and water (50 mL) by warming. Solution was then cooled to room temperature and ammonium thiocyanate (15.2 g, 100 mmol) was added. The mixture was heated on water bath for 6 h and then cooled to room temperature. The separated solid was filtered, washed with excess of cold water, dried and crystallized from aqueous ethanol to afford 4-aminosulfonylphenylthiourea (8) as white crystalline solid. Yield: 76%; m. p. 190-191°C; Lit.$^{27}$ m. p. 206 °C.

**Step 2: Synthesis of 2-aminobenzothiazole-6-sulfonamide (9)**

To a suspension of 4-aminosulfonylphenylthiourea (8, 125.5 mmol) in CHCl$_3$ (100 mL), bromine (Br$_2$, 10 mL) in CHCl$_3$ (100 mL) was added dropwise over 1 h duration and the resulting mixture was
refluxed for 1h. Excess chloroform was then distilled off and the orange colored semi-solid product thus obtained was treated with freshly prepared sulfurous acid (H₂SO₃) till the orange color was discharged. The solution was then refluxed for 1h, cooled to 5-10 °C and neutralized with cold aqueous ammonia. The solid separated out which was filtered, washed with excess of water, dried and crystallized from ethanol to afford 2-aminobenzothiazole-6-sulfonamide (9). Yield: 69%; m. p. 268-270 °C; Lit.²⁸ m. p. 277 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 8.12 (s, 1H, Ar), 7.87 (s, ex, 2H, NH₂), 7.66 (d, 1H, J = 8.4 Hz, Ar), 7.42 (d, 1H, J = 8.4 Hz, Ar), 7.21 (s, ex, 2H, SO₂NH₂).

Representative protocol for the synthesis of imidazo[2,1-b]benzothiazoles (IBTs 5a-5f and 6a-6c)

A mixture of 2-aminobenzothiazole-6-sulfonamide (9, 4 mmol) and appropriate p-substituted phenacyl bromide (10) or 6-substituted-3-bromoacetylcoumarin (11) (4.8 mmol) was refluxed in 2-methoxyethanol (15 mL) for 4h. 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 colored 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; 5a-5e) or from DMF-ethanol (1 : 1; 5f, 6a-6c) to afford the target IBTs.

2-Phenylimidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (5a)

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, C=C stretch), 1497 (s, N-H bend), 1335 & 1165 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 8.83 (s, 1H, imidazo C₄-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 = 7.5 Hz, Ar), 7.51 (s, ex, 2H, SO₂NH₂), 7.45 (t, 2H, J = 7.5 Hz, Ar), 7.31 (t, 1H, J = 7.5 Hz, Ar); ¹³C NMR (75.5 MHz, DMSO-d₆): 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)⁺, C₁₅H₁₁N₃O₂S₂H⁺ calcd. 330.02.
2-(4-Methylphenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (5b)

Yield: 62%; m. p. 300-302 °C (d); IR (KBr) cm⁻¹: 3356 & 3148 (m, N-H stretch), 1589 (s, C=N stretch), 1543 (m, C=C stretch), 1497 (s, N-H bend), 1335 & 1157 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 8.76 (s, 1H, imidazo C₄-H), 8.54 (s, 1H, Ar), 8.13 (d, 1H, J = 8.1 Hz, Ar), 8.00 (d, 1H, J = 8.1 Hz, Ar), 7.76 (d, 2H, J = 7.2 Hz, Ar), 7.50 (s, ex, 2H, SO₂NH₂), 7.25 (d, 2H, J = 7.2 Hz, Ar), 2.33 (s, 3H, CH₃); ¹³C NMR (75.5 MHz, DMSO-d₆): 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 (CH₃); DART-MS: m/z 344.08 (M+H)⁺, C₁₆H₁₃N₃O₂S₂H⁺ calcd. 344.02.

2-(4-Fluorophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (5c)

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, C=C stretch), 1497 (s, N-H bend), 1327 & 1165 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 8.78 (s, 1H, imidazo C₄-H), 8.55 (s, 1H, Ar), 8.12 (d, 1H, J = 7.8 Hz, Ar), 8.01 (d, 1H, J = 7.8 Hz, Ar), 7.88-7.92 (m, 2H, Ar), 7.52 (s, ex, 2H, SO₂NH₂), 7.28 (t, 2H, J = 7.5 Hz, Ar); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 162.0 (d, ¹JC₆ = 244.6 Hz), 148.5, 146.1, 140.7, 134.0, 130.2, 130.0, 127.1 (d, ¹JC₆ = 8.3 Hz), 124.8, 123.1, 115.9 (d, ²JC₆ = 22.6 Hz), 113.9, 109.2; DART-MS: m/z 348.06 (M+H)⁺, C₁₅H₁₀FN₃O₂S₂H⁺ calcd. 348.01.

2-(4-Chlorophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (5d)

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, C=C stretch), 1497 (s, N-H bend), 1327 & 1157 (s, SO₂ stretch); ¹H NMR (300 MHz, DMSO-d₆): δ 8.87 (s, 1H, imidazo C₄-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\textsubscript{2}NH\textsubscript{2}, Ar; \textsuperscript{13}C NMR (75.5 MHz, DMSO-\textit{d}\textsubscript{6}): \textit{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: \textit{m/z} 364.03 (M+H)+, C\textsubscript{13}H\textsubscript{10}ClN\textsubscript{3}O\textsubscript{2}S\textsubscript{2}H+ calcd. 363.99.

\textbf{2-(4-Bromophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (5e)}

Yield: 60%; m. p. 298-300 °C (d); IR (KBr) cm\textsuperscript{-1}: 3333 & 3240 (m, N-H stretch), 1643 (s, C=N stretch), 1558 (m, C=C stretch), 1528 (s, N-H bend), 1311 & 1157 (s, SO\textsubscript{2} stretch); \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}\textsubscript{6}): \textit{δ} 8.88 (s, 1H, imidazo C\textsubscript{4}-H), 8.56 (s, 1H, Ar), 8.13 (d, 1H, \textit{J} = 8.4 Hz, Ar), 8.01 (d, 1H, \textit{J} = 8.4 Hz, Ar), 7.82 (d, 2H, \textit{J} = 8.1 Hz, Ar), 7.64 (d, 2H, \textit{J} = 8.1 Hz, Ar), 7.51 (s, ex, 2H, SO\textsubscript{2}NH\textsubscript{2}); \textsuperscript{13}C NMR (75.5 MHz, DMSO-\textit{d}\textsubscript{6}): \textit{δ} 148.8, 146.1, 140.8, 132.1, 130.4, 127.2, 124.9, 121.0, 114.1, 110.0; DART-MS: \textit{m/z} 407.99 (M+H)+, C\textsubscript{13}H\textsubscript{10}BrN\textsubscript{3}O\textsubscript{2}S\textsubscript{2}H+ calcd. 407.93.

\textbf{2-(4-Nitrophenyl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (5f)}

Yield: 82%; m. p. >330 °C (d); IR (KBr) cm\textsuperscript{-1}: 3348 & 3140 (s, N-H stretch), 1597 (s, C=N stretch), 1543 (m, C=C stretch), 1504 (s, N-H bend), 1335 & 1165 (s, SO\textsubscript{2} stretch); \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}\textsubscript{6}): \textit{δ} 9.11 (s, 1H, imidazo C\textsubscript{4}-H), 8.57 (s, 1H, Ar), 8.31 (d, 1H, \textit{J} = 8.1 Hz, Ar), 8.16 (d, 1H, \textit{J} = 8.4 Hz, Ar), 8.11 (d, 2H, \textit{J} = 8.1 Hz, Ar), 8.02 (d, 2H, \textit{J} = 8.4 Hz, Ar), 7.53 (s, ex, 2H, SO\textsubscript{2}NH\textsubscript{2}); \textsuperscript{13}C NMR (75.5 MHz, DMSO-\textit{d}\textsubscript{6}): \textit{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: \textit{m/z} 375.06 (M+H)+, C\textsubscript{13}H\textsubscript{10}N\textsubscript{4}O\textsubscript{4}S\textsubscript{2}H+ calcd. 375.01.

\textbf{2-(2-Oxo-2\textsubscript{H}-chromen-3-yl)imidazo[2,1-b][1,3]benzothiazole-7-sulfonamide (6a)}

Yield: 85%; m. p. >330 °C (d); IR (KBr) cm\textsuperscript{-1}: 3271 & 3140 (m, N-H stretch), 1720 (s, lactone C=O stretch), 1605 (s, C=N stretch), 1497 (s, N-H bend), 1311 & 1157 (s, SO\textsubscript{2} stretch); \textsuperscript{1}H
NMR (300 MHz, DMSO-$d_6$): $\delta$ 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$_2$NH$_2$, coumarin), 7.37 (d, 1H, $J = 8.1$ Hz, coumarin), 7.30 (t, 1H, $J = 7.2$ Hz, coumarin); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): $\delta$ 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$_3$O$_4$S$_2$H$^+$ calcd. 398.01.

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

Yield: 80%; m. p. >330 °C (d); IR (KBr) cm$^{-1}$: 3348 & 3163 (m, N-H stretch), 1720 (s, lactone C=O stretch), 1597 (s, C=N stretch), 1497 (s, N-H bend), 1327 & 1157 (s, SO$_2$ stretch); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 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, coumarin C$_5$-H, Ar), 7.57 (d, 1H, $J = 8.7$ Hz, coumarin), 7.51 (s, ex, 2H, SO$_2$NH$_2$), 7.44 (d, 1H, $J = 8.4$ Hz, coumarin); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): $\delta$ 158.5, 151.6, 149.0, 141.4, 135.9, 134.0, 130.3, 128.0, 124.9, 123.3, 121.5, 118.3, 114.7; DART-MS: $m/z$ 432.09 (M+H)$^+$, C$_{18}$H$_{10}$ClN$_3$O$_4$S$_2$H$^+$ calcd. 431.98.

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

Yield: 76%; m. p. >330 °C (d); IR (KBr) cm$^{-1}$: 3340 & 3163 (m, N-H stretch), 1720 (s, lactone C=O stretch), 1597 (s, C=N stretch), 1497 (s, N-H bend), 1327 & 1157 (s, SO$_2$ stretch); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 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, Ar), 7.96 (d, 1H, $J = 8.4$ Hz, Ar), 7.73 (d, 1H, $J = 8.7$ Hz, coumarin), 7.51 (s, ex, 2H, SO$_2$NH$_2$), 7.42 (d, 1H, $J = 8.7$ Hz, coumarin); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): $\delta$ 158.4, 151.6, 149.0, 141.4, 140.2, 135.7, 134.0, 130.9, 130.3, 124.9, 123.3, 121.5, 118.3, 114.7; DART-MS: $m/z$ 425.09 (M+H)$^+$, C$_{18}$H$_{10}$BrN$_3$O$_4$S$_2$H$^+$ calcd. 425.01.
121.3, 118.4, 116.8, 114.6; DART-MS m/z 476.00 (M+H)^+, C_{24}H_{10}BrN_{3}O_{4}S_{2}H^+ calcd. 386.17.
3.5. References

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