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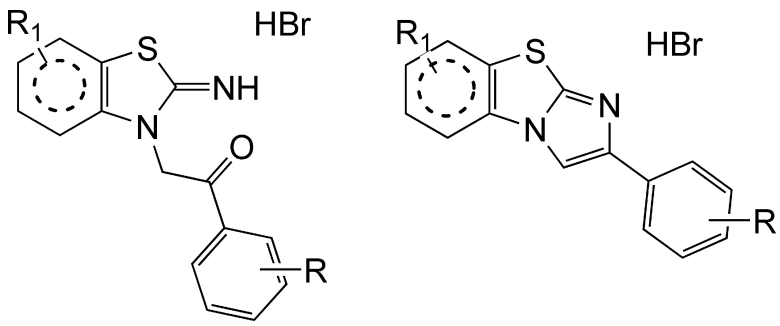

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Inhibitors of Apoptosis in Lymphocytes: Synthesis and Biological Evaluation of Compounds Related to Pifithrin-α

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The chemoprotection of cells from apoptosis induced by toxins or ionizing radiation could be important for biodefense and in the treatment of acute injuries. We describe a series of small heterocycles, including fused benzothiazoles, benzimidazoles, and related compounds, that abrogate thymocyte apoptosis induced by dexamethasone and γ-irradiation. To optimize the protective activity of the previously reported pifithrin-α (PFT-α, **1**), various derivatives and analogues of this and the corresponding ring-closed imidazobenzothiazole (IBT, **39**) were synthesized. The aromatic analogues of **39** were more protective than **39**, while the aromatic analogues of **1** were not active. Compound **19** containing a pyrrolidinyl substituent on the phenyl ring provided potent antiapoptotic activity (EC_{50} of 1.31 μM compared to 4.16 μM for **1**). Modification of aromatic **39** with a pyrrolidinyl para substituent (compound **60**) enhanced the activity, lowering the EC_{50} to 0.35 μM. Also, **60** provided significant protection against γ-irradiation-induced apoptosis, as expected. Compounds **19** and **60** may be promising for potential clinical development.

Introduction

The prevention of untoward cell death has become an important goal for pharmacologic intervention in a variety of clinical settings. Profound disability can result from apoptotic cell death and tissue injury due to ischemia, chemotherapeutic agents, ionizing radiation, or hyperthermia. Recently, a small molecule, 2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-*p*-tolylethanone hydrobromide (PFT-α, **1**), was originally identified from a broad screen of 10 000 compounds to inhibit cell death from γ-radiation.[1] In addition, this compound was shown to protect mice from lethal genotoxic stress,[1] focal cortical ischemic injury, and neuronal excitotoxic damage.[2,3] This protection by **1** has been attributed to inhibition of p53 transactivation. Supporting evidence has included diminished nuclear accumulation with decreased p53 DNA binding activity and decreased caspase activity and suppression of mitochondrial dysfunction.[2,3] Furthermore, the transcription of apoptosis-associated gene products p53, Bax, and p21 was inhibited.[4]

However, the activity of **1** is not limited to inhibiting the transactivation of p53. This small molecule has been found to also suppress the heat shock and glucocorticoid signaling pathways.[5] Glucocorticoid-induced cell death is independent of p53.[6] This pathway, however, also induces the transcription and activity of the proapoptotic BH3 (Bcl-2 homology)-only protein p53-upregulated modulator of apoptosis (PUMA).[7] When this death-inducing signal reaches the mitochondria, cell-death-related events ensue. The inner mitochondrial membrane loses its potential, and cytochrome *c* is released into the cytoplasm where it can associate with ATP, apoptosis-activating factor-1, and procaspase-9. This apoptosome complex cleaves procaspase-9 to caspase-9, which in turn cleaves procaspase-3, initiating the cascade of protease activation in the execution phase of apoptosis.[8,9]

Hence, **1** has been reported to diminish p53-dependent and -independent mitochondria-mediated cell death in vitro and in vivo.[1,5] The multiplicity of molecular pathways that are reportedly influenced by **1** suggested that the cytoprotective effect could be further optimized. Furthermore, **1** is not stable under physiological conditions and spontaneously undergoes ring closure to form the imidazo[2,1-*b*]benzothiazole (IBT) **39**.[10,11] Despite recent reports by Zhu[12] and Pietrancosta[11] describing a few of the compounds prepared here, the biologically active form of **1** (open versus closed ring) has not previously been formally determined. Elucidation of the active ring structure would allow investigation of further chemical modifications that may enhance the potency of the compound. In addition, alternative ring structures, such as the quaternary salts recently reported from our laboratories, may also provide greater potency.[13] Hence, we report the synthesis and structure−activity relationship of novel derivatives and analogues of **1** and corresponding closed ring counterparts with enhanced potency and stability. To determine activity, we used a p53-independent assay of cell death wherein mouse thymocytes were treated with dexamethasone in the presence or absence of test compounds. In addition, we confirmed the activity of the most potent compounds in a p53-dependent apoptosis assay.

Chemistry

With PFT-α (**1**) as the lead compound with known biological activity, lead optimization strategies were applied to establish a structure−activity relationship.

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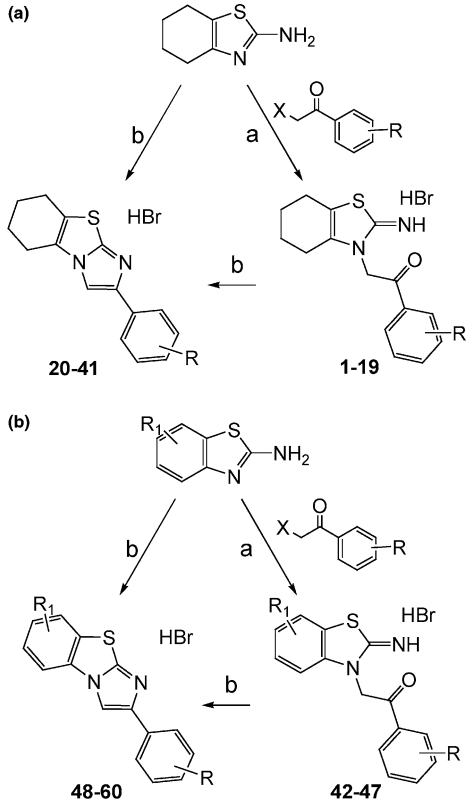
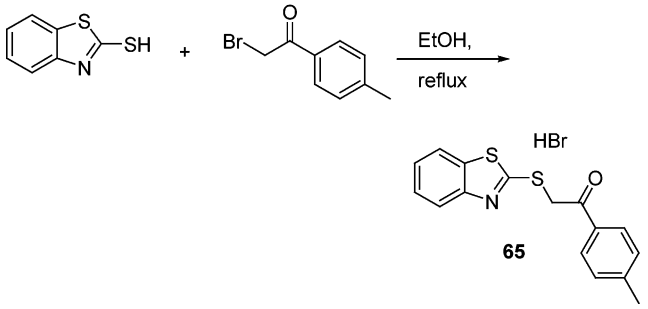
Scheme 1[a]



[a] (a) Toluene, room temp, 24−48 h; (b) ethanol or methoxyethanol, reflux, 90 min; (c) toluene, room temp, 24−48 h; (d) ethanol or methoxyethanol, reflux, 1.5 h.

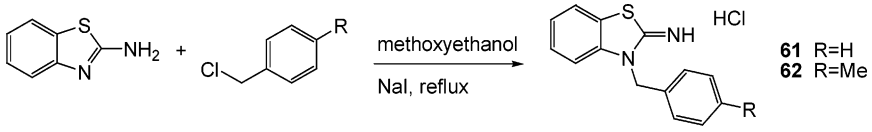
The starting 2-aminotetrahydrobenzothiazoles used to prepare PFT-α and derivatives **2**−**19** were obtained following the procedure described by Singh et al.[10] in which the appropriate cyclohexanone was reacted with thiourea in the presence of iodine (Scheme 1a). For the synthesis of the aromatic analogues of PFT-α, **42**−**47**, the starting 2-aminobenzothiazoles were commercially available (Scheme 1b). Alkylation of the 2-aminobenzothiazoles with various α-haloacetophenones provided the corresponding N-alkylated compounds **1**−**19** and **42**−**47**, with alkylation occurring exclusively at the ring nitrogen (Scheme 1). In protic solvents the ring alkylated fused thiazoles often formed aromatic ring-closed products with concomitant dehydration. Therefore, we elected to prepare the corresponding closed ring derivatives **20**−**41**. The corresponding IBT derivatives **20**−**41** and **48**−**60** were then prepared by ring closure in refluxing ethanol or in methoxyethanol when higher temperatures were needed (Scheme 1). Compounds **3**, **17**, **20**, and **21** have been reported by Pietrancosta[11] and **3**, **8**, **13**, **14**, **16**, **17**, and **66** by Zhu[12] as p53 inactivators and neuroprotective agents.

Scheme 4

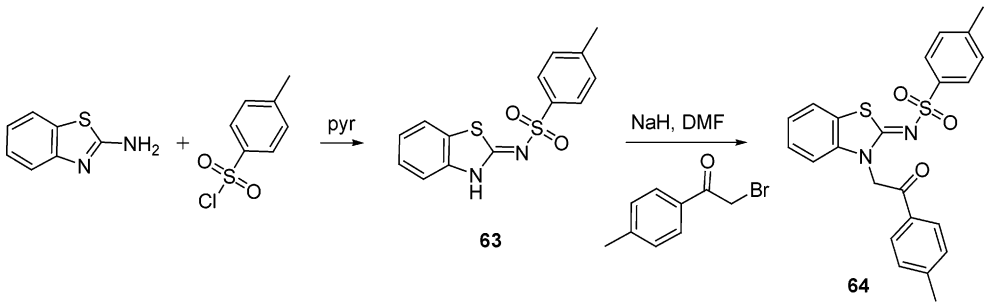


To study the effects of ring closure on activity, two strategies were employed. First, to prepare derivatives that would undergo limited spontaneous ring closure under normal physiological conditions, we alkylated 2-aminobenzothiazole with benzyl chloride to obtain **61**, a product incapable of ring closure in refluxing ethanol because it lacks the carbonyl group. In addition, the para methyl substituted product **62** was synthesized in the same manner, giving a molecule closer in shape to that of **1** (Scheme 2). In a second approach to hinder ring closure, we protected the exocyclic nitrogen of the 2-aminobenzothiazole using *p*-toluenesulfonyl choride to yield **63** before alkylating with 2-bromo-4′-methylacetophenone to give **64** (Scheme 3). In addition, a non-ring-closable PFT-α analogue **65** was prepared by reaction of 2-bromo-4′-methylacetophenone with 2-mercaptobenzothiazole, resulting exclusively in the S-alkylation product (Scheme 4). Compound **66**, which was synthesized by alkylation of 2-amino-4,5,6,7-tetrahydrobenzothiazole with ethyl bromoacetate, appeared to be resistant to ring closure in refluxing ethanol even though it possesses a carbonyl group β to the ring nitrogen and should in theory be able to form a ring (Scheme 5).

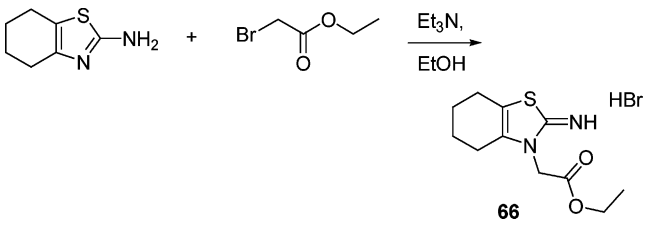
Scheme 2



Scheme 3



Scheme 5



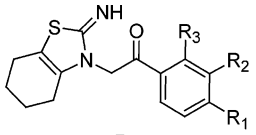
The effects of substitution at the para position of the phenyl ring of **1** and corresponding derivatives were investigated. A variety of substituents was selected on the basis of their electronic and lipophilic properties. The electronic (Hammett σ) and the lipophilic (Hansch π) values calculated for each substituent were plotted in a two-dimensional graph, the so-called Craig plot,[14] and substituents were selected from each quadrant for preparation. These para-substituted derivatives in both the open ring (Table 1) and closed ring series (Table 2) are indicated by the R_1 variable substitution.

Analogues in the PFT and IBT series still containing the thiazole moiety were synthesized in order to evaluate how modifications on parts of the molecule other than the para position of the phenyl group would influence the biological activity. The phenyl ring was replaced by a methyl group in the synthesis of **68**. Thus, the open ring structure **67** was prepared by reacting 2-aminobenzothiazole with propargyl bromide followed by addition of sodium to refluxing ethanol to effect ring closure (Scheme 6).[15] An isomeric version of IBT with the phenyl substituent attached to C-3 rather than C-2 was made by first forming the substituted 2-mercaptoimidazole **69** as described by Maeda et al.,[16] followed by reaction with 2-chlorocyclohexanone to form the imidazole **70** (Scheme 7). An isomer of **70**, compound **72**, was prepared by alkylation of a 2-mercaptobenzimidazole with the appropriate acetophenone to form exclusively the S-alkylated compound **71** that was further ring-closed using PPA, formed in situ as described by Mahfouz et al.[17] for a related compound (Scheme 8). Finally, the PFT-α and IBT analogues lacking the benzo ring, **73** and **74**, respectively, were prepared following the basic alkylation and ring-closure procedure described earlier.

Related ring systems were designed to maintain the overall shape relative to IBT. Heterocycles containing various combinations or arrangements of nitrogen atoms were prepared. Anthranilonitrile refluxed with methoxyethanol in the presence of formamidine acetate generated 4-aminoquinazoline,[18] which was subsequently used for the synthesis of the 1,2,4-triazolo[1,5-*c*]quinazoline **79** and imidazo[1,2-*c*]quinazoline **81** (Scheme 9). First, 4-aminoquinazoline was refluxed with hydrazine hydrate for 6 h to form quinazolin-4-ylhydrazine. A Schiff base **77** was obtained by reacting this substituted hydrazine derivative with tolualdehyde. Ring closure to **78** occurred by addition of acetic anhydride, and finally, deacetylation gave the desired quinazoline **79**. Second, 4-aminoquinazoline was alkylated with 2-bromo-4′-methylacetophenone to produce a mixture of the open ring **80** and closed ring **81** products, which were then separated by flash chromatography on silica gel.

Another fused quinazoline, the imidazo[1,2-*a*]quinazoline **85**, was prepared starting with the alkylation of isatoic anhydride using 2-bromo-4′-methylacetophenone

Table 1. Survival of Cells Treated with PFT-α and Analogues When Challenged with DEX



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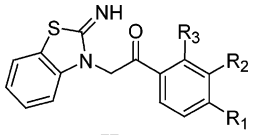
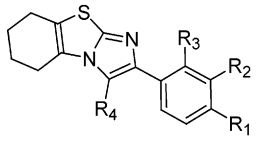


II

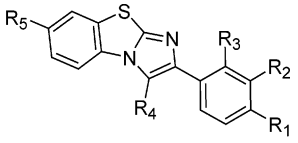
compd	class	R_1	R_2	R_3	average cell survival (%)[a]	standard deviation (%)	*P* (Mann-Whitney *U* test)
1	I	Me	H	H	72	9	0.0001
2	I	OCOPh	H	H	17	17	0.0364
3	I	NO_2	H	H	21	0	0.0553
4	I	Br	H	H	31	1	0.0910
5	I	CF_3	H	H	36	12	0.7037
6	I	CN	H	H	40	1	0.7182
7	I	F	F	H	40	18	0.2255
8	I	F	H	H	48	17	0.1070
9	I	H	F	H	54	17	0.0098
10	I	N_3	H	H	56	10	0.0027
11	I	Cl	Cl	H	57	3	0.0009
12	I	2-naphthyl	H	H	58	4	0.0003
13	I	H	H	H	59	9	0.0020
14	I	Cl	H	H	59	10	0.0017
15	I	H	H	Cl	65	1	0.0009
16	I	Ph	H	H	65[b]	5	0.0001
17	I	OMe	H	H	66	2	0.0019
18	I	Et_2N	H	H	70	9	0.0044
19	I	pyrrolidinyl	H	H	77	3	0.0009
42	II	H	H	H	20	14	0.0514
43	II	Me	H	H	21	11	0.0364
44	II	cyclopentyl	H	H	33	3	0.4273
45	II	2-naphthyl	H	H	62	5	0.0001
46	II	pyrrolidinyl	H	H	64	3	0.0009
47	II	Ph	H	H	66	3	0.0009

[a] Control average % cell survival: 72 ± 4. Dexamethasone average % cell survival: 36 ± 7. [b] Toxic at 10 μM. Tested at 5 μM.

Table 2. Survival of Cells Treated with IBT and Analogues When Challenged with DEX



III

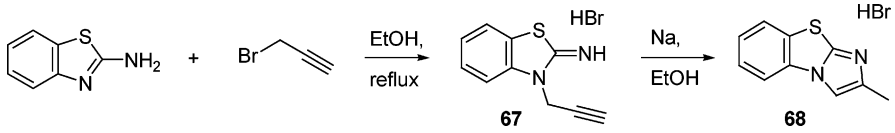


IV

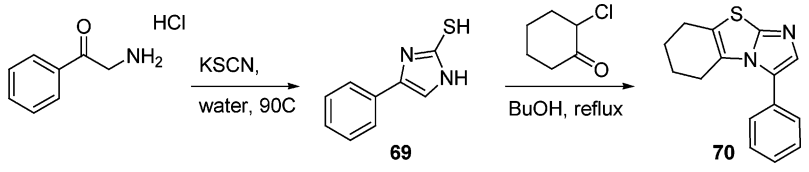
compd	class	R_1	R_2	R_3	R_4	R_5	average cell survival (%)[a]	standard deviation (%)	*P* (Mann-Whitney *U* test)
20	III	NO_2	H	H	H	H	11	2	0.0358
21	III	NH_2	H	H	H	H	18	14	0.0829
22	III	H	H	H	H	H	19	7	0.0173
23	III	COOH	H	H	H	H	20	8	0.0364
24	III	OH	H	H	H	H	21	1	0.0553
25	III	CN	H	H	H	H	21	11	0.0514
26	III	OCOPh	H	H	H	H	23	14	0.0829
27	III	CF_3	H	H	H	H	27	14	0.1265
28	III	Me	H	H	Br	H	27	16	0.4114
29	III	OMe	H	H	H	H	28	19	0.3167
30	III	F	H	H	H	H	33	5	0.2494
31	III	H	F	H	H	H	34	7	0.6481
32	III	Cl	H	H	H	H	35	22	0.7122
33	III	Br	H	H	H	H	35	7	0.4292
34	III	Cl	Cl	H	H	H	36	19	0.9273
35	III	H	H	Cl	H	H	37	8	0.7149
36	III	F	F	H	H	H	44	11	0.3154
37	III	N_3	H	H	H	H	50	5	0.3863
38	III	pyrrolidinyl	H	H	H	H	50	6	0.0057
39	III	Me	H	H	H	H	54	10	0.0051
40	III	2-naphthyl	H	H	H	H	57	1	0.0003
41	III	Ph	H	H	H	H	75	5	0.0019
48	IV	furanyl	H	H	H	H	16	13	0.0553
49	IV	Me	H	H	H	OH	19[b]	10	0.0009
50	IV	Br	H	H	H	H	22	10	0.0829
51	IV	Me	H	H	Br	H	24[b]	10	0.0829
52	IV	Me	H	H	H	NH_2	27	22	0.4114
53	IV	cyclopentyl	H	H	H	H	36	2	0.9517
54	IV	Me	H	H	H	Br	39	3	0.8286
55	IV	Ph	H	H	H	H	59	2	0.0009
56	IV	Me	H	H	H	OMe	68[b]	14	0.0009
57	IV	H	H	H	H	H	73	1	0.0019
58	IV	Me	H	H	H	H	73[b]	5	0.0003
59	IV	2-naphthyl	H	H	H	H	75[b]	2	0.0009
60	IV	pyrrolidinyl	H	H	H	H	78[b]	1	0.0003

[a] Control average % cell survival: 72 ± 4. Dexamethasone average % cell survival: 36 ± 7. [b] Toxic at 10 μM. Tested at 5 μM.

Scheme 6



Scheme 7

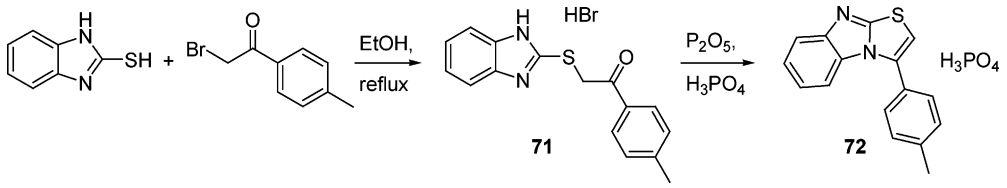


(Scheme 10). The product **82** was reacted with *S*-methylthiopseudourea, followed by refluxing in diglyme under basic conditions to give the ring-closed derivative **84** whose keto group was further transformed to a chloro by treatment with phosphorus oxychloride to give the imidazo[1,2-*a*]quinazoline **85** (Scheme 10). The 2-(5-chloro-2-iminobenzoxazol-3-yl)-1-*p*-tolylethanone **86**, an open-ring-related derivative, was prepared following the same procedure as that used for the alkylation of the thiazoles.

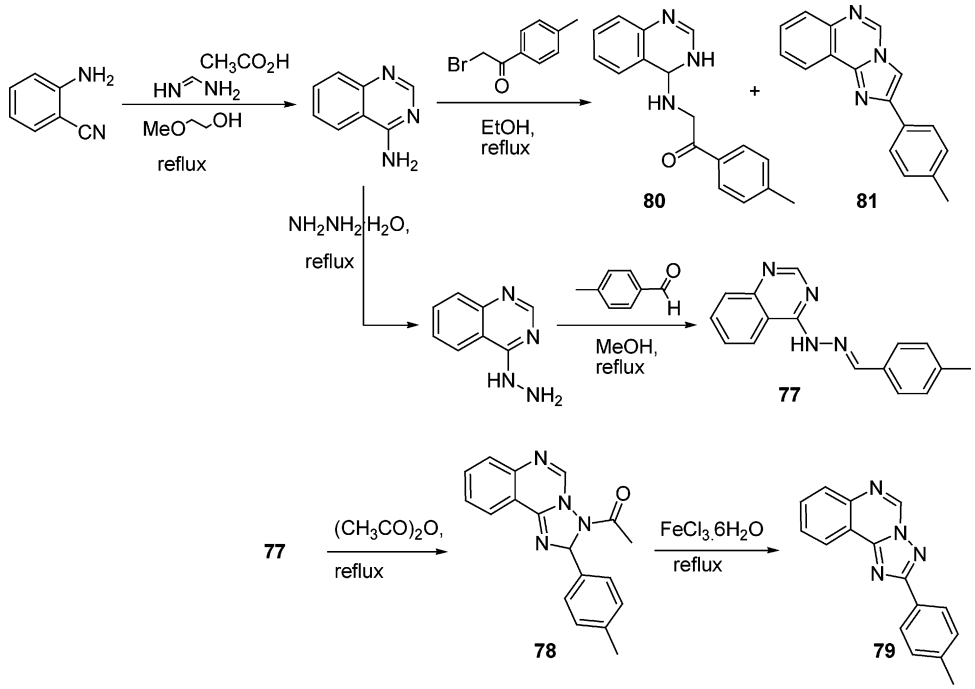
Biology Section

To examine the relative protective effect of the compounds, a reliable and relatively rapid screening assay was required. We and others have found that the structural similarity of PFT-α and IBT with luciferin rendered luciferase-based reporter gene assays for apoptosis unreliable.[19] Instead, we assayed fluorescent dye retention described below in thymocytes after dexamethasone-induced apoptosis. Dexamethasone (DEX) treatment of lymphocytes from the thymus initiates a

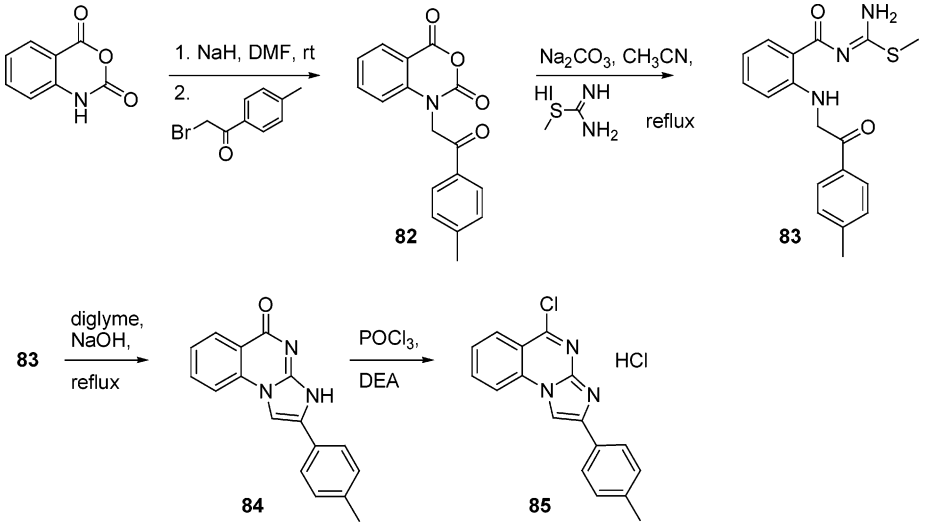
Scheme 8



Scheme 9



Scheme 10



signaling pathway that converges on the mitochondria-mediated molecular executioner cascade, leading to apoptosis within 6 h.[20] The 6 h time point was chosen because PFT-α was reported to inhibit DEX-induced apoptosis within this incubation period, and in our hands it was found to be reliable and reproducible.[5]

All of the compounds were screened in the thymocyte assay for cytotoxicity and their ability to prevent dexamethasone-induced cell death. Murine thymocytes were exposed to 10 or 5 μM of each compound in the presence or absence of DEX for 6 h and then assayed for their in vitro viability by 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) and propidium iodide (PI) staining and by flow cytometry. When cell death signals converge on the mitochondria, there is a loss of the inner mitochondrial membrane potential and cells can no longer retain the dye $DiOC_6$ as an indicator of apoptosis. Once cells lose the integrity of the outer cell membrane, then the dye propidium iodide is no longer excluded from these cells. The percentage of viable cells that retained $DiOC_6$ and excluded PI was compared to cells treated with DEX alone. Compounds that significantly enhanced survival

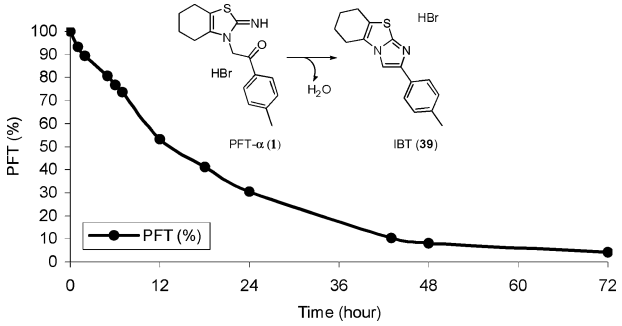


Figure 1. Kinetics of ring closure from PFT-α 1 to IBT **39**. A 200 μM solution of PFT-α in water with 0.1% DMSO was kept at room temperature and, at the indicated time points, quantitated by HPLC. IBT precipitated and was not reliably quantitated by this method.

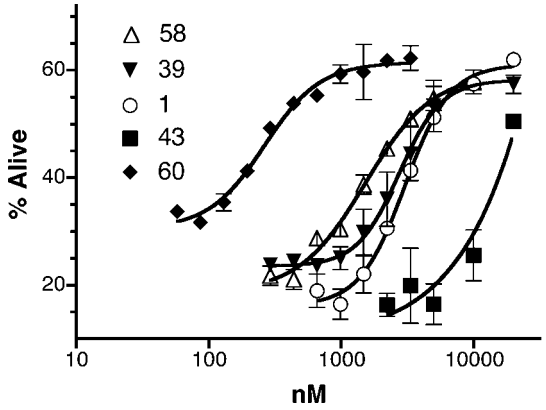


Figure 2. Inhibition of dexamethasone-induced thymocyte cell death. Murine thymocytes were pretreated with graded quantities of the indicated compounds. Apoptosis was then induced with 5 μM dexamethasone. After 6 h the cells were stained with $DiOC_6$ and PI and assayed for survival by flow cytometry. Shown are the percentages of living cells at the end of 6 h for three independent experiments.

in repeated determinations ($P \leq 0.01$) were then selected for a dose response study to determine the EC_{50} (Tables 5 and 6 and Figure 2).

Results and Discussion

PFT-α (**1**) has been described as a small-molecule inhibitor of programmed cell death.[1] However, as mentioned above, **1** does not remain in the open ring form in protic solution. After 16 h in solution under physiological conditions, **1** is largely ring-closed and converted to IBT (**39**) (Figure 1). Hence, a biological assay that could be performed during a relatively short period of time was necessary to investigate whether the closed ring or the open ring form of the compound was responsible for cytoprotective activity. It has been well established that murine thymocytes rapidly undergo apoptotic cell death after exposure to glucocorticoids such as DEX,[21] and recently glucocorticoid-induced thymocyte death was shown to be inhibited by **1**.[5] To evaluate the features associated with the cytoprotective effect of **1**, compounds were synthesized on the basis of the PFT-α or IBT structural platforms (summarized above and in Tables 1−4).

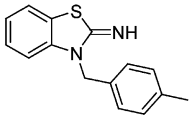
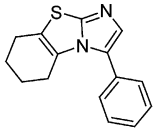
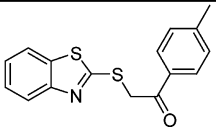
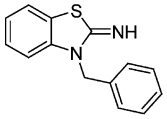
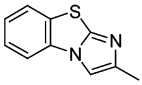
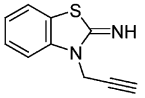
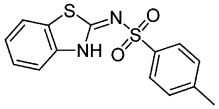
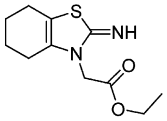
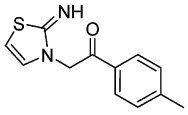
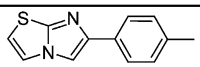
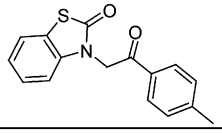
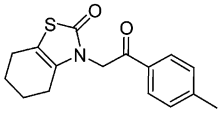
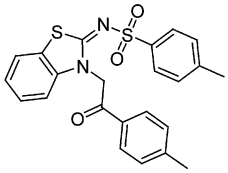
The PFT and IBT analogues were divided into four classes (Tables 1 and 2). Class I compounds included the open ring structure of PFT with a saturated benzo ring. Class II structures also had the open PFT platform but contained the aromatic benzo ring. Class III and class IV compounds had the closed ring structure of IBT bearing the saturated and aromatic rings, respectively. These series of compounds were used to compare the influence of an open versus closed ring structure, a saturated benzo ring versus an aromatic ring, and the characteristics of the para substituent on cytoprotective activity. Additional compounds were synthesized to evaluate other isoteric ring systems for activity (Tables 3 and 4). Several of these compounds were generated with obligately open ring structures to determine if this was the optimal scaffold to suppress mitochondrial death in DEX treated thymocytes.

As a primary screen for biological activity, each compound was tested for its ability to prevent DEX-induced cell death (Tables 1−4). Notably, all of the compounds that prevented DEX-induced cell death were derivatives of **1** and **39**. That is, none of the other isosteric ring analogues were cytoprotective (see Tables 3 and 4). The open PFT structure with a saturated benzo ring proved to be the best scaffold. While PFT-α showed impressive cytoprotective activity, with an average of 72% cells remaining alive at the end of the assay, compound **19**, bearing a pyrrolidinyl substituent at the para position of the phenyl, was even more protective with 77% cell survival. Another compound (**18**) bearing a tertiary amine substituent, the diethylamino group, also enhanced survival significantly compared to control cells treated with DEX. Moreover, compounds **15** (*o*-Cl), **16** (*p*-Ph), and **17** (*p*-OMe) were also fairly active. Compounds with similar substituents proved to be active in the class II series (Table 1). While none of the compounds in this class were as protective as PFT-α, three of them showed a cell survival average higher than 60%. Compounds **45** (*p*-naphthyl), **46** (*p*-pyrrolidinyl), and **47** (*p*-phenyl) were also titrated to obtain their EC_{50}.

Some compounds in the ring closed IBT series also enhanced survival of thymocytes treated with DEX (Table 2). Here again, a phenyl substituent (**41**) improved cytoprotective activity in the class III series. However, there were compounds in the aromatic series with greater activity (class IV, Table 2). Again, the pyrrolidinyl (**60**), naphthyl (**59**), and methyl (**58**) substituents were among the most active. Compound **57** without a susbstituent on the phenyl, as well as compound **56** with a methoxyl group on the benzo ring, also showed enhanced cytoprotective activity.

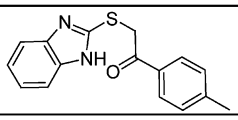
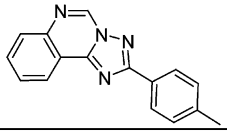
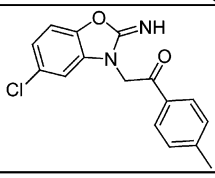
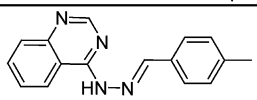
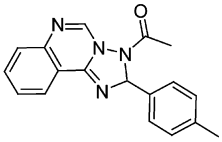
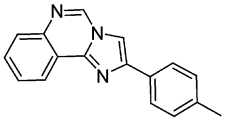
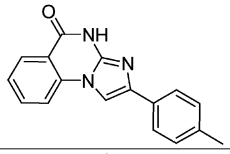
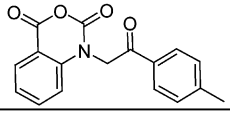
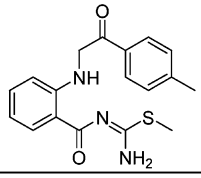
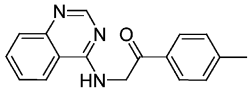
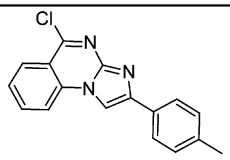
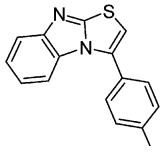
Titration of those compounds that provided cytoprotection of at least 60%, a value arbitrarily chosen, allowed for a more precise comparison using EC_{50} values (Tables 5 and 6). Overall, the same substituents seemed to improve activity in several classes. Compounds with lipophilic substituents such as tertiary amines (**18**, **19**, and **60**) or large aromatic groups (**12**, **16**, **41**, and **59**) showed similar and even greater cytoprotection than that of PFT-α or IBT. To explore the contribution of the para pyrrolidinyl group to the activity of the most potent compounds, we selected an isosteric mimic of this group, the cyclopentyl group, and prepared derivatives in classes II and IV (**44** and **53**, respectively) for evaluation in the thymocyte protection assay. Neither compound

Table 3. Survival of Cells Treated with PFT-α and IBT Analogues When Challenged with DEX

compd	structure	average cell survival (%)[a]	standard deviation (%)	Mann-Whitney U test
62		8	3	0.0358
70		15	11	0.0553
65		16	8	0.0553
61		20	18	0.1267
68		22	7	0.0253
67		23	4	0.0364
63		26	6	0.0358
66		27	4	0.1208
73		27	9	0.1208
74		27	6	0.0829
75		30	5	0.1296
76		40	3	0.4306
64		48	24	0.4273

[a] Control average % cell survival: 72 ± 4. Dexamethasone average % cell survival: 36 ± 7.

Table 4. Survival of Cells Treated with Various Compounds with Ring Structures Related to PFT-α and IBT When Challenged with DEX

compd	structure	average cell survival (%)[a]	standard deviation (%)	Mann-Whitney U test
71		15	13	0.0358
79		17	11	0.5228
86		19	19	0.0358
77		23	9	0.0829
78		23	11	0.0829
81		26	16	0.0829
84		28	4	0.2354
82		28	3	0.5228
83		31	6	0.0713
80		32	10	0.1017
85		46	12	0.0358
72		48	0	0.2011

[a] Control average % cell survival: 72 ± 4. Dexamethasone average % cell survival: 36 ± 7.

Table 5. EC_{50} (μM) of Compounds in Thymocyte Apoptosis Assay

compd	EC_{50} (μM)	95% confidence interval (μM)
9	9.69	ND[a]
10	5.68	4.61−7.00
11	7.28	3.86−13.70
13	6.06	4.64−7.91
14	5.37	3.26−8.82
15	5.72	4.08−8.01
17	7.54	6.71−8.48
18	1.84	1.51−2.24
56	4.44	2.86.6.89
57	10.23	8.49−12.32

[a] ND: 95% confidence interval not determined.

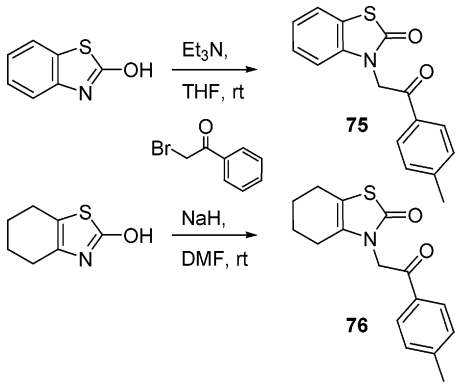
Table 6. EC_{50} (μM) of Compounds in Thymocyte Apoptosis Assay

R_1 substituent	class	compd	EC_{50} (μM)	95% confidence interval (μM)
methyl	I	**1**	4.16	2.95−5.88
methyl	II	**43**	>10	
methyl	III	**39**	2.01	1.45−2.80
methyl	IV	**58**	1.22	0.95−1.57
phenyl	I	**16**	2.29	1.76−2.98
phenyl	II	**47**	7.26	6.02−8.76
phenyl	III	**41**	3.79	2.78−5.16
phenyl	IV	**55**	8.12	4.08−13.75
2-naphthyl	I	**12**	4.02	3.07−5.28
2-naphthyl	II	**45**	>10	
2-naphthyl	III	**40**	4.77	3.95−5.75
2-naphthyl	IV	**59**	4.02	3.45−4.69
pyrrolidinyl	I	**19**	1.31	0.94−1.82
pyrrolidinyl	II	**46**	9.84	2.78−34.83
pyrrolidinyl	III	**38**	>10	
pyrrolidinyl	IV	**60**	0.35	0.11−1.08

was found to have significant activity. Thus, the presence of the amine nitrogen appears to be essential for enhanced bioactivity.

Following leads for the most biologically active compounds in the primary screen, families of compounds were evaluated for structural features that optimized activity (Table 6). Thus, aromatic and nonaromatic derivatives of **1** and **39** that were para-substituted on the phenyl moiety with a methyl, phenyl, naphthyl, or pyrrolidinyl group were synthesized. The aromatic analogues of **39** (class IV) were highly active, whereas this modification in the aromatic PFT-α series (class II) diminished the activity. The addition of large aryl para substituents modestly enhanced activity except for the para phenyl group in the class IV series, which paradoxically diminished cytoprotective ability. Adding the para pyrrolidinyl or diethylamino group largely improved activity as opposed to the primary amine **21** (Table 2). Interestingly, the para pyrrolidinyl on the IBT template (class III) abrogated all cytoprotective activity (compound **38**). Hence, modifying IBT with an aromatic ring (class IV) and a para pyrrolidinyl group revealed two features that in combination improved thymocyte survival. Compound **39** was modestly more cytoprotective than **1** as judged by the EC_{50} values, but over half of the 10 most potent compounds were derivatives of **1** (class I) (Tables 5 and 6). However, it is unlikely that the biological activity of **1** is limited to its conversion to the ring closed **39** counterpart during the 6 h assay because only about 25% conversion takes place within this time period (Figure 1). In addition, other compounds specifically designed to maintain an open ring structure did not necessarily promote thymocyte survival, as can be seen in the following examples. In one case, the imine at C-2 of an open ring compound (**63**) was protected by a tosyl group in order to prevent formation of the imidazole ring. With or without alkylation by the usual acetophenone, compounds **63** or **64**, respectively, did not show significant ability to protect cells against DEX-induced apoptosis (Table 3). Another example is illustrated by the alkylation of 2-amino-4,5,6,7-tetrahydrobenzothiazole with a benzyl (**61**) or tolyl group (**62**) (Table 3). With just the carbonyl group missing, these structures were much closer in shape to that of **1** but were less active. Also, replacement of the 4-methylacetophenone by a propargyl group (**67**) led to a compound showing diminished cytoprotective activity (**68**, Table 3).

Scheme 11



In general aromatic closed ring analogues demonstrated improved cytoprotective activity, with **60** and **58** being the most potent compounds in this assay. In contrast, the aromatic series of PFT-α analogues exhibited weaker protective ability. However, in both **1** and **39** the saturated fused benzo ring was a feature that conferred protective ability, since both **73** and **74**, lacking this ring, were devoid of protective activity (Table 3). An additional critical feature for bioactivity was the position of the phenyl substituent. Compound **70**, similar to **39** except that the phenyl ring is attached to C-3 instead of the usual C-2, was not active (Table 3). The fully aromatic **72**, an isomer of **58** but having the sulfur and nitrogen ring atoms interchanged, showed a much weaker ability to protect the cells relative to **58** (Tables 3 and 4).

Finally, to examine the influence of the imine nitrogen in the open ring compounds (classes I and II), an isostere of the imino group, the oxo function was considered. Thus, the 2-oxo derivatives in both classes (compounds **76** and **75**, respectively) were prepared (Scheme 11). Both compounds showed very weak activity, suggesting that the imine function plays a vital role in the bioactivity of the open ring compounds, particularly for those of class I, such as **1**.

Several compounds had greater cytoprotective activity compared with **1**, particularly a few members of classes I and IV. The three most potent new compounds were **19**, **58**, and **60**, which had antiapoptotic activity in the thymocyte protection assay at low micromolar or submicromolar concentrations while displaying direct cytotoxic activity at 20-fold higher concentrations. Moreover, **60** protected thymocytes from apoptosis induced by γ-irradiation (Figure 3), as expected for a p53-

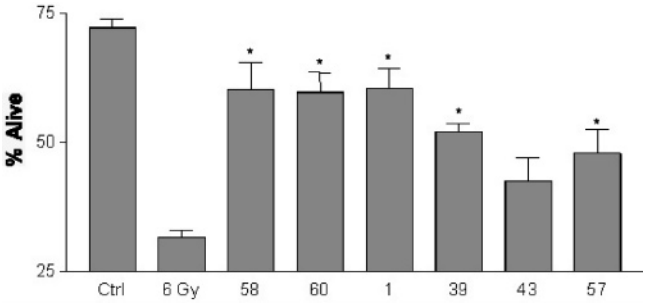


Figure 3. Inhibition of γ-irradiation-induced apoptosis. Ten million thymocytes per well were pretreated with 5 μM of the indicated compounds for 30 min and then irradiated with 6 Gy. After 6 h the cells were stained with $DiOC_6$ and PI and assayed for survival by flow cytometry. Shown are the average percent survival data pooled from two experiments and the SEM. Compounds that significantly improved viability after irradiation are indicated by an asterisk ($P < 0.01$ by Students t test).

mediated death pathway. Similar compounds were previously tested by Zhu[12] and Pietrancosta[11] for their neuroprotective ability. Recently, Zhu and co-workers found that oxazole analogues of PFT-α as well as compound **17** improved p53 inactivation by 2- to 4-fold[12] in a neuroprotection assay. Moreover, Pietrancosta and co-workers found that an analogue of **17** with a cyano group adjacent to the ketone, as well as compound **20**, had a 10-fold greater potency in protecting mouse embryo cortical neurons against DNA damage than PFT-α.[11] Interestingly, no mention was made of the tendency of these PFT-α derivatives to cyclize, even during recrystallization.

The mechanism of action of these compounds reported here is still unknown. PFT-α has been reported to diminish p53 transactivation and disrupt other death-inducing pathways. While cell death induced by irradiation is p53-dependent, dexamethasone-induced cell death is not.[6] Thus, it is unlikely that the compounds directly target either the glucocorticoid receptor or p53 directly.

Alternative candidates for the molecular target of these compounds would include the proapoptotic BH3-only proteins, which converge on mitochondrial mechanisms to initiate cell death.[22] Dexamethasone and p53 both induce the transcription of one of these proteins, namely, PUMA/bbc3.[7,23,24] In addition, PUMA deficiency has been reported to protect thymocytes from apoptosis induced by a variety of stimuli including irradiation, cytokine withdrawal, staurosporine, and dexamethasone.[25] Indeed, dexamethasone-treated p53 deficient thymocytes have augmented intracellular PUMA levels (Figure 4). Prior treatment of these thymocytes with either **1** or **60** restricted the levels of PUMA expression (Figure 4), which correlated with enhanced survival as assayed by $DiOC_6$ and PI staining (data not shown).

These data suggest that the regulation of cell death by the compounds described here is not restricted to p53 as originally described. Regulation of other independent proapoptotic mechanisms such as the BH3-only proteins should also be examined. The divergence in the structures presented here also suggests that a variety of signaling intermediates may be influenced before the convergence on the mitochondrial death cascade.

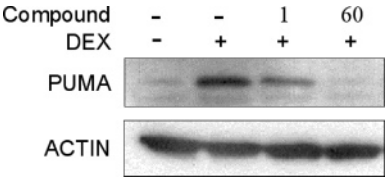


Figure 4. Effect of compounds **1** and **60** on PUMA protein expression in mouse thymocytes. Thymocytes were pretreated with 10 μM of the indicated compounds and then exposed to 5 μM of dexamethasone for 6 h. The cytosolic proteins were fractionated and then separated on a 4−12% gradient gel by SDS−PAGE and transferred to a PDVF membrane, which was probed with antibodies to PUMA and actin.

Experimental Section

Chemistry. Melting points were obtained on a Mel-temp II capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were obtained on a Varian Unity 500 at 499.8 MHz or on a Varian Mercury at 400.06 MHz. The chemical shifts are expressed as δ values (parts per million) relative to tetramethylsilane (TMS) as the internal standard. High-resolution mass spectrometric analyses were performed on a Finnigan MAT900XP high-resolution double-focusing mass spectrometer using electron impact. Elemental analyses were performed by NuMega Resonance Labs, San Diego, CA. Thin-layer chromatography was performed on silica gel 60 F-254 plates (EM Reagents). E Merck silica gel (230−400 mesh) was used for flash column chromatography.

Biological Materials. Mice. C57Bl/6 and $p53^{-/-}$ mice were purchased from The Jackson Laboratories (Bar Harbor, ME). The mice were bred and maintained under standard conditions at the University of California San Diego Animal Facility that is accredited by the American Association for Accreditation of Laboratory Animal Care. All animal protocols receive prior approval by the institutional review board.

Reagents. Dexamethasone (DEX) was purchased from Sigma-Aldrich (St. Louis, MO). Other chemicals were purchased from Maybridge plc (Trevillett, Tintagel, Cornwall, U.K.) and Lancaster Synthesis (Windham, NH).

Apoptosis Assays. Thymocytes were harvested from young C57Bl/6 mice and cultured at 37 °C in 5% CO_2 in RPMI 1640 containing 10% FBS, 1% penicillin/streptomycin (Gibco BRL, Rockville, MD). Thymocytes were plated at a density of 10^7 cells/mL and preincubated with 5−10 μM of each compound (from 10 mM stock in DMSO) for 30 min before induction of apoptosis. Apoptosis was induced with 5 μM dexamethasone or by exposure to 6 Gy of γ-radiation. After 6 h, cell apoptosis was assayed by propidium iodide (PI) and 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) staining. The cells were removed from the plate and incubated for 30 min in medium with 40 nM $DiOC_6$ and 5 μg/mL PI and then analyzed by flow cytometry in a FACS caliber (Beckton-Dickinson, San Jose, CA). Viable cells had high $DiOC_6$ (FL-1) and low PI (FL-3), whereas apoptotic cells had low $DiOC_6$ (FL-1) and low PI (FL-3). To evaluate the EC_{50} values, the thymocytes were pre-exposed to graded concentrations of selected compounds for 30 min and then apoptosis was induced with 5 μM dexamethasone. After 6 h the cells were harvested and stained as above. This time point was selected on the basis of previously reported assay conditions[5] and confirmed for our own system (data not shown).

EC_{50} Determination. The concentration (EC_{50}) of each compound that inhibited dexamethasone-induced cell death by 50% was determined by nonlinear regression fitting of the data to a one-site model. Pseudo Hill slopes were determined by nonlinear regression fit of the data to a sigmoidal dose response equation (variable slope):

$$\%\ \text{viability} = \min\ \%\ \text{viability} + \frac{\max - \min\ \%\ \text{viability}}{1 + 10(\log \text{EC}_{50} - X)^n}$$

where X is the logarithm of inhibitor concentration and n is the pseudo Hill slope. The maximum % viability and minimum % viability were experimentally determined after dexamethasone exposure and drug treatment. EC_{50} values and 95% confidence intervals (CI) were derived from the sigmoid fits to the percent control transformed data shown using GraphPad Prism, version 4.0b, for Macintosh (GraphPad Software, San Diego, CA).

Immunoblotting. After removal of medium, cells were disrupted in lysis buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.6% NP-40) on ice. The nuclei were separated by centrifugation, and the cytoplasmic sample was removed. The nuclear pellets were resuspended in 20 mM HEPES, pH 7.9, 0.4 M NaCl, 1.0 mM EDTA, 1.0 mM EGTA, 1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride, and the insoluble material was removed by centrifugation. The samples were heated to 70 °C for 10 min in loading buffer with 10 mM DTT. Each lane of an SDS−PAGE gel was loaded with 20 μg of protein. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, blocked with 2% I-blockTM (Tropix Inc, Bedford, MA) containing 0.05% Tween-20 in PBS, and then incubated with anti-PUMA (AbCam, Inc., Cambridge, MA). Horseradish peroxidase conjugated anti-IgG (Santa Cruz Laboratories, Santa Cruz, CA) was used as the secondary antibody. The membranes were developed using a chemiluminescence system (ECL detection reagent: Amersham Life Science, Aylesbury, U.K.). The membranes were reprobed with anti-actin (Sigma, St Louis, MO) to ensure equivalent loading.

Method A: General Procedure for Synthesis of PFT-α Derivatives and Aromatic Analogues: Compounds 1−19 and 42−47. Following a procedure reported by Singh,[10] a mixture of 2-amino-4,5,6,7-tetrahydrobenzothiazole (1 equiv) and the appropriate phenacyl bromide (1.1 equiv) was stirred in toluene at room temperature for 24−48 h. The product crystallized out and was collected by filtration. The same procedure was used to synthesize aromatic analogues starting from commercially available 2-aminobenzothiazole, some bearing a substituent on C-6.

General Procedure for Synthesis of IBT Derivatives and Aromatic Analogues: Compounds 20−41 and 48−60. Method B: One-Step Ring Closure. A mixture of 2-amino-4,5,6,7-tetrahydrobenzothiazole (1 equiv) and the appropriate phenacyl bromide (1.1 equiv) was refluxed in ethanol for 90 min. The ring-closed product crystallized out upon cooling and was separated by filtration.

Method C: Two-Step Ring Closure. 2-Imino-3-phenacyl-4,5,6,7-tetrahydrobenzothiaole hydrobromide or analogue was refluxed in ethanol for 90 min. The ring-closed product crystallized out upon cooling and was separated by filtration.

The aromatic analogues were made using the same procedures.

Benzoic Acid 4-[2-(2-Imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)acetyl]phenyl Ester Hydrobromide Salt (2). 2 was prepared from 4-(bromoacetyl)phenyl benzoate and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 65% yield: mp 175−177 °C; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 4H), 2.34 (s, 2H), 2.54 (s, 2H), 5.74 (s, 2H), 5.59 (d, 2H), 7.63 (t, 2H), 7.81 (t, 1H), 8.16 (d, 4H), 9.49 (s, 2H); HR-MS (EI) *m/z* 392.1197 (M^+).

2-(2-Imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-(4-trifluoromethylphenyl)ethanone Hydrobromide Salt (5). 5 was prepared from 4-(trifuoromethyl)phenacyl bromide (Maybridge) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 79% yield: mp 272−282 °C; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 4H), 3.42 (s, 4H), 5.81 (s, 2H), 8.03 (d, 2H), 8.25 (d, 2H), 9.55 (s, 2H); HR-MS (EI) *m/z* 340.0856 (M^+).

4-[2-(2-Imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)acetyl]-benzonitrile Hydrobromide Salt (6).[12] was prepared from 4-cyanophenacyl bromide (Maybridge) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 75% yield: mp 322−325 °C; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 4H), 2.36 (s, 2H), 2.55 (s, 2H), 5.79 (s, 2H), 8.14 (d, 2H), 8.20 (d, 2H), 9.55 (s, 2H); HR-MS (EI) *m/z* 297.0934 (M^+).

1-(3,4-Difluorophenyl)-2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)ethanone Hydrobromide Salt (7). was prepared from 3,4-difluorophenacyl bromide (Maybridge) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 71% yield: mp 270 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.72 (s, 4H), 2.40 (t, 2H), 2.49 (t, 2H), 5.73 (s, 2H), 7.74 (dd, 1H), 7.96 (s, 1H), 8.13 (dt, 1H), 9.52 (s, 2H); HR-MS (EI) *m/z* 308.0794 (M^+).

1-(3-Fluorophenyl)-2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)ethanone Hydrobromide Salt (9). 9 was prepared from 3-fluorophenacyl bromide (Maybridge) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 72% yield: mp 265−365 °C; ^{1}H NMR (DMSO-d_6) δ 1.72 (s, 4H), 2.34 (m, 2H), 2.49 (m, 2H), 5.75 (s, 2H), 7.64 (t, 1H), 7.69 (m, 1H), 7.88 (dd, 1H), 7.91 (dd, 1H), 9.52 (s, 2H). Anal. ($C_{15}H_{16}BrFN_2OS$) C, H, N.

1-(4-Azidophenyl)-2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)ethanone Hydrobromide Salt (10). was prepared from *p*-azidophenacyl bromide (Sigma) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 67% yield: mp 179−182 °C; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 4H), 2.36 (s, 2H), 2.55 (m, 2H), 5.71 (s, 2H), 7.37 (t, 2H), 8.08 (t, 2H), 9.51 (s, 2H). Anal. ($C_{15}H_{16}BrN_5OS$) C, H, N.

1-(3,4-Dichlorophenyl)-2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)ethanone Hydrobromide Salt (11). 11 was prepared from 3,4-dichlororophenacyl bromide (Maybridge) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 71% yield: mp 294−299 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 4H), 2.35 (s, 2H), 2.55 (s, 2H), 5.76 (s, 2H), 7.96 (m, 2H), 8.29 (d, 1H), 9.52 (s, 2H); HR-MS (EI) *m/z* 340.0201 (M^+).

1-(2-Chlorophenyl)-2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)ethanone Hydrobromide Salt (15). 15 was prepared from 2-chlororophenacyl bromide (Maybridge) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 49% yield: mp 205−209 °C; ^{1}H NMR (DMSO-d_6) δ 1.74 (m, 4H), 2.40 (m, 2H), 2.56 (d, 2H), 5.64 (s, 2H), 7.68 (d, 2H), 8.09 (d, 2H), 9.56 (s, 2H); HR-MS (EI) *m/z* 306.0578 (M^+).

1-(4-Diethylaminophenyl)-2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)ethanone Hydrobromide Salt (18). 18 was prepared from α-bromo-4-(diethylamino)acetophenone (Lancaster) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 45% yield: mp 189−191 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 6H), 1.72 (s, 4H), 2.30 (s, 2H), 2.54 (m, 2H), 3.47 (q, 4H), 5.55 (s, 2H), 6.78 (d, 2H), 7.83 (d, 2H), 9.42 (s, 2H). Anal. ($C_{19}H_{26}BrN_3OS$) C, H, N.

2-(2-Imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-(4-pyrrolidin-1-yl-phenyl)ethanone Hydrobromide Salt (19). 19 was prepared from α-bromo-4-(1-pyrrolidino)acetophenone (Lancaster) and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 57% yield: mp 224−226 °C; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 4H), 1.99 (m, 4H), 2.29 (m, 2H), 2.54 (s, 2H), 3.36 (s, 4H), 5.56 (s, 2H), 6.66 (d, 2H), 7.86 (d, 2H), 9.43 (s, 2H). Anal. ($C_{19}H_{24}BrN_3OS$) C, H, N.

4-(5,6,7,8-Tetrahydroimidazo[2,1-*b*]benzothiazol-2-yl)-phenylamine (21).[11] A mixture of **20** (239 mg, 0.63 mmol), NaO_2CH (136 mg, 2.00 mmol), and KH_2PO_4 (142 mg, 1.04 mmol) was refluxed for 6 h in 1-methyl-2-pyrrolidinone (5 mL). The mixture was filtered, and the filtrate was dissolved in EtOAC and washed with brine. Preparative TLC (5% MeOH/CH_2Cl_2) was used to separate the product in 33% yield: mp >150 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.87 (m, 4H), 2.66 (m, 4H), 6.57 (d, 2H), 7.48 (d, 2H), 7.85 (s, 1H); HR-MS (EI) *m/z* 269.0984 (M^+). Anal. Calcd for $C_{15}H_{18}N_3O_4PS$: C, 49.04; H, 4.94; N, 11.44. Found: C, 49.23; H, 5.24; N, 13.59.

4-(5,6,7,8-Tetrahydroimidazo[2,1-*b*]benzothiazol-2-yl)-benzoic Acid Hydrochloride Salt (23). A solution of **25** (359 mg, 1.0 mmol) in methanol (25 mL) was refluxed with 25% aqueous NaOH (8.5 mL) for 3.5 h. The solvent was evaporated under vacuum, and the solution was made acidic by addition of concentrated HCl. A precipitate dropped out and was filtered to give a white solid in 47% yield: mp 315−319 °C; ^{1}H NMR

(DMSO-d_6) δ 1.89 (d, 4H), 2.73 (s, 5H), 7.95 (m, 4H), 8.51 (d, 1H); HR-MS (EI) *m/z* 298.0779 (M^+).

4-(5,6,7,8-Tetrahydroimidazo[2,1-*b*]benzothiazol-2-yl)-phenol Hydrobromide Salt (24). To a solution of **26** (248 mg, 0.55 mmol) in dry methanol (6 mL) was added a catalytic amount of sodium methoxide. The mixture was stirred overnight. A cation-exchange resin (H^+ form) was added to the mixture and stirred for 10 min. The resin was removed by filtration, and the solvent was evaporated to give a white solid in 39% yield: mp 239−343 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.89 (d, 4H), 2.76 (s, 4H), 6.88 (d, 2H), 7.62 (d, 2H), 8.38 (s, 1H), 9.86 (s, 1H); HR-MS (EI) *m/z* 270.0823 (M^+). Anal. Calcd for $C_{15}H_{15}BrN_2OS$: C, 51.29; H, 4.30; N, 7.98. Found: C, 48.38; H, 4.55; N, 7.63.

4-(5,6,7,8-Tetrahydroimidazo[2,1-*b*]benzothiazol-2-yl)-benzonitrile Hydrobromide Salt (25). 25 was prepared from **6** using method C in 74% yield: mp >310 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.87 (m, 4H), 2.70 (m, 4H), 7.86 (d, 2H), 8.00 (d, 2H), 8.49 (s, 1H). Anal. ($C_{16}H_{14}BrN_3S$) C, H, N.

Benzoic Acid 4-(5,6,7,8-Tetrahydrobenzo[*d*]imidazo-[2,1-*b*]thiazol-2-yl)phenyl Ester Hydrobromide Salt (26). 26 was prepared from **2** using method C in 76% yield: mp 255−257 °C; ^{1}H NMR (DMSO-d_6) δ 1.88 (m, 4H), 2.73 (d, 4H), 7.40 (d, 2H), 7.62 (t, 2H), 7.76 (t, 1H), 7.91 (d, 2H), 8.14 (d, 2H), 8.45 (s, 1H). Anal. ($C_{22}H_{19}BrN_2O_2S\cdot H_2O$) C, H, N.

2-(4-Trifluoromethylphenyl)-5,6,7,8-tetrahydrobenzo-[*d*]imidazo[2,1-*b*]thiazole Hydrobromide Salt (27). 27 was prepared from **5** using method C in 43% yield: mp 294−296 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.88 (d, 4H), 2.73 (d, 4H), 7.79 (d, 2H), 8.04 (d, 2H), 8.51 (s, 1H). Anal. ($C_{16}H_{14}BrF_3N_2S$) C, H, N.

3-Bromo-2-*p*-tolyl-5,6,7,8-tetrahydroimidazo[2,1-*b*]benzothiazole (28). To a suspension of **39** (372 mg, 1.07 mmol) in DMF (40 mL) at room temperature was slowly added dropwise a solution of *N*-bromosuccinimide (210 mg, 1.18 mmol) in DMF (5 mL). The solvent was removed under high vacuum, and the residue was slurried in ice−water/ethanol (50 mL, 50%). The solid that dropped out was collected by filtration and rinsed with water to give **28** in 100% yield: mp >165 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.85 (m, 4H), 2.33 (s, 3H), 2.68 (s, 2H), 3.04 (s, 2H), 7.25 (d, 2H), 7.79 (d, 2H); HR-MS (EI) *m/z* 346.0138 (M^+).

2-(3-Fluorophenyl)-5,6,7,8-tetrahydroimidazo[2,1-*b*]-benzothiazole Hydrobromide Salt (31). 31 was prepared from **9** using method C in 75% yield: mp 293−297 °C; ^{1}H NMR (DMSO-d_6) δ 1.87 (m, 4H), 2.71 (t, 4H), 7.16 (dt, 1H), 7.49 (dt, 1H), 7.65 (m, 2H), 8.49 (s, 1H). Anal. ($C_{15}H_{14}BrFN_2S$) C, H, N.

2-(3,4-Dichlorophenyl)-5,6,7,8-tetrahydroimidazo[2,1-*b*]benzothiazole Hydrobromide Salt (34). 34 was prepared from **11** using method C in 75% yield: mp 314−319 °C; ^{1}H NMR (DMSO-d_6) δ 1.89 (d, 4H), 2.70 (s, 4H), 7.68 (d, 1H), 7.80 (dd, 1H), 8.07 (d, 1H), 8.47 (s, 1H). Anal. ($C_{15}H_{13}BrCl_2N_2S$) C, H, N.

2-(2-Chlorophenyl)-5,6,7,8-tetrahydroimidazo[2,1-*b*]-benzothiazole Hydrobromide Salt (35). 35 was prepared from **15** using method C in 19% yield: mp 189−191 °C; ^{1}H NMR (DMSO-d_6) δ 1.86 (q, 4H), 2.70 (m, 4H), 7.28 (dt, 1H), 7.39 (dt, 1H), 7.50 (dd, 1H), 8.12 (dd, 1H), 8.21 (s, 1H). Anal. ($C_{15}H_{14}BrClN_2S\cdot{}^1/_2H_2O$) C, H, N. HR-MS (EI) *m/z* 288.0486 (M^+).

2-(3,4-Difluorophenyl)-5,6,7,8-tetrahydroimidazo[2,1-*b*]benzothiazole Hydrobromide Salt (36). 36 was prepared from **7** using method C in 68% yield: mp 301−305 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.89 (d, 4H), 2.72 (s, 4H), 7.54 (qt, 1H), 7.68 (m, 1H), 7.87 (m, 1H), 8.48 (d, 1H). Anal. ($C_{15}H_{13}BrF_2N_2S$) C, H, N.

2-(4-Azidophenyl)-5,6,7,8-tetrahydro-imidazo[2,1-*b*]benzothiazole Hydrobromide Salt (37). 37 was prepared from **10** using method C in 62% yield: mp >200 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.88 (m, 4H), 2,73 (s, 4H), 7.23 (d, 2H), 7.86 (d, 2H), 8.43 (s, 1H). Anal. ($C_{15}H_{14}BrN_5OS$) C, H, N.

2-(4-Pyrrolidin-1-ylphenyl)-5,6,7,8-tetrahydroimidazo-[2,1-*b*]benzothiazole Hydrobromide Salt (38). A mixture of 2-amino-4,5,6,7-tetrahydrobenzothiazole (584 mg, 3.79 mmol) and α-bromo-4-(1-pyrrolidinyl)acetophenone (Lancaster) (1.109 g, 4.14 mmol) was refluxed in methoxyethanol (30 mL) for 2 h. The solvent was removed under vacuum, and the residue was purified by flash chromatography on silica gel (1% MeOH in CH_2Cl_2) to give **38** as a solid in 25% yield: mp 255−257 °C; ^{1}H NMR (DMSO-d_6) δ 1.86 (m, 4H), 2.50 (m, 4H), 2.66 (s, 4H), 3.24 (s, 4H), 6.54 (d, 2H), 7.62 (d, 2H), 7.90 (s, 1H). Anal. ($C_{19}H_{22}BrN_3S\cdot{}^1/_5H_2O$) C, H, N.

2-Naphthalen-2-yl-5,6,7,8-tetrahydroimidazo[2,1-*b*]benzothiazole Hydrobromide Salt (40). 40 was prepared from 2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-naphthalen-2-ylethanone hydrobromide salt using method C in 73% yield: mp >300 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.91 (m, 4H), 2.76 (m, 4H), 7.55 (q, 2H), 7.94 (m, 3H), 8.02 (d, 1H), 8.37 (s, 1H), 8.57 (s, 1H). Anal. ($C_{19}H_{17}BrN_2S$) C, H, N.

1-(4-Cyclopentylphenyl)-2-(2-iminobenzothiazol-3(2*H*)-yl)ethanone Hydrobromide Salt (44). 44 was prepared from α-bromo-4-(1-cyclopentyl)acetophenone[26,27] and 2-aminobenzothiazole using method A in 16% yield: mp 186−190 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.59 (m, 2H), 1.69, (m, 2H), 1.81 (m, 2H), 2.07 (m, 2H), 3.12 (m, 1H), 6.07 (s, 2H), 7.44 (d, 2H), 7.52 (m, 2H), 7.69 (t, 1H), 7.84 (t, 1H), 8.03 (d, 2H), 9.13 (s, 1H); HR-MS (FAB) *m/z* 337.1376 (M^+). Anal. Calcd for $C_{20}H_{21}BrN_2OS$: C, 57.56; H, 5.07; N, 6.71. Found: C, 50.82; H, 4.21; N, 7.65.

2-(2-Iminobenzothiazol-3-yl)-1-(4-pyrrolidin-1-ylphenyl)-ethanone Hydrobromide Salt (46). 46 was prepared from α-bromo-4-(1-pyrrolidino)acetophenone and 2-amino-4,5,6,7-tetrahydrobenzothiazole using method A in 43% yield: mp 285−290 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 4H), 3.38 (m, 4H), 5.93 (s, 2H), 6.68 (d, 2H), 7.43 (t, 1H), 7.50 (t, 1H), 7.57 (d, 1H), 7.90 (d, 2H), 8.03 (d, 1H). Anal. ($C_{19}H_{20}BrN_3OS$) C, H, N.

2-Furan-2-ylimidazo[2,1-*b*]benzothiazole Hydrobromide Salt (48). 48 was prepared from 2-bromo-1-furan-2-ylethanone and 2-aminobenzothiazole using method B in 30% yield: mp >250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 6.63 (s, 1H), 6.78 (s, 1H), 7.48 (t, 1H), 7.60 (t, 1H), 7.77 (s, 1H), 8.09 (d, 1H), 8.13 (d, 1H), 8.68 (s, 1H). Anal. ($C_{13}H_9BrN_2OS\cdot{}^2/_3H_2O$) C, H, N.

2-*p*-Tolylbenzo[*d*]imidazo[2,1-*b*]thiazol-7-ol (49). A mixture of **54** (362 mg, 0.845 mmol) and copper(I) oxide (46 mg, 0.321 mmol) in a 30% aqueous solution of NaOH (4 mL) was heated in a bomb for 4 h. The mixture was neutralized and extracted with EtOAc. The solid recovered upon evaporation was purified by preparative TLC to give **49** in 47% yield: mp 275−277 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 6.95 (dd, 1H), 7.22 (d, 2H), 7.38 (d, 1H), 7.72 (d, 2H), 7.77 (d, 1H), 8.60 (s, 1H), 9.95 (s, 1H); HR-MS (EI) *m/z* 280.0662 (M^+). Anal. Calcd for $C_{16}H_{13}BrN_2OS\cdot{}^1/_2HBr$: C, 59.90; H, 3.93; N, 8.73. Found: C, 59.47; H, 4.43; N, 8.55.

3-Bromo-2-*p*-tolylimidazo[2,1-*b*]benzothiazole (51). To a suspension of **58** (1.643 g, 4.76 mmol) in DMF (150 mL) at room temperature was slowly added dropwise a solution of *N*-bromosuccinimide (933 mg, 5.24 mmol) in DMF (6 mL). The solvent was removed under high vacuum, and the residue was slurried in ice−water/ethanol (100 mL, 50%). A solid dropped out and was collected by filtration and rinsed with water to give **51** in 82% yield: mp >180 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H), 7.31 (d, 2H), 7.51 (t, 1H), 7.61 (t, 1H), 7.90 (d, 2H), 8.11 (d, 1H), 8.44 (d, 1H). Anal. ($C_{16}H_{11}BrN_2S\cdot{}^1/_3H_2O$) C, H, N.

2-*p*-Tolylimidazo[2,1-*b*]benzothiazol-7-ylamine (52). To a suspension of 7-nitro-2-*p*-tolylimidazo[2,1-*b*]benzothiazole hydrobromide salt (128 mg, 0.328 mmol) in water at 80 °C were added four portions (10 equiv) of sodium dithionite over 30 min whereupon the suspension become clear and decolorized to give **52** in 22% yield: mp >210 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 6.80 (dd, 1H), 7.13 (d, 1H), 7.23 (d, 2H), 7.65 (d, 1H), 7.72 (d, 2H), 8.53 (s, 1H); HR-MS (EI) *m/z* 279.0821 (M^+). Anal. Calcd for $C_{16}H_{13}N_3S\cdot{}^7/_3HBr$: C, 41.05; H, 3.30; N, 8.98. Found: C, 41.81; H, 2.86; N, 6.10.

2-(4-Cyclopentylphenyl)imidzxo[2,1-*b*]benzothiazoles Hydrobromide Salt (53). 53 was prepared from **44** using method method C in 65% yield: mp 250 °C (dec); ^{1}H NMR

(DMSO-d_6) δ 1.57 (m, 2H), 1.66, (m, 2H), 1.79 (m, 2H), 2.03 (m, 2H), 3.00 (q, 1H), 7.33 (d, 2H), 7.45 (t, 1H), 7.59 (t, 1H), 7.78 (d, 2H), 8.01 (d, 1H), 8.06 (d, 1H), 8.77 (s, 1H); HR-MS (FAB) *m/z* 319.1267 (M^+). Anal. Calcd for $C_{20}H_{18}N_2S \cdot {}^1/_4HBr$: C, 70.93; H, 5.43; N, 8.27. Found: C, 70.01; H, 5.82; N, 10.97.

7-Bromo-2-*p*-tolylimidazo[2,1-*b*]benzothiazole Hydrobromide Salt (54). 54 was prepared from 2-bromo-4′-methylacetophenone and 2-amino-6-bromobenzothiazole using method B in 24% yield: mp 205−208 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 7.23 (d, 2H), 7.73 (m, 3H), 7.91 (d, 1H), 8.32 (s, 1H), 8.70 (s, 1H); HR-MS (EI) *m/z* 341.9822 (M^+).

7-Methoxy-2-*p*-tolylimidazo[2,1-*b*]benzothiazole Hydrobromide Salt (56). 56 was prepared from 2-bromo-4′-methylacetophenone and 2-amino-6-bromobenzothiazole using method B in 62% yield: mp 274−276 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 3H), 3.84 (s, 3H), 7.18 (dd, 1H), 7.26 (d, 2H), 7.72 (m, 3H), 7.92 (d, 1H), 8.71 (s, 1H). Anal. ($C_{17}H_{15}BrN_2OS$) C, H, N.

2-(4-Pyrrolidin-1-ylphenyl)benzo[*d*]imidazo[2,1-*b*]thiazole Hydrobromide Salt (60). 60 was prepared from α-bromo-4-(1-pyrrolidino)acetophenone (Lancaster) and 2-aminobenzothiazole using method B and 10 h of reflux in 16% yield: mp 283−287 °C; ^{1}H NMR (DMSO-d_6) δ 1.98 (m, 4H), 3.31 (m, 4H), 6.68 (m, 2H), 7.51 (d, 1H), 7.66 (d, 3H), 8.11 (m, 2H), 8.71 (s, 1H). Anal. ($C_{19}H_{18}BrN_3S$) C, H, N.

3-Benzyl-3*H*-benzothiazol-2-ylideneamine Hydrochloride Salt (61).[12] A mixture of 2-aminobenzothiazole (2.116 g, 13.7 mmol), benzyl chloride (2.1 mL, 18.2 mmol), and sodium iodide (200 mg) was refluxed for 8 h in methoxyethanol (25 mL). Crystallization occurred upon cooling. The crystals were filtered off and thoroughly washed with ether to give yellow crystals in 39% yield: mp 275−278 °C; ^{1}H NMR (DMSO-d_6) δ 5.65 (s, 2H), 7.28, (d, 2H), 7.36 (m, 4H), 7.50 (t, 1H), 7.55 (d, 1H), 8.02 (s, 1H); HR-MS (EI) *m/z* 240.0719 (M^+).

3-(4-Methylbenzyl)-3*H*-benzothiazol-2-ylideneamine Hydrochloride Salt (62). A mixture of 2-aminobenzothiazole (2.104 g, 13.6 mmol) and 4-methylbenzyl chloride (2.25 mL, 12.5 mmol) was refluxed for 8 h in methoxyethanol in the presence of a catalytic amount of sodium iodide (220 mg, 1.46 mmol). After the mixture was cooled, a precipitate formed that was filtered and rinsed with ether to give 35% yield: mp 243−248 °C; ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 5.65 (s, 2H), 7.20 (dd, 4H), 7.39 (t, 1H), 7.48 (t, 1H), 7.55 (d, 1H), 8.02 (d, 1H); HR-MS (EI) *m/z* 254.0870 (M^+).

***N*-(3*H*-Benzothiazol-2-ylidene)-4-methyl-benzenesulfonamide (63).** To a solution of 2-aminobenzothiazole (1.018 g, 6.78 mmol) in dry pyridine (4 mL) was added portionwise *p*-toluenesulfonyl chloride (1.42 g, 7.48 mmol). The solution turned yellow upon addition. After 5 min of stirring at room temperature, the mixture was heated (70−80 °C) for 5 min. The mixture was poured on a bed of ice, and the resulting precipitate was filtered off and dried overnight in a desiccator under vacuum to give a yellow powder in 93% yield: mp 246−249 °C; ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H), 7.26, (dt, 1H), 7.30 (d, 1H), 7.37 (d, 2H), 7.40 (dt, 1H), 7.75 (d, 2H), 7.81 (d, 1H). Anal. ($C_{14}H_{12}N_2O_2S_2$) C, H, N.

4-Methyl-*N*-[3-(2-oxo-2-*p*-tolylethyl)-3*H*-benzothiazol-2-ylidene]benzenesulfonamide (64). To a solution of **63** (930 mg, 3.01 mmol) in DMF (15 mL) at room temperature was added NaH. After the effervescence subsided, 2-bromo-4′-methylacetophenone (775 mg, 3.36 mmol) was added. The reaction mixture was first stirred at room temperature for an hour and then heated at 80 °C until completion (by TLC). The mixture was allowed to cool before being poured on ice (400 mL). A precipitate dropped out. It was filtered and washed with cold water and dried to give a pale-yellow powder in quantitative yield: mp 180−182 °C; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H), 2.43 (s, 3H), 5.86 (s, 2H), 7.33 (m, 3H), 7.42 (m, 3H), 7.57 (d, 1H), 7.64 (d, 2H), 7.91 (d, 1H), 7.97 (d, 2H). Anal. ($C_{23}H_{20}N_2O_3S_2$) C, H, N.

2-(Benzothiazol-2-ylsulfanyl)-1-*p*-tolylethanone Hydrobromide Salt (65). A mixture of 2-mercaptobenzothiazole (570 mg, 3.34 mmol) and 2-bromo-4′-methylacetophenone (870 mg, 3.67 mmol) was refluxed in ethanol (15 mL) for 90 min. The solution was cooled in the fridge to give a yellow solid in 18% yield: mp 76−78 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 5.15 (s, 2H), 7.36 (t, 1H), 7.41 (d, 2H), 7.44 (t, 1H), 7.78 (d, 1H), 8.00 (m, 3H). Anal. ($C_{16}H_{14}BrNOS_2 \cdot {}^1/_2H_2O$) C, H, N.

(2-Imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)acetic Acid Ethyl Ester Hydrobromide Salt (66).[12] A mixture of 2-amino-4,5,6,7-tetrahydrobenzothiazole (2.296 g, 14.9 mmol) and ethyl bromoacetate (2.747 g, 16.4 mmol) was refluxed in ethanol (50 mL) containing three drops of Et_3N for 90 min. The reaction mixture was concentrated to half volume in vacuo, and the resulting precipitate was filtered and rinsed with cold ethanol and then ether to give a white powder in 79% yield: mp 222−224 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H), 1.74 (d, 4H), 2.39 (s, 2H), 2.52 (m, 2H), 4.22 (q, 2H), 4.95 (s, 2H), 9.67 (s, 2H). Anal. ($C_{11}H_{17}BrlN_2O_2S$) C, H, N. HR-MS (EI) *m/z* 240.0931 (M^+).

4-Phenyl-1*H*-imidazole-2-thiol (69) was synthesized following the procedure described by Maeda et al.[16]

3-Phenyl-5,6,7,8-tetrahydroimidazo[2,1-*b*]benzothiazole Hydrochloride Salt (70). A solution of 4-phenyl-1*H*-imidazole-2-thiol **69**[16] and 2-chlorocyclohexanone was refluxed in butanol for 3 h. After the mixture was cooled, the resulting precipitate was filtered to give **70** in 75% yield: mp 274−278 °C; ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 4H), 2.76 (m, 4H), 7.38 (t, 1H), 7.49 (t, 2H), 7.88 (d, 2H), 8.51 (s, 1H); HR-MS (EI) *m/z* 254.0874 (M^+).

2-(2-Iminothiazol-3-yl)-1-*p*-tolylethanone Hydrobromide Salt (73). 73 was prepared from 2-bromo-4′-methylacetophenone and 2-aminothiazole using method A in 64% yield: mp >220 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 5.79, (s, 2H), 7.06 (d, 1H), 7.35 (d, 1H), 7.44 (d, 2H), 7.92 (d, 2H), 9.55, (s, 2H). Anal. ($C_{12}H_{13}BrN_2OS \cdot {}^1/_3H_2O$) C, H, N.

6-*p*-Tolylimidazo[2,1-*b*]thiazole Hydrobromide Salt (74). 74 was prepared from **73** using a modification of method C with methoxyethanol instead of ethanol. The solvent was evaporated, and the residue was recrystallized from ethanol in 49% yield: mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H), 7.31 (d, 2H), 7.55 (d, 1H), 7.72 (d, 2H), 8.16 (d, 1H), 8.41 (s, 1H). Anal. ($C_{12}H_{11}BrN_2S$) C, H, N.

3-(2-Oxo-2-*p*-tolylethyl)benzothiazol-2(3*H*)-one (75). To a solution of 2-hydroxybenzothiazole (1.17 g, 7.58 mmol) in THF (10 mL) was added Et_3N (1.1 mL, 7.89 mmol). A solution of 2-bromo-4′-methylacetophenone (1.67 g, 7.06 mmol) in THF (10 mL) was added dropwise. Another equivalent of Et_3N was added after 24 h. Purification by flash chromatography on silica gel (CH_2Cl_2) gave a white powder in 67% yield: mp 168−170 °C; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H), 5.62 (s, 2H), 7.21 (t, 1H), 7.27 (d, 1H), 7.32 (t, 1H), 7.42 (d, 2H), 7.70 (d, 1H), 8.01 (d, 2H). Anal. ($C_{16}H_{13}NO_2S$) C, H, N.

3-(2-Oxo-2-*p*-tolylethyl)-4,5,6,7-tetrahydrobenzothiazol-2(3*H*)-one (76). To a solution of 2-hydroxy-4,5,6,7-tetrahydrobenzothiazole (335 mg, 2.16 mmol) in DMF (3 mL) was added NaH (101 mg, 2.52 mmol). After the mixture was stirred for 10 min at room temperature, a solution of 2-bromo-4′-methylacetophenone (668 mg, 2.82 mmol) in DMF (5 mL) was added and the mixture was stirred overnight. Water was added, and the mixture was extracted with EtOAc and dried over $MgSO_4$. The solvent was evaporated. The oil recovered was purified by flash chromatography on silica gel (CH_2Cl_2) to give **76** as a solid in 65% yield: mp 108−110 °C; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 4H), 2.19 (s, 2H), 2.40 (m, 5H), 5.23 (s, 2H), 7.39 (d, 2H), 7.95 (d, 2H). Anal. ($C_{16}H_{17}NO_2S$) C, H, N.

***N*-(4-Methylbenzylidene)-*N*′-quinazolin-4-yl-hydrazine (77).** A solution of quinazolin-4-ylhydrazine[28] (265 mg, 1.65 mmol) was refluxed with tolualdehyde (400 μL, 3.39 mmol) in methanol for 1 h. The solvent was evaporated and the resulting solid was triturated with water and filtered to give a yellow powder in 87% yield: mp 211−214 °C; ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 7.29 (d, 2H), 7.47 (dt, 2H), 7.67 (t, 1H), 7.88 (d, 3H), 8.20 (m, 1H), 8.51 (s, 1H), 11.65 (s, 1H); HR-MS (EI) *m/z* 262.1223 (M^+).

1-(2-*p*-Tolyl-2*H*-[1,2,4]triazolo[1,5-*c*]quinazolin-3-yl)-ethanone (78). A solution of **77** (273 mg, 1.04 mmol) in acetic anhydride (5 mL) was refluxed for 12 h. After cooling, the mixture was poured on ice and extracted with CH_2Cl_2, washed

with aqueous Na_2CO_3, and dried over $MgSO_4$. The solvent was removed under vacuum, and the product was cooled to promote crystallization. The solid was triturated with methanol to give 47% yield: mp 166−169 °C; ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H), 2.29 (s, 3H), 7.22 (m, 3H), 7.34 (d, 2H), 7.47 (m, 2H), 7.67 (dt, 1H), 7.79 (s, 1H), 7.88 (dd, 1H). Anal. ($C_{18}H_{16}N_4O$) C, H, N.

2-*p*-Tolyl[1,2,4]triazolo[1,5-*c*]quinazoline (79). A mixture of **78** (124 mg, 0.407 mmol) and ferric chloride (260 mg, 0.961 mmol) in water (5 mL) was heated under reflux for 4 h. The insoluble salts were filtered off while hot, and the solution was cooled. The resulting crystals were filtered to give 50% yield: mp 249−251 °C; ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H), 7.40 (d, 2H), 7.86 (t, 1H), 7.96 (t, 1H), 8.09 (d, 1H), 8.19 (d, 2H), 8.53 (d, 1H), 9.65 (s, 1H). Anal. ($C_{16}H_{12}N_4 \cdot H_2O$) C, H, N.

2-(Quinazolin-4-ylamino)-1-*p*-tolylethanone Hydrobromide Salt (80) and 2-*p*-Tolylimidazo[1,2-*c*]quinazoline Hydrobromide Salt (81). To a hot solution of 4-aminoquinazoline (420 mg, 2.89 mmol) in ethanol (50 mL) was added 2-bromo-4′-methylacetophenone (931 mg, 3.93 mmol). The mixture was refluxed for 4 h. After the mixture was cooled, the precipitate was filtered to give **80** in 44% yield: mp 312−313 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H), 6.30 (s, 2H), 7.47 (d, 2H), 7.86 (m, 2H), 8.05 (m, 3H), 8.54 (d, 1H), 8.90 (s, 1H), 10.12 (s, 2H). Anal. ($C_{17}H_{16}BrN_3O$) C, H, N. Flash chromatography on silica gel (3:7 EtOAc/hex) separated the ring closed **81** in 20% yield: mp 215−218 °C; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H), 7.29 (d, (2H), 7.74 (m, 2H), 7.94 (t, 3H), 8.47 (m, 2H), 9.27 (s, 1H); HR-MS (EI) *m/z* 259.1106 (M^+). Anal. Calcd for $C_{16}H_{13}N_3S \cdot {}^1/_4H_2O$: C, 77.40; H, 5.16; N, 15.93. Found: C, 77.74; H, 6.18; N, 15.31.[18]

1-(2-Oxo-2-*p*-tolylethyl)-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (82). To a solution of isatoic anhydride (2.084 g, 12.3 mmol) in *N,N*-dimethylacetamide (30 mL) was added NaH (500 mg, 12.5 mmol) in small portions. After the effervescence subsided, 4′-methyl-4-bromoacetophenone (2.64 g, 12.4 mmol) was added, and the solution was stirred overnight at room temperature. The reaction mixture was concentrated to half the volume under vacuum, and water (35 mL) was added. A precipitate formed and was filtered in 86% yield: mp 182−184 °C; ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H), 5.71 (s, 2H), 7.37 (m, 2H), 7.44 (d, 2H), 7.80 (t, 1H), 8.05 (d, 2H), 8.09 (d, 1H); HR-MS (EI) *m/z* 295.0842 (M^+).

Methyl-1-[2-(2-oxo-2-*p*-tolylethylamino)benzoyl]isothiourea (83). A suspension of **82** (2.317 g, 7.85 mmol), Na_2CO_3 (915 mg, 8.63 mmol), and 2-methyl-2-thiopseudourea sulfate (1.092 g, 3.92 mmol) was refluxed in acetonitrile (30 mL) for 30 min. TLC showed starting material left and $^1/_3$ more Na_2CO_3 and 2-Methyl-2-thiopseudourea sulfate was added, and the mixture was refluxed for an additional 30 min. The solvent was removed under vacuum, and the residue was triturated in methylene chloride. The insoluble salts were removed by filtration, and the filtrate was concentrated under vacuum. The residue was recrystallized from methanol to give 17% yield: mp 305 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H), 2.50 (s, 3H), 4.84 (d, 2H), 6.59 (t, 1H), 6.79 (d, 1H), 7.33 (t, 1H), 7.40 (d, 2H), 8.01 (d, 2H), 8.28 (d, 1H), 9.20 (m, 3H); HR-MS (EI) *m/z* 341.1239 (M^+). Anal. Calcd for $C_{18}H_{19}N_3O_2S$: C, 63.32; H, 5.61; N, 12.31. Found: C, 68.94; H, 5.17; N, 13.88.

2-*p*-Tolyl-4*H*-imidazo[1,2-*a*]quinazolin-5-one (84). A solution of **83** (413 mg, 1.21 mmol) in diglyme (5 mL) was refluxed for 2 h with a catalytic amount of NaOH. A precipitate formed upon cooling and was collected by filtration and rinsed with EtOAC to give **84** in 65% yield: mp 325−328 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 3H), 3.50 (br, 1H), 7.24 (d, 2H), 7.49 (t, 1H), 7.74 (d, 2H), 7.90 (t, 1H), 8.03 (d, 1H), 8.19 (d, 1H), 8.50 (d, 1H); HR-MS (EI) *m/z* 275.1069 (M^+).

5-Chloro-2-*p*-tolylimidazo[1,2-*a*]quinazoline (85). A solution of **84**, $POCl_3$, and *N,N*-diethylaniline was refluxed for 90 min. The mixture was poured onto ice, stirred for 10 min, and filtered. The residue was recrystallized to give **85** in 20% yield: mp 190 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 7.35 (d, 2H), 7.86 (t, 1H), 7.94 (d, 2H), 8.24 (t, 1H), 8.41 (d, 1H), 8.55 (d, 1H), 9.35 (s, 1H); HR-MS (EI) *m/z* 293.0716 (M^+). Anal. Calcd for $C_{17}H_{13}Cl_2N_3$: C, 61.83; H, 3.97; N, 12.73. Found: C, 40.84; H, 2.90; N, 8.58.

2-(5-Chloro-2-iminobenzooxazol-3-yl)-1-*p*-tolylethanone Hydrobromide Salt (86). A solution of 2-amino-5-chlorobenzoxazole 1.172 g, 6.74 mmol) and 2-bromo-4′-methylacetophenone (1.81 g, 7.64 mmol) was refluxed in ethanol (50 mL) for 90 min. A precipitate formed. It was filtered and rinsed with ether to give 33% yield: mp 288−290 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H), 5.86 (s, 2H), 7.47 (d, 3H), 7.81 (d, 1H), 7.97 (m, 3H). Anal. ($C_{16}H_{14}BrClN_2O_2$) C, H, N.

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