# Design and Synthesis of Novel Cyclic Amine Benzimidazoles for the Treatment of Pancreatic Cancer

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**ABSTRACT:** The Wnt/β-Catenin signaling pathway has a very important role in pancreatic ductal adenocarcinoma (PDAC) initiation and progression. This signaling pathway has been implicated in angiogenesis, maintenance of resistant cancer-initiating cells (CICs), metastasis, regulation of cell cycle, apoptosis and chemoresistance. We investigated the activity of a chemical series of phenyl benzimidazoles, small molecule inhibitors of Wnt/β-catenin signaling in LDL-Receptor Related Protein-6 (LRP6)-expressing HEK 293 cells and against PDAC cells. A series of benzimidazoles were designed and synthesized from which several were potent inhibitors of Wnt signaling and cytotoxic to PDAC cells under both adherent and CIC conditions.

Two compounds in particular, the piperidine 6-Dichloro-2-(4-(piperidin-1-yl)phenyl)-1H-benzo[d]imidazole (**43)** and pyrrolidine 55,6-dichloro-2-(4-(pyrrolidin-1-yl)phenyl)-1H-benzo[d]imidazole (**48)** shown above exhibited excellent Wnt inhibitory activity with $IC_{50}$ values of 2 nM and potent cytotoxicity activity in the PDAC cellular assays with $IC_{50}$ values averaging 1 μM. The Wnt inhibitory activity and cytotoxicity of these novel benzimidazole analogs are described.

**INTRODUCTION**

The Wnt/β-catenin signaling pathway is one of the major signaling pathways in stem and progenitor cells, and plays an important role in cancer initiation and progression.[1-3] The low-density lipoprotein receptor-related protein 6 (LRP6) acts as an essential co-receptor for Wnt/β-catenin signaling.[1-3] When Wnt proteins interact with LRP6 and the seven transmembrane receptor of the Frizzled (Fzd) family, signaling from the cell surface proceeds through the proteins, dishevelled (Dvl) and axin, and results in the inhibition of glycogen synthase kinase-3β (GSK3β) and the stabilization of cytosolic β-catenin. The β-catenin then translocates into the nucleus where it interacts with T-cell factor/lymphoid enhancing factor (TCF/LEF) to induce the expression of specific target genes.[1-3]

In addition, the Wnt/β-catenin signaling pathway plays an important role in pancreatic ductal adenocarcinoma (PDAC) initiation and progression and has been implicated in angiogenesis, maintenance of resistant cancer initiating cells (CICs), metastasis, regulation of cell cycle, apoptosis and chemoresistance.[4-18] A growing body of evidence indicates that CICs are characterized as a subpopulation of stem cells in a tumor that are capable of unlimited self-renewal, resistance to standard chemotherapy or radiation therapy, and high tumorigenicity.[19-22] CICs initiate and propagate PDAC as well as other types of tumors.[19, 23-25] In addition, CICs have been linked to epithelial-to-mesenchymal transition (EMT) in various solid tumors, including PDAC.[26, 27] EMT has also been linked to cancer metastasis in PDAC.[28-30]

It has been shown that elimination of both differentiated and differentiating cells which form the bulk of the tumor and the CICs should ultimately halt neoplastic expansion.[31-33] Since pancreatic CICs display an aberrant activation of the Wnt/β-catenin signaling pathway,[11, 34-36] its

pharmacologic blockade represents a potential therapeutic strategy to inhibit metastatic spread in PDAC. [1-3, 36]

To date, there are no small molecule Wnt inhibitors yet approved by the FDA for cancer treatment. There is one small molecule inhibitor, 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-*N*-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (LGK974), of Porcupine (PORCN), a Wnt-specific acyltransferase, that is currently in Phase I clinical trials for the treatment of malignancies that are dependent on Wnt ligands.[37, 38] This compound may provide a path forward for targeting Wnt-driven cancers through the inhibition of PORCN. Also, the only current therapy for PDAC, a leading cause of cancer-related deaths worldwide,[39-41] is surgical resection which can potentially provide a five year survival, but only 20% of these patients are eligible. Therefore, it is imperative to develop novel therapeutic strategies for the inhibition of Wnt/β-catenin signaling and the identification of novel therapeutics for the treatment of PDAC.

Niclosamide, an anthelminthic approved for use in humans for more than 50 years,[42,43] has recently shown excellent anti-cancer *in vitro* activity. [44] Previously we reported that Niclosamide suppressed Wnt/β-catenin signaling by promoting Wnt co-receptor LRP6 degradation in breast, prostate, ovarian and pancreatic cancer cells.[45-47] However, Niclosamide is not selective and also affects other signaling pathways in cancer cells, such as the target of rapamycin complex 1 (mTORC1) and signal transducer and activator of transcription 3 (STAT3) pathways.[48-50] The *in vivo* activity of Niclosamide in anti-tumor studies has also been modest. [46,51,52] This could be due to several factors, including the off-target activities of Niclosamide and its less than desirable pharmacokinetic properties, such as metabolic stability ($t\frac{1}{2} = 29$ min, rat liver microsomes) and solubility (1.6 μM, pH 7.4), data that was determined in our laboratories. Targeted values for these parameters should be at least a $t\frac{1}{2} = 60$ min (rat liver microsomes)

which usually translates to BID dosing and > 10 μM solubility (pH 7.4) which gives some leverage in formulating compounds for *in vivo* animal studies. We found Niclosamide to have good inhibition of Wnt/β-catenin signaling, with an $IC_{50}$ value of 0.22 μM, and activity against the two pancreatic cancer cell lines, S2VP10 (adherent, $IC_{50}$ = 0.7 μM; CIC, $IC_{50}$ = 0.05 μM) and Suit-2 (adherent, $IC_{50}$ = 7.5 μM; CIC, $IC_{50}$ = 2.7 μM).

Using Niclosamide as a starting point, we designed several potential novel derivatives. The first approach was the evaluation of compounds that constrained the amide bond of Niclosamide while varying its substituents, such as nitro- and hydroxyl- on the phenyl rings, and targeting improved drug-like properties (e.g., metabolic stability and solubility) as well as less toxicities than Niclosamide. This would include carcinogenicity, hepatotoxicity, mutagenicity, and bone-marrow suppression. [53-55] It was anticipated that by forming a ring system (i.e., benzimidazole) which adds rigidity to the Niclosamide scaffold, would affect the potency and drug-like properties of the compound and enhance specificity. In addition, replacing the nitro ($NO_2$) substituent, as well as replacing the phenyl hydroxy (OH) group, potentially a site of first pass metabolism, would be a good starting point. Figure 1 illustrates the amide bond of Niclosamide being constrained by its integration into the five membered ring to give the benzimidazole scaffold of which several analogs are described within.
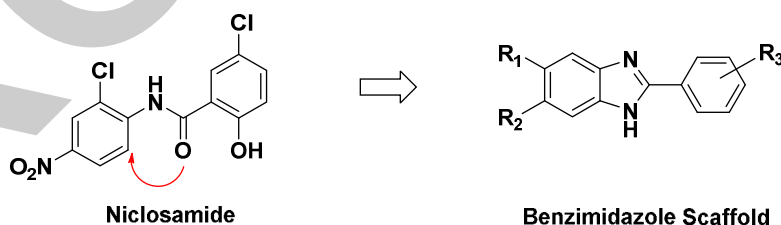


Figure 1. Benzimidazole Analogs

## RESULTS AND DISCUSSION

**Chemistry.** The design and synthesis of the novel Wnt inhibitors focused on the identification of selective benzimidazole analogs that were more potent than Niclosamide and possessed improved drug-like properties with less potential toxicities. These benzimidazoles were synthesized via standard coupling reaction conditions utilizing Method A or Method B as illustrated in Scheme 1.

**Scheme 1. Synthesis of Substituted Phenyl Benzimidazoles**[a]
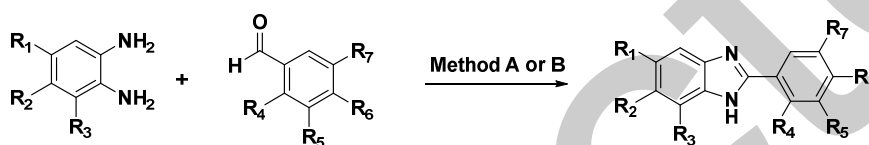


[a]Method A: NaHSO$_3$, DMSO, 210$^o$C, 1h; Method B: Na$_2$S$_2$O$_5$. DMF, 170$^o$C, 15 min, MW

Method B was preferred versus Method A due to shorter reaction times (15 min) and higher yields. Another Method C for the synthesis of several 4-trifluoromethyl phenyl analogs involved Suzuki coupling of bromo-intermediate A with boronic acids or esters (Scheme 2). Using these synthetic routes, approximately 60 benzimidazole analogs[56] were synthesized and tested for Wnt inhibition.

**Scheme 2. Synthesis of Substituted Phenyl Benzimidazoles via Suzuki Coupling**[a]
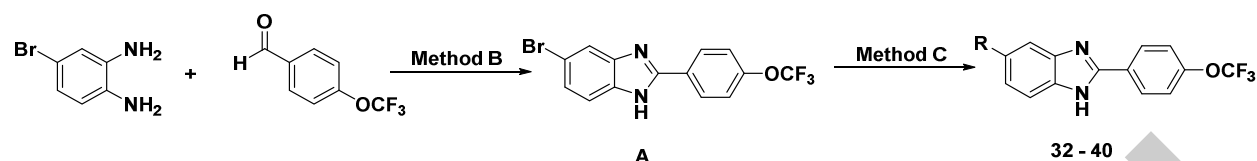
[a]Method B: $Na_2S_2O_5$, DMF, $170^oC$, 15 min, MW; Method C: $R-B(OH)_2$, $K_2CO_3$, $Pd(PPh_3)_4$, Dioxane-Water, Reflux, 15 h

**Biological Evaluation.** Compounds were tested for inhibition of Wnt/β-catenin signaling in LRP6-expressing HEK293 cells using a luciferase assay system and expressed as an $IC_{50}$ determination. Compounds were then tested in an *in vitro* cytotoxicity assay in two pancreatic cancer cell lines, Suit-2 and S2VP10, in adherent conditions and in non-adherent conditions using CIC medium. After a 3 day treatment, cell viability was assessed using an ATP-dependent luciferase assay. ATP levels were measured relative to untreated control cells with values derived from 4 or 6 replicate wells for adherent or CIC conditions, respectively. All cytotoxicity experiments were performed at least two times.

In our previous studies, we demonstrated that HEK293 displayed a high level of Wnt reporter activity after the cells were transiently transfected with LRP6 plasmid and Super8XTOPFlash Wnt reporter, and that the Wnt reporter activity was significantly suppressed by treatment with Niclosamide.[45] Therefore, in this study, we used the Super8XTOPFlash Wnt reporter assay in LRP6-expressing HEK293 cells as the primary assay to determine effects of our synthesized compounds on Wnt/β-catenin signaling.

The activity of the phenyl benzimidazole analogs was determined at two concentrations (2 and 10 µM). The active compounds were then tested at 8-12 different concentrations in serial doubling dilutions to obtain an $IC_{50}$ value. Compounds with $IC_{50}$ values less than 10 µM against Wnt/β-catenin signaling in LRP6-expressing HEK293 cells were then tested for cytotoxicity in PDAC cells using standard cell culture conditions [adherent cells in complete medium containing

10% fetal bovine serum (FBS)]. To examine whether the active compounds were able to kill PDAC CICs, cytotoxicity assays were also performed in PDAC cells that were exposed to compound in conditions designed to enrich CICs. All of these results are summarized in Tables 1-4 which include Wnt inhibition and cytotoxicity activity ($IC_{50}$ values) against PDAC Suit-2 and S2VP10 cells under adherent and CIC conditions.

Compounds illustrated in Table 1 mimicked that of Niclosamide, having a 2,5-disubstituted phenyl ring with a hydroxyl group in the 2-position and a halogen (e.g., Cl) in the 5-position. Di-substitution (ortho in relationship to one another) on the benzimidazole moiety mainly included dichloro-substituents while mono-substitution included nitro- (like Niclosamide), chlorine- and trifluoro-methyl groups. Of the di-substituted analogs (Table 1, **1-5),** compound **1** gave the best overall activity profile. Replacing the fluorine substituent of **1** with a chlorine on the benzimidazole ring gave **4** which had a decrease in **both** Wnt inhibition and cytotoxicity in the PDAC cell lines by at least 2-fold. Replacing the 2-hydroxyl group of **4** with a trifluoromethyl group gave **3** which was completely inactive. For similar mono-substituted benzimidazoles (Table 1, **6 – 13**), such as compound **6** and **9**, replacement of the nitro group of **6** with a trifluoromethyl group yielded **9** which had a comparable activity profile to **6**. However, when the 2- hydroxyl, 5-chloro-disubstituted pattern of **9** is reversed to 2-chloro, 5-hydroxyl-to give **12**, no activity was observed. Inactivity was also observed for compound **13** which also has no hydroxyl group in the 2-position. These data indicate that a 2-hydroxy-phenyl group (such as in Niclosamide) for the di- and mono-substituted benzimidazoles is important for activity.

**Table 1. 2,5-Disubstituted Phenyl Benzimidazoles**
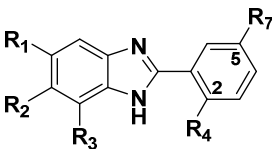


| # | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_7$ | Wnt inhib[a] $IC_{50}$ ($\mu$M) | Suit-2 / $IC_{50}$ ($\mu$M)[b] | | S2VP10 / $IC_{50}$ ($\mu$M)[b] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Adherent | CIC | Adherent | CIC |
| **1** | **F** | **Cl** | **H** | **OH** | **F** | **2.8** | **3.8** | **1.8** | **1.1** | **0.3** |
| 2 | Cl | Cl | H | OH | Cl | 3.8 | >10 | >10 | 1.6 | 0.6 |
| 3 | Cl | Cl | H | $OCF_3$ | F | $IA^c$ | --- | --- | --- | --- |
| 4 | Cl | Cl | H | OH | F | 6.1 | 9.2 | 4.0 | 2.5 | 1.4 |
| 5 | Cl | H | Cl | OH | F | 1.3 | 9.1 | 2.8 | 1.6 | 0.9 |
| **6** | **H** | **NO₂** | **H** | **OH** | **Cl** | **0.9** | **4.8** | **2.3** | **1.1** | **0.5** |
| 7 | H | Cl | H | OH | F | 5.0 | >10 | >10 | >10 | 2.6 |
| 8 | H | NO₂ | H | OH | NO₂ | 3.8 | 4.8 | 2.1 | 2.7 | 0.5 |
| 9 | H | $CF_3$ | H | OH | Cl | 2.1 | 3.4 | 1.5 | 2.7 | 0.2 |
| 10 | H | $CF_3$ | H | OH | F | 3.2 | 8.2 | 6.5 | 2.2 | 1.1 |
| 11 | H | NO₂ | H | OH | F | 1.2 | 0.4 | >10 | >10 | 5.8 |
| 12 | H | NO₂ | H | Cl | OH | $IA^c$ | --- | --- | --- | --- |
| 13 | H | $CF_3$ | H | Cl | OH | $IA^c$ | --- | --- | --- | --- |

See Experimental Section for assay descriptions. [a] Wnt Reporter Assay. Each compound tested in triplicate to give $IC_{50}$ value. [b] *In Vitro* Cytotoxicity Assay. Each compound tested in duplicate to give $IC_{50}$ value.

[c] IA=Inactive.

To further explore the effect of phenylbenzimidazole substitution, a group of 3,4-disubstituted benzimidazoles (Table 2) was evaluated. Overall, the compounds were inactive across all assays for with the exception of **16** which bears a 3-F, 4-OCF$_3$-substitution pattern and showed only modest activity across assays.

**Table 2. 3,4-Disubstituted Phenyl Benzimidazoles**
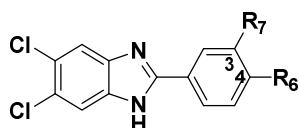


| # | R$_6$ | R$_7$ | Wnt inhib[a] IC$_{50}$ (μM) | Suit-2 / IC$_{50}$ (μM)[b] | | S2VP10 / IC$_{50}$ (μM)[b] | |
|---|---|---|---|---|---|---|---|
| | | | | Adherent | CIC | Adherent | CIC |
| 14 | OCH$_3$ | OCH$_3$ | IA[c] | --- | --- | --- | --- |
| 15 | OCH$_3$ | F | IA[c] | --- | --- | --- | --- |
| 16 | OCF$_3$ | F | 7.7 | 4.2 | 1.0 | 3.6 | 0.9 |
| 17 | F | OCF$_3$ | IA[c] | --- | --- | --- | --- |
| 18 | Cl | OCF$_3$ | IA[c] | --- | --- | --- | --- |
| 19 | OCH$_3$ | OCF$_3$ | IA[c] | --- | --- | --- | --- |

[a, b, c] See Table 1 notes.

Since variations of di-substitutions on the phenyl ring significantly affected the activity of the compounds (Tables 1 and 2), we prepared a series of compounds with a single substitution at the 4-position of the phenyl ring (Table 3). From this selection of compounds, it was observed that the 4-OCF$_3$ group was preferred for both di- and mono-substituted benzimidazoles (**20**, **28**, and **30**), with compound **20** having significant Wnt inhibition and activity in both PDAC cell lines. Keeping this 4-OCF$_3$ phenyl substitution constant, a variety of aryl substituted benzimidazoles (**31-39**) showed no activity.

**Table 3. 4-Substituted Phenyl Benzimidazoles**
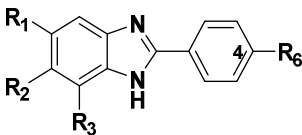


| # | $R_1$ | $R_2$ | $R_3$ | $R_6$ | Wnt inhib[a] $IC_{50}$ (μM) | Suit-2 / $IC_{50}$ (μM)[b] | | S2VP10 / $IC_{50}$ (μM)[b] | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Adherent | CIC | Adherent | CIC |
| **20** | **Cl** | **Cl** | **H** | **OCF₃** | **0.4** | **3.7** | **1.6** | **3.7** | **1.6** |
| 21 | Cl | Cl | H | CF₃ | 1.1 | 3.7 | 0.7 | 3.3 | 0.8 |
| 22 | Cl | Cl | H | F | IA[c] | --- | --- | --- | --- |
| 23 | Cl | Cl | H | Me | 3.0 | 3.0 | 2.0 | 3.7 | 2.8 |
| 24 | Cl | Cl | H | OMe | IA[c] | --- | --- | --- | --- |
| 25 | Cl | Cl | H | N(Me)₂ | IA[c] | --- | --- | --- | --- |
| 26 | Cl | H | Cl | CF₃ | 6.0 | 4.5 | 0.6 | 3.4 | 0.8 |
| 27 | Cl | H | Cl | OCF₃ | 7.9 | 7.7 | 0.8 | 5.4 | 0.8 |
| **28** | **CF₃** | **H** | **Cl** | **OCF₃** | **1.7** | **1.4** | **0.3** | **2.5** | **0.4** |
| 29 | H | Cl | H | CF₃ | 3.0 | 2.4 | 1.4 | 2.3 | 1.6 |
| **30** | **H** | **Cl** | **H** | **OCF₃** | **1.7** | **2.6** | **2.1** | **2.5** | **1.6** |
| 31 | 3-F-Phenyl | H | H | OCF₃ | IA[c] | --- | --- | --- | --- |
| 32 | 4-F-Phenyl | H | H | OCF₃ | IA[c] | --- | --- | --- | --- |
| 33 | 3-*t*-Bu-Phenyl | H | H | OCF₃ | IA[c] | --- | --- | --- | --- |
| 34 | 3-Me-Pyridine | H | H | OCF₃ | IA[c] | --- | --- | --- | --- |
| 35 | 3-Pyridine | H | H | OCF₃ | IA[c] | --- | --- | --- | --- |
| 36 | 4-Cl-Phenyl | H | H | OCF₃ | IA[c] | --- | --- | --- | --- |
| 37 | 4-Pyrazole | H | H | OCF₃ | IA[c] | --- | --- | --- | --- |

| 38 | 4-*N*-Me-Pyrazole | H | H | OCF$_3$ | IA$^c$ | --- | --- | --- | --- |
| 39 | 5-Pyrimidine | H | H | OCF$_3$ | IA$^c$ | --- | --- | --- | --- |

$^{a, b, c}$ See Table 1 notes.

This data seems to indicate that a 3,4-dichloro-substituted benzimidazole and a 4-OCF$_3$–substituted phenyl are optimal for activity across all the assays (Table 3, **20**). Keeping the di-chloro-substituted benzimidazole ring constant, the 4-OCF$_3$-phenyl substituent was replaced with a variety of cyclic amino groups, such as *N*-Me-piperazine, morpholine, pyrrolidine, imidazole and azapane (Table 4). A cycloalkyl group could help evaluate the size that can be tolerated in the 4-phenyl position and a nitrogen atom within the ring could potentially improve the solubility and affect the metabolic stability. Once again, compounds with mono- and di-substitutions (e.g., **40**, **43**, **48**, **53**, **58**, Table 4) exhibited activity across all the assays. However, the Wnt inhibition for these cyclic amino analogs was significantly improved and ranged from 5-fold to 200-fold more potent than **20.** The compounds also had similar, if not better activity across all cell based cytotoxicity assays. The most potent compounds versus Wnt inhibition are **43** and **48, (**piperidine and pyrrolidine analogs, respectively), with each compound demonstrating an IC$_{50}$ = 2 nM and having similar PDAC cellular activity that ranges from approximately 1-1.5 μM across all cell lines. Another amine variant, **53**, an azapane derivative, and keeping the di-Cl-substituted benzimidazole, maintained significant Wnt inhibition (IC$_{50}$ = 5 nM) as well as moderate cellular activity.

We continued in this vein and focused on the pyrrolidine analog, **48**. First we replaced the di-Cl-substitution on the benzimidazole ring with -CF$_3$, Cl to give **52** which was totally inactive. This loss of activity was also observed when the di-Cl substitution of **40** was replaced with -CF$_3$, -Cl to give inactive **46**. In addition to the benzimidazole substitution having an effect

on activity, various cyclic amines contributed to a range of activities, such that replacing a mono-

nitrogen ring (such as piperidine **43** and pyrrolidine **48)** with a di-nitrogen ring (such as

piperazine **41**, morpholine **44** and imidazole **49**) completely abolished all activity.

**Table 4. 4-Aryl-Substituted Phenyl Benzimidazoles**
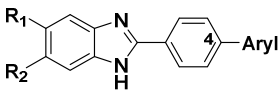


| # | $R_1$ | $R_2$ | Aryl | Wnt inhib[a] IC$_{50}$ (μM) | Suit-2 / IC$_{50}$(μM)[b] | | S2VP10 / IC$_{50}$ (μM)[b] | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Adherent | CIC | Adherent | CIC |
| **40** | **Cl** | **Cl** | *N*-Me-Piperazine | **0.018** | **5.3** | **1.5** | **5.2** | **1.3** |
| 41 | Cl | Cl | Piperazine | IA[c] | --- | --- | --- | --- |
| 42 | Cl | Cl | *N*-Et-Piperazine | IA[c] | --- | --- | --- | --- |
| **43** | **Cl** | **Cl** | **Piperidine** | **0.002** | **1.2** | **1.1** | **1.5** | **0.9** |
| 44 | Cl | Cl | Morpholine | IA[c] | --- | --- | --- | --- |
| 45 | CF$_3$ | F | *N*-Me-Piperazine | IA[c] | --- | --- | --- | --- |
| 46 | CF$_3$ | Cl | *N*-Me-Piperazine | IA[c] | --- | --- | --- | --- |
| 47 | F | Cl | *N*-Me-Piperazine | IA[c] | --- | --- | --- | --- |
| **48** | **Cl** | **Cl** | **Pyrrolidine** | **0.002** | **1.7** | **1.2** | **2.0** | **1.5** |
| 49 | Cl | Cl | Imidazole | IA[c] | --- | --- | --- | --- |
| 50 | Cl | Cl | 2-Me-Imidazole | IA[c] | --- | --- | --- | --- |
| 51 | F | Cl | Pyrrolidine | 0.33 | 0.8 | 0.7 | 0.6 | 0.6 |
| 52 | CF$_3$ | Cl | Pyrrolidine | IA[c] | --- | --- | --- | --- |
| **53** | **Cl** | **Cl** | **Azapane** | **0.005** | **5.9** | **2.1** | **7.6** | **1.4** |
| 54 | H | F | Piperidine | 0.21 | 0.2 | 0.2 | 0.2 | 0.2 |
| 55 | H | CF$_3$ | *N*-Me-Piperazine | IA[c] | --- | --- | --- | --- |
| 56 | H | F | *N*-Me-Piperazine | IA[c] | --- | --- | --- | --- |
| 57 | H | CF$_3$ | Piperidine | IA[c] | --- | --- | --- | --- |

| 58 | H | F | Pyrrolidine | 0.087 | 0.3 | 0.3 | 0.3 | 0.2 |
| 59 | H | CF$_3$ | Pyrrolidine | IA$^c$ | --- | --- | --- | --- |

$^{a, b, c}$ See Table 1 notes.

Several readily available mono-substituted benzimidazoles were also tested (**54 – 59**, Table 4) against Wnt and PDAC cells. Interestingly, the mono-substituted benzimidazole compounds were about 50-to100-fold less active (**58**, IC$_{50}$ = 87 nM and **54**, IC$_{50}$ = 210 nM) versus the comparable di-substituted analogs (**48**, IC$_{50}$ = 2 nM and **43**, IC$_{50}$ = 2 nM), but still exhibiting significant Wnt inhibition and had increased activity against PDAC cells. These data in combination with the data described above of the di-substituted analogs may indicate that size and electronic effects could be a key component in the activity of these benzimidazoles.

Many of the benzimidazoles described overall have a better activity profile than Niclosamide. For example, the cyclic amine analogs (**43** and **48**) are very potent, in that they are 100-fold more potent than Niclosamide) and overall display better cellular activity. Using Niclosamide as a starting point, we were able to design a series of novel and potent benzimidazoles (Figure 1). The analogs reported have several advantages over Niclosamide. In addition to having potent Wnt inhibitory and cellular activity, several of the compounds e.g., **43**, also were shown to be less cytotoxic than Niclosamide in non-cancerous cells and also had diminished activity on other signaling, [48-50] such as STAT3 and mTORC1. [57]

In addition to differentiating the benzimidazoles from Niclosamide, data was generated to support that the inhibition of Wnt/β-catenin signaling is responsible for the observed anticancer activity. For example, **41** (Table 4), is inactive against Wnt/β-catenin signaling and had very weak activity against pancreatic cancer Suit-2 and S2VP10 cell proliferation (See Supplemental Figure S1), whereas compounds that were active against Wnt/β-catenin signaling (e.g., **43, 48,**

**53, 54, 58**, Table 40) were very effective against pancreatic cancer Suit-2 and S2VP10 cell proliferation. These data provides evidence that inhibition of Wnt/β-catenin signaling does result in the inhibition of cancer cell growth, and adds support to Wnt/β-catenin signaling as an attractive target for pancreatic cancer therapy.

We evaluated the ADME (absorption, distribution, metabolism, and excretion) properties of several cyclic amine benzimidazoles to determine solubility, LogD and metabolic stability in both mouse and human microsomes (Table 5). These data provide information to further guide lead optimization, with the ultimate goal of testing compounds in animals and identification of a preclinical candidate. We are targeting solubility of greater than 10 μM (in pH 7.4 buffer), Log D determinations within a range of 2-4, and microsomal stability of approximately 60 min half-life for early chemical leads but a half-life of >2-hours for compounds to be evaluated *in vivo*.

**Table 5. ADME Properties of Selected 4-Aryl Substituted Phenyl Benzimidazoles**

| # | Solubility[d] (μM) | LogD[d] | Microsome Stability Mouse t ½ (min)[d] | Microsome Stability Human t ½ (min)[d] | Wnt inhib[a] $IC_{50}$(μM) | Suit-2 / $IC_{50}$(μM)[b] | | S2VP10 / $IC_{50}$ (μM)[b] | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Adherent | CIC | Adherent | CIC |
| 40 | 2.4 | 5 | 10 | 57 | 0.018 | 5.3 | 1.5 | 5.2 | 1.3 |
| 43 | 1.2 | 3 | 16 | 39 | 0.002 | 1.2 | 1.1 | 1.5 | 0.9 |
| 48 | 1.2 | 3 | 26 | 35 | 0.002 | 1.7 | 1.2 | 2.0 | 1.5 |
| 51 | 1.4 | 3.3 | 10 | 18 | 0.33 | 0.8 | 0.7 | 0.6 | 0.6 |
| 53 | 1.2 | 3.2 | 24 | 37 | 0.005 | 5.9 | 2.1 | 7.6 | 1.4 |
| 58 | 2.0 | 4 | 5 | 9 | 0.087 | 0.3 | 0.3 | 0.3 | 0.2 |

*a, b* See Table 1 notes. *d*See Experimental Section for assay descriptions.

The compounds containing a di-chloro-substitution pattern on the phenyl benzimidazole ring (**43**, **48**, and **53**) versus a fluoro-chloro- (**51**) and mono-fluoro- (**58**) substituted analogs had better stability in mouse microsomes. These data are supported by having less sites on the ring

(di-substitution, **43**, **48**, **53** versus **58**) or larger groups Cl *vs* F (**48** versus **51**) which allows for less metabolism. In addition, **43**, **48**, and **53** do not have an *N*-methyl group as in **40** which can be readily metabolized. The log D values for most analogs fall within the desired range of 2-4, but the solubility is low for these compounds (3 and 5 μM). Increasing solubility to above 10 μM would be preferred and would help with identifying acceptable formulations for animal efficacy studies.

Future work on this series of compounds will focus on improving the solubility by synthesizing compounds with polar substituents on the benzimidazole framework and/or to the phenyl ring while maintaining the activity profile observed for these compounds. Also, microsomal stability will need to be improved to a $t\frac{1}{2} > 60$ min. and can be achieved by exploring a variety of different substituents on the benzimidazoles. These could include bulkier groups, additional rings, or multiple substituents. Thus, a desired candidate to pursue for preclinical development would have a similar potency and activity profile as **43** or **48**, solubility at least 10 μM and a half-life of greater than 60 min in mouse microsomal stability.

**CONCLUSION**

This study of the benzimidazole compounds presented here led us to a select group of cyclic amine benzimidazole analogs (Table 4) that are the most promising novel inhibitors within this chemical series. These cyclic amines offer a significant increase in potency in inhibiting Wnt/β-catenin signaling and overall cellular cytotoxicity across the pancreatic cell lines versus the other modifications as illustrated in Tables 1-3.

Importantly, **43** and **48** are very potent inhibitors with $IC_{50}$ values of 2 nM against Wnt/β-catenin signaling in LRP6-expressing HEK293 cells, and potent activity against PDAC ranging

from 1-1.5 μM under both adherent and CIC conditions. Unlike Niclosamide which inhibits multiple signaling pathways, **43**, for example, has no effect on STAT3 and mTORC1 signaling and has an improved profile versus non-cancerous cells.[57] In addition, the lead compounds **43** and **48** have improved drug-like properties versus Niclosamide and can be further optimized. We also noted that compounds **20**, **28** and **30** exhibit less of an inhibition of Wnt/β-catenin signaling with IC$_{50}$ values of 0.4 – 1.7 μM, but display similar potency against PDAC cell viability when compared to compounds **43** and **48** which may suggest that compounds **20**, **28** and **30** could mimic Niclosamide and thus inhibit other signaling pathways. More studies in the future are required to address this issue.

In summary, the study of various substituents on these benzimidazoles indicated that di-substitution of the benzimidazole ring as well as the phenyl ring resulted in compounds with inhibition of the Wnt/β-catenin pathway as well as activity against the PDAC cell lines tested. By constraining the amide functionality of Niclosamide into a ring (Figure 1), a series of benzimidazoles was identified that can be for further studied *in vivo* in human pancreatic cancer and other Wnt-dependent cancers. These efforts will lend to the continued search for a novel therapeutic for the treatment of PDAC.

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## EXPERIMENTAL SECTION

### Chemistry General Procedures and Synthetic Methods.

The reactions were performed under a dry argon atmosphere and reaction temperatures were measured externally. Anhydrous solvents over molecular sieves were purchased from Fluka and used as such in reactions. Reactions were performed in CEM Discover Labmate System with

Intelligent Technology for FocusedTM Microwave Synthesizer (Explorer 48). The reactions

were monitored by thin-layer chromatography (TLC) on pre-coated silica gel ($60F_{254}$) aluminium

plates (0.25 mm) from E. Merck and visualized using UV light (254 nm). Purification of

compounds was performed on an Isco Teledyne Combiflash Rf200 with four channels to

carryout sequential purification. Universal RediSep solid sample loading pre-packed cartridges

(5.0 g silica) were used to absorb crude product and purified on 12 g silica RediSep Rf Gold

Silica (20–40 μm spherical silica) columns using appropriate solvent gradients. Pure samples

were dried overnight under high vacuum over $P_2O_5$ at 78$^\circ$C before analyses. The HR–mass

spectral data were obtained on an Agilent LC-MSTOF by electrospray ionization (ESI). [1]H

NMR spectra were recorded at 400 MHz on Agilent/Varian MR-400 spectrometer in DMSO-$d_6$

as solvent. The chemical shifts (δ) are in ppm downfield from standard tetramethylsilane (TMS).

Purity of final compounds was checked by HPLC using Agilent 1100 LC equipped with a diode

array UV detector and monitored at multiple wavelengths on Bondclone 10μ C18 column using

Solvent A: $H_2O$, solvent B: MeOH, 1.0 mL/min; 30 min linear gradient from 10–90% B or on

Waters HPLC equipped with a 3100 Mass Detector using Sunfire C18 column (5μm, 4.6x150

mm) using ACN-$H_2O$ (both containing 0.1% formic acid) 10-90% in 15 min. Final compounds

are ≥95% pure.

**Method A (Scheme 1).** A mixture of an appropriate 1,2-phenylenediamine (1.0 eq), appropriate

aldehyde (1.0 eq), and sodium bisulfite (1.0 eq) in 10 mL of DMSO was heated at 210$^\circ$C for 1 h.

The reaction mixture was filtered, concentrated *in vacuo* and purified on a purification system

using 10-50% hexanes and EtOAc.

**Method B (Scheme 2).** A mixture of an appropriate 1,2-phenylenediamine (1.0 eq), appropriate

aldehyde (1.0 eq), and sodium metabisulfite (1.0 eq) in 8 mL of *N,N*--Dimethylformamide

(DMF) was heated in a CEM Discover Labmate System with Intelligent Technology for Focused™ Microwave Synthesizer (Explorer 48) microwave at 170°C for 15 min. The reaction mixture was filtered, concentrated *in vacuo* and purified on a purification system using 10-50% hexanes and EtOAc.

**Method C (Scheme 3).** To a stirred solution of 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole (0.14 mmol) in dioxane/water (4 mL/0.4 mL) was added an appropriately substituted boronic acid or boronic ester (0.28 mmol) and potassium carbonate (38.7 mg, 0.28 mmol). The reaction mixture was purged with argon and stirred for 15 min at room temperature. Tetrakis-triphenylphosphine palladium (0.02 mg, 0.01 mmol) was added and the reaction mixture was heated in microwave at 80°C for 1h. The reaction mixture was then cooled to room temperature, diluted with water (10 mL) and extracted with ethyl acetate (2 x10 mL). The organic layer was separated, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue obtained was purified on ISCO purification system using hexanes–EtOAc (0–70%) as eluent to obtain the desired product.

**2-(5-Chloro-6-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-4-fluorophenol (1).** This compound was prepared from 4-chloro-5-fluorobenzene-1,2-diamine and 5-fluoro-2-hydroxybenzaldehyde using Method A. Yield 64%. TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 12.60 (s, 1H), 8.54 (s, 1H), 8.22 – 8.11 (m, 2H), 7.82 (d, $J$ = 8.9 Hz, 1H), 7.24 – 7.09 (m, 1H). HRMS *m/z* calcd for $C_{13}H_7ClF_2N_2O$ [M+H]$^+$: 280.0215, found: 280.0215.

**4-Chloro-2-(5,6-dichloro-1*H*-benzo[*d*]imidazol-2-yl)phenol (2).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 5-chloro-2-hydroxybenzaldehyde using Method B. Yield 92%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 12.99 (s, 1H), 8.24 (s, 1H), 7.95 (s, 1H), 7.70 (q, $J$ = 1.8 Hz, 1H), 7.45 - 7.40 (m, 2H), 7.08 (dd, $J$ = 8.9,

2.4 Hz, 1H).  HRMS $m/z$ calcd for $C_{13}H_7Cl_3N_2O$ [M+H]$^+$: 312.9697, found: 312.9696.  HPLC: 100% ($t_R$ = 3.05 min).

**5,6-Dichloro-2-(5-fluoro-2-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole** **(3).**

This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 5-fluoro-2-(trifluoromethoxy)benzaldehyde using Method B.  Yield 27%.  TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.95 - 7.87 (m, 2H), 7.73 - 7.64 (m, 1H), 7.56 (ddd, $J$ = 9.1, 7.7, 3.2 Hz, 1H), 3.22 (s, 1H).  HRMS $m/z$ calcd for $C_{14}H_6Cl_2F_4N_2O$ [M+H]$^+$: 363.9793, found: 363.9795.  HPLC: 95% ($t_R$ = 2.75 min).

**2-(5,6-Dichloro-3*H*-benzo[*d*]imidazol-2-yl)-4-fluorophenol (4).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 5-fluoro-2-hydroxybenzaldehyde using Method A. Yield 17%.  TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.24 (s, 1H), 7.14 (dd, $J$ = 8.9, 3.2 Hz, 1H), 7.06 (dd, $J$ = 9.1, 4.8 Hz, 1H), 6.99 (dd, $J$ = 9.0, 4.6 Hz, 1H), 6.86 (td, $J$ = 8.6, 3.1 Hz, 1H), 6.70 (dd, $J$ = 8.9, 4.8 Hz, 1H), 6.34 (dd, $J$ = 9.2, 3.1 Hz, 1H). HRMS $m/z$ calcd for $C_{13}H_7Cl_2FN_2O$ [M+H]$^+$: 296.9992, found: 296.9993.

**2-(5,7-Dichloro-3*H*-benzo[*d*]imidazol-2-yl)-4-fluorophenol (5).** This compound was prepared from 3,5-dichlorobenzene-1,2-diamine and 5-fluoro-2-hydroxybenzaldehyde using Method A. Yield 21%.  TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.40 (s, 1H), 7.95 (s, 1H), 7.71 (s, 1H), 7.48 (s, 2H), 7.30 (ddd, $J$ = 9.2, 8.1, 3.1 Hz, 1H), 7.09 (dd, $J$ = 9.1, 4.7 Hz, 1H).  HRMS $m/z$ calcd for $C_{13}H_7Cl_2FN_2O$ [M+H]$^+$: 296.9992, found: 296.9993.

**4-Chloro-2-(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)phenol (6).** This compound was prepared from 4-nitrobenzene-1,2-diamine and 5-chloro-2-hydroxybenzaldehyde using Method A. Yield 45%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.60 – 8.55 (m, 2H),

8.25 – 8.14 (m, 2H), 7.85 (d, $J$ = 8.9 Hz, 1H), 7.48 (dd, $J$ = 8.8, 2.7 Hz, 1H), 7.13 (d, $J$ = 8.8 Hz, 1H), 2.09 (s, 1H).  HRMS $m/z$ calcd for $C_{13}H_8ClN_3O_3$ [M+H]$^+$: 290.0327, found: 290.0326.

**2-(5-Chloro-1$H$-benzo[$d$]imidazol-2-yl)-4-fluorophenol (7).**  This compound was prepared from 4-chlorobenzene-1,2-diamine and 5-fluoro-2-hydroxybenzaldehyde using Method A.  Yield 10%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.73 (s, 2H), 7.92 (dd, $J$ = 9.7, 3.1 Hz, 1H), 7.76 (s, 1H), 7.69 (d, $J$ = 8.6 Hz, 1H), 7.35 – 7.22 (m, 2H), 7.07 (dd, $J$ = 9.1, 4.8 Hz, 1H).   HRMS $m/z$ calcd for $C_{13}H_8ClFN_2O$ [M+H]$^+$: 263.0382, found: 263.0383.

**4-Nitro-2-(5-nitro-1$H$-benzo[$d$]imidazol-2-yl)phenol (8).** This compound was prepared from 4-nitrobenzene-1,2-diamine and 2-hydroxy-5-nitrobenzaldehyde using Method A. Yield 78%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.65 (s, 2H), 9.14 (d, $J$ = 2.9 Hz, 1H), 8.61 (d, $J$ = 2.2 Hz, 1H), 8.29 (dd, $J$ = 9.2, 2.9 Hz, 1H), 8.19 (dd, $J$ = 8.9, 2.2 Hz, 1H), 7.88 (d, $J$ = 8.9 Hz, 1H), 7.25 (d, $J$ = 9.2 Hz, 1H).   HRMS $m/z$ calcd for $C_{13}H_8N_4O_5$ [M+H]$^+$: 301.0568, found: 301.0569.

**4-Chloro-2-(5-(trifluoromethyl)-1$H$-benzo[$d$]imidazol-2-yl)phenol (9).** This compound was prepared from 4-(trifluoromethyl)benzene-1,2-diamine and 5-chloro-2-hydroxybenzaldehyde using Method A. Yield 45%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 2:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.08 (s, 1H), 8.22 (d, $J$ = 2.6 Hz, 1H), 8.07 (s, 1H), 7.87 (d, $J$ = 8.4 Hz, 1H), 7.61 (dd, $J$ = 8.6, 1.7 Hz, 1H), 7.46 (dd, $J$ = 8.8, 2.6 Hz, 1H), 7.11 (d, $J$ = 8.8 Hz, 1H). HRMS $m/z$ calcd for $C_{14}H_8ClF_3N_2O$ [M+H]$^+$: 313.0350, found: 313.0349.

**4-Fluoro-2-(6-(trifluoromethyl)-3$H$-benzo[$d$]imidazol-2-yl)phenol                    (10).** This compound was prepared from 4-(trifluoromethyl)benzene-1,2-diamine and 5-fluoro-2-

hydroxybenzaldehyde using Method A. Yield 26%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1). [1]H

NMR (400 MHz, DMSO-$d_6$) δ 13.40 (s, 1H), 8.07 (s, 1H), 7.96 (dd, $J$ = 9.6, 3.1 Hz, 1H), 7.90 –

7.83 (m, 1H), 7.61 (dd, $J$ = 8.5, 1.8 Hz, 1H), 7.30 (ddd, $J$ = 9.2, 8.1, 3.1 Hz, 1H), 7.10 (dd, $J$ =

9.1, 4.7 Hz, 1H). HRMS $m/z$ calcd for $C_{14}H_8F_4N_2O$ [M+H]$^+$: 297.0646, found: 297.0645.

HPLC: 100% ($t_R$ = 2.85 min).

**4-Fluoro-2-(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)phenol (11).** This compound was prepared

from 4-nitrobenzene-1,2-diamine and 5-fluoro-2-hydroxybenzaldehyde using Method A. Yield

56%. TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 13.50 (s, 1H),

8.16 (d, $J$ = 8.9 Hz, 2H), 7.95 (dd, $J$ = 9.7, 3.1 Hz, 1H), 7.83 (d, $J$ = 8.7 Hz, 1H), 7.30 (ddd, $J$ =

9.1, 8.1, 3.2 Hz, 1H), 7.10 (dd, $J$ = 9.1, 4.7 Hz, 1H). HRMS $m/z$ calcd for $C_{13}H_8FN_3O_3$ [M+H]$^+$:

273.0550, found: 273.0548. HPLC: 100% ($t_R$ = 2.30 min).

**4-Chloro-3-(6-nitro-3*H*-benzo[*d*]imidazol-2-yl)phenol (12).** This compound was prepared

from 4-nitrobenzene-1,2-diamine and 2-chloro-5-hydroxybenzaldehyde using Method A. Yield

24%. TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 13.35 (s, 1H),

8.32 – 8.24 (m, 2H), 7.88 (s, 2H), 7.58 (dq, $J$ = 7.8, 1.1 Hz, 2H). HRMS $m/z$ calcd for

$C_{13}H_8ClN_3O_3$ [M+H]$^+$: 289.0254, found: 289.0254. HPLC: 99% ($t_R$ = 1.84 min).

**4-Chloro-3-(6-(trifluoromethyl)-3*H*-benzo[*d*]imidazol-2-yl)phenol (13).**

This compound was prepared from 4-(trifluoromethyl)benzene-1,2-diamine and 2-chloro-5-

hydroxybenzaldehyde using Method A. Yield 21%. TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1). [1]H

NMR (400 MHz, DMSO-$d_6$) δ 13.38 (s, 1H), 8.30 – 8.22 (m, 2H), 7.85 (s, 2H), 7.56 (dq, $J$ = 7.8,

1.1 Hz, 2H). HRMS $m/z$ calcd for $C_{14}H_8ClF_3N_2O$ [M+H]$^+$: 312.0277, found: 312.0277.

**5,6-Dichloro-2-(3,4-dimethoxyphenyl)-1*H*-benzo[*d*]imidazole (14).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 3,4-dimethoxybenzaldehyde using Method A. Yield 4.0%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.06 (s, 1H), 7.24 – 7.15 (m, 1H), 7.06 (s, 1H), 7.00 (dd, $J$ = 8.2, 2.0 Hz, 1H), 6.94 – 6.87 (m, 1H), 6.78 (t, $J$ = 7.3 Hz, 1H), 3.79 (d, $J$ = 8.1 Hz, 3H), 3.44 (t, $J$ = 5.1 Hz, 3H). HRMS *m/z* calcd for $C_{15}H_{12}Cl_2N_2O_2$ [M+H]$^+$: 322.0276, found: 322.0277.

**5,6-Dichloro-2-(3-fluoro-4-methoxyphenyl)-1*H*-benzo[*d*]imidazole (15).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 3-fluoro-4-methoxybenzaldehyde using Method B. Yield 20%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.02 - 7.93 (m, 3H), 7.81 (s, 2H), 7.37 (t, $J$ = 8.9 Hz, 1H), 3.93 (s, 3H). HRMS *m/z* calcd for $C_{14}H_9Cl_2FN_2O$ [M+H]$^+$: 311.0149, found: 311.0149. HPLC: 100% ($t_R$ = 2.42 min).

**5,6-Dichloro-2-(3-fluoro-4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole (16).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 3-fluoro-4-(trifluoromethoxy)benzaldehyde using Method B. Yield 12%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.50 (s, 1H), 8.24 (dt, $J$ = 11.4, 1.6 Hz, 1H), 8.16 - 8.08 (m, 1H), 7.98 - 7.85 (m, 1H), 7.92 (s, 1H), 7.89 - 7.76 (m, 1H). HRMS *m/z* calcd for $C_{14}H_6Cl_2F_4N_2O$ [M+H]$^+$: 364.9837, found: 364.9838. HPLC: 100% ($t_R$ = 2.80 min).

**5,6-Dichloro-2-(4-fluoro-3-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole (17).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-fluoro-3-(trifluoromethoxy)benzaldehyde using Method B. Yield 39%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.34 - 8.21 (m, 2H), 7.90 (d, $J$ = 1.4 Hz, 2H), 7.75 (ddd,

$J$ = 10.1, 8.7, 1.4 Hz, 1H).   HRMS $m/z$ calcd for $C_{14}H_6Cl_2F_4N_2O$ [M+H]$^+$: 364.9866, found: 364.9866.  HPLC: 100% ($t_R$ = 2.4 min).

**5,6-Dichloro-2-(4-chloro-3-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole   (18).**   This compound   was   prepared   from   4,5-dichlorobenzene-1,2-diamine   and   4-chloro-3-(trifluoromethoxy)benzaldehyde using Method B. Yield 51%.  TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.30 (s, 1H), 8.22 (dd, $J$ = 8.5, 2.0 Hz, 1H), 7.93 (d, $J$ = 9.0 Hz, 1H), 7.91 (s, 2H).   HRMS $m/z$ calcd for $C_{14}H_6Cl_3F_3N_2O$ [M+H]$^+$: 380.9570, found: 380.9569.  HPLC: 100% ($t_R$ = 2.91 min).

**5,6-Dichloro-2-(4-methoxy-3-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole   (19).**   This compound   was   prepared   from   4,5-dichlorobenzene-1,2-diamine   and   4-methoxy-3-(trifluoromethoxy)benzaldehyde using Method B. Yield 42%.  TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.23 - 8.10 (m, 2H), 7.84 (s, 2H), 7.46 (dd, $J$ = 8.8, 1.3 Hz, 1H), 3.96 (d, $J$ = 1.3 Hz, 3H).   HRMS $m/z$ calcd for $C_{15}H_9Cl_2F_3N_2O_2$ [M+H]$^+$: 375.9993, found: 375.9994.  HPLC: 100% ($t_R$ = 2.68 min).

**5,6-Dichloro-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole   (20).**  This compound was   prepared   from   4,5-dichlorobenzene-1,2-diamine   and   4-(trifluoromethoxy)benzaldehyde using Method A. Yield 47%.   TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1).   $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.35 (s, 1H), 8.32 – 8.24 (m, 2H), 7.88 (s, 2H), 7.58 (dq, $J$ = 7.8, 1.1 Hz, 2H).  HRMS $m/z$ calcd for $C_{14}H_7Cl_2F_3N_2O$ [M+H]$^+$: 345.9888, found: 345.9879.  HPLC: 99% ($t_R$ = 2.67 min).

**5,6-Dichloro-2-(4-(trifluoromethyl)phenyl)-1*H*-benzo[*d*]imidazole                           (21).**   This   compound   was   prepared   from   4,5-dichlorobenzene-1,2-diamine   and   4-

(trifluoromethyl)benzaldehyde using Method A. Yield 42%. TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.30 (s, 1H), 8.30 – 8.21 (m, 2H), 7.85 (s, 2H), 7.55 (dq, $J$ = 7.8, 1.1 Hz, 2H). HRMS $m/z$ calcd for $C_{14}H_7Cl_2F_3N_2$ [M+H]$^+$: 329.9938, found: 329.9933. HPLC: 99% ($t_R$ = 2.51 min).

**5,6-Dichloro-2-(4-fluorophenyl)-1$H$-benzo[$d$]imidazole (22).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-fluorobenzaldehyde using Method A. Yield 24%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.24 (s, 1H), 8.25 – 8.16 (m, 2H), 7.92 (s, 1H), 7.75 (s, 1H), 7.46 – 7.37 (m, 2H). HRMS $m/z$ calcd for $C_{13}H_7Cl_2FN_2$ [M+H]$^+$: 279.9970, found: 279.9971. HPLC: 100% ($t_R$ = 2.23 min).

**5,6-Dichloro-2-(p-tolyl)-1$H$-benzo[$d$]imidazole (23).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-methylbenzaldehyde using Method A. Yield 23%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.13 (s, 1H), 8.08 – 8.00 (m, 1H), 7.89 (s, 2H), 7.72 (s, 2H), 7.37 (d, $J$ = 8.0 Hz, 1H), 2.38 (s, 3H). HRMS $m/z$ calcd for $C_{14}H_{10}Cl_2N_2$ [M+H]$^+$: 276.0221, found: 276.0221. HPLC: 95% ($t_R$ = 2.19 min).

**5,6-Dichloro-2-(4-methoxyphenyl)-1$H$-benzo[$d$]imidazole (24).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-methoxybenzaldehyde using Method A. Yield 14%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.05 (s, 1H), 8.14 – 8.05 (m, 2H), 7.86 (s, 1H), 7.69 (s, 1H), 7.15 – 7.07 (m, 2H), 3.83 (d, $J$ = 0.5 Hz, 3H). HRMS $m/z$ calcd for $C_{14}H_{10}Cl_2N_2O$ [M+H]$^+$: 292.0170, found: 292.0169. HPLC: 95% ($t_R$ = 2.09 min).

**4-(5,6-Dichloro-1H-benzo[d]imidazol-2-yl)-N,N-dimethylaniline (25).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(dimethylamino)benzaldehyde using

Method A. Yield 25%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$)

δ 12.81 (s, 1H), 8.00 – 7.93 (m, 1H), 7.78 (s, 1H), 7.62 (s, 2H), 6.86 – 6.79 (m, 2H), 2.99 (s, 6H).

HRMS $m/z$ calcd for $C_{15}H_{13}Cl_2N_3$ [M+H]$^+$: 305.0847, found: 305.0849.

**4,6-Dichloro-2-(4-(trifluoromethyl)phenyl)-1*H*-benzo[*d*]imidazole (26).** This compound was

prepared from 3,5-dichlorobenzene-1,2-diamine and 4-(trifluoromethyl)benzaldehyde using

Method A. Yield 73%. TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1). ^{1}H NMR (400 MHz, DMSO-$d_6$)

δ 13.35 (s, 1H), 8.41 (s, 2H), 7.96 (d, $J$ = 8.1 Hz, 2H), 7.78 – 7.32 (m, 2H). HRMS $m/z$ calcd for

$C_{14}H_7Cl_2F_3N_2$ [M+H]$^+$: 329.9938, found: 329.9938.

**4,6-Dichloro-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole (27).** This compound

was prepared from 3,5-dichlorobenzene-1,2-diamine and 4-(trifluoromethoxy)benzaldehyde

using Method A. Yield 52%. TLC $R_f$ = 0.45 (Hexanes–EtOAc, 2:1). ^{1}H NMR (400 MHz,

DMSO-$d_6$) δ 8.33 (d, $J$ = 8.5 Hz, 4H), 7.58 (d, $J$ = 8.5 Hz, 2H). HRMS $m/z$ calcd for

$C_{14}H_7Cl_2F_3N_2O$ [M+H]$^+$: 345.9886, found: 345.9888. HPLC: 98% ($t_R$ = 2.57 min).

**7-Chloro-2-(4-(trifluoromethoxy)phenyl)-5-(trifluoromethyl)-1*H* benzo[*d*]imidazole (28).**

This compound was prepared from 3-chloro-5-(trifluoromethyl)benzene-1,2-diamine and 4-

(trifluoromethoxy)benzaldehyde using Method B. Yield 19%. TLC $R_f$ = 0.35 (Hexanes–EtOAc,

1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 8.40 - 8.33 (m, 2H), 7.89 (s, 1H), 7.68 - 7.56 (m, 3H).

HRMS $m/z$ calcd for $C_{15}H_7ClF_6N_2O$ [M+H]$^+$: 381.0224, found: 381.0222.

**5-Chloro-2-(4-(trifluoromethyl)phenyl)-1*H*-benzo[*d*]imidazole (29).** This compound was

prepared from 4-chlorobenzene-1,2-diamine and 4-(trifluoromethyl)benzaldehyde using Method

A. Yield 73%. TLC $R_f$ = 0.35 (hexane- Hexanes–EtOAc, 2:1). ^{1}H NMR (400 MHz, DMSO-$d_6$)

δ 13.37 (s, 1H), 8.38 (d, $J$ = 8.2 Hz, 2H), 7.95 (d, $J$ = 8.3 Hz, 2H), 7.73 – 7.62 (m, 2H), 7.28 (dd,

$J$ = 8.6, 2.1 Hz, 1H).  HRMS $m/z$ calcd for $C_{14}H_8ClF_3N_2$ [M+H]$^+$: 296.0328, found: 296.0323.

HPLC: 95% ($t_R$ = 2.26 min).

**5-Chloro-2-(4-(trifluoromethoxy)phenyl)-1$H$-benzo[$d$]imidazole (30).** This compound was

prepared from 4-chlorobenzene-1,2-diamine and 4-(trifluoromethoxy)benzaldehyde using

Method A. Yield 10%.  TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1).  $^1$H NMR (400 MHz, DMSO-$d_6$)

δ 13.18 (s, 1H), 8.31 − 8.22 (m, 2H), 7.75 − 7.63 (m, 1H), 7.60 − 7.51 (m, 3H), 7.23 (ddd, $J$ =

13.3, 8.5, 2.0 Hz, 1H).  HRMS $m/z$ calcd for $C_{14}H_8ClF_3N_2O$ [M+H]$^+$: 312.0277, found: 312.0277.

HPLC: 98% ($t_R$ = 2.36 min).

**5-(3-Fluorophenyl)-2-(4-(trifluoromethoxy)phenyl)-1$H$-benzo[$d$]imidazole (31).** This

compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1$H$-benzo[$d$]imidazole

and (3-fluorophenyl)boronic acid using Method C. Yield 48%.  TLC $R_f$ = 0.40 (Hexanes–EtOAc,

1:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.16 - 8.08 (m, 2H), 7.77 (d, $J$ = 1.7 Hz, 1H), 7.64 (d, $J$

= 8.4 Hz, 1H), 7.49 - 7.31 (m, 4H), 7.35 - 7.26 (m, 2H), 7.03 (dtt, $J$ = 11.5, 5.6, 2.8 Hz, 1H).

HRMS $m/z$ calcd for $C_{20}H_{12}F_4N_2O$ [M+H]$^+$: 372.0886, found: 372.0885.  HPLC: 100% ($t_R$ = 2.50

min).

**5-(4-Fluorophenyl)-2-(4-(trifluoromethoxy)phenyl)-1$H$-benzo[$d$]imidazole (32).** This

compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1$H$-benzo[$d$]imidazole

and (4-fluorophenyl)boronic acid using Method C. Yield 58%.  TLC $R_f$ = 0.40 (Hexanes–EtOAc,

1:1).  $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.07 (s, 1H), 7.71 - 7.66 (m, 1H), 7.59 (d, $J$ = 8.4 Hz,

1H), 7.53 - 7.45 (m, 2H), 7.38 (dt, $J$ = 8.4, 1.3 Hz, 1H), 7.30 - 7.22 (m, 3H), 7.09 - 7.00 (m, 2H).

HRMS $m/z$ calcd for $C_{20}H_{12}F_4N_2O$ [M+H]$^+$: 372.0886, found: 372.0886. HPLC: 100% ($t_R$ = 2.45

min).

**5-(3-(*tert*-butyl)phenyl)-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole (33).** This compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1H-benzo[d]imidazole and (3-(tert-butyl)phenyl)boronic acid using Method C. Yield 45%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.07 (s, 1H), 7.74 (q, $J$ = 1.6 Hz, 1H), 7.64 - 7.56 (m, 2H), 7.49 - 7.41 (m, 1H), 7.40 - 7.27 (m, 4H), 7.29 - 7.23 (m, 1H), 7.24 (s, 1H), 1.35 - 1.27 (m, 9H). HRMS *m/z* calcd for $C_{24}H_{21}F_3N_2O$ [M+H]$^+$: 410.1606, found: 410.1602. HPLC: 100% ($t_R$ = 2.71 min).

**5-(6-Methylpyridin-3-yl)-2-(4-(trifluoromethoxy)phenyl)-1*H* benzo[*d*]imidazole (34).** This compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1H-benzo[d]imidazole and (6-methylpyridin-3-yl)boronic acid using Method C. Yield 45%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.79 (d, $J$ = 2.5 Hz, 1H), 8.35 - 8.26 (m, 2H), 7.99 (dd, $J$ = 8.1, 2.5 Hz, 1H), 7.86 (s, 1H), 7.69 (d, $J$ = 8.3 Hz, 1H), 7.60 - 7.48 (m, 2H), 7.33 (d, $J$ = 8.0 Hz, 2H), 3.23 (s, 3H). HRMS *m/z* calcd for $C_{20}H_{14}F_3N_3O$ [M+H]$^+$: 369.1089, found: 369.1092. HPLC: 100% ($t_R$ = 1.68 min).

**5-(Pyridin-3-yl)-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole (35).** This compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole and pyridin-3-ylboronic acid using Method C. Yield 60%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1), $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.94 (dd, $J$ = 2.5, 0.9 Hz, 1H), 8.54 (dd, $J$ = 4.8, 1.6 Hz, 1H), 8.30 (s, 1H), 8.11 (ddd, $J$ = 8.0, 2.5, 1.6 Hz, 1H), 7.91 (s, 1H), 7.72 (d, $J$ = 8.4 Hz, 1H), 7.57 (dd, $J$ = 8.4, 1.6 Hz, 3H), 7.48 (ddd, $J$ = 7.9, 4.7, 0.9 Hz, 2H). HRMS *m/z* calcd for $C_{19}H_{12}F_3N_3O$ [M+H]$^+$: 355.0932, found: 355.0933. HPLC: 100% ($t_R$ = 1.66 min).

**5-(4-Chlorophenyl)-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole (36).** This compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole

and (4-chlorophenyl)boronic acid using Method C. Yield 51%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 8.35 - 8.24 (m, 2H), 7.86 - 7.64 (m, 3H), 7.60 - 7.47 (m, 3H), 7.41 - 7.34 (m, 3H). HRMS *m/z* calcd for $C_{20}H_{12}ClF_3N_2O$ [M+H]$^+$: 388.0590, found: 388.0592. HPLC: 100% ($t_R$ = 2.60 min).

**5-(1H-Pyrazol-4-yl)-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole       (37).**       This compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole and (1*H*-pyrazol-4-yl)boronic acid using Method C. Yield 33%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 8.31 - 8.23 (m, 4H), 7.59 - 7.51 (m, 4H), 7.47 (d, *J* = 7.9 Hz, 2H). HRMS *m/z* calcd for $C_{17}H_{11}F_3N_4O$ [M+H]$^+$: 344.0885, found: 344.0887. HPLC: 100% ($t_R$ = 2.58 min).

**5-(1-Methyl-1H-pyrazol-4-yl)-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole       (38).** This compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[d]imidazole and (1-methyl-1*H*-pyrazol-4-yl)boronic acid using Method C. Yield 30%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 8.26 (s, 1H), 8.13 (d, *J* = 0.8 Hz, 1H), 7.87 (d, *J* = 0.8 Hz, 1H), 7.71 (s, 1H), 7.60 - 7.51 (m, 4H), 7.42 (dd, *J* = 8.4, 1.6 Hz, 1H), 3.86 (s, 3H). HRMS *m/z* calcd for $C_{18}H_{13}F_3N_4O$ [M+H]$^+$: 358.1041, found: 358.1043. HPLC: 100% ($t_R$ = 1.81 min).

**5-(Pyrimidin-5-yl)-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole       (39).**       This compound was prepared from 5-bromo-2-(4-(trifluoromethoxy)phenyl)-1*H*-benzo[*d*]imidazole and pyrimidin-5-ylboronic acid using Method C. Yield 44%. TLC $R_f$ = 0.25 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 9.18 (d, *J* = 12.1 Hz, 2H), 8.37 - 8.25 (m, 2H), 8.03 (s, 2H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.65 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.57 (dq, *J* = 7.9, 1.1 Hz, 2H).

HRMS $m/z$ calcd for $C_{18}H_{11}F_3N_4O$ [M+H]$^+$: 356.0885, found: 356.0886. HPLC: 100% ($t_R$ = 1.98 min).

**5,6-Dichloro-2-(4-(4-methylpiperazin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole (40).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(4-methylpiperazin-1-yl)benzaldehyde using Method B. Yield 17%. TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1), $^1$H NMR (400 MHz, DMSO-d$_6$) δ 12.88 (s, 1H), 8.00 (dd, $J$ = 9.0, 1.8 Hz, 2H), 7.74 (s, 2H), 7.08 (dd, $J$ = 9.0, 1.8 Hz, 2H), 3.28 (d, $J$ = 5.2 Hz, 4H), 2.46 (t, $J$ = 4.9 Hz, 4H), 2.23 (d, $J$ = 1.7 Hz, 3H). HRMS $m/z$ calcd for $C_{18}H_{18}Cl_2N_4$ [M+H]$^+$: 361.0982, found: 361.0979. HPLC: 100% ($t_R$ = 1.48 min).

**5,6-Dichloro-2-(4-(piperazin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole (41).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(piperazin-1-yl)benzaldehyde using Method B. Yield 11%. TLC $R_f$ = 0.25 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.95 (s, 1H), 8.01 (s, 2H), 7.83 (s, 2H), 7.68 (s, 2H), 7.10 (s, 8H). HRMS $m/z$ calcd for $C_{17}H_{16}Cl_2N_4$ [M+H]$^+$: 347.0835, found: 347.0845. HPLC: 96% ($t_R$ = 3.76 min).

**5,6-Dichloro-2-(4-(4-ethylpiperazin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole (42).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(4-ethylpiperazin-1-yl)benzaldehyde using Method B. Yield 14%. TLC $R_f$ = 0.25 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.92 (s, 1H), 8.02 - 7.94 (m, 2H), 7.73 (s, 3H), 7.10 - 7.02 (m, 3H), 3.31 - 3.23 (m, 5H), 2.47 (s, 1H), 2.36 (q, $J$ = 7.2 Hz, 2H), 1.02 (t, $J$ = 7.2 Hz, 3H). HRMS $m/z$ calcd for $C_{19}H_{20}Cl_2N_4$ [M+H]$^+$: 375.1137, found: 375.1155. HPLC: 100% ($t_R$ = 3.98 min).

**5,6-Dichloro-2-(4-(piperidin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole (43).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(piperidin-1-yl)benzaldehyde using

Method B. Yield 25%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 8.01 - 7.94 (m, 2H), 7.74 (s, 2H), 7.11 - 7.01 (m, 2H), 3.41-3.46 (m, 4H), 1.61 (d, $J$ = 5.5 Hz, 6H). HRMS $m/z$ calcd for C$_{18}$H$_{17}$Cl$_2$N$_3$ [M+H]$^+$: 346.0876, found: 346.0884. HPLC: 100% ($t_R$ = 9.04 min).

**4-(4-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)phenyl)morpholine (44).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-morpholinobenzaldehyde using Method B. Yield 66%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 12.95 (s, 1H), 8.05 - 7.96 (m, 2H), 7.73 (s, 2H), 7.12 - 7.03 (m, 2H), 3.78 - 3.70 (m, 4H), 3.28 - 3.20 (m, 4H). HRMS $m/z$ calcd for C$_{17}$H$_{15}$Cl$_2$N$_3$O [M+H]$^+$: 348.0665, found: 348.0661. HPLC: 100% ($t_R$ = 6.47 min).

**6-Fluoro-2-(4-(4-methylpiperazin-1-yl)phenyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (45).** This compound was prepared from 4-fluoro-5-(trifluoromethyl)benzene-1,2-diamine and 4-(4-methylpiperazin-1-yl)benzaldehyde using Method B. Yield 15%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 13.15 (s, 1H), 13.11 (s, 1H), 8.01 (d, $J$ = 8.4 Hz, 2H), 7.91 (d, $J$ = 6.5 Hz, 1H), 7.66 (dd, $J$ = 18.1, 9.1 Hz, 1H), 7.50 (d, $J$ = 10.8 Hz, 1H), 7.08 (d, $J$ = 8.7 Hz, 2H), 3.27 (s, 2H), 2.44 (s, 2H), 2.44 (d, $J$ = 10.1 Hz, 2H), 2.22 (s, 3H). HRMS $m/z$ calcd for C$_{19}$H$_{18}$F$_4$N$_4$ [M+H]$^+$: 379.1540, found: 379.1530. HPLC: 100% ($t_R$ = 4.01 min).

**6-Chloro-2-(4-(4-methylpiperazin-1-yl)phenyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (46).** This compound was prepared from 4-chloro-5-(trifluoromethyl)benzene-1,2-diamine and 4-(4-methylpiperazin-1-yl)benzaldehyde using Method B. Yield 7%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 13.16 (s, 1H), 8.01 (d, $J$ = 8.4 Hz, 4H), 7.87 (s, 1H), 7.81 (s, 1H), 7.69 (s, 1H), 7.09 (d, $J$ = 8.6 Hz, 3H), 3.37 (q, $J$ = 6.9 Hz, 1H), 3.27 (s, 1H),

2.46 (s, 2H), 2.23 (s, 4H). HRMS *m/z* calcd for $C_{19}H_{18}ClF_3N_4$ [M+H]$^+$: 395.1245, found: 398.1238. HPLC: 100% ($t_R$ = 4.01 min).

**6-Chloro-5-fluoro-2-(4-(4-methylpiperazin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole (47).** This compound was prepared from 4-chloro-5-fluorobenzene-1,2-diamine and 4-(4-methylpiperazin-1-yl)benzaldehyde using Method B. Yield 35%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.99 (d, *J* = 7.9 Hz, 2H), 7.64 - 7.55 (m, 1H), 7.08 (d, *J* = 8.8 Hz, 2H), 3.28 (t, *J* = 5.0 Hz, 4H), 2.46 (s, 3H), 2.46 (d, *J* = 10.2 Hz, 2H), 2.23 (s, 3H). HRMS *m/z* calcd for $C_{18}H_{18}ClFN_4$ [M+H]$^+$: 345.1277, found: 345.1278. HPLC: 100% ($t_R$ = 2.83 min).

**5,6-Dichloro-2-(4-(pyrrolidin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole (48).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(pyrrolidin-1-yl)benzaldehyde using Method B. Yield 20%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.01 - 7.93 (m, 2H), 7.70 (s, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 3.41-3.46 (m, 4H), 2.01 - 1.95 (m, 4H). HRMS *m/z* calcd for $C_{17}H_{15}Cl_2N_3$ [M+H]$^+$: 332.0116, found: 332.0730. HPLC: 100% ($t_R$ = 7.83 min).

**2-(4-(1H-Imidazol-1-yl)phenyl)-5,6-dichloro-1*H*-benzo[*d*]imidazole (49).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(1*H*-imidazol-1-yl)benzaldehyde using Method B. Yield 89%. TLC $R_f$ = 0.25 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.43 (s, 1H), 8.28 (s, 2H), 7.88 (s, 6H), 7.18 (s, 1H). HRMS *m/z* calcd for $C_{16}H_{10}Cl_2N_4$ [M+H]$^+$: 329.0355, found: 329.0369. HPLC: 100% ($t_R$ = 4.82 min).

**5,6-Dichloro-2-(4-(2-methyl-1*H*-imidazol-1-yl)phenyl)-1H-benzo[*d*]imidazole (50).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(2-methyl-1*H*-imidazol-1-yl)benzaldehyde using Method B. Yield 63%. TLC $R_f$ = 0.30 (Hexanes–EtOAc, 1:1). $^1$H NMR

(400 MHz, DMSO-$d_6$) δ 13.37 (s, 1H), 8.31 (d, $J$ = 8.2 Hz, 2H), 7.90 (s, 2H), 7.67 (d, $J$ = 8.0 Hz, 2H), 7.40 (s, 1H), 7.35 (s, 1H), 2.37 (s, 3H). HRMS $m/z$ calcd for $C_{17}H_{12}Cl_2N_4$ [M+H]$^+$: 343.0512, found: 345.0524. HPLC: 100% ($t_R$ = 4.87 min).

**6-Chloro-5-fluoro-2-(4-(pyrrolidin-1-yl)phenyl)-1$H$-benzo[$d$]imidazole (51).** This compound was prepared from 4-chloro-5-fluorobenzene-1,2-diamine and 4-(pyrrolidin-1-yl)benzaldehyde using Method B. Yield 43%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.76 (s, 1H), 7.98 - 7.90 (m, 2H), 7.53 (s, 2H), 6.68 - 6.60 (m, 2H), 3.29 (d, $J$ = 6.6 Hz, 2H), 2.87 (s, 2H), 2.71 (d, $J$ = 0.6 Hz, 2H), 2.02 - 1.90 (m, 2H). HRMS $m/z$ calcd for $C_{17}H_{15}ClFN_3$ [M+H]$^+$: 316.1015, found: 316.1015. HPLC: 100% ($t_R$ = 5.54 min).

**6-Chloro-2-(4-(pyrrolidin-1-yl)phenyl)-5-(trifluoromethyl)-1$H$-benzo[$d$]imidazole (52).** This compound was prepared from 4-chloro-5-(trifluoromethyl)benzene-1,2-diamine and 4-(pyrrolidin-1-yl)benzaldehyde. Yield: 23%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 2:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.17 (s, 1H), 8.04 (d, $J$ = 8.3 Hz, 2H), 7.70 (s, 1H), 7.51 (s, 1H), 6.68 (d, $J$ = 8.5 Hz, 2H), 3.30 (m, 4H), 1.97 (q, $J$ = 4.8, 3.2 Hz, 4H). HRMS $m/z$ calcd for $C_{18}H_{15}ClF_3N_3$ [M+H]$^+$: 366.0979 found: 366.0981. HPLC: 100% ($t_R$ = 12.48 min).

**2-(4-(Azepan-1-yl)phenyl)-5,6-dichloro-1$H$-benzo[$d$]imidazole (53).** This compound was prepared from 4,5-dichlorobenzene-1,2-diamine and 4-(azepan-1-yl)benzaldehyde using Method B. Yield 7%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.78 (s, 1H), 7.92 (d, $J$ = 8.8 Hz, 2H), 7.68 (s, 2H), 6.80 (d, $J$ = 9.0 Hz, 2H), 3.52 (t, $J$ = 6.0 Hz, 2H), 2.51 - 2.45 (m, 6H), 1.73 (s, 2H), 1.46 (p, $J$ = 2.7 Hz, 2H). HRMS $m/z$ calcd for $C_{19}H_{19}Cl_2N_3$ [M+H]$^+$: 360.1029, found: 360.1025. HPLC: 100% ($t_R$ = 7.51 min).

**6-Fluoro-2-(4-(piperidin-1-yl)phenyl)-1H-benzo[d]imidazole (54).** This compound was prepared from 4-fluorobenzene-1,2-diamine and 4-(piperidin-1-yl)benzaldehyde. Yield: 21%.; TLC $R_f$ 0.65 (Hexane:EtOAc, 2:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.67 (d, J = 8.2 Hz, 1H), 7.99 - 7.90 (m, 2H), 7.37 (ddd, J = 27.0, 9.3, 3.7 Hz, 2H), 7.06 - 7.00 (m, 2H), 7.00 - 6.92 (m, 1H), 3.27 (d, J = 5.6 Hz, 4H), 1.58 (s, 6H). HRMS $m/z$ calcd for $C_{18}H_{18}FN_3$ + $H^+$ [M+H]$^+$: 295.1485 found: 295.1490. HPLC: 100% ($t_R$ = 4.90 min).

**2-(4-(4-Methylpiperazin-1-yl)phenyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (55).** This compound was prepared from 4-(trifluoromethyl)benzene-1,2-diamine and 4-(4-methylpiperazin-1-yl)benzaldehyde using Method B. Yield 20%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.03 (s, 1H), 8.04 (d, $J$ = 8.5 Hz, 2H), 7.92 (d, $J$ = 1.6 Hz, 1H), 7.80 - 7.71 (m, 1H), 7.64 (d, $J$ = 8.3 Hz, 1H), 7.50 - 7.42 (m, 1H), 7.10 (d, $J$ = 8.6 Hz, 2H), 3.30 (s, 3H), 3.30 (d, $J$ = 10.3 Hz, 1H), 2.46 (s, 3H), 2.50 - 2.43 (m, 2H), 2.24 (s, 3H). HRMS $m/z$ calcd for $C_{19}H_{19}F_3N_4$ [M+H]$^+$: 361.1625, found: 361.1629. HPLC: 95% ($t_R$ = 3.06 min).

**6-Fluoro-2-(4-(4-methylpiperazin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole (56).** This compound was prepared from 4-fluorobenzene-1,2-diamine and 4-(4-methylpiperazin-1-yl)benzaldehyde using Method B. Yield 25%. TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.72 (d, $J$ = 7.7 Hz, 1H), 8.03 - 7.95 (m, 2H), 7.57 (dd, $J$ = 8.7, 5.0 Hz, 1H), 7.43 (dd, $J$ = 8.6, 4.9 Hz, 1H), 7.11 - 7.04 (m, 2H), 7.00 (s, 1H), 3.28 (t, $J$ = 5.0 Hz, 4H), 2.47 (s, 4H), 2.47 (d, $J$ = 10.0 Hz, 1H), 2.24 (s, 3H). HRMS $m/z$ calcd for $C_{18}H_{19}FN_4$ [M+H]$^+$: 311.1666, found: 311.1663. HPLC: 98% ($t_R$ = 1.29 min).

**2-(4-(Piperidin-1-yl)phenyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (57).** This compound was prepared from 4-(trifluoromethyl)benzene-1,2-diamine and 24-(piperidin-1-yl)benzaldehyde

using Method B. Yield: 56%.  TLC $R_f$ = 0.40 (Hexanes–EtOAc, 2:1).  ^{1}H NMR (400 MHz,

DMSO-$d_6$) δ 12.67 (d, $J$ = 8.2 Hz, 1H), 7.99 - 7.90 (m, 2H), 7.37 (ddd, $J$ = 27.0, 9.3, 3.7 Hz, 2H),

7.06 - 7.00 (m, 2H), 7.00 - 6.92 (m, 1H), 3.27 (d, $J$ = 5.6 Hz, 4H), 1.58 (s, 6H).  HRMS $m/z$ calcd

for $C_{19}H_{18}F_3N_3$ [M+H]$^+$: 345.1453 found: 345.1447.  HPLC: 100% ($t_R$ = 6.64 min).

**6-Fluoro-2-(4-(pyrrolidin-1-yl)phenyl)-1$H$-benzo[$d$]imidazole  (58).**  This compound was

prepared from 4-fluorobenzene-1,2-diamine and 4-(pyrrolidin-1-yl)benzaldehyde using Method

B. Yield 23%.  TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1).  ^{1}H NMR (400 MHz, DMSO-$d_6$) δ 12.57

(d, $J$ = 9.4 Hz, 1H), 7.98 - 7.90 (m, 2H), 7.38 (dd, $J$ = 8.7, 4.9 Hz, 1H), 7.31 (dd, $J$ = 10.0, 2.5

Hz, 1H), 6.99 - 6.89 (m, 1H), 6.67 - 6.60 (m, 2H), 3.30 (t, $J$ = 1.6 Hz, 4H), 1.99 - 1.94 (m, 4H).

HRMS $m/z$ calcd for $C_{17}H_{16}FN_3$ [M+H]$^+$: 282.1400, found: 282.1400.  HPLC: 100% ($t_R$ = 4.05

min).

**2-(4-(Pyrrolidin-1-yl)phenyl)-6-(trifluoromethyl)-1$H$-benzo[$d$]imidazole  (59).**  This

compound was prepared from 4-(trifluoromethyl)benzene-1,2-diamine and 4-(pyrrolidin-1-

yl)benzaldehyde using Method B. Yield 43%.  TLC $R_f$ = 0.35 (Hexanes–EtOAc, 1:1).  ^{1}H NMR

(400 MHz, DMSO-$d_6$) δ 12.90 (s, 1H), 8.04 - 7.95 (m, 2H), 7.79 (s, 1H), 7.64 (d, $J$ = 8.4 Hz,

1H), 7.42 (dt, $J$ = 8.4, 1.2 Hz, 1H), 6.71 - 6.62 (m, 2H), 2.87 (d, $J$ = 0.5 Hz, 2H), 2.71 (d, $J$ = 1.2

Hz, 2H),  2.03 - 1.91 (m, 4H).  HRMS $m/z$ calcd for $C_{18}H_{16}F_3N_3$ [M+H]$^+$: 332.1374, found:

332.1375. HPLC: 100% ($t_R$ = 5.56 min).

**ADME Evaluation. Solubility.** Standard kinetic solubility assay done at pH 7.5

(simulated intestinal fluid). The assay measures solubility of test compound (in triplicate) using

an LC-MS method to quantify the samples and Estradiol (haloperidol) as a control standard.

**LogD.** The distribution coefficient (or LogD) is the ratio of the sum of the concentrations

of all forms of the test compound (ionized and un-ionized). The assay measures test compound

concentrations (in triplicate) after partitioning between an organic solvent (octanol) and aqueous buffer in a 96-well plate. LC-MS method is used to quantify the samples, and Microsoft Excel is used to calculate the logD. The assay is run at pH 7.5 (PBS buffer) with Verapamil as a control standard.

**Microsomal stability.** For the stability studies, test compounds are incubated with liver microsomes (from mouse or human) in the presence of NADPH. Compound concentrations are then measured via analytical methods, such as LC-MS/MS, at various time points, such as 0, 5, 10, 20, and 45 min. The half-life or intrinsic clearance of the test compound is then calculated. Diclofenac is used as a positive control.

**Biological Evaluation. Cell culture**. HEK293 cells were obtained from ATCC, and were cultured in high glucose DME containing 10% FBS. Pancreatic cancer Suit-2 and S2-VP10 PDAC cells were obtained from Dr. Michael A. Hollingsworth at The University of Nebraska Medical Center, and were cultured with RPMI containing 4.5 g/L glucose, 1 mM sodium pyruvate, 10 mM HEPES, and 10% FBS. All cell lines were expanded upon receipt to prepare frozen cell stocks that were tested to confirm lack of mycoplasma contamination and cultured *in vitro* for no longer than 3 months after thawing. For CIC experiments, cell lines were grown using standard cell culture conditions, then trypsinized, washed with PBS, and incubated in CIC medium (serum free RPMI supplemented with 20 ng/mL EGF, 10 ng/mL basic FGF, 25 µg/mL insulin, 5 µg/mL transferrin, 5 ng/mL sodium selenite, 16 µg/mL putrescene, and 7.3 ng/mL progesterone) using ultra-low attachment plates or flasks (Corning, Tewksbury, MA) to enrich for cells with CIC characteristics.

**Wnt Reporter Assay**. The synthesized compounds were first tested for inhibition of Wnt/β-catenin signaling in LRP6-expressing HEK293 cells. HEK293 cells were plated onto 24-

well plates. After overnight culture, the cells were transiently transfected with 0.06 µg of the Super8XTOPFlash luciferase construct (kindly provided by Dr. Randall T. Moon at University of Washington, Seattle), 0.06 µg of β-galactosidase-expressing vector, and 0.06 µg of pCS-Myc-hLRP6 or control vector (kindly provided by Dr. Christof Niehrs at Deutsches Krebsforschungszentrum, Heidelberg, Germany). After 24 h incubation, cells were treated with each individual compound in triplicate for 24 h. Cells were then lysed, and the luciferase and β-galactosidase activities were determined by the luciferase assay system (Promega) and β-galactosidase assay system (Promega), respectively. The luciferase activity was normalized to the β-galactosidase activity.

*In Vitro* **Cytotoxicity Assay**. Pancreatic cancer cell lines were trypsinized, washed with PBS, and seeded at a density of 2,000 cells/well in 96 well plates. Cells were evaluated in adherent conditions using DME containing 10% FBS and tissue culture treated, optically clear 96 well black plates (Corning #3904) or in non-adherent conditions using CIC medium and ultra-low attachment 96 well plates (Corning #3474). After plating, cells were incubated overnight at 37°C in 5% $CO_2$ before starting treatments. Compounds were prepared as 10 mM stock solutions in DMSO and diluted in serum free medium to obtain final concentrations of 0.1 to 10 µM. Final DMSO concentration was $\leq$ 0.1%. Cells were treated for 3 days and then examined microscopically to assess morphological alterations. Cell viability was assessed using an ATP-dependent luciferase assay (ATPlite, Perkin Elmer, Waltham, MA). ATP levels were reported relative to untreated control cells with values derived from 4 or 6 replicate wells for adherent or CIC conditions, respectively. All cytotoxicity experiments were performed at least two times.

SUPPORTING INFORMATION

'Effect of **41** on pancreatic cancer cell proliferation' is available free of charge on the ACS Publications website at DOI.

Molecular Formula Strings (CSV)

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NOTES

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ABBREVIATIONS USED

Pancreatic ductal adenocarcinoma (PDAC)
Cancer-initiating cells (CIC)
LDL-Related Protein-6 (LRP6)
Frizzled (Fzd)
Dishevelled (Dvl)
Glycogen synthase kinase-3 β (GSK3β)
T-cell factor/lymphoid enhancing factor (TCF/LEF)
Epithelial-to-mesenchymal transition (EMT)
Porcupine (PORCN)
Target of rapamycin complex 1 (mTORC1)
Signal transducer and activator of transcription 3 (STAT3)
Nitro ($NO_2$)
Hydroxy (OH)
Absorption, distribution, metabolism, and excretion (ADME)
Thin-layer chromatography (TLC)
Electrospray ionization (ESI)
Tetramethylsilane (TMS)
Dimethyl fumarate (DMF)
Fetal bovine serum (FBS)
LDL-Receptor Related Protein-6 (LRP6)

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20: $IC_{50} = 0.40$ μM
(Wnt Inhib: HEK293 cells)

40: $IC_{50} = 0.018$ μM
(Wnt Inhib: HEK293 cells)

43: $IC_{50} = 0.002$ μM
((Wnt Inhib: HEK293 cells)