Checkpoint Kinase Inhibitors: SAR and Radioprotective Properties of a Series of 2-Arylbenzimidazoles

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The discovery of a series of novel, potent, and highly selective inhibitors of the DNA damage control kinase chk2 is disclosed. Here we report the first SAR study around inhibitors of this kinase. High-throughput screening of purified human chk2 led to the identification of a novel series of 2-arylbenzimidazole inhibitors of the kinase. Optimization was facilitated using homology models of chk2 and docking of inhibitors, leading to the highly potent 2-arylbenzimidazole **2h** (IC_{50} 15 nM). Compound **2h** is an ATP-competitive inhibitor of chk2 that dose dependently protects human $CD4^+$ and $CD8^+$ T-cells from apoptosis due to ionizing radiation. This work suggests that a selective small molecule inhibitor of chk2 could be a useful adjuvant to radiotherapy, increasing the therapeutic window of such treatment.

Introduction

Side effects of cancer therapy represent a major clinical problem, which seriously affects both the quality of life of cancer patients and the outcome of treatment. In the case of radiotherapy, clinical complications vary depending on the tissue being treated and include lung fibrosis and pneumonitis, GI toxicity with diarrhea, mucositis, and hematological effects. Because side effects from radiation are often serious enough to be dose limiting, a large number of studies have been undertaken to develop adjuvants to improve the therapeutic index of radiation therapy. Such treatments may either sensitize tumor tissue or protect normal tissue from the effects of radiation. A major challenge in these endeavors has been to develop radiosensitizers that do not increase the toxicity to normal tissue or, conversely, radioprotectants that do not protect tumor cells.

It is generally agreed that proliferating cells are more sensitive to radiation than nondividing cells. Thus, in most cases tumor cells will be more efficiently killed by radiation than cells in the surrounding tissues. However, despite this the clinical outcome is often compromised by radiation toxicity to normal tissue. This is likely due to a combination of several factors. With respect to the tumor, successful treatment generally requires all tumor cells be eliminated, requiring high cumulative radiation doses, a problem that is often compounded by the inherent resistance of tumor cells to apoptosis.[1] In addition, radiosensitive tissue is generally composed of rapidly proliferating cells that are prone to apoptosis, and loss of a smaller fraction of the cells leads to serious impairment of organ function. A good example of the latter is the severe effects of radiation on the intestinal mucosa. Typical strategies for radio-protection/sensitization involve making use of differential physical properties of the tumor, such as lower pH, or lower oxygen content.[2,3]

Alternatively, the targeting of specific differential biological pathways in tumors is also an attractive strategy for radio-protection/sensitization. For instance, the lower sensitivity of tumor cells to apoptosis depends on deficiencies in particular pathways. The p53 system is considered the most important of these, and it is noteworthy that p53 is mutated in 50% of all tumors.[4] In addition, alterations in systems that control p53, such as MDM2, are also common in tumors.[5] Thus, whereas activation of the p53 pathway is responsible for apoptosis in many normal tissues in response to stresses such as ionizing radiation, there is frequently no response through this system in tumor cells. If p53-dependent apoptosis could be suppressed in normal cells, radiation toxicity could probably be decreased for a number of different cell types. As an example a recent report of compounds that interfere with nuclear trafficking of p53 demonstrated a radioprotective effect in mice.[6]

The p53 response to DNA breaks induced by radiation and certain chemical agents is controlled by the serine/threonine kinase chk2. It was recently shown that targeted disruption of the chk2-encoding gene leads to increased survival of mice exposed to radiation (apparently through suppression of apoptosis).[7,8] Several functions of chk2 (a homologue of the yeast Cds1 checkpoint kinase) relating to cell cycle control and DNA repair, such as phosphorylation of Cdc25A and BRCA1, have also been described.[9,10] However, chk2-deficient mice did not show a marked phenotype with the exception of their resistance to apoptosis after radiation exposure, possibly indicating that the other postulated activities of chk2 are less important or are redundant. In addition, $chk2^{-/-}$ mice did not appear prone to spontaneous tumor development, which is seen in p53-deficient mice. Thus, suppression of side effects from radiation and other therapies that induce double-strand DNA breaks by targeting chk2 with a small molecule inhibitor may be a viable approach. Because radiotherapy is administered

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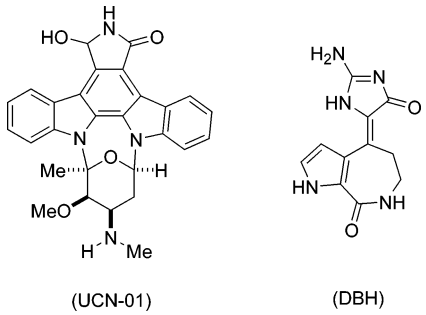


Figure 1. Previously reported chk2 inhibitors.

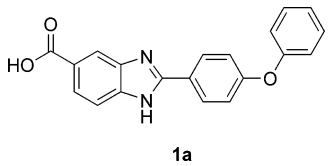


Figure 2. Initial high-throughput screening lead.

to approximately 50% of all cancer patients, such a compound would have a major impact on the treatment of cancer.

Very limited reports of small molecules which inhibit chk2 are available in the literature.[11] Currently, the only characterized inhibitors of the chk2 kinase are the indolocarbazole UCN-01[12] and the alkaloid natural product debromohymenialdisine (DBH) (Figure 1).[13,14] Although UCN-01 was reported to be a potent inhibitor of chk2 (10 nM) isolated by immunoprecipitation,[12] this potency was not confirmed with expressed and purified chk2.[15] In addition, UCN-01 also inhibits a variety of other kinases involved in cell cycle control, making this compound, and other similar indolocarbazoles, poor tools for exploring the pharmacology of specific chk2 inhibition. DBH is a moderately potent inhibitor of chk2 (3.5 uM); however, it also has similar activity at the cell cycle control kinase chk1 (3.0 uM).[13]

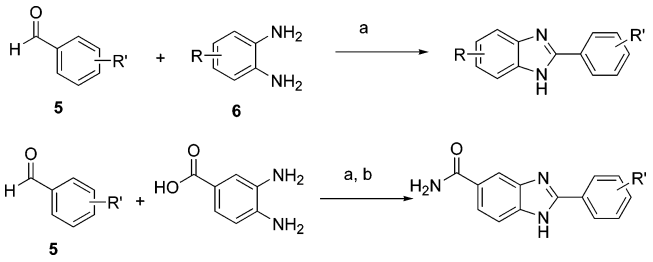
Here we disclose a novel class of benzimidazole-based chk2 inhibitors which show selectivity for chk2 over other kinases including chk1.[16] These compounds also demonstrate clear, dose-dependent, radioprotective effects on human $CD4^+$ and $CD8^+$ primary cells.

Chemistry

High-throughput screening of our corporate compound collection identified the benzimidazole compound **1a** (Figure 2) as a moderately potent inhibitor of the chk2 kinase (IC_{50} = 640 nM). The compound represents a unique class of kinase inhibitors, which appear to be remarkably selective for the chk2 kinase.

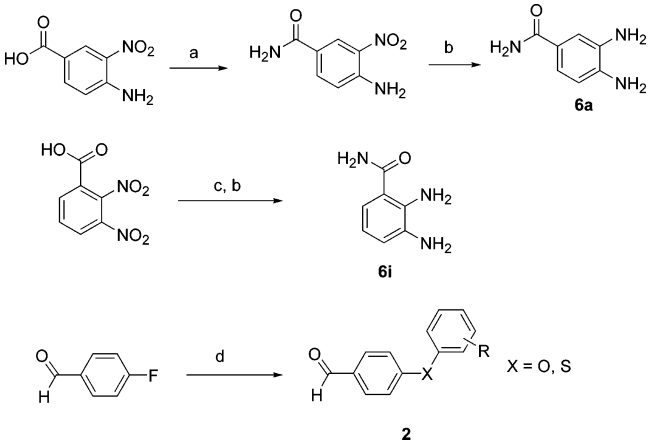
Analogues of benzimidazole **1a** were synthesized by the coupling of phenylenediamines with aromatic aldehydes in the presence of $Na_2S_2O_5$ as an oxidizing agent (Scheme 1).[17] This procedure was found to be milder than the more common oxidative coupling using hot nitrobenzene.[18] Most of the analogues were made by this route from commercially available aldehydes and phenylenediamines. Additionally, appropriate aromatic aldehydes (**5**) and phenylenediamines (**6**) were synthesized using the procedures outlined in Scheme 2 and combined using the $Na_2S_2O_5$ coupling reaction. Alternatively, a number of analogues were synthesized by coupling the appropriate aldehyde with 3,4-diaminobenzoic acid, followed by conversion of the acid to a primary amide using EDCI and $(NH_4)_2CO_3$.

Scheme 1[a]



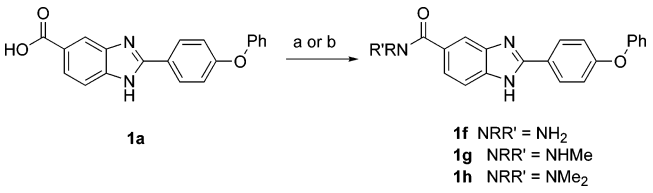
[a] Reagents and conditions: (a) $Na_2S_2O_5$, DMF, 100 °C; (b) EDCI; *N,N*-diisopropylethylamine, $(NH_4)_2CO_3$, THF, rt.

Scheme 2[a]



[a] Reagents and conditions: (a) EDCI; *N,N*-diisopropylethylamine, $(NH_4)_2CO_3$, THF, rt; (b) Pd/C, H_2, EtOH/DMF, rt; (c) CDI; $(NH_4)_2CO_3$, DMF; (d) Cs_2CO_3, ArXH, DMF, 80 °C.

Scheme 3[a]

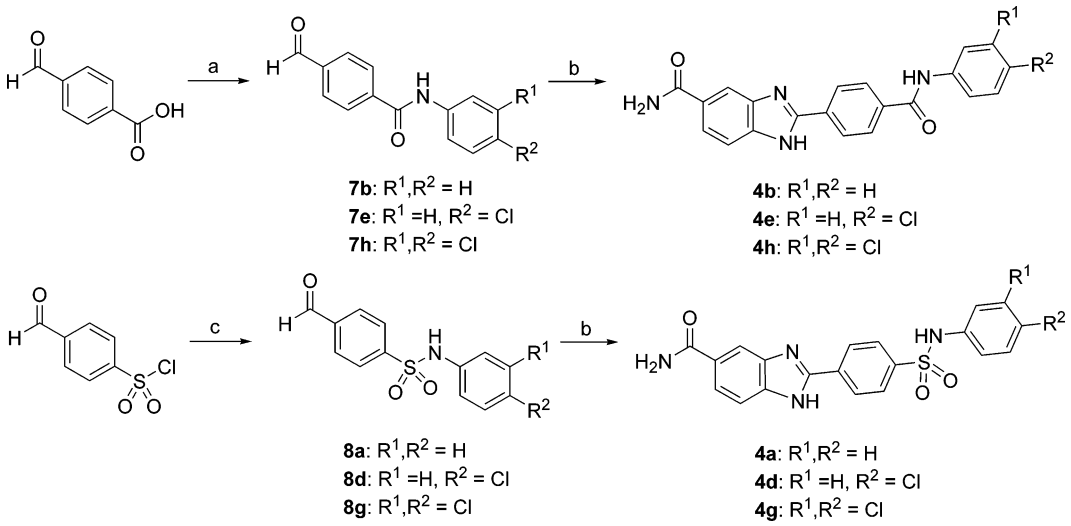


[a] Reagents and conditions: (a) CDI; $(NH_4)_2CO_3$, DMF, rt; (b) CDI, DBU, NH_2Me or $NHMe_2$, DMF, rt.

The starting material 3,4-diaminobenzamide (**6a**) was originally commercially available, but an alternate synthesis was developed when the material was discontinued (Scheme 2). Commercially available 4-amino-3-nitrobenzoic acid was converted to 4-amino-3-nitrobenzamide using the EDCI coupling with ammonium carbonate. Subsequent reduction of the nitro group afforded the desired phenylene diamine **6a**. A similar procedure was used for the synthesis of **6i**. The biaryl ether aldehydes required for the synthesis of compounds **3a**−**e**, **3i**, **3j**, **3l**, **3m**, **3o**, and **4i** were obtained by S_nAr reactions of 4-fluorobenzaldehyde with commercially available phenols or thiophenols using Cs_2CO_3 as base. Amides **1f**−**h** were obtained by simple CDI coupling of acid **1a** with the appropriate amines (Scheme 3).

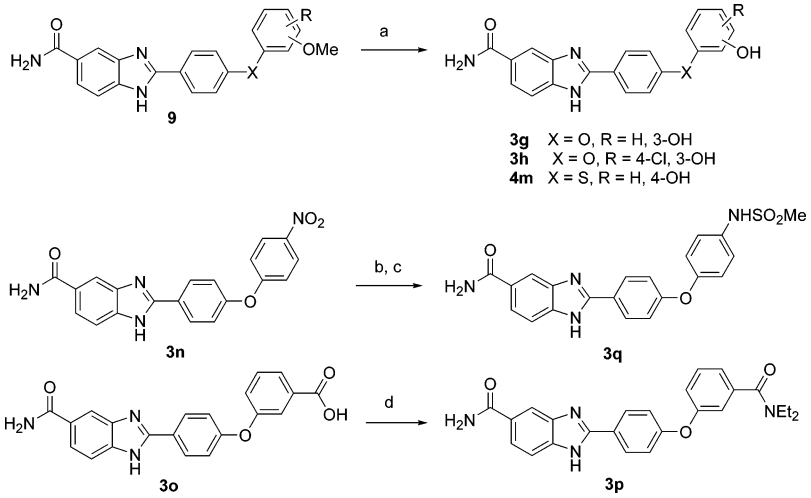
Benzimidazoles **4a**, **4b**, **4d**, **4e**, **4g**, and **4h** were synthesized by the methods described in Scheme 4. 4-Carboxybenzaldehyde was coupled with anilines using isobutyl chloroformate to provide amides of type **7**. Coupling of these aldehydes with 3,4-diaminobenzamide

Scheme 4[a]



[a] Reagent and conditions: (a) isobutyl chloroformate, NH_2Ar,Et_3N, DCE, 5 °C; (b) **6a**, $Na_2S_2O_5$, DMA, 90 °C; (c). NH_2Ar, pyridine, DCM, rt.

Scheme 5[a]



[a] Reagents and conditions: (a) BBr_3, CH_2Cl_2; (b) Pd/C, H_2, EtOH, rt; (c) $MeSO_2Cl$, pyridine, THF, rt; (d) EDCI, HOBt, Et_2NH, DMF, rt.

6a provided benzimidazoles **4b**, **4e**, and **4h**. Additionally 4-formylbenzenesulfonyl chloride was coupled to anilines to provide aldehydes **8**, which were also converted to benzimidazoles in the same manner to afford **4a**, **4d**, and **4g**.

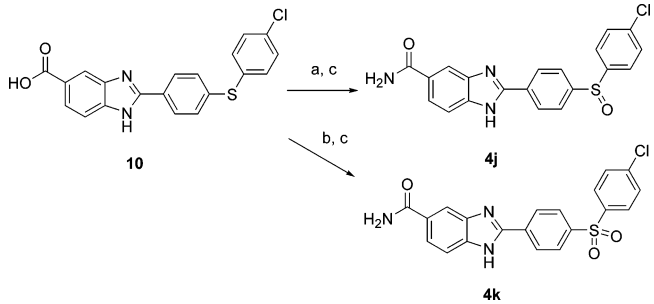
Phenols **3g**−**h** and **4m** were synthesized by demethylation of the corresponding methoxy compounds (**9**) using BBr_3 (Scheme 5). The sulfonamide **3q** was synthesized from the corresponding aniline which was in turn obtained by reduction of the nitro group of **3n** by hydrogenation. Compound **3p** was synthesized by coupling of acid **3o** with diethylamine using a standard EDCI coupling procedure.

The thioether linker of benzimidazole **10** (prepared as described in Schemes 1 and 2) was oxidized to either the sulfoxide or the sulfone using oxone or TeO_2 respectively. The resulting carboxylates were then converted to the amides **4j** or **4k** using $(NH_4)_2CO_3$, and CDI (Scheme 6).

Results and Discussion

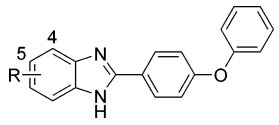
The initial high-throughput screening of chk2 provided benzimidazole **1a** (IC_{50} = 640 nM) which was a reasonable starting point for SAR studies around chk2 inhibition. Because the analogous 5-unsubstituted benzimidazole was inactive at chk2 (data not shown) a number of analogues of **1a** in which the 5-carboxy substituent was altered were investigated (Table 1). These analogues resulted in the identification of a more potent compound **1f** (IC_{50} = 55 nM) possessing a primary amide as a replacement for the carboxylate.

Scheme 6[a]



[a] Reagents and conditions: (a) TeO_2, H_2O_2, HCl (cat); (b) oxone, CH_3OH, H_2O; (c) CDI, $(NH_4)_2CO_3$, DMF, rt.

Table 1. Chk2 Binding Data for Benzimidazole Substitution[a]



no.	R	IC_{50}	no.	R	IC_{50}
1a	5-CO_2H	640 ± 210	**1f**	5-$CONH_2$	55 ± 31
1b	5-CN	1900 ± 610	**1g**	5-CONHMe	1400 ± 550
1c	5-NO_2	1500 ± 720	**1h**	5-$CONMe_2$	>10000
1d	5-NH_2	2400 ± 1200	**1i**	4-$CONH_2$	6000 ± 700
1e	5-SO_2NH_2	290 ± 90			

[a] IC_{50} values expressed in nM ± SEM, all values are the mean of at least six replicate experiments.

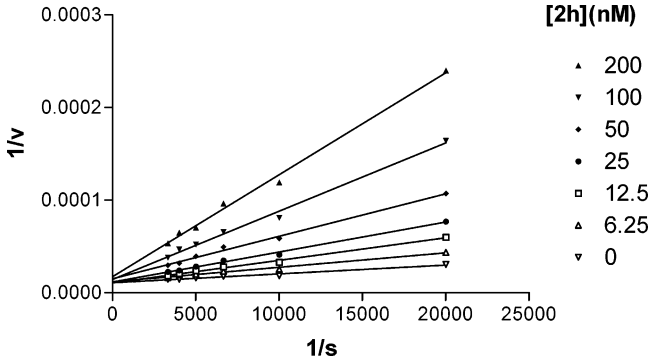


Figure 3. Lineweaver–Burke plot of chk2 activity at differing ATP concentrations in the presence of increasing **2h** concentrations.

ATP-competitive inhibition of chk2 by **2h** (Table 2) was confirmed by Lineweaver–Burke analysis using varying concentrations of ATP (K_i = 37 nM) (Figure 3). Based on the above data, a homology model of the ATP binding domain of chk2 was created.[19] The crystal structures of cyclic AMP dependent protein kinase (2cpk)[22] and phosphorylase kinase (2phk)[21] were used as reference structures to build this model. These kinases were selected based upon their scores in a threading calculation using the SeqFold program.[22] The threading results were also used to aid in the critical alignment of the target sequence with the reference structures. The MODELER program[23] was then used to build the homology model.

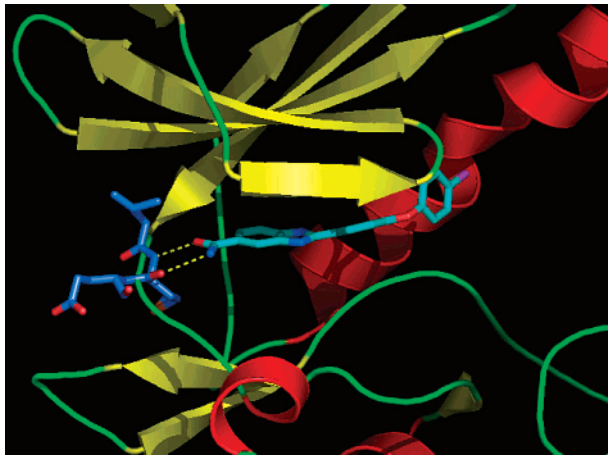


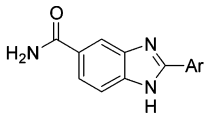
Figure 4. Model of **2h** docked in the ATP binding site of chk2. Key hydrogen bonding interactions of the primary amide with MET 90 are shown.

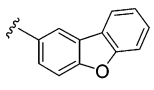
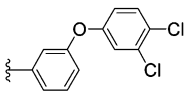
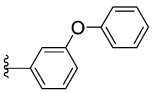
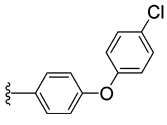
Initially, we docked **1f** in the ATP binding site of this homology model by hand. The docked structure was refined using energy minimization performed with the cvff force field[24] and the Discover molecular mechanics program.[25] The resulting docked structure served as a starting point for an automated docking procedure, which was used to search for additional low-energy poses of the inhibitor. The Affinity[26] program was used to perform these calculations, which utilizes a Monte Carlo procedure to randomly change the molecule orientation or internal degrees of freedom followed by energy minimization with the cvff force field.

Renderings of inhibitor **2h** (Table 2) are shown in Figures 4 and 5. Figure 4 illustrates the key hydrogen bonding interactions predicted to occur between the 5-amide group of the benzimidazole moiety and the backbone amide nitrogen and carbonyl group of MET 90. This type of donor–acceptor interaction is common for the binding of ATP, and many known ATP competitive kinase inhibitors.[27] Figure 5 depicts the solvent accessible surface of chk2 and how the inhibitor complements the size and shape of the ATP pocket.

The benzimidazole binds roughly in the same location that the adenosine of ATP would bind. The 5-amide

Table 2. Chk2 Binding: Variations in the 2-Aryl Substituent[a]



No.	Ar	IC_{50}	No.	Ar	IC_{50}
2a	Phenyl	1200 ± 10	**2e**	4-CH_3-Phenyl	1100 ± 330
2b	4-OEt-Phenyl	200 ± 36	**2f**	2-Naphthyl	200 ± 100
2c		500 ± 170	**2g**		530 ± 150
2d		930 ± 250	**2h**		15 ± 6.9

[a] IC_{50} values expressed in nM ± SEM, all values are the mean of at least six replicate experiments.

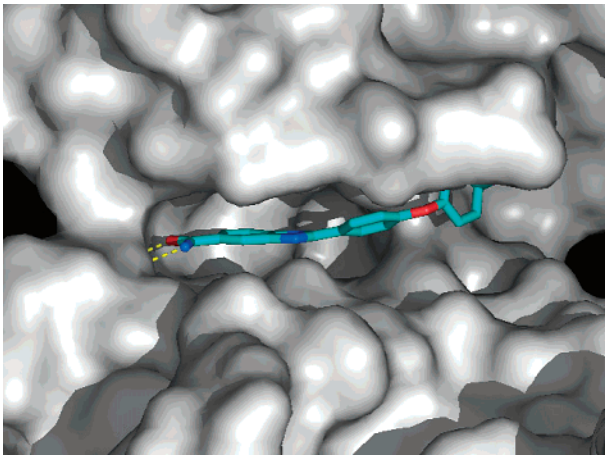


Figure 5. Model of **2h** docked in the ATP site of chk2 with the solvent accessible surface shown.

group begins to emerge from the end of the ATP site and is in a region that is partially solvent exposed in our model (Figure 5). The aryl ring attached to the 2-carbon of the benzimidazole lies roughly in the same plane as the benzimidazole and further complements the adenosine binding site. Finally, the terminal phenolic ether group remains in the ATP cleft but its conformation is twisted relative to the preceding phenyl ring by almost 90°. This distinct bending of the molecule follows a similar turn in the ATP cleft, allowing for good hydrophobic contact to be maintained.

The SAR in Table 1 is consistent with the docking model. For example, compounds with groups such as 5-C(O)NH_2 and 5-SO_2NH_2 which are capable of a donor−acceptor interaction with MET-90, are more active, while compounds containing 5-CN, 5-NO_2, and 5-NH_2 groups are less active. The 5-carboxy containing compound **1a** has intermediate potency. This is consistent with the model, in that this group can serve the hydrogen bond accepting function of **1f**, but lacks the donor function at physiological pH. Further evidence of the consistency of the model is exhibited by the loss of activity observed with the sequential methylation of the amide nitrogen (**1g**, **1h**). Methylation results in a 20-fold reduction in binding and dimethylation results in an additional >10-fold reduction. This is in accordance with the loss of ability to form the critical H-bond donor interaction required for the tight binding of **1f**, and the increasing steric demand of these methyl groups. In addition, when the amide is moved from the 5-position (**1f**) to the 4-position (**1i**) significant activity is also lost, suggesting that the precise geometry of the amide group is critical for good affinity.

In the original automated docking of **1f** into the homology model two unique confirmations were identified which were equally attractive (Figure 6). The difference between these poses lies in how the specific hydrogen bond donor and acceptor group interact with the ATP binding site. Pose **A** is preferred because it is more consistent with the high affinity of the sulfonamide analogue **1e**. The sulfonamide is capable of making the desired donor−acceptor interactions in either pose. However, pose **A** places the polar sulfonamide in a solvent exposed position which would be more capable of accommodating the highly polar nature of this group.

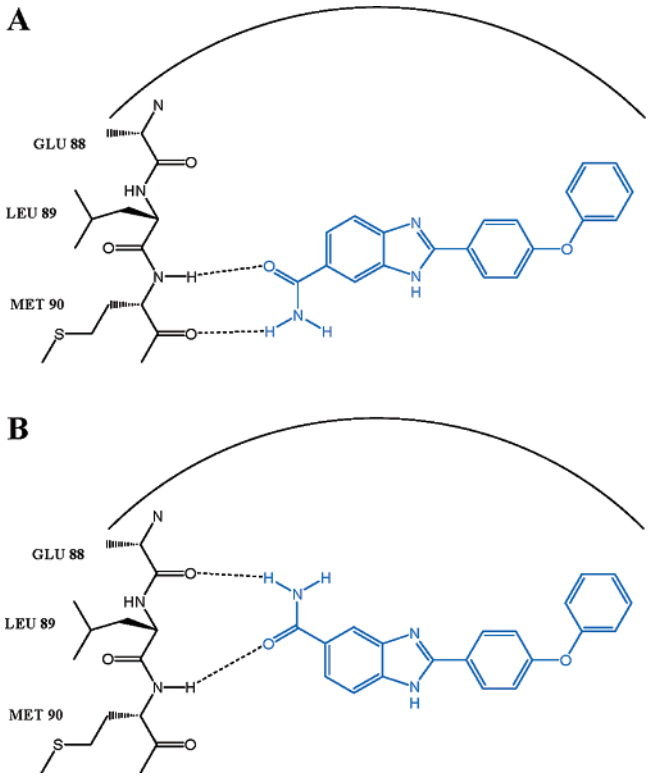


Figure 6. Schematic representation of the two potential docking poses of **1f** in the ATP site of Chk2.

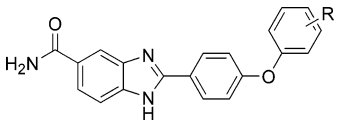
Alternatively, pose **B** buries the highly polar sulfonamide into a hydrophobic environment.

Modifications were next made to the substituents on the central phenyl ring of **1f** (Table 2). Removal of the 4-phenoxy group (**2a**, **2b**) resulted in a significant decrease in affinity. This is consistent with a reduction of lipophilic contacts made by the 4-phenoxy group of **1f**. Meta-substitution of the central aryl ring would be expected to reduce affinity because this would orient the substituent into either an unfavorable steric interaction with the back of the binding cleft, or more likely into solvent. Accordingly, moving the aryl ring from the 4- to the 3-position as in analogues **2d**, and **2g** resulted in a significant decrease in activity. Constraining the ring as in analogue **2c** does not allow the critical 90° orientation of the two rings found in **1f** and also resulted in decreased affinity. Addition of a 4-chloro group to **1f** afforded the more potent analogue **2h** presumably due to increasing hydrophobic interactions.

The increased affinity of **2h** prompted a further investigation of substitution on the terminal phenyl ring (Table 3). A variety of groups at any position on the terminal ring appear to be tolerated. In general, more lipophilic groups result in increased activity. Conversely, compounds with hydrophilic groups on the terminal phenyl ring generally resulted in lower affinity, supporting the notion that this region of the binding cleft is primarily hydrophobic.

The effect of changes in the linkage between the aryl rings was also investigated (Table 4). These data suggests that the ether linkage can be replaced by a sulfonamide without loss of chk2 affinity. Interestingly the affinity trends for these two linkages parallel one another (**3a** > **2h** > **1f**; **4g** > **4d** > **4a**) with increased lipophilicity resulting in increased affinity. In contrast,

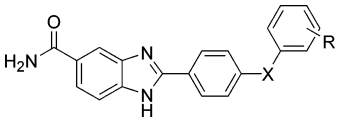
Table 3. Chk2 Inhibition: Evaluation of Terminal Aryl Substitution[a]



no.	R	IC_{50}	no.	R	IC_{50}
3a	3,4-diCl	5.5 ± 1.1	**3j**	3-Cl	17 ± 2.2
3b	4-Me, 3-Cl	4.4 ± 1.3	**3k**	4-OMe	23 ± 9.5
3c	4-Cl, 3-CF_3	4.5 ± 2.1	**3l**	4-Me	24 ± 1.1
3d	2-F, 5-CF_3	12 ± 3.2	**3m**	3,4-diOMe	31 ± 8.9
3e	3,4-diMe	12 ± 3.8	**3n**	4-NO_2	81 ± 34
3f	4-$CONH_2$	47 ± 4.3	**3o**	3-CO_2H	715 ± 240
3g	3-OH	61 ± 31	**3p**	3-$CONEt_2$	820 ± 120
3h	4-Cl, 3-OH	9.8 ± 1.0	**3q**	4-$NHSO_2CH_3$	41 ± 34
3i	2-naphthyl[b]	12 ± 1.1			

[a] IC_{50} values expressed in nM ± SEM, all values are the mean of at least six replicate experiments. [b] The naphthyl group includes the aryl ring attached to R depicted above the table.

Table 4. Chk2 Inhibition: Evaluation of Biaryl Linker[a]



no.	X	R	IC_{50}	no.	X	R	IC_{50}
1f	-O-	H	55 ± 31	**3a**	-O-	3,4-diCl	5.5 ± 1.1
4a	-SO_2NH-	H	18 ± 0.8	**4g**	-SO_2NH-	3,4-diCl	3.4 ± 1.3
4b	-CONH-	H	410 ± 70	**4h**	-CONH-	3.4-diCl	>10000
4c	-bond-	H	100 ± 26	**4i**	-S-	4-Cl	8.2 ± 2.0
2h	-O-	4-Cl	16 ± 6.9	**4j**	-S(O)-	4-Cl	13 ± 6.6
4d	-SO_2NH-	4-Cl	14 ± 4.1	**4k**	-$S(O)_2$-	4-Cl	6.6 ± 3.0
4e	-CONH-	4-Cl	680 ± 13	**4l**	-OCH_2-	H	32 ± 4.4
4f	-bond-	4-Cl	250 ± 190	**4m**	-S-	4-OH	2.3 ± 0.5

[a] IC_{50} values expressed in nM ± SEM, all values are the mean of at least six replicate experiments.

when the ether oxygen is replaced by an amide or direct bonding of the two aryl rings, not only does the activity across the series decrease, but the affinity trend is precisely opposite to that of an ether or sulfonamide linker (**4h** < **4e** < **4b**). One critical structural difference between the ether/sulfonamide and amide linkages is the ability of the former to accommodate the sharp bend required for maintaining good hydrophobic contact with the binding cleft in the model. In contrast, the amide prefers a linear and planar alignment, which orients the terminal phenyl ring into a solvent exposed position rather than establishing van der Waals contact with the binding cleft (Figure 7). Therefore, it would be expected that increasing the lipophilicity of the substituents in the amide series would result in increasingly unfavorable solvent interactions. In this respect, the lack of additivity seen when one changes from an ether to an amide linkage is in excellent agreement with the proposed binding model. In addition, other functionalities, such as thioether (**4i**), sulfone (**4j**), and sulfoxide (**4k**) that can accommodate the necessary sharp bend, also demonstrate good affinity for chk2.

Survival studies conducted with $chk^{-/-}$ mice demonstrated that these animals have a clear radioresistant phenotype. Significant protection of splenic lymphocytes, thymocytes, and neurons were demonstrated in the knockout animal subjected to ionizing radiation.[7,8] To test the effectiveness of our chk2 inhibitors as potential radioprotectants, the ability of **2h** to prevent

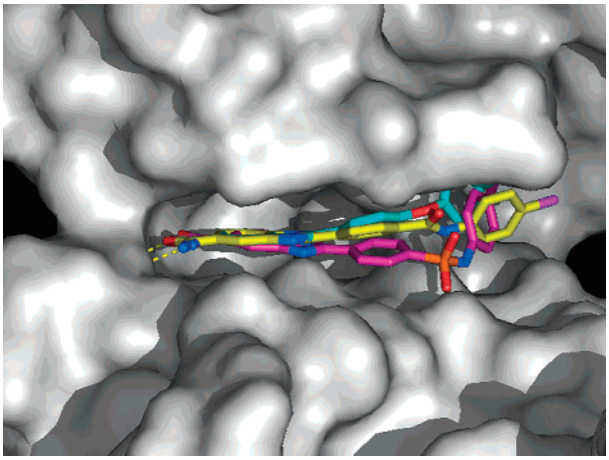


Figure 7. Alignment of **2h** (blue), **4d** (pink), and **4e** (yellow) in the chk2 homology model, demonstrating increased solvent exposure of the terminal chloride in amide **4e**.

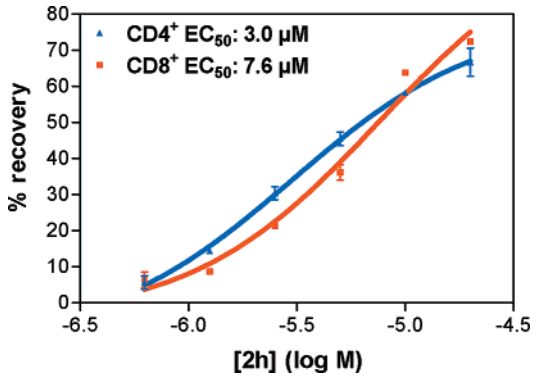


Figure 8. Dose dependent radioprotection of human primary T-cells by **2h**.

apoptosis of human T-cells subjected to ionizing radiation was measured. Peripheral $CD4^+$ and $CD8^+$ T-cells were isolated from human blood. The cells were treated with varying concentrations of **2h** for 1 h prior to administration of 10Gy of γ-irradiation. The cells were then incubated for an additional 24 h after irradiation. Viable, apoptotic, and dead cells were quantified with FACS after staining with Annexin V and propidium iodide. As shown in Figure 8, **2h** efficiently rescued both T-cell populations from radiation-induced apoptosis in a dose-dependent manner with an observed EC_{50} of 3−7.6 μM. The concentration of **2h** required for radioprotection is consistent with the biochemical measurement of chk2 inhibition. Providing the K_m of ATP for chk2 was determined to be 99 μM and the K_i for **2h** is 37 nM, and assuming that the intracellular ATP concentration is 10 mM, a 5 μM concentration of **2h** would be expected to produce 42% inhibition of intracellular chk2 ($v/V_{max} = [S]/([S] + K_m(1 + [I]/K_i))$).

Conclusions

In conclusion a novel series of potent, selective, ATP competitive chk2 kinase inhibitors was discovered. Inhibitors in this class were evaluated through docking into a homology model of the chk2 ATP binding site, and the SAR was found to be remarkably consistent with this model. The disclosed compounds also show effective radioprotection of human T-cells subjected to ionizing radiation. These compounds represent important tools for the investigation of chk2 pharmacology

and could lead to potentially important therapeutics as an adjuvant to radiation therapy. Further pharmacological and pharmacokinetic evaluation of these compounds will be disclosed in future publications.

Experimental Section

General. Reagents were purchased from commercial suppliers and were used without further purification, unless otherwise noted. THF, Et_2O, DMF, CH_2Cl_2, DMA, and MeOH were dried by filtration through alumina according to the procedure of Grubbs.[28] Flash chromatography was performed using prepacked ISCO RediSep silica cartridges. Reversed-phase HPLC analyses were carried out on an Agilent HP-1100. HPLC retention times are reported in minutes, using the methods and conditions reported below. Method A: Solvent: acetonitrile (0.05% TFA)/H_2O (0.05% TFA); flow rate at 0.75 mL/min; gradient: 1 min at 1% H_2O; 7 min linear ramp to 99% H_2O; 4 min at 99% H_2O. Column: Zorbax Eclipse XDB-C_8 (5 um, 4.6 × 150 mm), temperature: 35 °C, wavelength: dual detection at 220 nM and 254 nM. Method B: Solvent: acetonitrile (0.05% TFA)/H_2O (0.05% TFA); flow rate at 1.5 mL/min; gradient: 1 min at 1% H_2O; 3.5 min linear ramp to 99% H_2O; 1.5 min at 99% H_2O; Column: XTterra RP18 (4.6 × 50 mm), temperature: 35 °C; Wavelength: Duel detection at 220 nM and 254 nM. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX400 or DPX500. Chemical shifts are reported in parts per million downfield from the internal Me_4Si standard. Spin multiplets are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constants (*J*) are given in hertz (Hz). Mass spectra were recorded on a Hewlett-Packard 1100 MSD using electrospray ionization in either positive or negative mode as indicated. High-resolution mass spectra (HRMS) were acquired using a Micromass LCT time-of-flight spectrometer using electrospray ionization in positive mode (ESI+). Combustion analyses were performed by Desert Analytics.

3,4-Diaminobenzamide (6a). To a stirred suspension of 4-amino-3-nitrobenzoic acid (10.0 g, 55.0 mmol), 1-hydroxybenzotriazole (8.20 g, 60.5 mmol), and EDCI (11.6 g, 60.5 mmol, 1.10 equiv) in anhydrous THF (500 mL) was added *N,N*-diisopropylethylamine (7.80 g, 60.5 mmol, 1.10 equiv). The reaction mixture was stirred at ambient temperature for 10 min. Ammonium carbonate (15.8 g, 165 mmol) was added in one portion, and the resulting suspension was stirred at ambient temperature for 16 h. The reaction mixture was concentrated to a yellow-brown paste, 1:1 saturated aqueous $NaHCO_3$/H_2O (400 mL) was added, and stirring was continued for 2 h. The suspension was filtered through a glass frit, and the solids were dried in a vacuum oven at 40 °C for 24 h to afford crude 4-amino-3-nitrobenzamide (9.7 g, 98%, HPLC purity 99%) as a yellow-brown solid. This was used in the subsequent step without further purification.

A solution of the above 4-amino-3-nitrobenzamide in ethanol (75 mL) and DMF (50 mL) along with 10% Pd/C (1.9 g) was hydrogenated on a Parr shaker at 50 psi for 20 h. The reaction mixture was filtered through a pad of Celite, and the Celite pad was washed with EtOH (3 × 25 mL). The combined filtrate was concentrated to a dark brown solid. Trituration of the solid with 5% MeOH/Et_2O (200 mL) afforded the title compound **6a** as a brown powder (7.4 g, 94%), which was used without further purification. ^1H NMR (400 MHz, DMSO-d_6) δ 7.40 (br s, 1H), 7.05 (d, J = 1.9 Hz, 1H), 6.94 (dd, J = 1.9, 8.0 Hz, 1H), 6.72 (br s, 1H), 6.43 (d, J = 8.0 Hz, 1H), 4.95 (br s, 2H), 4.50 (br s, 2H).

2,3-Diaminobenzamide (6i). Ammonium carbonate (203 mg, 2.12 mmol, 4.50 equiv) was added to a solution of 2,3-dinitrobenzoic acid (100 mg, 0.470 mmol, 1.00 equiv) and CDI (191 mg, 1.18 mmol, 2.50 equiv) in DMF (2.5 mL) and was stirred for 30 min at room temperature. The reaction mixture was stirred for an additional 24 h, after which it was diluted with water (5 mL) and extracted with ethyl acetate (3 ×10 mL). Combined organic layers were dried with Na_2SO_4, and solvent was removed under reduced pressure. The resulting yellow oil was purified by silica gel chromatography (10% MeOH/CH_2Cl_2), to afford 2,3-dinitrobenzamide (41.6 mg, 42%). To a solution of 2,3-dinitrobenzamide (41.6 mg, 0.20 mmol, 1.0 equiv) and DMF (0.1 mL) was added anhydrous EtOH (1 mL). Pd/C (5.0 mg, 10% on carbon) was added, and the reaction was stirred under hydrogen at 1 atm. After 16 h, the resulting mixture was diluted with Et_2O (15 mL). The mixture was filtered through Celite and rinsed with Et_2O. Combined organic phases were evaporated to yield **6i** (24 mg, 79%). Diamine **6i** was used without further purification. ^1H NMR (500 MHz, CD_3OD): δ 6.93 (d, J = 10.0 Hz, 1H), 6.72 (d, J = 7.6, 1H), 6.46−6.42 (m, 1H).

(Method A) 2-(4-Phenoxyphenyl)-1*H*-benzoimidazole-5-carboxylic Acid (1a). To a mixture of 4-phenoxybenzaldehyde (130 mg, 0.65 mmol, 1.0 equiv) and 3,4-diaminobenzoic acid (100 mg, 0.650 mmol, 1.00 equiv) in DMF (1.5 mL) was added $Na_2S_2O_5$ (162 mg, 0.850 mmol, 1.30 equiv). The reaction was heated at 100 °C for 18 h, at which time it was allowed to cool to room temperature. The solution was filtered and the filtrate concentrated under reduced pressure. The residue was dissolved in 1.5 mL of DMF and purified by reverse phase HPLC (C18, acetonitrile/water/0.1% TFA) giving the title compound as a white solid, TFA salt (182 mg, 85%): ^1H NMR (400 MHz, DMSO-d_6) δ 8.15−8.10 (m, 3H), 7.82 (dd, J = 8.4, 1.5 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.41−7.36 (m, 2H), 7.18−7.05 (m, 5H); ^{13}C NMR (125 MHz, CD_3OD) δ 168.19, 159.25, 156.01, 153.53, 130.62, 129.08, 124.84, 124.82, 124.65, 123.84, 119.90, 118.66; MS *m/z* (ESI+): 331.1 $(M + H)^+$; Anal. ($C_{20}H_{14}N_2O_3$) C, H, N.

2-(4-Phenoxyphenyl)-1*H*-benzoimidazole-5-carbonitrile (1b). Prepared according to method A (21%): ^1H NMR (400 MHz, DMSO-d_6) δ 8.30 (m, 2H), 8.19 (s, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.55 (m, 2H), 7.31 (t, J = 7.4 Hz, 1H), 7.23 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.6, 155.8, 154.5, 130.6, 129.3, 126.0, 124.7, 124.1, 120.3, 120.0, 118.6, 104.3; MS *m/z* (ESI+): 312.1 $(M+H)^+$; Anal. ($C_{20}H_{13}N_3O$) C, H, N.

5-Nitro-2-(4-phenoxyphenyl)-1*H*-benzoimidazole (1c). Prepared according to method A (98%): ^1H NMR (500 MHz, CD_3OD) δ 8.44 (d, J = 2.0 Hz, 1H), 8.19 (dd, J = 9.0, 2.2 Hz, 1H), 8.00−8.03 (m, 2H), 7.68 (d, J = 9.0 Hz, 1H), 7.35−7.38 (m, 2H), 7.16 (t, J = 7.5 Hz, 1H), 7.08 (d, J = 8.1, 2H), 7.02 (d, J = 3.0, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 162.02, 156.19, 155.96, 144.90, 141.54, 137.56, 130.52, 129.78, 125.12, 121.77, 120.41, 119.58, 118.65, 114.64, 111.46. HRMS $(M + H)^+$ calcd, 332.1033; found, 332.1035; HPLC (method A): t_R = 6.04, >98% pure.

2-(4-Phenoxyphenyl)-1*H*-benzoimidazole-5-sulfonic Acid Amide (1e). Prepared according to method A (95%): ^1H NMR (500 MHz, CD_3OD) δ 8.26 (d, J = 1.2 Hz, 1H), 8.13−8.16 (m, 2H), 8.02 (dd, J = 8.6, 1.6 Hz, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.47−7.50 (m, 2H), 7.27−7.30 (m, 1H), 7.22−7.24 (m, 2H), 7.14−7.16 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.76, 155.35, 152.86, 139.02, 136.78, 130.33, 129.30, 124.55, 122.29, 120.76, 119.71, 118.34, 114.78, 112.83; HRMS $(M + H)^+$ calcd, 366.0899; found, 366.0912; HPLC (method A): t_R = 4.83, >97% pure.

2-(4-Phenoxyphenyl)-1*H*-benzoimidazole-5-carboxylic Acid Amide (1f). Prepared according to method A (65%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, J = 6.8 Hz, 2H), 8.17 (s, 1H), 8.06 (br s, 1H), 7.82 (d, J = 6.4 Hz, 1H), 7.62 (d, J = 6.4 Hz, 1H), 7.46 (t, J = 6.0 Hz, 2H), 7.30 (bs, 1H), 7.23 (t, J = 6.0 Hz, 1H), 7.16 (d, J = 6.8 Hz, 2H), 7.13 (d, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.7, 162.3, 158.5, 155.0, 151.6, 129.7, 128.5, 128.2, 123.8, 123.1, 121.8, 119.0, 117.7; MS *m/z* (ESI+): 330.1 $(M+H)^+$; Anal. ($C_{20}H_{15}N_3O_2$·TFA.) C, H, N.

2-Phenyl-1*H*-benzoimidazole-5-carboxylic Acid Amide (2a). Prepared according to method A (65%): ^1H NMR (400 MHz, DMSO-d_6) δ 8.20 (m, 3H), 8.07 (br s, 1H), 7.88 (d, J = 6.8 Hz, 1H), 7.70 (d, J = 6.8 Hz, 1H), 7.61 (m, 3H), 7.38 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.2, 159.0, 152.5, 131.8, 130.1, 129.6, 127.7, 127.5, 123.6, 117.4, 115.1, 114.4; MS (ESI+): *m/z* 238.1 $(M + H)^+$; Anal. ($C_{14}H_{11}N_3O$·TFA·$0.5H_2O$) C, H, N.

2-[4-(4-Carbamoylphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3f). Prepared according to method A above (9.6%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26−8.24 (m 2H), 8.20 (d, J = 1.3 Hz, 1H), 8.11 (br, s, 1H), 7.99−7.96 (m, 3H), 7.91 (dd, J = 8.6, 1.3 Hz, 1H), 7.28 (d, J = 8.6 Hz, 1H), 7.43 (br, s, 1H), 7.34 (br, s, 1H), 7.30−7.33 (m, 2H), 7.17−7.19 (m, 2H). MS (ESI+): *m/z* 373.1 (M + H)$^+$. Anal. ($C_{21}H_{16}N_4O_3$) C, H, N.

2-[4-(4-Methoxyphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide. (3k). Prepared according to method A (30%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35−8.33 (m, 3H), 8.26 (br s, 1H), 8.06 (d, J = 8.5 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.32−7.27 (m, 4H), 7.22−7.19 (m, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 159.1, 154.2, 149.4, 145.8, 135.4, 133.3, 127.9, 127.3, 121.4, 119.4, 118.2, 115.1, 113.2, 111.9, 111.6, 53.3; MS (ESI+) *m/z* 360.1 (M + H)$^+$; Anal. ($C_{21}H_{17}N_3O_3$·TFA) C, H, N.

2-[4-Phenylphenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4c). Prepared according to method A (34%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.3 (d, J = 8.4 Hz, 2H), 8.23 (s, 1H), 8.12 (br s, 1H), 7.98 (d, J = 8.4 Hz, 2H), 7.92 (dd, J = 8.5, 1.7 Hz, 1H), 7.82 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 1H), 7.53 (t, J = 7.6 Hz, 2H), 7.44 (t, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 150.1, 141.2, 137.1, 136.6, 134.7, 128.1, 127.4, 126.6, 126.1, 125.7, 125.1, 124.4, 121.7, 115.5, 113.2; MS (ESI+): *m/z* 314.1 (M + H)$^+$; Anal. ($C_{20}H_{15}N_3O$·TFA·H_2O) C, H, N.

2-[4-(4-Chlorophenyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4f). Prepared according to method A (26%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, J = 8.4 Hz, 2H), 8.21 (s, 1H), 8.09 (br s, 1H), 7.97 (d, J = 9.35 Hz, 2H), 7.89−7.84 (m, 3H), 7.83 (d, J = 8.5 Hz, 1H), 7.61−7.57 (m, 2H), 7.39 (br, s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 158.7, 158.4, 152.3, 141.5, 139.5, 138.0, 133.4, 129.9, 129.4, 128.9, 128.0, 127.6, 127.4, 123.4; MS (ESI+): *m/z* 348.0 (M + H)$^+$; Anal. ($C_{20}H_{14}ClN_3O_2$·TFA·0.5 H_2O) C, H, N.

(Method B) 2-[4-(3-Chloro-4-methylphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3b). To a solution of 4-fluorobenzaldehyde (38 mg, 0.30 mmol, 1.0 equiv) in DMF (1.5 mL) was added 3-chloro-4-ethylphenol (0.66 mmol, 2.2 equiv), followed by Cs_2CO_3 (220 mg, 0.66 mmol, 2.2 equiv). The mixture was heated at 90°C for 24 h. The reaction mixture was then cooled to room temperature, and MP-carbonate scavenger resin (Argonaut, 311 mg, loading = 2.64 mmol/g) was added. The mixture was shaken for 24 h, and the MP-carbonate resin was removed by filtration to provide crude 4-[(3-chloro-4-methyl)phenoxy]benzaldehyde.

The crude aldehyde above was treated with a solution of 3,4-diaminobenzamide (50 mg, 0.33 mM, 1.0 equiv) in DMF (1.0 mL), followed by $Na_2S_2O_5$ (94 mg, 0.50 mmol, 1.5 equiv). The mixture was heated to 95 °C for 24 h. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in DMF (1.5 mL) and purified by reverse phase HPLC (C_{18}, water/acetonitrile/0.1% TFA) to provide the title compound as a white solid (TFA-salt) (55 mg, 33% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (d, J = 8.9 Hz, 2H), 8.03 (d, J = 1.0 Hz, 1H), 7.93 (br s, 1H), 7.72 (dd, J = 8.5, 1.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 7.24 (br s, 1H), 7.10 (s, 1H), 7.08 (d, J = 8.9 Hz, 2H), 6.91 (dd, J = 8.3, 2.6 Hz, 1 H), 2.19 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.4, 162.9, 162.7, 155.3, 153.4, 137.9, 136.2, 135.6, 133.9, 133.3, 132.2, 131.0, 125.7, 121.8, 120.8, 119.8, 119.6, 115.3, 114.9, 19.4; MS (ESI+): *m/z* 378.09 (M + H)$^+$; Anal. ($C_{21}H_{16}ClN_3O_2$·TFA) C, H, N.

2-[4-(3,4-Dichlorophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3a). Prepared according to the procedure described in Method B (65%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 8.6 Hz, 2H), 7.99 (s, 1H), 7.88 (br s, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.51 (t, J = 8.9 Hz, 2H), 7.30 (d, J = 2.8 Hz, 1H), 7.19 (br s, 1H), 7.12 (d, J = 8.6 Hz, 2H), 6.97 (dd, J = 8.8, 2.8 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 172.8, 160.0, 157.2, 154.9, 134.3, 132.6, 130.1, 129.6, 128.4, 126.4, 123.6, 122.3, 120.3, 120.2; HRMS (M + H)$^+$ calcd, 398.0463; found, 398.0468; Anal. ($C_{20}H_{13}Cl_2N_3O_2$·0.5H_2O) C, H, N.

2-[4-(4-Chloro-3-trifluoromethylphenoxy)phenyl]-1*H*-benzoimidazole −5-carboxylic Acid Amide (3c). Prepared according to the procedure described in Method B (60%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (br s, 1H), 8.13 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 7.6 Hz, 1H), 7.61 (br s, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 2.8 Hz, 1H), 7.29−7.26 (dd, J = 8.8, 2.7 Hz, 1H), 7.20 (d, J = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.7, 157.6, 155.5, 133.8, 129.1, 128.6, 128.3, 126.3, 125.5, 124.6, 123.7, 121.6, 119.6, 118.8; MS (ESI+): *m/z* 432.0 (M + H)$^+$; Anal. ($C_{21}H_{13}ClF_3N_3O_2$) C, H, N.

2-[4-(2-Fluoro-5-trifluoromethylphenoxy)phenyl]-1*H*-benzoimidazole −5-carboxylic Acid Amide (3d). Prepared according to the procedure described in Method B (51%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 8.9 Hz, 2H), 8.16 (s, 1H), 8.06 (br s, 1H), 7.85 (dd, J = 8.5, 1.4 Hz, 1H), 7.77−7.71 (m, 3H), 7.67 (d, J = 8.5 Hz, 1H), 7.37 (br s, 1H), 7.28 (d, J = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.6, 162.0, 159.0, 157.0, 153.4, 144.0, 138.4, 136.1, 132.0, 131.1, 129.2, 128.9, 125.6, 123.7, 121.9, 121.7, 119.7, 119.5, 118.7, 115.5, 115.0; HRMS (M + H)$^+$ calcd, 416.1022; found, 416.1023; Anal. ($C_{21}H_{13}F_4N_3O_2.C_2HF_3O_2$) C, H, N.

2-[4-(3,4-Dimethylphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3e). Prepared according to the procedure described in Method B (61%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, J = 8.9 Hz, 2H), 8.04 (s, 1H), 7.91 (br s, 1H), 7.70 (dd, J = 8.4, 1.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.21 (br s, 1H), 7.12 (d, J = 8.3 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 2.5 Hz, 1H), 6.77 (dd, J = 8.1, 2.6 Hz, 1H), 2.14 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.1, 159.7, 153.1, 152.3, 138.3, 132.4, 130.8, 128.7, 123.1, 122.2, 120.8, 117.6, 117.0, 19.4, 18.6; HRMS (M + H)$^+$ calcd, 358.1556; found, 358.1562; Anal. ($C_{22}H_{19}N_3O_2$) calculated C, 73.93; H, 5.36; N, 11.76; found: 67.77% C, 5.02% H, 10.13% N. HPLC (Method A): t_R = 7.10, 94% pure.

2-[4-(2-Naphthyloxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3i). Prepared according to the procedure described in Method B (56%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, J = 8.7 Hz, 2H), 8.32 (s, 1H), 8.22 (br s, 1H), 8.19 (d, J = 9.1 Hz, 1H), 8.12 (d, J = 7.7 Hz, 1H), 8.03 (m, 2H), 7.83 (d, J = 8.6 Hz, 1H), 7.75 (s, 1H), 7.67 (m, 2H), 7.53 (dd, J = 8.8, 2.4 Hz, 2H), 7.44 (d, J = 8.7 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 162.6, 153.0, 152.1, 136.7, 134.7, 131.5, 131.0, 130.1, 128.2, 127.7, 127.3, 126.0, 125.2, 120.5, 119.0, 116.9, 114.5, 114.3; HRMS (M + H)$^+$ calcd, 380.1399; found, 380.1407; Anal. ($C_{24}H_{17}N_3O_2$·TFA·H_2O) C, H, N.

2-[4-(3-Chlorophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3j). Prepared according to the procedure described in Method B (43%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, J = 8.6 Hz, 2H), 8.19 (s, 1H), 8.09 (br s, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.49 (t, J = 8.2 Hz, 1H), 7.40 (br s, 1H), 7.32 (s, 1H), 7.29 (d, J = 8.6 Hz, 2H), 7.25 (m, 1H), 7.12 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.5, 162.0, 156.6, 152.2, 136.8, 136.0, 134.4, 131.6, 131.5, 130.4, 128.9, 125.7, 125.5, 120.9, 119.7, 119.5, 118.9, 117.4, 114.7, 114.4; HRMS (M + H)$^+$ calcd, 364.0853; found, 364.0851; Anal. ($C_{20}H_{14}ClN_3O_2$) calcd C, 66.03; H, 3.88; N, 11.55; found: C, 56.36; H, 3.89; N, 7.70; HPLC (method A): t_R = 7.30, 91% pure.

2-[4-(4-Methylphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3l). Prepared according to the procedure described in Method B (70%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 9.0 Hz, 2H), 8.11 (br s, 1H), 7.89 (dd, J = 8.5, 1.5 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.42 (br s, 1H), 7.28 (d, J = 8.6 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 7.06 (d, J = 8.5 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.6, 164.6, 154.5, 153.6, 137.3, 136.7, 135.1, 133.0, 132.2, 131.7, 131.4, 129.6, 126.5, 121.8, 120.7, 119.5, 119.4, 119.0, 115.5, 115.2, 21.2; MS (ESI+): *m/z* 344.1 (M + H)$^+$; Anal. ($C_{21}H_{17}N_3O_2$) calcd C, 73.45; H, 4.99; N, 12.24: found C, 55.78; H, 3.84; N, 7.87. HPLC (method A): t_R = 7.27, 92% pure.

2-[4-(3,4-Dimethoxyphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3m). Prepared according to the procedure described in Method B (63%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 8.9 Hz, 2H), 8.15 (s, 1H), 8.06

(br s, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.38 (br s, 1H), 7.15 (d, J = 8.9 Hz, 2H), 7.01 (d, J = 8.8 Hz, 1H), 6.83 (d, J = 2.7 Hz, 1H), 6.67 (dd, J = 8.7, 2.7 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.6, 164.9, 153.7, 152.3, 150.4, 148.6, 137.5, 135.3, 132.9, 131.4, 126.4, 119.5, 119.2, 115.5, 115.2, 114.3, 113.7, 107.1, 57.3, 57.0; MS (ESI+): m/z 390.10 $(M + H)^+$; Anal. ($C_{22}H_{19}N_3O_4 \cdot 0.6$TFA) C, H, N.

2-[4-(3-Carboxyphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3o). Prepared according to the procedure described in Method B (35%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 (br s, 1H), 8.05 (d, J = 8.8 Hz, 2H), 7.96 (br s, 1H), 7.80 (br s, 1H), 7.59 (m, 2H), 7.7 (br s, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.36 (m, 1H), 7.23 (m, 1H), 7.09 (br s, 1H), 7.06 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.3, 166.5, 157.9, 156.2, 132.8, 130.5, 128.6, 125.3, 124.7, 123.6, 119.0; HRMS $(M + H)^+$ calcd, 374.1141; found, 374.1142; Anal. ($C_{21}H_{15}N_3O_4 \cdot 3H_2O$) C, H, N.

2-[4-(4-Chlorophenylsulfanyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4i). Prepared according to the procedure described in Method B (58%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 8.7 Hz, 3H), 8.07 (br s, 1H), 7.85 (t, J = 8.5 Hz, 2H), 7.68 (d, J = 8.4 Hz, 1H), 7.56−7.48 (m, 6H), 7.37 (br s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.9, 152.7, 145.2, 138.6, 137.3, 136.3, 136.0, 135.1, 133.0, 131.9, 131.4, 130.7, 130.0, 129.2, 128.9, 128.4, 125.4, 123.2, 114.9, 114.5; HRMS $(M + H)^+$ calcd, 380.0624; found, 380.0623; Anal. ($C_{20}H_{14}ClN_3OS \cdot$TFA) C, H, N.

2-(4-Phenoxyphenyl)-1*H*-benzoimidazole-4-carboxylic Acid Amide (1i). Prepared according to the procedure described in Method B from **6i** (25%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.06 (d, J = 8.4 Hz, 2H), 7.88 (dd, J = 7.7, 1.0 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 7.34−7.37 (m, 2H), 7.15 (tt, J = 7.4, 1.0 Hz, 1H), 7.08 (d, J = 8.1 Hz, 2H), 7.02−7.04 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.69, 159.69, 155.90, 151.78, 139.92, 135.70, 130.65, 129.73, 124.76, 123.41, 122.96, 122.35, 120.19, 119.92, 118.68, 115.82; HRMS $(M + H)^+$ calcd, 330.1243; found, 330.1243; HPLC (method A): t_R = 5.05, 88% pure.

4-Formyl-*N*phenyl-benzamide (7b). To a cooled solution (0 °C) of 4-formylbenzoic acid (1.0 g, 6.6 mmol, 1.0 equiv) and triethylamine (1.0 mL, 7.3 mmol, 1.1 equiv) in CH_2Cl_2 (17 mL) was added isobutyl chloroformate (0.95 mL, 7.3 mmol, 1.1 equiv) dropwise. The reaction mixture was stirred for 40 min at 5 °C. Phenylamine (668 mL, 7.30 mmol, 1.10 equiv) was then added, and the solution was stirred for 18 h at room temperature. CH_2Cl_2 (50 mL) was added, and the organics were washed with water (1 × 75 mL) and brine (1 × 75 mL), dried over Na_2SO_4, and filtered. The CH_2Cl_2 layer was evaporated under reduced pressure. The crude solid was dissolved in a minimum amount of ethyl acetate, and the product was precipitated by the addition of hexanes (120 mL). The resulting solid was collected and dried under reduced pressure yielding **7b** (1.0 g, 70%) as a light yellow solid. The title compound was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.11 (s, 1H), 8.04−8.00 (m, 4H), 7.84 (br s, 1H), 7.65 (d, J = 7.7 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.19 (t, J = 7.4 Hz, 1H); MS (ESI−): m/z 224.1 $(M - H)^-$.

***N*-(4-Chlorophenyl)-4-formyl-benzamide (7e).** Prepared according to the procedure described in for **7b** (59%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.15 (s, 1H), 7.99 (s, 4H), 7.97 (br s, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H); MS (ESI+): m/z 260.3, 262.4 $(M + H)^+$.

***N*-(3,4-Dichlorophenyl)-4-formylbenzamide (7h).** Prepared according to the procedure described in for **7b** (53%). ^{1}H NMR (500 MHz, acetone-d_6) δ 10.12 (s, 1H), 7.96 (d, J = 8.2 Hz, 2H), 7.87 (br s, 1H), 7.49 (d, J = 8.3 Hz, 1H), 7.44 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 2.2 Hz, 1H), 7.12 (dd, J = 8.4, 2.4 Hz, 2H); MS (ESI−): m/z 292.0, 294.0 $(M - H)^-$; HPLC (Method A): t_R = 9.4 min.

***N*-(3,4-Dichlorophenyl)-4-formylbenzenesulfonamide (8g).** To a stirred solution of 3,4-dichloroaniline (174 mg, 1.07 mmol, 1.10 equiv) and pyridine (0.080 mL, 1.1 mmol, 1.1 equiv) in CH_2Cl_2 (4.0 mL) was added 4-formylbenzenesulfonyl chloride (200 mg, 0.980 mmol, 1.00 equiv). The mixture was stirred at room temperature for 16 h under N_2. The solvent was removed under reduced pressure to yield a red semisolid. Purification by chromatography (silica gel, 5% MeOH/CH_2Cl_2) afforded **8g** (242 mg, 75%) as a pale tan solid. ^{1}H NMR (400 MHz, CD_3OD) δ 10.61 (s, 1H), 8.09 (d, J = 8.6 Hz, 2H), 8.04 (d, J = 8.5 Hz, 2H), 7.42 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 2.5 Hz, 1H), 7.06−7.04 (dd, J = 8.6, 2.6 Hz, 2H); MS (ESI−) m/z 328.0 $(M - H)^-$; HPLC (method A): t_R = 9.33.

4-Formyl-*N*phenylbenzenesulfonamide (8a). Prepared according to the procedure described for **8g** (98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 7.97−7.89 (m, 4H), 7.84 (d, J = 8.5, 1H), 7.41 (t, J = 8.0, 2H), 7.20 (d, J = 7.3, 1H), 7.07 (t, J = 7.3, 1H), 6.64 (br s, 1H); MS (ESI−) m/z 260.0 $(M - H)^-$; HPLC (Method A): t_R = 8.39.

***N*-(4-Chlorophenyl)-4-formylbenzenesulfonamide (8d).** Prepared according to the procedure described for **8a** (65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.07 (s, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 6.64 (br s, 1H); MS (ESI−): m/z 294.0, 296.0 $(M - H)^-$.

2-(4-Phenylsulfamoylphenyl)-1*H*-benzoimidazole-5-carboxylic Acid Amide (4a). Prepared using aldehyde **8a** according to the procedure described in Method B (51%): ^{1}H NMR (400 MHz, DMSO-d_6) + TFA δ 10.58 (s, 1H), 8.40−8.38 (m, 3H), 8.34 (br s, 1H), 8.16 (d, J = 8.6 Hz, 3H), 7.95 (d, J = 8.7 Hz, 1H), 7.64 (br s, 1H), 7.29 (t, J = 8.5 Hz, 2H), 7.21 (d, J = 7.5 Hz, 2H), 7.08 (t, J = 7.3 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) + TFA δ 167.3, 149.6, 143.4, 137.4, 134.6, 132.6, 132.5, 129.4, 129.3, 128.0, 127.7, 125.9, 124.7, 120.8, 114.2; HRMS $(M + H)^+$ calcd, 393.1021; found, 393.1019. Anal. ($C_{20}H_{16}N_4O_3S \cdot$TFA$\cdot H_2O$) C, H, N.

2-(4-Phenylcarbamoylphenyl)-1*H*-benzoimidazole-5-carboxylic Acid Amide (4b). Prepared using aldehyde **7b** according to the procedure described in Method B (86%). ^{1}H NMR (500 MHz, DMSO-d_6) + TFA δ 10.45 (s, 1H), 8.32 (d, J = 8.6 Hz, 2H), 8.28 (s, 1H), 8.25 (d, J = 8.5 Hz, 3H), 8.07−8.05 (dd, J = 8.5, 1.5 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.76 (d, J = 7.6 Hz, 2H), 7.55 (br s, 1H), 7.33 (t, J = 7.9 Hz, 2H), 7.08 (d, J = 7.4 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 193.0, 192.9, 166.7, 165.2, 139.4, 139.2, 138.3, 135.8, 131.1, 130.7, 130.0, 129.6, 129.5, 128.6, 125.6, 121.7, 120.4; MS (ESI+): m/z 357.1 $(M + H)^+$; Anal. ($C_{21}H_{16}N_4O_2 \cdot 2H_2O$) C, H, N.

2-[4-(4-Chlorophenylsulfamoyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide. (4d). Prepared using aldehyde **8b** according to the procedure described in Method B (84%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (d, J = 8.7 Hz, 3H), 7.89 (d, J = 8.7 Hz, 2H), 7.87−7.80 (m, 1H), 7.65−7.63 (br m, 1H), 7.20 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.8, 151.7, 141.3, 137.1, 134.4, 129.7, 129.3, 129.1, 127.7, 127.6, 122.9; HRMS $(M + H)^+$ calcd, 427.0632; found, 427.0637; Anal. ($C_{20}H_{15}ClN_4O_3S$) calcd; C, 56.27; H, 3.54; N, 13.12. found; C, 55.87; H, 3.55; N, 12.36; HPLC (Method A): t_R = 7.20, >98% pure.

2-[4-(4-Chlorophenylcarbamoyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4e). Prepared using aldehyde **7e** according to the procedure described in Method B (68%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (d, J = 8.5 Hz, 2H), 8.20 (br s, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.86−7.84 (m, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.65 (br s, 1H), 7.37 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.6, 165.3, 152.3, 138.4, 136.1, 132.9, 129.1, 128.9, 128.7, 127.7, 126.9, 122.2; MS (ESI−) m/z 389.2 $(M - H)^-$; Anal. ($C_{21}H_{15}ClN_4O_2 \cdot$TFA$\cdot H_2O$) C, H, N.

2-[4-(3,4-Dichlorophenylsulfamoyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4g). Prepared using aldehyde **7h** according to the procedure described in Method B (85%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (d, J = 8.6 Hz, 2H), 8.19 (br s, 1H), 7.93 (d, J = 8.6 Hz, 2H), 7.82 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 7.7 Hz, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.28 (d, J = 2.5 Hz, 1H), 7.06−7.03 (dd, J = 8.7, 2.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.6, 162.7, 151.5, 140.2, 138.2, 134.3, 131.8, 131.6, 129.3, 127.9, 127.8, 126.6, 122.9,

121.6, 120.3; MS (ESI+) *m/z* 461.0, 463.3 $(M + H)^+$; HPLC (Method A): $t_R = 7.53$, >98% pure.

2-[4-(3,4-Dichlorophenylcarbamoyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4h). Prepared using aldehyde **7h** according to the procedure described in Method B (75%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.29 (d, $J = 8.3$ Hz, 2H), 8.23 (br s, 1H), 8.13 (d, $J = 2.52$ Hz, 1H), 8.08 (d, $J = 8.6$ Hz, 2H), 7.95 (br s, 1H), 7.75 (br s, 1H), 7.72 (dd, $J = 8.8$, 2.3 Hz, 2H), 7.58 (d, $J = 8.8$ Hz, 2H), 7.23 (br s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.5, 152.0, 143.7, 139.6, 135.7, 133.2, 131.3, 131.0, 128.8, 127.0, 126.9, 125.6, 121.8, 120.7; MS (ESI+): *m/z* 425.0, 427.0 $(M + H)^+$; HPLC (method A): $t_R = 7.47$, >98% pure.

(Method C) 2-[4-(4-Chlorophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (2h). To a solution of 4-(4-chloro-phenoxy)benzaldehyde (7.3 g, 31.5 mmol, 1.0 equiv) in DMA (100 mL) were added 3,4-diaminobenzoic acid (4.8 g, 31.5 mmol, 1.0 equiv) and $Na_2S_2O_5$ (7.2 g, 37.8 mmol, 1.2 equiv) at room temperature. The mixture was heated then to 100° C for 6.5 h. The reaction mixture was then cooled, diluted with ethyl acetate (2 L), washed with water (5 × 500 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was tritrated with CH_2Cl_2 and MeOH (50 mL, 20:1). The gray solid was collected on a sintered-glass filter, washed with CH_2Cl_2, and dried to provide (8.0 g, 70%) of 2-[4-(4-chlorophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic acid. The crude product was dissolved in DMF (100 mL), and 1,1′-carbonyldiimidazole (5.1 g, 31.5 mmol, 1 equiv) was added. The mixture was stirred for 30 min at room temperature and then cooled to 0 °C. Ammonium carbonate (6.0 g, 63 mmol, 2 equiv) was added, and the reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was taken up in ethyl acetate (30 mL), washed with water (3 × 10 mL), and dried ($MgSO_4$). The organics were then collected and concentrated. The residue was purified by silica gel chromatography (5% MeOH/CH_2Cl_2) yielding the title compound as a light yellow solid (4.2 g, 86%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, $J = 9.0$ Hz, 2H), 8.20 (d, $J = 0.78$ Hz, 1H), 8.13 (br, s, 1H), 7.92 (dd, $J = 8.4$, 1.6 Hz, 1H), 7.73 (d, $J = 8.4$ Hz, 1H), 7.51 (d, $J = 8.8$ Hz, 2H), 7.44 (br, s, 1H), 7.26 (d, $J = 8.8$ Hz, 2H), 7.18 (d, $J = 9.0$ Hz, 2H), 6.76 (br, s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.69, 159.75, 154.19, 151.40, 137.53, 135.53, 130.24, 130.16, 129.65, 128.54, 123.65, 121.59, 121.42, 118.54, 114.18, 113.85; MS (ESI+): *m/z* 364.1 $(M + H)^+$; Anal. ($C_{20}H_{14}ClN_3O_2$) C, H, N.

2-(4-Ethoxyphenyl)-1*H*-benzoimidazole-5-carboxylic Acid Amide (2b). Prepared according to method C (51%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.93 (d, $J = 9.7$ Hz, 1H), 8.12 (t, $J = 8.6$ Hz, 2H), 8.11 (d, $J = 9.7$ Hz, 1H), 7.96 (d, $J = 21.7$ Hz, 1H), 7.75 (ddd, $J = 18.8$, 8.4, 1.2 Hz, 1H), 7.57 (dd, $J = 18.8$, 8.4 Hz, 1H), 7.24 (d, $J = 18.5$ Hz, 1H), 7.11 (d, $J = 8.2$ Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.40, 168.26, 153.40, 152.73, 145.99, 143.37, 137.03, 134.52, 128.23, 128.14, 128.09, 127.80, 122.10, 122.04, 121.19, 117.99, 117.53, 114.73, 110.79, 110.29, 63.23, 14.49; HRMS $(M + H)^+$ calcd, 282.1243; found, 282.1243; Anal. ($C_{16}H_{15}N_3O$) calcd C, 68.31; H, 5.37; N, 14.94; found: C, 64.19; H, 5.37; N, 14.94. HPLC (Method B): $t_R = 4.56$, >99% pure.

2-Dibenzofuran-2-yl-1*H*-benzoimidazole-5-carboxylic Acid Amide (2c). Prepared according to method C (99%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.70 (s, $J = 1.8$ Hz, 1H), 8.20 (d, $J = 1.0$, 1H), 8.13 (dd, $J = 8.7$, 2.0 Hz, 1H), 8.04 (d, $J = 7.5$ Hz, 1H), 7.94 (dd, $J = 8.6$, 1.5 Hz, 1H), 7.79 (d, $J = 8.7$ Hz, 1H), 7.72 (d, $J = 8.6$ Hz, 1H), 7.57 (d, $J = 8.3$ Hz, 1H), 7.48−7.51 (m, 1H), 7.36−7.39 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.84, 157.94, 156.47, 152.31, 138.38, 136.34, 129.86, 128.59, 124.47, 123.85, 123.37, 122.89, 122.41, 121.79, 121.38, 120.56, 114.34, 113.94, 112.67, 112.05; HRMS $(M + H)^+$ calcd, 328.1086; found, 328.1086; Anal. ($C_{20}H_{13}N_3O_2$) calcd C, 59.87; H, 3.20; N, 9.52; F, 12.91; found: C, 54.66; H, 2.64; N, 8.49; F, 9.94. HPLC (Method B): $t_R = 4.53$, 89% pure.

2-(3-Phenoxyphenyl)-1*H*-benzoimidazole-5-carboxylic Acid Amide (2d). Prepared according to method C (73%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (d, $J = 0.9$ Hz, 1H), 7.92 (dd, $J = 8.6$, 1.6 Hz, 1H), 7.77 (ddd, $J = 7.8$, 1.6, 0.8 Hz, 1H), 7.67−7.69 (m, 2H), 7.56 (t, $J = 8.0$ Hz, 1H), 7.32−7.36 (m, 2H), 7.21−7.23 (m, 1H), 7.12−7.14 (m, 1H), 7.00−7.03 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 172.01, 160.54, 158.13, 153.87, 139.04, 136.86, 132.81, 132.46, 131.68, 129.10, 125.93, 125.84, 123.86, 123.55, 120.97, 118.57, 116.09, 115.60; HRMS $(M + H)^+$ calcd, 330.1243; found, 330.1243; Anal. ($C_{20}H_{15}N_3O_2 \cdot H_2O$) C, H, N.

2-*p*-Tolyl-1*H*-benzoimidazole-5-carboxylic Acid Amide (2e). Prepared according to method C (58%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (s, 1H), 8.00 (d, $J = 8.2$ Hz, 2H), 7.81 (dd, $J = 8.5$, 1.6 Hz, 1H), 7.63 (d, $J = 8.4$ Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.34, 153.07, 139.07, 129.48, 128.17, 127.01, 126.84, 126.51, 121.87, 117.87, 110.72, 20.91; HRMS $(M + H)^+$ calcd, 252.1137; found, 252.1137; Anal. ($C_{15}H_{13}N_3O \cdot 0.5H_2O$) C, H, N.

2-Naphthalen-2-yl-1*H*-benzoimidazole-5-carboxylic Acid Amide (2f). Prepared according to method C (51%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.62 (s, 1H), 8.23 (s, 1H), 8.19 (dd, $J = 8.6$, 1.6 Hz, 1H), 8.05 (d, $J = 8.6$ Hz, 1H), 7.99−8.02 (m, 1H), 7.93−7.96 (m, 1H), 7.86 (dd, $J = 8.4$, 1.5 Hz, 1H), 7.69 (d, $J = 8.5$, 1H), 7.57−7.61 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.77, 157.94, 152.22, 133.87, 132.46, 129.55, 128.84, 128.60, 127.87, 127.81, 127.24, 127.16, 124.97, 123.69, 123.13, 114.47, 113.99; HRMS $(M + H)^+$ calcd, 288.1137; found, 288.1137; Anal. ($C_{18}H_{13}N_3O \cdot 2H_2O$) C, H, N.

2-[3-(3,4-Dichlorophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (2g). Prepared according to method C (98%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.16 (dd, $J = 1.5$, 0.5 Hz, 1H), 7.89 (dd, $J = 8.2$, 1.6 Hz, 1H), 7.84−7.86 (m, 1H), 7.70 (t, $J = 2.0$ Hz, 1H), 7.67 (dd, $J = 8.5$, 0.5 Hz, 1H), 7.61 (t, $J = 8.0$ Hz, 1H), 7.47 (d, $J = 8.8$ Hz, 1H), 7.27−7.30 (m, 1H), 7.20 (d, $J = 2.8$, 1H), 6.97 (dd, $J = 2.8$, 8.2 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.71, 159.14, 159.40, 153.29, 138.74, 136.56, 134.62, 132.92, 132.89, 132.27, 129.11, 128.75, 125.77, 124.34, 124.23, 122.44, 120.34, 118.82, 115.88, 115.39; HRMS $(M + H)^+$ calcd, 398.0463; found, 398.0463; Anal. ($C_{20}H_{13}Cl_2N_3O_2 \cdot TFA \cdot 0.5H_2O$) C, H, N.

2-[4-(4-Nitrophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3n). Prepared according to method C (77%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.4−8.31 (m, 4H), 8.21 (s, 1H), 8.08 (br s, 1H), 7.87 (d, $J = 8.5$ Hz, 1H), 7.70 (d, $J = 8.5$ Hz, 1H), 7.45 (d, $J = 8.6$ Hz, 2H), 7.39 (br s, 1H), 7.32−7.29 (m, 2H), 4.28 (br s, 2H); ^{13}C (125 MHz, DMSO-d_6) δ 114.19, 119.03, 119.44, 120.85, 121.50, 124.96, 126.71, 130.70, 131.57, 133.99, 135.95, 143.67, 150.97, 158.82, 161.57, 167.59; HRMS $(M + H)^+$ calcd, 375.1093; found, 375.1109; Anal. ($C_{20}H_{14}N_4O_4$.TFA) calcd C, 54.11; H, 3.10; N, 11.47; F, 11.67; found: C, 55.63; H, 2.93; N, 11.25; F, 11.88; HPLC (Method A): $t_R = 6.99$, >99% pure.

2-(4-Phenoxyphenyl)-1*H*-benzoimidazol-5-ylamine (1d). To a solution of **1c** (39 mg, 0.13 mmol, 1.0 equiv) and DMF (1 mL) was added anhydrous ethanol (1 mL). Pd/C (5 mg, 10%) was added, and the reaction was stirred under hydrogen (1 atm). After 16 h, the resulting mixture was diluted with ethyl ether (15 mL). The mixture was filtered through celite and rinsed with ethyl ether (3 ×10 mL). Combined organic phases were concentrated under reduced pressure to yield **1d** (12 mg, 30%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.92−7.96 (m, 2H), 7.47 (d, $J = 8.8$ Hz, 1H), 7.35−7.39 (m, 2H), 7.17 (t, $J = 7.4$ Hz, 2H), 7.07−7.12 (m, 3H), 6.99−7.05 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 163.61, 156.77, 150.01, 135.73, 131.55, 130.67, 128.39, 126.38, 121.55, 119.69, 119.35, 118.30, 115.54, 100.88; HRMS $(M + H)^+$ calcd, 302.1293; found, 302.1293; Anal. ($C_{19}H_{15}N_3O \cdot TFA$) calcd C, 60.72; H, 3.88; N, 10.12; F, 13.72; found: C, 54.63; H, 3.37; N, 8.54; F, 12.60; HPLC (Method B): $t_R = 4.37$, 89% pure.

2-(4-Phenoxyphenyl)-1*H*-benzoimidazole-5-carboxylic Acid Dimethylamide (1h). A solution of **1a** (100 mg, 0.30 mmol, 1.0 equiv) and CDI (122 mg, 0.750 mmol, 2.50 equiv) in DMA (1.5 mL) was stirred in a sealed tube for 30 min, after

which it was treated with DBU (0.60 mL, 4.1 mmol, 14 equiv) and dimethylamine hydrochloride (110 mg, 1.35 mmol, 4.5 equiv). After an additional 16 h, the resulting mixture was filtered and purified by reverse phase HPLC (C_{18}, water/acetonitrile/0.1% TFA) to yield **1h** (96 mg, 66%). ^{1}H NMR (500 MHz, (DMSO-d_6) δ 8.24 (d, J = 8.8 Hz, 2H), 7.76−7.78 (m, 2H), 7.44−7.51 (m, 3H), 7.24−7.27 (m, 3H), 6.86−7.18 (m, 2H), 3.0 (d, J = 15 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.83, 160.31, 155.10, 150.91, 135.55, 134.83, 132.38, 130.58, 130.37, 130.14, 129.62, 124.74, 123.35, 120.53, 119.88, 118.27, 114.10, 113.35, 35.76, 34.67; HRMS $(M + H)^+$ calcd, 358.1556; found, 358.1556; HPLC (Method B): t_R = 4.66, >97% pure.

2-(4-Phenoxyphenyl)-1*H*-benzoimidazole-5-carboxylic Acid Methylamide (1g). Prepared according to the procedure described for **1h** (89%): ^{1}H NMR (500 MHz, (DMSO-d_6) δ 8.51 (d, J = 4.5 Hz,1H), 8.20−8.23 (m, 2H), 8.13 (d, J = 1.0, 1H), 7.83 (dd, J = 8.5, 1.4 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.46−7.50 (m, 2H), 7.21−7.27 (m, 3H), 7.15−7.17 (m, 2H), 2.82 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.47, 159.91, 155.26, 151.26, 137.99, 136.03, 130.34, 130.11, 129.42, 124.61, 122.87, 121.59, 119.75, 118.29, 113.95, 113.72, 26.38; HRMS $(M + H)^+$ calcd, 344.1399; found, 344.1399; HPLC (Method B): t_R = 4.65, >94% pure.

2-[4-(3-Hydroxyphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3g). To a solution of 2-[4-(3-methoxyphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic acid amide (65 mg, 0.18 mmol, 1.0 equiv, prepared through Method C) in dichloromethane (3 mL) at −78 °C was added boron tribromide (0.20 mL, 2.1 mmol, 12 equiv). The mixture was allowed to warm to room temperature over 15 h, cooled to 0 °C, and quenched with aqueous saturated ammonium chloride. The solid was filtered and rinsed with water to afford **3g** (58 mg, 93%): ^{1}H (500 MHz, CD_3OD) δ 8.21 (s, 1H), 8.02 (m, 2H), 8.00 (dd, J = 8.6, 1.5 Hz, 1H), 7.74 (d, J = 8.6 Hz, 1H), 7.16 (m, 3H), 6.60 (m, 1H), 6.49 (m, 1H), 6.45 (m, 1H); ^{13}C (125 MHz, CD_3OD) δ 109.07, 112.58, 113.89, 115.12, 115.28, 118.32, 119.97, 127.12, 131.75, 132.27, 133.81, 133.88, 136.04, 153.19, 157.71, 161.02, 164.79, 171.23; HRMS $(M + H)^+$ calcd, 346.1192; found, 346.1181; Anal. ($C_{20}H_{16}N_3O_3 \cdot 3H_2O$) calcd C, 60.14; H, 5.30; N, 10.52. found: C, 60.07; H, 4.23; N, 8.85; HPLC (method A): t_R = 6.48, 95% pure.

2-[4-(4-Chloro-3-hydroxyphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3h). Prepared according to the procedure described in for **3g** and then purified by reverse phase HPLC (C18, water/acetonitrile/0.1% TFA) giving the title compound (49% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 (s, 1H), 8.04 (d, J = 9.0 Hz, 2H), 7.95 (dd, J = 8.6, 1.6 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.27 (d, J = 8.7 Hz, 1H), 7.16 (d, J = 8.9 Hz, 2H), 6.61 (d, J = 2.7 Hz, 1H), 6.51 (d, J = 8.6, 2.7 Hz, 1H); ^{13}C (125 MHz, CD_3OD) δ 109.97, 113.35, 115.28, 115.62, 118.55, 120.14, 120.58, 126.34, 131.43, 132.40, 132.93, 135.58, 137.81, 153.72, 156.27, 156.57, 163.60, 171.70; HRMS $(M + H)^+$ calcd, 380.8042; found, 380.0795; Anal. ($C_{20}H_{14}ClN_3O_3 \cdot TFA \cdot 0.5H_2O$) C, H, N, F.

2-[4-(4-Methanesulfonylaminophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3q). To a solution of **3n** (0.10 g, 0.29 mmol) in 1:1 DMF/EtOH (6 mL), was added palladium (5 mg, 10% on carbon). The mixture was stirred under H_2 (1 atm) at room temperature for 15 h and was then filtered through a pad of Celite. The filtrate was concentrated under reduced pressure and purified by silica gel chromatography (1% methanol saturated with ammonia/9% methanol/CH_2Cl_2) to afford (40 mg, 40%) of 2-[4-(4-aminophenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic acid amide.

To a solution of the above amide (40 mg, 0.12 mmol) in THF (0.5 mL) was added pyridine (0.5 mL), followed by methanesulfonyl chloride (0.5 mL of 0.2 M in THF). The mixture was stirred for 15 h at room temperature and was concentrated and then purified by reverse phase HPLC (C_{18}, water/acetonitrile/0.1% TFA) to afford **3q** (10 mg, 20%):^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (br s, 1H), 8.03 (d, J = 8.9 Hz, 2H), 7.95 (dd, J = 8.6, 1.5 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.26 (d, J = 8.9 Hz, 2H), 7.14 (d, J = 8.9 Hz, 2H), 7.04 (d, J = 8.9 Hz, 2H), 2.89 (s, 3H); ^{13}C (125 MHz, CD_3OD) δ 39.66, 115.24, 115.54, 119.78, 119.95, 122.83, 124.48, 126.47, 131.51, 133.06, 135.25, 136.98, 137.48, 153.62, 153.78, 164.21, 171.62; HRMS (M + H)+ calcd: 423.4127, found: 423.1142; Anal. ($C_{21}H_{18}N_4O_4S \cdot TFA \cdot H_2O$) C, H, N, S.

2-[4-(3-Diethylcarbamoylphenoxy)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (3p). Into a flask charged with **3o** (50 mg, 0.13 mmol, 1.0 equiv), EDCI (36 mg, 0.19 mmol, 1.46 equiv), HOBt hydrate (25 mg, 0.19 mmol, 1.46 equiv), and DMF (5 mL) was added *N,N*-diethylamine (33 mg, 0.26 mmol, 2.0 equiv). The mixture was stirred for 4 h at room temperature and then diluted with ethyl acetate, washed with saturated $NaHCO_3$ (5 mL) and water (4 × 5 mL), and dried with Na_2SO_4. Solvent was removed under reduced pressure, and the crude material was purified by chromatography (silica gel, EtOAc/hexanes) to provide the title compound (45%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 8.9 Hz, 2H), 8.12 (s, 1H), 8.02 (br s, 1H), 7.82 (dd, J = 8.5, 1.4 Hz, 1H), 7.63 (d, J = 8.5 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.33 (br s, 1H), 7.21 (d, J = 8.9 Hz, 2H), 7.14 (m, 2H), 6.98 (m, 1H), 3.35 (br s, 2H), 3.11 (br s, 2H), 1.05 (br s, 3H), 0.97 (br s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.2, 168.1, 159.8, 158.8, 158.5, 155.8, 151.9, 139.7, 131.0, 130.3, 129.9, 123.8, 122.4, 120.4, 119.2, 117.3, 115.1, 114.6, 114.2; HRMS: $(M+H)^+$ calcd 429.1926; found, 429.1928; Anal. ($C_{25}H_{24}N_4O_3$) calculated C, 70.08; H, 5.65; N, 13.08; found: C, 58.81; H, 5.00; N, 9.72; HPLC (Method A): t_R = 6.78, 98% pure.

2-[4-(4-Chlorobenzenesulfinyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4j). To a flask containing **10** (190 mg, 0.5 mmol, 1.0 equiv) in methanol (6 mL) were added tellurium dioxide (80 mg, 0.5 mmol, 1.0 equiv), 30% H_2O_2 (0.11 mL), and a drop of concentrated HCl. The mixture was stirred at room temperature for 72 h. Water (10 mL) was then added, and the resulting precipitate was collected by filtration. The precipitate was washed with water (2 × 5 mL) and purified by reverse phase HPLC (C18; water, acetonitrile, 0.01% TFA) to afford 2-[4-(4-chlorobenzenesulfinyl)phenyl]-1*H*-benzoimidazole-5-carboxylic acid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, J = 8.6 Hz, 2H), 8.35 (s, 1H), 8.10 (d, J = 8.6 Hz, 2H), 8.03 (dd, J = 8.5, 1.5 Hz, 1H), 7.95 (d, J = 8.6 Hz, 2H), 7.85 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 8.7 Hz, 2H); MS (ESI+): m/z 397.0 $(M + H)^+$.

4j was prepared according to the procedure described in Method C from the above acid (62%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, J = 8.6 Hz, 2H), 8.14 (s, 1H), 8.01 (br s, 1H), 7.91 (d, J = 8.6 Hz, 2H), 7.80 (dd, J = 8.0, 1.6 Hz, 1H), 7.76 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.0, 1H), 7.58 (d, J = 8.0, 2H), 7.32 (br s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.9, 158.4, 158.1, 151.1, 147.8, 144.4, 136.1, 130.9, 129.7, 129.4, 127.9, 126.1, 124.8, 123.0. HRMS: $(M + H)^+$ calcd; 396.0573 found; 396.0573; Anal. ($C_{20}H_{14}ClN_3O_2S \cdot TFA \cdot H_2O$) calcd: C, 50.05,; H, 3.25; N, 7.96. found: C, 47.58; H, 3.74; N, 8.81; HPLC (method A): t_R = 6.65, 98% pure.

2-[4-(4-Chlorobenzenesulfonyl)phenyl]-1*H*-benzoimidazole-5-carboxylic Acid Amide (4k). To a flask containing **10** (100 mg, 0.260 mmol, 1.00 equiv) were added oxone (480 mg, 0.780 mmol, 3.00 equiv), MeOH (1.0 mL), and water (1.0 mL). The mixture was stirred at room temperature for 24 h followed by collection of the resulting precipitate by filtration. The precipitate was washed with water (3 × 20 mL) and dried to afford 2-[4-(4-chlorobenzenesulfonyl)phenyl]-1*H*-benzoimidazole-5-carboxylic acid (105 mg, 98%): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.36 (d, J = 8.7 Hz, 2H), 8.17 (m, 1H), 8.13 (d, J = 8.7 Hz, 2H), 7.98 (d, 8.7 Hz, 2H), 7.83 (dd, J = 8.5, 1.5 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.66 (m, 1H); MS (ESI+): m/z 413.0 $(M + H)^+$. **4k** was prepared according to the procedure described in Method C from the above acid (70%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (d, J = 8.6 Hz, 2H), 8.19 (br s, 1H), 8.18 (d, J = 8.6 Hz, 2H), 8.04 (d, J = 8.7 Hz, 2H), 8.03 (br s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 8.7 Hz, 2H), 7.66 (br s, 1H), 7.33 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 139.8, 138.0, 137.4, 133.0, 128.4, 127.8, 126.7, 126.1; MS (ESI+): m/z 412.0 $(M + H)^+$; Anal. ($C_{20}H_{14}ClN_3O_3S \cdot 0.5H_2O$) C, H, N.

In Vitro Assays. Inhibition of Purified chk2. Activity of inhibitors of chk2 was determined by incubating inhibitory compounds with recombinant full-length chk2: 5 nM recombinant human Chk2, 50 mM HEPES (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$, 25 μM synthetic peptide substrate (biotin-SGLYRSPSMPENLNRPR, 1 μM ATP, 50 μCi/mL [γ-^{33}P] ATP, and a protease inhibitor mixture. The reaction mixtures were incubated at 37 °C for 3 h, and the peptide substrate was captured on streptavidin conjugated to agarose beads. The agarose beads were washed repeatedly with a 0.1% solution of Tween-20 in phosphate-buffered saline, pH 7.4. Enzyme activity at different chk2 inhibitory compound concentrations was determined by measuring the amount of radioactive phosphate bound to the substrate peptide by scintillation counting. In kinetic experiments ATP concentration was varied while the ratio between unlabeled and [γ-^{33}P] labeled ATP was kept constant. Reactions were stopped at different time points by addition of 50 mM cold ATP and samples were kept on ice during further processing.

Isolation of Human CD4$^+$ and CD8$^+$ T-Cells. Human CD4$^+$ and CD8$^+$ T-cells were isolated from heparinized whole blood by positive selection with CD4 or CD8 microbeads (Miltenyi) and an AutoMacs automated cell purification system. Blood was briefly subject to density gradient centrifugation on Ficoll. Buffy coats were harvested, pooled, and washed in PBS containing 2% FBS (binding buffer). The cells were resuspended in 800 μL of binding buffer to which 200 μL of anti-CD4$^+$ microbeads was added. This mixture was rotated slowly end over end for 20 min at 4 °C. The cells were then brought to 5 mL with binding buffer and purified by positive selection. Unbound cells were subjected to a second round of purification to isolate CD8$^+$ T-cells (as above). Purified CD4$^+$ and CD8$^+$ cells were cultured in RPMI (CellGro) containing 10% FBS, pen/strep, and glutamine.

Human CD4$^+$ and CD8$^+$ T-Cell Apoptosis Assay. To determine the radioprotective effect of Chk2 inhibitors, purified T-cells were incubated at 100 000 cells per well in compounds or vehicle (DMSO) at varying concentrations in 96-well stripwells (Costar) for 1 h. Cells were then exposed to a dose of 0 or 10 Gy gamma irradiation from a ^{137}Cs source (Nordion Gamma Cell) at a dose rate of 3.65 Gy/min and then returned to the incubator for a further 24 h. Cells were stained with Annexin V-FITC (Oncogene Research Products, La Jolla, CA) and propidium iodide, according to the manufacturers protocol. Apoptotic and surviving cells were quantitated with a FACSCalibur FACS machine (Becton Dickinson, San Jose, CA). Data are reported as percent recovery-or the number of survivors from treatment groups minus the number of cells surviving in the irradiated control group divided by the number of surviving cells in the untreated control groups.

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