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Benzimidazole derivatives as potential dual inhibitors for PARP-1 and DHODH

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ABSTRACT

Poly (ADP-ribose) polymerases (PARPs) play diverse roles in various cellular processes that involve DNA repair and programmed cell death. Amongst these polymerases is PARP-1 which is the key DNA damage-sensing enzyme that acts as an initiator for the DNA repair mechanism. Dihydroorotate dehydrogenase (DHODH) is an enzyme in the pyrimidine biosynthetic pathway which is an important target for anti-hyperproliferative and anti-inflammatory drug design. Since these enzymes share a common role in the DNA replication and repair mechanisms, it may be beneficial to target both PARP-1 and DHODH in attempts to design new anti-cancer agents.

Benzimidazole derivatives have shown a wide variety of pharmacological activities including PARP and DHODH inhibition. We hereby report the design, synthesis and bioactivities of a series of benzimidazole derivatives as inhibitors of both the PARP-1 and DHODH enzymes.

1. Introduction

Poly (ADP-ribose) polymerases (PARPs) comprise 18 putative family members of the nuclear enzymes that have significant roles in multifunctional cellular processes, including detection and repair of damaged DNA and RNA.[1] PARP-1, PARP-2 and PARP-3 are the best studied members of this family of enzymes due to their role in DNA repair.[24] Other distinct biochemical activities of PARP-1 are epigenetic chromatic modifications, genomic stability regulations,[7,22] replication and transcription of DNA[18] and distinctive cell death formation known as parthanatos.[10,14] PARP-1 is known to be the trigger point in the DNA repair mechanism for single strand breaks where it acts as the DNA damage-sensing enzyme. In response to DNA damage that may have occurred due to radiation or chemotherapeutic agents, PARP-1 initiates its repair process by binding to the damaged site and catalyzing the synthesis of long, branched poly (ADP-ribose) chains using nicotinamide adenine dinucleotide (NAD+) as the substrate. These actions of PARP-1 result in the resistance that frequently develops after cancer therapy. Hence inhibition of the PARP-1 enzyme is believed to enhance sensitivity towards radiotherapy and certain kinds of DNA targeting cancer chemotherapies.[17]

To date, a significant number of potent PARP-1 inhibitors have been reported accentuating the role of PARP-1. Inhibition of PARP in homologous recombination (HR) deficient tumor cells have also exclusively explained the crucial role of PARP-1 in DNA repair.[11,28] These inhibitors generally bind to the nicotinamide binding site of the PARP-1 catalytic domain, thus inhibiting automodification and subsequent release of the enzyme from the site of DNA damage as well as preventing the access of other repair proteins to the site of DNA damage. The binding of these inhibitors mimics the binding mode of nicotinamide towards PARP-1 with key interactions to Ser243 (C=O to O—H Ser) and Gly202 (C=O to N—H Gly and N—H to C=O Gly) through hydrogen-bonding and π–π stacking with Tyr246, which is approximately coplanar with the benzimidazole moiety of the ligand.[29] In addition, Griffin and co-workers reported an intramolecular hydrogen bond between the carboxamide hydrogen on C2 of the indole ring to the nitrogen in the indole ring. This intramolecular H-bond resulted in a pseudo-6-membered ring, creating a rigid 6:6:5 tricyclic system which improved its potency.[15,25] A water molecule also plays a crucial role at the

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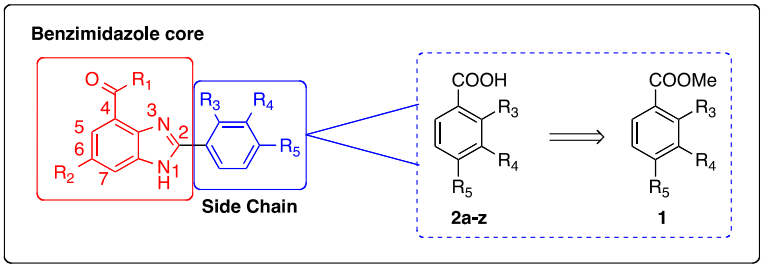
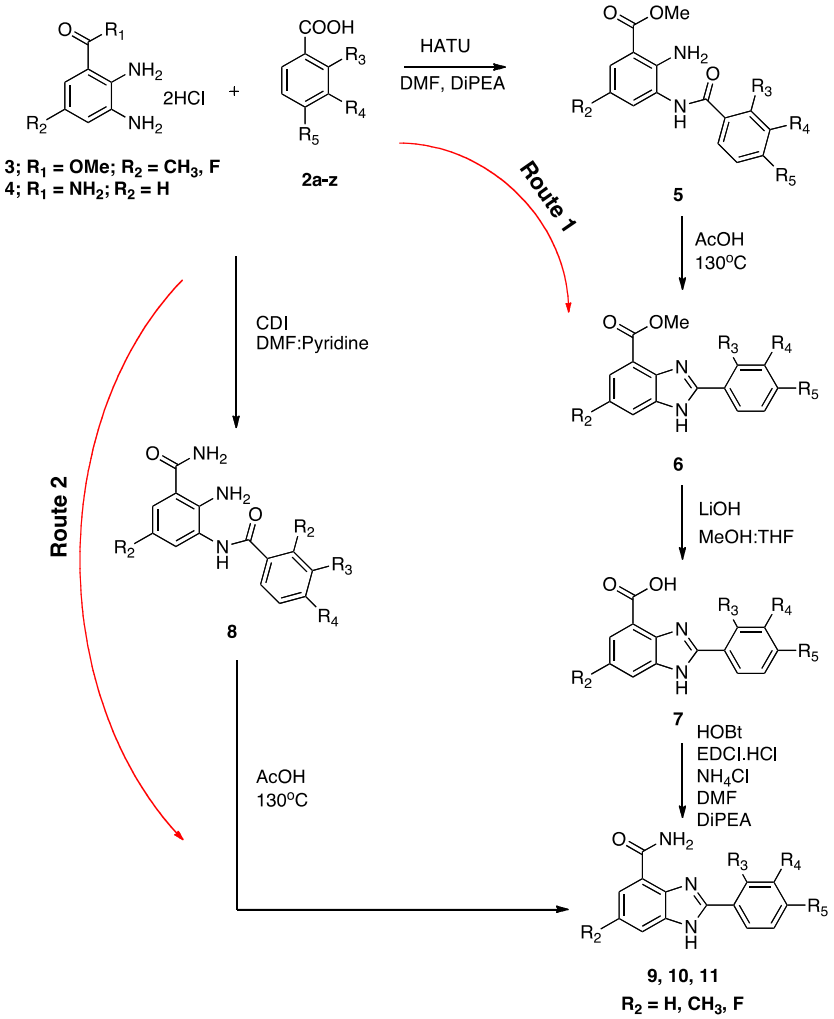


Scheme 1. Side chain preparation from phenyl ester **1**.

active site by interacting with the catalytically important carboxylate group of Glu327 which forms a hydrogen bond with the NH indole of benzimidazole of the ligand.[29,30]

Inhibition of pyrimidine biosynthesis has been shown to have an efficacious anti-proliferative effect on cells that are dividing rapidly.[13] Mitochondrial enzyme, dihydroorotate dehydrogenase (DHODH) catalyzes the fourth step in the de novo biosynthetic pathway of pyrimidines, converting dihydroorotate to orotate by oxidative reaction, with flavin mononucleotide (FMN) and ubiquinone (CoQ) acting as co-factors.[13] This enzyme has been identified as a therapeutic target for treatment of cancer,[9,12] as well as several autoimmune disorders such as rheumatoid arthritis and multiple sclerosis.[16] Inhibition of enzymatic activities has been reported on *h*DHODH and *Pf*DHODH by X-ray crystallographic studies with known inhibitors such as leflunomide, teriflunomide (the active metabolite of leflunomide) and brequinar. These inhibitors are positioned in the suggested ubiquinone binding site where polar and hydrophobic residues contribute to the binding. The carboxylic acid group from the inhibitors shows good hydrogen-bonding interactions with the guanidinyl moiety of Arg136 and an additional hydrogen bonding interaction to the side chain of Gln47.

Targeting both PARP-1 and DHODH for anti-cancer therapy would certainly be beneficial as these enzymes share a common role in the DNA replication and repair mechanisms which are involved in the hyper-proliferation of cancer cells. Since benzimidazole-containing compounds have been reported to show good



Scheme 2. General strategy for the synthesis of benzimidazole carboxamide and carboxylic acid derivatives.

pharmacological activity against these targets,[6,8,23] we have chosen them as lead structures in the search for dual PARP-1/DHODH inhibitors described in this study.

2. Results and discussion

2.1. Chemistry

Numerous reports on benzimidazole ring system construction have been published.[3,20,21]

We started off with the preparation of methyl ester **1** with different functional groups placed at the *ortho* (R_3), *meta* (R_4) and *para* (R_5) positions of the phenyl ring moiety to be connected at position 2 of the benzimidazole core as shown in Scheme 1. Saponification of **1** led to carboxylic acids **2a–z** (Supporting information). Two different routes were employed to prepare the benzimidazole compounds (Scheme 2). Route 1 involved coupling of diamine **3** using standard 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) in dimethylformamide and the presence of *N*,*N*-diisopropylethylamine (DiPEA) as base[31] for the formation of **5**. Subsequent thermal cyclization of **5** in AcOH gave intermediate **6**. Saponification of ester **6** with LiOH provided carboxylic acid derivatives **7a–g** (Table 1), which were then converted to the corresponding carboxamide benzimidazole derivatives **9a–i**, **10a–i** and **11a–j** with hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI).

Alternatively, **9a–i**, **10a–i** and **11a–j** (Table 2) could be prepared via Route 2. Reacting 2,3-diaminobenzamide (**4**) with **2a–z** in the presence of 1,1′-carbonyldiimidazole (CDI) in pyridine and dimethylformamide (DMF) (1:1 v/v) gave amide **8** which could be cyclized to benzimidazole **9**, **10** and **11** by refluxing in AcOH as reported by Penning and co-workers.[20]

2.2. Pharmacological evaluation

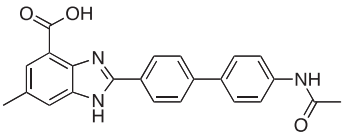
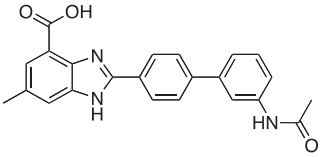
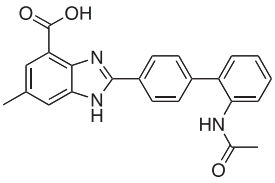
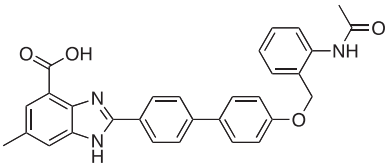
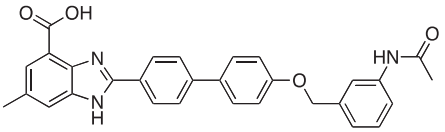
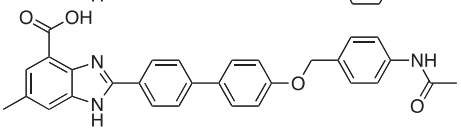
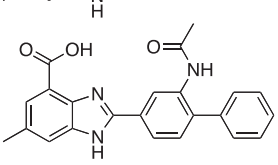
2.2.1. Poly (ADP-ribose) polymerase (PARP) colorimetric assay

The inhibitory effect of compounds **7a–g**, **9a–i**, **10a–i** and **11a–j** on PARP-1 activity was measured using an HT Universal Colorimetric PARP assay kit (Trevigen, Gaithersburg, MD, USA). With slight modification, the assays were carried out by quantifying the incorporation of biotinylated poly (ADP-ribose) onto histone proteins in 96-well plate. In brief, the experiment began with pre-incubating 10 μl of PARP-1 enzyme (0.25 U) with 15 μl of tested compounds in rehydrated histone-coated wells followed by addition of 25 μl of PARP cocktail mixture containing 2 μl of 10× PARP Cocktail, 2 μl of 10× activated DNA and 21 μl of 1× PARP buffer into the wells. After 60 min of incubation, the wells were washed twice with 1× PBS + 0.1% Triton X-100 followed by 1× PBS. 50 μl of 1× Strep-HRP was then added and incubated for 60 min. The wells were washed again as in the previous step. 50 μl of pre-warmed TACS-Sapphire substrate was added and the mixture was incubated for 15 min in the dark. The reactions were terminated with 50 μl 0.2 M HCl. The absorbance reading at 450 nm was measured using a VICTOR X5 2030 Multilabel Reader (Perkin–Elmer, Waltham, MA, USA). IC_{50} values were determined by fitting the activity data at different concentrations of the compound to a sigmoidal dose–response curve using GraphPad Prism software version 6.00.

2.2.2. Dihydroorotate dehydrogenase (DHODH) enzymatic assay

Compounds **7a–g**, **9a–i**, **10a–i** and **11a–j** were evaluated for their potency to inhibit DHODH in a coupled enzymatic spectrophotometric assay. The assay is based on the decrease in absorbance at 610 nm resulting from the oxidation of L-dihydrooroticacid (L-DHO) facilitated by the reduction of 2,6-dichloroindophenol (DCIP) and decylubiquinone (DUQ).[2] The decrease in absorbance at 610 nm is proportional to the reduction of DCIP. The assay buffer was 50 mM TrisHCl, 150 mM KCl and 0.8% Triton, pH 8.0. A 100 μl reaction mixture was used in 96-well plates at room temperature. A mixture of 82 μl of enzyme (25 ng) in buffer and 5 μl of test compounds were pre-incubated for 30 min and the reaction was started by adding 13 μl of substrate mixture (20 mM of L-DHO, 2 mM of DUQ and 2 mM of DCIP). The final concentration of DMSO used was 1%. The absorbance of each well was measured after 20 min of the reaction at 610 nm using a VICTOR X5 Multilabel Reader (Perkin–Elmer) every 10 min for 1 h. IC_{50} values were determined from the dose response plot using GraphPad Prism software version 6.00.

Table 1
Chemical structure of benzimidazole carboxylic acid derivatives synthesized via Scheme 2

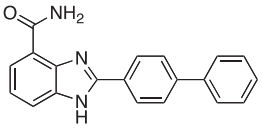
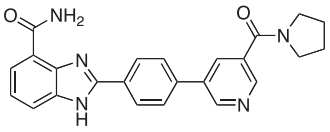
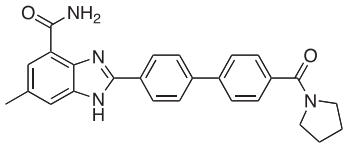
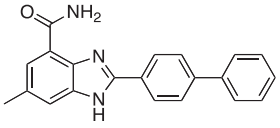
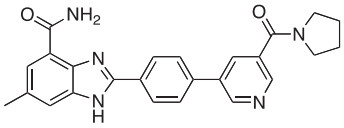
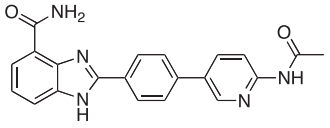
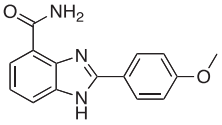
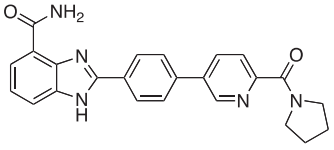
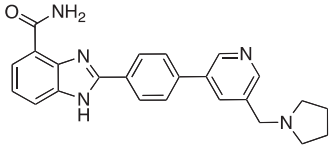
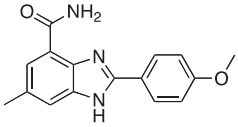
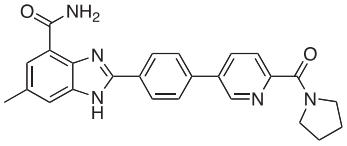
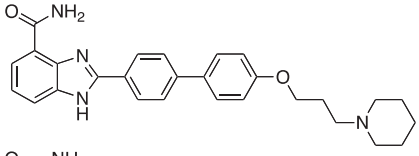
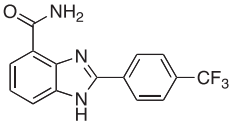
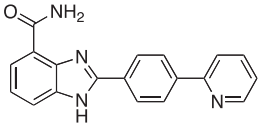
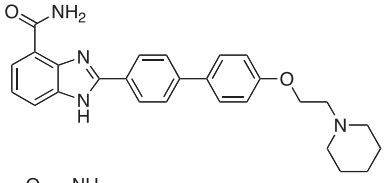
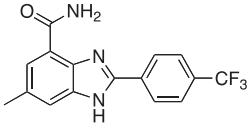
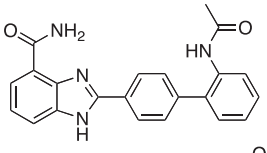
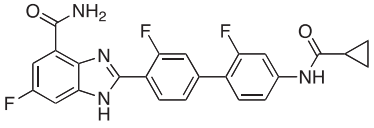
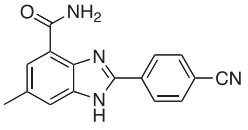
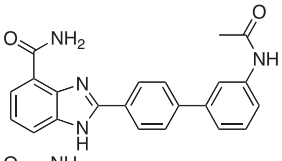
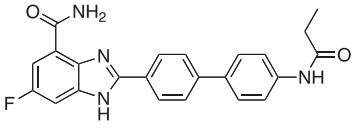
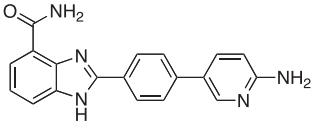
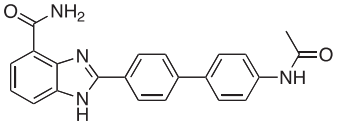
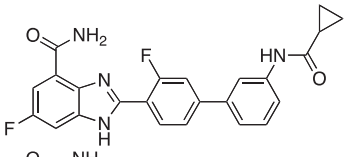
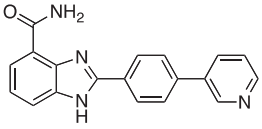
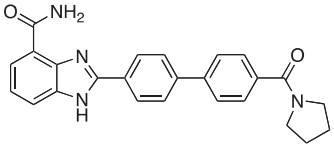
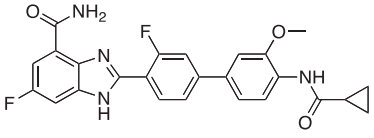
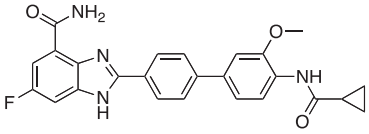
Entry	Compound
7a	
7b	
7c	
7d	
7e	
7f	
7g	

The preliminary activity for both the PARP and DHODH assays was measured at 10 μM concentration of the synthesized compounds (**7a–g**, **9a–i**, **10a–i** and **11a–j**) along with veliparib and brequinar as reference inhibitors (Table 3).

2.4. Structure–activity relationship (SAR) studies

In this work, we designed and synthesized diversified compounds (as shown in Table 3) by substituting various electron-

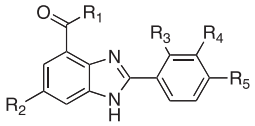
Table 2
Chemical structure of benzimidazole carboxamide derivatives synthesized via Scheme 2

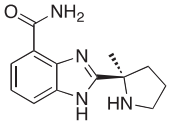
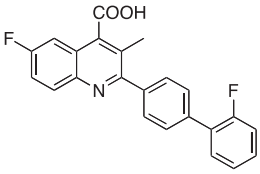
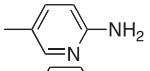
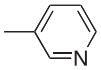
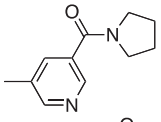
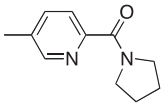
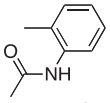
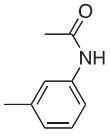
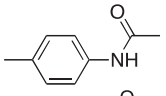
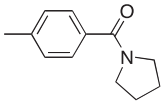
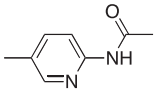
Entry	Product	Entry	Product	Entry	Product
9a[a]		**10a**		**11a**	
9b		**10b**		**11b**	
9c[b]		**10c**		**11c**	
9d		**10d**		**11d**	
9e[b]		**10e**[a]		**11e**	
9f		**10f**		**11f**	
9g		**10g**		**11g**	
9h		**10h**		**11h**	
9i[a]		**10i**		**11i**	
				11j	

[a] Compounds reported by Tong and group.[27]
[b] Compounds reported by Alex and group.[29]

donating and electron-withdrawing groups into the benzimidazole scaffold and evaluated their biological activities for the two targets, PARP-1 and DHODH. The compounds were docked into PARP-1 and DHODH, and their interactions with the active site residues were analyzed in order to rationalize their potencies in the two targets. Compound **I1** (Fig. 1) has been reported by Thunuguntla's group to inhibit DHODH[26] with IC_{50} of 0.75 μM. Replacing the carboxylic acid substituent at the C4 position of the benzimidazole ring in this compound with an amide functionality (**9a**) gave improved activity against PARP-1 (IC_{50} = 0.71 μM), but at the same time the DHODH potency was reduced by several folds (IC_{50} = 9.80 μM). Introduction of the amide group probably would have reduced

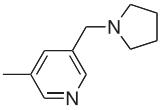
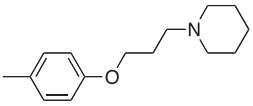
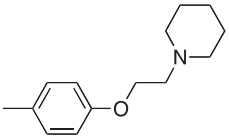
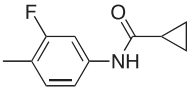
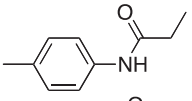
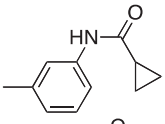
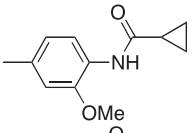
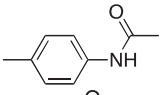
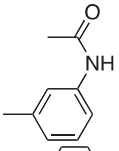
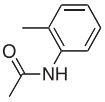
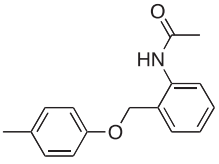
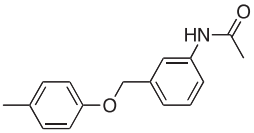
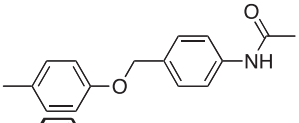
Table 3
PARP-1 and DHODH inhibition by benzimidazole derivatives



Entry	R_1	R_2	R_3	R_4	R_5	PARP-1 IC_{50} (μM)/% inhibition at 10 μM	DHODH IC_{50} (μM)/% inhibition at 10 μM
Veliparib (ABT-888)						0.005	–
Brequinar						–	0.012
9a[a]	NH_2	H	H	H		0.71	9.80
9b	NH_2	CH_3	H	H		28%	7.80
9c[b]	NH_2	H	H	H	$-OCH_3$	NA	NA
9d	NH_2	CH_3	H	H		NA	NA
9e[b]	NH_2	H	H	H	$-CF_3$	NA	NA
9f	NH_2	CH_3	H	H		NA	NA
9g	NH_2	CH_3	H	H	–CN	NA	NA
9h	NH_2	H	H	H		0.032	20
9i[a]	NH_2	H	H	H		0.022	19
10a	NH_2	H	H	H		0.029	28
10b	NH_2	CH_3	H	H		NA	50
10c	NH_2	H	H	H		0.012	NA
10d	NH_2	CH_3	H	H		17	37
10e[a]	NH_2	H	H	H		0.083	28
10f	NH_2	H	H	H		0.14	51
10g	NH_2	H	H	H		0.46	42
10h	NH_2	H	H	H		16	56
10i	NH_2	H	H	H		0.31	20
11a	NH_2	CH_3	H	H		NA	NA
11b	NH_2	H	H	H		0.049	40

(*continued on next page*)

Table 3 (*continued*)

Entry	R_1	R_2	R_3	R_4	R_5	PARP-1 IC_{50} (μM)/% inhibition at 10 μM	DHODH IC_{50} (μM)/% inhibition at 10 μM
11c	NH_2	H	H	H		0.061	55
11d	NH_2	CH_3	H	H		2.28	NA
11e	NH_2	H	H	H		0.72	31
11f	NH_2	F	F	H		0.084	48
11g	NH_2	F	H	H		1.72	NA
11h	NH_2	F	F	H		1.28	38
11i	NH_2	F	F	H		0.98	23
11j	NH_2	F	H	H		5.96	NA
7a	OH	CH_3	H	H		NA	0.21
7b	OH	CH_3	H	H		NA	1.38
7c	OH	CH_3	H	H		NA	32
7d	OH	CH_3	H	H		10.63	0.30
7e	OH	CH_3	H	H		NA	0.028
7f	OH	CH_3	H	H		44	0.013
7g	OH	CH_3	H	NHAc		NA	47

The activity is expressed as percent inhibition at 10 μM or IC_{50} values.
*NA = not active (less than 15% inhibition @ 10 μM).
[a] Compounds reported by Tong and group.[27]
[b] Compounds reported by Alex and group.[29]

the strength of H-bonding in this crucial region of the DHODH active site, which appears to prefer a purely acceptor moiety (like the COOH group which gets deprotonated at pH 7.4) to form H-bonds with the donor groups of Arg136 and Gln47, and a

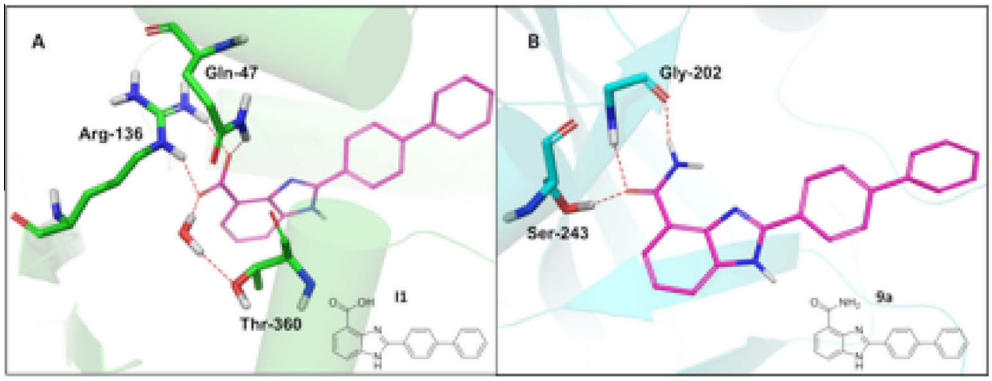
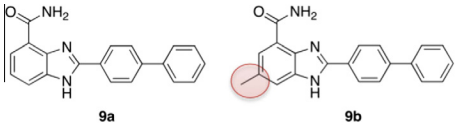


Figure 1. Docking modes showing the interactions of compounds **I1** (A) and **9a** (B) in DHODH (pdb ID: 4IGH) and PARP-1 (pdb ID: 4HHZ), respectively.



9a
PARP-1: IC_{50} = 0.71 uM
DHODH : IC_{50} = 9.80 uM

9b
PARP-1: 28% @ 10 uM
DHODH : IC_{50} = 7.80 uM

Figure 2. Structure and biochemical activity of compounds **9a** and **9b**.

water-mediated H-bond with Thr360 (Fig. 1A). This observation indicated that the COOH group at C4 position favors DHODH activity, while the $CONH_2$ substituent favors PARP-1 activity (by gaining polar contacts with the donor and acceptor groups of Ser243 and Gly202 as shown in Fig. 1B). Incorporating a CH_3 group at the R_2 position of the benzimidazole ring (**9b**) proved detrimental for PARP-1 activity, but it slightly improved the DHODH potency (Fig. 2). This might be due to the availability of enough space to accommodate a methyl group at this particular site of the DHODH pocket, in comparison to that in the PARP-1 active site where the same group could not be endured well.

Compounds **9c**–**g**, with functional groups other than a phenyl ring at the *para* position (R_5) were found to be completely inactive for both targets. Substituting a strongly electron-donating methoxy group at R_5 position (**9c** and **9d**), produced a devastating effect on the activity. Another approach was then carried out by substituting strong electron-withdrawing groups at this position such as $–CF_3$ (**9e** and **9f**) and –CN (**9g**), but these also proved to be inactive. These observations indicated that instead of small electron donating or withdrawing groups, a relatively bulkier system (e.g., in the form of a second phenyl ring) is required to sufficiently occupy the space available around this position in both PARP-1 and DHODH enzymes.

Further investigations were performed on the second phenyl ring in order to study the effect of substituting various functional groups at its *ortho*, *meta* and *para* positions. Incorporation of an acetamide group (NHAc) at the *ortho* (**10f)** and *meta* (**10g**) positions showed significant improvement especially in PARP-1 potency, but *para* substitution (**10h**) resulted in the loss of activity compared to the parent compound **9a**. These *o*- and *m*-acetamide substitutions showed H-bonding interactions with Arg217 (water-mediated) and Asp105, respectively, but the *p*-substitution failed to fetch any such interaction in PARP-1. In case of DHODH, the *o*-(**10f**) and *p*-(**10h**) acetamide substituted compounds did show H-bonding with Tyr38 and Leu67, respectively, but they only seemed to result in around 50% inhibition of the activity at 10 μM concentration (compound **10g** also demonstrated 45% inhibition of the DHODH activity at a relatively lower concentration of 1 μM). The interactions between compound **10g** and the active site residues of DHODH and PARP-1 is shown in Figure 3.

Substituting pyrrolidinyl methanone at the *para* position of the second phenyl ring as in the compounds **10c** and **10i** showed comparable potency in PARP-1 but poor activity in DHODH enzyme. As observed with compound **9b**, methyl substitution at the R_2 position for compounds **10d** and **11a** resulted in diminished activities, particularly for PARP-1 enzyme. When pyrrolidinyl methanone was substituted at position 3 of the pyridine ring as depicted by compounds **10a** and **10b**, a reduction in the activity was observed against both PARP-1 and DHODH as compared to the parent compound **9i**. Compound **10b** showed poor activity in PARP-1 probably due to the presence of the unfavorable $–CH_3$ group at the R_2 position. It is noteworthy that in all these analogs, the pyrrolidinyl methanone moiety could not gain any polar contact in the active site of both PARP-1 and DHODH. Increasing the chain length at *para* position of the second phenyl ring of the benzimidazole in compound **11d** did not result in good inhibitory activities. Compound **11e**, with one carbon atom less than **11d** however, exhibited reasonable activity. In both these compounds, the piperidinyl nitrogen got protonated at pH 7.4 and entered into H-bonding with Ile218 and Tyr38 in PARP-1 and DHODH, respectively.

Substituting the R_5 position of the benzimidazole scaffold with a hetero-aromatic moiety and removing the methyl substituent from the R_2 position dramatically improved the PARP-1 activity as exhibited by compound **9i** (IC_{50} = 0.022 μM). A slight decrease in activity was observed with electron donating groups attached to the hetero-aromatic moiety, for example, in compounds **9h** ($–NH_2$, IC_{50} = 0.032 μM) and **11b** ($–NHCOCH_3$, IC_{50} = 0.049 μM). Compound **10e** with a 2-pyrinyl moiety at R_5 position showed a drop in activity in PARP-1 with IC_{50} of 0.083 μM. The compounds **9h** and **11b** with polar groups (NH_2 and NHAc, respectively) towards the solvent exposed region could not fetch any polar interaction in PARP-1 active site. Though the pyridyl nitrogen (in most of the acetamide analogs) and the $–NHCOCH_3$ group in **11b** did form H-bond with Tyr38 and Leu67, respectively, in the DHODH pocket, but they failed to enhance the potency. In addition, a 3-fold drop in PARP-1 activity was observed with compound **11c** having a methyl-pyrrolidine moiety at position 3 of the pyridine ring (IC_{50} = 0.061 μM), but at the same time there is an increase in the DHODH activity to 55% at 10 μM concentration. However, the protonated nitrogen of the methyl-pyrrolidine group in this compound could not gain any polar contact in both the PARP-1 and DHODH enzymes.

The effects of fluoro and cyclopropane substitutions as bioisosteres were also investigated while maintaining the carboxamide group at the R_4 position of the benzimidazole scaffold. The only compound which demonstrated modest PARP-1 and DHODH dual activities was **11f** with fluorine substituted at R_3 and *meta* position of the second phenyl ring, in addition of the cyclopropanecarboxamide group at the *para* position. However, other compounds in

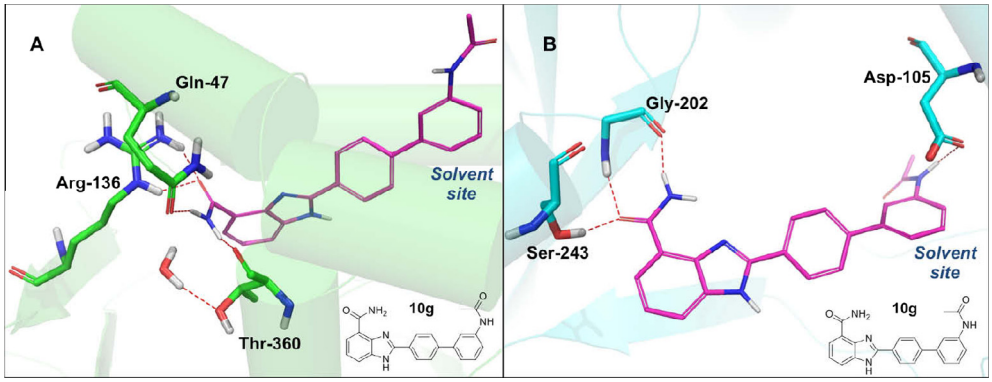


Figure 3. Docking modes showing the interactions of compound **10g** in DHODH (A, pdb ID: 4IGH) and PARP-1 (B, pdb ID: 4HHZ), respectively.

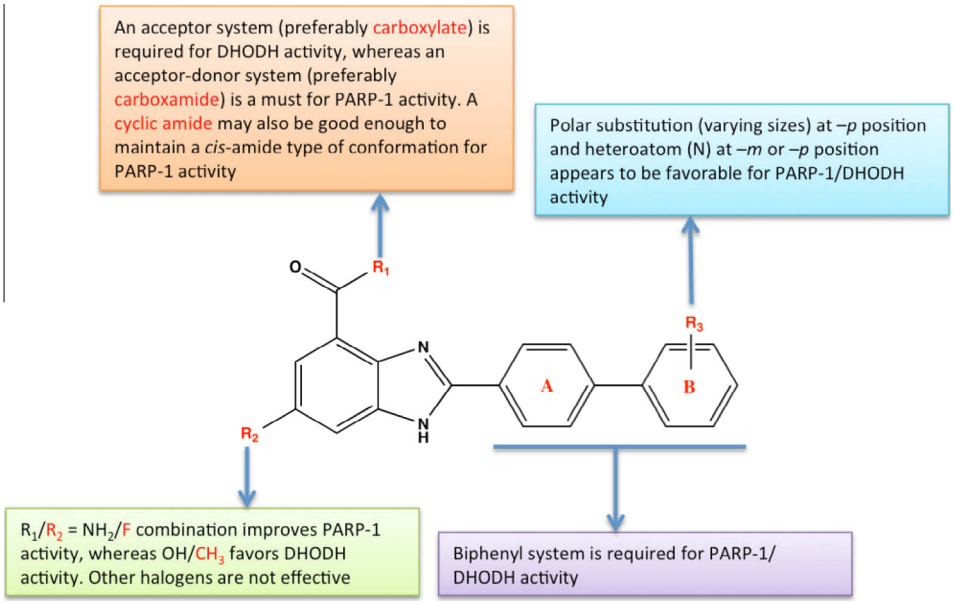


Figure 4. Summary of the SAR points for possible dual inhibition of DHODH and PARP-1 enzymes.

the same class, **11g**, **11h**, **11i** and **11j**, failed to exhibit any improvement in the dual activity. Compared to the methyl group, fluoro was well tolerated at R_2 position and resulted in improved PARP-1 activity. The cyclopropanecarboxamide group in most of these analogs formed H-bonding with Tyr49 and Leu67 in PARP-1 and DHODH, respectively.

Since it has been reported that the –COOH group at the R_4 position is favorable for DHODH activity,[4,5,19] we proceeded to synthesize seven benzimidazole carboxylic acid derivatives (Table 1). All these compounds have a methyl group at R_2 position, along with varied substitutions primarily on the second phenyl ring. Compound **7a** with an acetamide group at the *para* position of the second phenyl ring showed reasonably good DHODH activity (IC_{50} = 0.21 μM), compared to the *ortho* (**7c**) and *meta* (**7b**) substituted analogs. Similarly, compounds **7d**, **7e**, and **7f**, with a much bulkier substitution at the *para* position of the second phenyl ring displayed modest DHODH inhibitory activities (IC_{50} = 0.30 μM, 0.028 μM, and 0.013 μM, respectively). However, all the compounds shown in Table 1 had weak activity for PARP-1 except for compound **7f** which exhibited 44% inhibition at 10 μM concentration. The diminished PARP-1 activity of all these compounds may be attributed partly to the detrimental effect of methyl group at R_2 position, as well as to the absence of the favorable carboxamide moiety at R_4 position of the benzimidazole moiety. A summary of our SAR findings with respect to PARP-1 and DHODH dual inhibition is shown in Figure 4.

3. Conclusion

We have studied various benzimidazole derivatives as prospective inhibitors of both PARP-1 and DHODH enzymes. All the compounds have been synthesized in good yields. Several compounds, namely **7f**, **10g**, **11c** and **11f** showed dual potencies, albeit with relatively lower activity for both the targets. These compounds, however, can be considered for further study to understand the binding mechanisms and enhance their potential as dual inhibitors of PARP-1 and DHODH.

4. Experimental

4.1. Modeling

4.1.1. Ligand and protein preparation

The 2D structures of the ligands were sketched using ChemBioDraw Ultra 12.0 program and then converted into 3D format with the *LigPrep* utility of Schrödinger Software Suite 2014-1.

Similarly the protein–ligand complexes, obtained from the Protein Data Bank (PDB ID: 4IGH for DHODH and 4HHZ for PARP-1) were prepared using the *Protein Preparation Wizard* utility of the software. Hydrogen atoms were added, bond orders assigned, missing side-chains filled, and water molecules outside the active site deleted, followed by restrained minimization to relieve the strain and steric clashes in the protein–ligand complexes.

4.1.2. Receptor-grid generation

Using the *Glide* module of the Schrödinger Software Suite, the active site was defined by constructing a receptor grid spanning amino acid residues within a distance of around 10 Å from the co-crystallized ligand in the protein complexes. Some key hydrogen-bonding constraints (involving residues such as Gln47 and Arg136 in DHODH and Gly202 and Ser243 in PARP-1) were also defined while generating the receptor grid, to be employed in protein–ligand docking.

4.1.3. Protein–ligand docking

The prepared ligands (in sdf format) and protein structures (as receptor grids) were supplied as input files to the *Glide* module of the software for docking. During *Glide* docking, extra precision (XP) mode was employed. The protein was kept rigid, while ligands were given full flexibility in addition to sampling of nitrogen inversions and ring conformations. However the amide torsions were restricted to the *trans*-conformation only. Other parameters included adding *Epik* state penalties to docking scores, rewarding intramolecular hydrogen bonds, and enhancing planarity of the conjugated pi groups. The docking protocol was set to report at least 10 poses per ligand, which were viewed and analyzed within the protein active site for desired interactions using the *Maestro* viewer of the Schrodinger Software Suite.

4.2. Chemistry

Chemicals and reagents were either purchased from Merck, Sigma–Aldrich or provided by Aurigene Discovery Technologies Limited and used without further purification. NMR spectra were recorded on JEOL Lambda 400 and ECA 400. Thin layer chromatography (TLC) were carried out by using aluminum sheets TLC silica 60 F_{254} and flash column chromatography used were silica gel (40–60 μm) purchased from Merck. Anhydrous tetrahydrofuran (THF) used were purified from PureSolv solvent purification system and dichloromethane (CH_2Cl_2) were distilled from CaH_2 prior to use. Melting point were measured with Stuart Melting 30 (SMP 30) apparatus. Semi-preparative HPLC was performed on Waters HPLC Binary PUMP 1525, Waters Photodiode Array Detector 2998 with Merck Chromolith® Performance Reverse-phase C-18 column (100–4.6 mm). LC/MS was run on Agilent 1200 Series/Agilent Technologies 6530 Q-TOF (ESI) with Agilent Zorbax C-18 column.

4.2.1. General procedure for synthesis of 5

To a round bottom flask equipped with a magnetic stir bar was added diamine benzoate (**3**; $R_2 = CH_3$; F) (1 mmol), carboxylic acid **2a**–**z** (1 mmol), HATU (1.3 mmol) and dissolved in DMF (6 ml) stirred under N_2 gas. DiPEA (2 ml) was then added to the mixture and reaction mixture was left to stir for 3 h. Water (100 ml) was added to the reaction mixture, filtered and dried to afford **5** which was used without further purification.

4.2.2. General procedure for synthesis of 6

Compound **5** was dissolved in acetic acid (10 ml/mmol) and refluxed at 130 °C until reaction is fully completed under TLC analysis monitoring. Flash column chromatography with EtOAc/Hex (3:1 v/v) elution yielded purified compound **6**.

4.2.3. General procedure for synthesis of 7

Compound **6** was dissolved in mixture of MeOH/THF (1:1 v/v) followed by the addition of $LiOH{\cdot}H_2O$ (3–5 equiv) per mol and the reaction mixture was allowed to stir overnight. Upon reaction completion, solvents were removed in vacuo. The resulted aqueous mixture pH was adjusted to 2 with 10% HCl to obtain the desired product **7** which was filtered and dried and used without further purification.

4.2.3.1. 2-(4′-Acetamido-[1,1′-biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxylic acid (7a). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, NH), 8.33 (d, J = 8.3, 2H), 7.91 (d, J = 8.0, 2H), 7.83 (s, 1H), 7.81 (s, 1H), 7.75 (d, J = 9.0, 2H), 7.72 (d, J = 8.8, 2H), 2.50 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.10, 166.12, 151.38, 143.58, 139.96, 135.99, 134.90, 133.14, 131.05, 129.69, 128.22, 127.49, 126.71, 123.07, 119.71, 119.44, 117.08, 24.23, 21.12. HRMS (ESI) calculated for $C_{23}H_{19}N_3O_3$ $(M+H)^+$: 386.1504, found 386.1499.

4.2.3.2. 2-(3′-Acetamido-[1,1′-biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxylic acid (7b). ^{1}H NMR (270 MHz, DMSO-d_6): δ 10.08 (s, NH), 8.37 (d, J = 8.1, 2H), 7.97 (s, 1H), 7.76 (d, J = 8.4, 2H), 7.70 (s, 1H), 7.65 (s, 1H), 7.60 (br s, 1H), 7.41 (s, 1H), 7.39 (s, 1H), 2.46 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (67 MHz, DMSO-d_6): δ 168.67, 166.83, 152.52, 141.74, 140.04, 139.82, 129.53, 128.60, 128.12, 126.91, 125.80, 121.57, 118.67, 117.32, 24.12, 21.02. HRMS (ESI) calculated for $C_{23}H_{19}N_3O_3$ $(M+H)^+$: 386.1504, found 386.1502.

4.2.3.3. 2-(2′-Acetamido-[1,1′-biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxylic acid (7c). ^{1}H NMR (270 MHz, CD_3OD): δ 8.03 (d, J = 8.3, 2H), 7.84 (s, 1H), 7.53 (d, J = 8.3, 1H), 7.40 (d, J = 8.3, 2H), 7.38 (d, J = 5.9, 1H), 7.30–7.21 (m, 3H), 2.33 (s, 3H), 1.88 (s, 3H). ^{13}C NMR (67 MHz, CD_3OD): δ 172.44, 167.62, 154.55, 145.16, 142.97, 138.17, 135.47, 133.25, 131.39, 130.50, 129.48, 128.58, 128.52, 128.46, 127.96, 127.44, 124.14, 115.25, 22.92, 21.29. HRMS (ESI) calculated for $C_{23}H_{19}N_3O_3$ $(M+H)^+$: 386.1504, found 386.1510.

4.2.3.4. 2-(4′-((2-Acetamidobenzyl)oxy)-[1,1′-biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxylic acid (7d). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.66 (s, NH), 8.40 (d, J = 8.5, 2H), 7.96 (d, J = 7.9, 2H), 7.89 (br s, 2H), 7.80 (d, J = 8.5, 2H), 7.47 (d, J = 7.3, 1H), 7.43 (d, J = 7.9, 1H), 7.30 (t, J = 7.9, 1H), 7.20 (t, J = 7.3, 1H), 7.12 (d, J = 8.5, 2H), 5.16 (s, 2H), 2.54 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.71, 165.65, 158.94, 150.77, 144.10, 135.75, 130.88, 130.76, 130.04, 129.97, 129.49, 128.71, 128.33, 128.02, 126.44, 125.42, 125.30, 121.20, 118.51, 117.25, 115.50, 66.24, 23.31, 20.87. HRMS (ESI) calculated for $C_{30}H_{25}N_3O_4$ $(M+H)^+$: 492.1923, found 492.1929.

4.2.3.5. 2-(4′-((3-Acetamidobenzyl)oxy)-[1,1′-biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxylic acid (7e). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.26 (s, NH), 8.40 (d, J = 8.3, 2H), 7.89 (d, J = 8.3, 2H), 7.84 (s, 1H), 7.83 (s, 1H), 7.78 (s, 1H), 7.73 (d, J = 8.5, 2H), 7.60 (d, J = 8.0, 1H), 7.31 (t, J = 7.8, 1H), 7.13 (d, J = 10.2, 1H), 7.10 (d, J = 8.8, 2H), 5.12 (s, 2H), 2.51 (s, CH_3), 2.09 (s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.66, 165.72, 158.89, 150.65, 143.78, 139.67, 137.47, 135.24, 134.37, 130.83, 129.99, 129.80, 128.86, 128.41, 128.23, 126.34, 122.19, 121.55, 118.62, 118.08, 117.09, 115.46, 115.42, 69.42, 24.12, 20.96. HRMS (ESI) calculated for $C_{30}H_{25}N_3O_4$ $(M+H)^+$: 492.1923, found 492.1922.

4.2.3.6. 2-(4′-((4-Acetamidobenzyl)oxy)-[1,1′-biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxylic acid (7f). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.30 (s, NH), 8.34 (d, J = 8.5, 2H), 7.88 (d, J = 8.5, 2H), 7.78 (br s, 2H), 7.75 (d, J = 8.8, 2H), 7.60 (d, J = 8.5,

2H), 7.38 (d, J = 8.5, 2H), 7.12 (d, J = 8.8, 2H), 5.09 (s, 2H), 2.03 (s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.41, 166.14, 158.77, 151.65, 143.06, 139.09, 133.69, 131.29, 131.14, 129.11, 128.48, 128.14, 127.34, 126.37, 124.12, 120.01, 118.95, 116.60, 115.50, 69.18, 24.11, 20.91. HRMS (ESI) calculated for $C_{30}H_{25}N_3O_4$ $(M+H)^+$: 492.1923, found 492.1928.

4.2.3.7. 2-(2-Acetamido-[1,1′-biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxylic acid (7g). ^1H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, NH), 8.40 (s, 1H), 8.22 (d, J = 7.8, 1H), 7.74 (s, 1H), 7.71 (s, 1H), 7.52 (d, J = 8.1, 1H), 7.49–7.46 (m, 5H), 1.95 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.06, 166.51, 151.76, 139.21, 138.29, 135.32, 132.22, 130.79, 128.66, 128.55, 127.73, 127.55, 127.05, 126.43, 125.49, 116.10, 22.97, 20.98. HRMS (ESI) calculated for $C_{23}H_{19}N_3O_3$ $(M+H)^+$: 386.1504, found 386.1505.

4.2.4. General procedure for synthesis of 8

To a solution of DMF/pyridine (1:1 v/v) was added compound **2a–z** (0.9 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.9 mmol) stirred at 60 °C under N_2 gas atmosphere for 3 h. After the reaction mixture was cooled down to room temperature, 2,3-diaminobenzamide **4** (1 mmol) was added and left to stir overnight until the completion of the reaction as monitored by TLC analysis. Water (100 ml) was added and the resulting product **8** was filtered and dried without further purification.

4.2.5. General procedure for synthesis of 9, 10 and 11

4.2.5.1. From compound 7. To a solution of DMF (6 ml) was added EDCI·HCl (2 mmol), HOBt (2 mmol), NH_4Cl (5 mmol), compound **7** (1 mmol) followed by DiPEA (1 ml) and was left to stir overnight until reaction was completed judged by TLC analysis. Water (100 ml) was added to the reaction mixture which the resulting product was filtered and dried to afford compound **9**, **10** and **11**.

4.2.5.2. From compound 8. Compound **8** was dissolved in acetic acid (AcOH) (10 ml/mmol) and refluxed at 130 °C until reaction was fully completed under TLC analysis monitoring. Flash column chromatography with EtOAc/Hex (3:1 v/v) elution yielded purified compound **9**, **10** and **11**.

4.2.5.3. 2-([1,1′-Biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (9a). Compound reported by Tong et al.[27]

4.2.5.4. 2-([1,1′-Biphenyl]-4-yl)-6-methyl-1*H*-benzimidazole-4-carboxamide (9b). ^1H NMR (400 MHz, DMSO-d_6): δ 13.29 (s, $CONH_2$), 9.34 (s, NH), 8.31 (d, J = 7.1, 2H), 7.91 (d, J = 8.1, 1H), 7.79 (d, J = 7.8, 2H), 7.72 (s, 1H), 7.54 (s, 1H), 7.51 (t, J = 7.8, 2H), 7.43 (t, J = 7.3, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.33, 151.19, 141.89, 139.83, 139.18, 135.76, 132.02, 129.14, 128.23, 128.11, 127.37, 127.33, 126.80, 124.39, 121.87, 114.82, 21.34. HRMS (ESI) calculated for $C_{21}H_{17}N_3O$ $(M+H)^+$: 328.1449, found 328.1461.

4.2.5.5. 2-(4-Methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (9c). Compound reported by White et al.[29]

4.2.5.6. 2-(4-Methoxyphenyl)-6-methyl-1*H*-benzimidazole-4-carboxamide (9d). ^1H NMR (270 MHz, DMSO-d_6): δ 12.99 (s, $CONH_2$), 9.28 (s, NH), 8.09 (d, J = 8.9, 2H), 7.61 (s, 1H), 7.41 (s, 1H), 7.07 (d, J = 8.9, 2H), 3.79 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (67 MHz, DMSO-d_6): δ 166.33, 161.03, 151.59, 139.84, 135.63, 131.35, 128.39, 123.97, 121.72, 121.53, 114.52, 114.47, 55.41, 21.25. HRMS (ESI) calculated for $C_{16}H_{15}N_3O_2$ $(M+H)^+$: 282.1242, found 282.1239.

4.2.5.7. 2-(4-(Trifluoromethyl)phenyl)-1*H*-benzimidazole-4-carboxamide (9e). Compound reported by White et al.[29]

4.2.5.8. 6-Methyl-2-(4-(trifluoromethyl)phenyl)-1*H*-benzimidazole-4-carboxamide (9f). ^1H NMR (400 MHz, DMSO-d_6): δ 13.52 (br s, $CONH_2$), 9.24 (s, NH), 8.44 (d, J = 8.1, 2H), 7.96 (d, J = 8.1, 2H), 7.74 (s, 1H), 7.57 (s, 1H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.09, 149.81, 139.51, 135.78, 133.10, 132.54, 130.16, 129.84, 128.14, 127.42, 126.00, 122.72, 122.19, 115.19, 21.25. HRMS (ESI) calculated for $C_{16}H_{12}N_3F_3O$ $(M+H)^+$: 320.1010, found 320.1009.

4.2.5.9. 2-(4-Cyanophenyl)-6-methyl-1*H*-benzimidazole-4-carboxamide (9g). ^1H NMR (400 MHz, DMSO-d_6): δ 13.45 (br s, $CONH_2$), 9.29 (s, NH), 8.31 (d, J = 9.7, 2H), 8.06 (d, J = 7.3, 2H), 7.72 (d, J = 1.8, 1H), 7.55 (d, J = 1.7, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.81, 166.67, 151.10, 140.12, 136.18, 135.99, 132.81, 132.15, 128.72, 127.03, 125.12, 122.48, 115.45, 21.77. HRMS (ESI) calculated for $C_{16}H_{12}N_4O$ $(M+H)^+$: 277.1089, found 277.1092.

4.2.5.10. 2-(4-(6-Aminopyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (9h). ^1H NMR (400 MHz, DMSO-d_6): δ 13.39 (s, $CONH_2$), 9.39 (s, NH), 8.39 (s, 1H), 8.27 (d, J = 8.3, 2H), 7.88–7.79 (m, 4H), 7.74 (d, J = 8.3, 1H), 7.35 (t, J = 7.6, 1H), 6.58 (d, J = 8.7, 1H), 6.28 (s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.21, 159.46, 151.83, 145.83, 141.58, 140.06, 135.48, 135.36, 127.46, 126.75, 125.65, 122.89, 122.70, 122.28, 114.90, 108.16. HRMS (ESI) calculated for $C_{19}H_{15}N_5O$ $(M+H)^+$: 330.1354, found 330.1348.

4.2.5.11. 2-(4-(Pyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (9i). Compound reported by Tong et al.[27]

4.2.5.12. 2-(4-(5-(Pyrrolidine-1-carbonyl)pyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (10a). ^1H NMR (400 MHz, DMSO-d_6): δ 9.22 (s, 1H), 8.90 (s, 1H), 8.80 (br s, NH), 8.71 (s, 1H), 8.37 (d, J = 8.1, 2H), 8.07 (d, J = 8.1, 2H), 7.91 (d, J = 7.7, 1H), 7.86 (d, J = 8.6,1H), 7.47 (t, J = 8.1, 1H), 3.48–3.43 (m, 4H), 1.82–1.79 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.20, 164.47, 150.96, 144.55, 143.21, 138.35, 138.20, 136.65, 135.39, 134.44, 129.64, 128.42, 126.31, 124.98, 124.76, 122.06, 117.21 49.18, 46.67, 26.19, 24.24. HRMS (ESI) calculated for $C_{24}H_{21}N_5O_2$ $(M+H)^+$: 412.1773, found 412.1765.

4.2.5.13. 6-Methyl-2-(4-(5-(pyrrolidine-1-carbonyl)pyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (10b). ^1H NMR (400 MHz, DMSO-d_6): δ 9.10 (br s, 1H), 9.04 (br s, NH), 8.76 (br s, 1H), 8.38 (s, 1H) 8.33 (d, J = 8.6, 2H), 8.03 (d, J = 8.6, 2H), 7.71 (s, 1H), 7.57 (s, 1H), 3.48–3.44 (m, 4H), 1.86–1.81 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.89, 165.91, 151.09, 147.94, 146.50, 138.65, 135.85, 134.30, 134.18, 133.55, 130.42, 129.35, 128.62, 128.24, 126.62, 125.43, 122.07, 115.99, 49.29, 46.63, 26.43, 24.46, 21.69. HRMS (ESI) calculated for $C_{25}H_{23}N_5O_2$ $(M+H)^+$: 426.1929, found 426.1946.

4.2.5.14. 2-(4-(6-(Pyrrolidine-1-carbonyl)pyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (10c). ^1H NMR (400 MHz, DMSO-d_6): δ 9.03 (br s, NH), 8.66 (br s, 1H), 8.37 (d, J = 8.6, 2H), 8.33 (s, 1H), 8.07 (d, J = 8.6, 2H), 7.95 (d, J = 7.7, 1H), 7.90 (d, J = 8.1, 1H), 7.83 (br s, 1H), 7.53 (t, J = 8.1, 1H), 3.62 (m, 2H), 3.49 (m, 2H), 1.82 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.90, 165.35, 153.71, 151.07, 146.51, 140.53, 136.24, 134.03, 133.79, 129.98, 128.10, 125.30, 124.89, 124.25, 122.28, 117.00, 49.02, 46.97, 26.53, 23.98. HRMS (ESI) calculated for $C_{24}H_{21}N_5O_2$ $(M+H)^+$: 412.1773, found 412.1761.

4.2.5.15. 6-Methyl-2-(4-(6-(pyrrolidine-1-carbonyl)pyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (10d). ^1H NMR

(400 MHz, DMSO-d_6): δ 9.10 (s, NH), 9.00 (s, 1H), 8.33 (d, J = 8.6, 2H), 8.30 (dd, J = 2.3, 8.2, 1H), 8.01 (d, J = 8.2, 2H), 7.82 (d, J = 8.2, 1H), 7.70 (s, 1H), 7.55 (s, 1H), 3.64 (t, J = 6.3, 2H), 3.49 (t, J = 6.8, 2H), 2.50 (t, J = 5.4, 3H), 1.85–1.83 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.81, 165.75, 153.97, 151.21, 146.66, 138.91, 136.09, 135.80, 135.64, 133.22, 128.69, 128.44, 128.16, 125.26, 124.17, 122.18, 115.83, 49.09, 47.03, 26.65, 24.07, 21.75. HRMS (ESI) calculated for $C_{25}H_{23}N_5O_2$ (M+H)$^+$: 426.1929, found 426.1935.

4.2.5.16. 2-(4-(Pyridin-2-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (10e). Compound reported by Tong et al.[27]

4.2.5.17. 2-(2′-Acetamido-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (10f). ^{1}H NMR (270 MHz, CD_3OD): δ 8.19 (d, J = 8.3, 2H), 7.92 (d, J = 7.8, 1H), 7.68 (d, J = 7.8, 1H), 7.52 (d, J = 8.3, 2H), 7.48 (d, J = 8.1, 1H), 7.36–7.30 (m, 3H), 7.22 (t, J = 7.5, 1H), 1.98 (s, 3H). ^{13}C NMR (67 MHz, CD_3OD): δ 172.54, 153.64, 143.00, 138.24, 135.64, 131.40, 130.63, 129.55, 129.47, 128.45, 128.08, 127.97, 124.39, 123.50, 123.27, 122.56, 118.58, 22.90. HRMS (ESI) calculated for $C_{22}H_{18}N_4O_2$ (M+H)$^+$: 371.1507, found 371.1505.

4.2.5.18. 2-(3′-Acetamido-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (10g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.49 (s, $CONH_2$), 10.10 (s, NH), 9.38 (s, NH), 8.33 (d, J = 8.3, 2H), 7.99 (s, 1H), 7.88 (d, J = 7.7, 1H), 7.83 (d, J = 8.3, 2H), 7.74 (d, J = 7.8, 1H), 7.60 (s, 1H), 7.42 (d, J = 4.8, 2H), 7.34 (t, J = 7.8, 1H), 2.07 (s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.56, 166.22, 151.60, 142.07, 141.55, 140.02, 139.66, 135.41, 129.48, 128.17, 127.54, 127.26, 123.02, 122.42, 121.54, 118.35, 117.28, 115.05, 24.07. HRMS (ESI) calculated for $C_{22}H_{18}N_4O_2$ (M+H)$^+$: 371.1507, found 371.1509.

4.2.5.19. 2-(4′-Acetamido-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (10h). ^{1}H NMR (270 MHz, DMSO-d_6): δ 8.32 (d, J = 8.4, 2H), 8.22 (d, J = 7.8, 1H), 8.14 (d, J = 8.1, 1H), 7.93 (d, J = 8.4, 2H), 7.75 (d, J = 8.6, 2H), 7.69 (d, J = 8.6, 2H), 7.54 (br s, 1H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 168.98, 167.10, 153.10, 142.02, 139.85, 133.94, 129.92, 129.04, 128.67, 127.79, 127.48, 126.84, 126.31, 125.17, 122.55, 119.85, 116.65, 24.54. HRMS (ESI) calculated for $C_{22}H_{18}N_4O_2$ (M+H)$^+$: 371.1507, found 371.1508.

4.2.5.20. 2-(4′-(Pyrrolidine-1-carbonyl)-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (10i). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38 (br s, NH), 8.37 (d, J = 8.4, 2H), 7.96 (d, J = 8.4, 2H), 7.90 (d, J = 7.2, 1H), 7.84 (d, J = 8.4, 2H), 7.78 (d, J = 8.0, 1H), 7.65 (d, J = 8.0, 2H), 7.37 (t, J = 7.2, 1H), 3.51–3.48 (m, 4H), 1.90–1.83 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.34, 166.79, 152.07, 141.63, 140.77, 137.14, 128.99, 128.75, 128.43, 128.36, 128.08, 127.94, 127.16, 127.05, 123.48, 122.92, 115.68, 49.10, 46.51, 26.53, 24.44. HRMS (ESI) calculated for $C_{25}H_{22}N_4O_2$ (M+H)$^+$: 411.1820, found 411.1817.

4.2.5.21. 6-Methyl-2-(4′-(pyrrolidine-1-carbonyl)-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (11a). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.33 (s, $CONH_2$), 9.33 (s, NH), 8.31 (d, J = 8.5, 2H), 7.93 (d, J = 8.3, 2H), 7.83 (d, J = 8.3, 2H), 7.70 (s, 1H), 7.64 (d, J = 8.1, 2H), 7.53 (s, 1H), 3.48–3.26 (m, 4H, overlap), 1.87–1.82 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.86, 166.20, 151.02, 140.95, 140.28, 139.77, 136.61, 135.72, 132.02, 128.59, 128.23, 127.90, 127.39, 126.51, 124.38, 121.90, 114.82, 48.95, 46.00, 25.99, 23.92, 21.29. HRMS (ESI) calculated for $C_{26}H_{24}N_4O_2$ (M+H)$^+$: 425.1977, found 425.1979.

4.2.5.22. 2-(4-(6-Acetamidopyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (11b). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.48 (s, $CONH_2$), 10.67 (s, NH), 9.38 (s, NH), 8.77 (s, 1H), 8.34 (d, J = 8.0, 2H), 8.21 (s, 2H), 7.97 (d, J = 8.0, 2H), 7.89 (d, J = 7.3, 1H), 7.81–7.75 (m, 1H), 7.36 (t, J = 8.0, 1H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.96, 166.70, 152.32, 152, 03, 146.42, 142.03, 139.24, 136.72, 135.88, 130.42, 128.63, 128.06, 127.39, 123.54, 122.91, 115.54, 113.72, 24.46. HRMS (ESI) calculated for $C_{21}H_{17}N_5O_2$ (M+H)$^+$: 372.1460, found 372.1461.

4.2.5.23. 2-(4-(5-(Pyrrolidin-1-ylmethyl)pyridin-3-yl)phenyl)-1*H*-benzimidazole-4-carboxamide (11c). ^{1}H NMR (400 MHz, CD_3OD): δ 8.79 (s, 1H), 8.52 (s, 1H), 8.26 (d, J = 8.3, 2H), 8.15 (s, 1H), 7.92 (bd, 1H), 7.83 (d, J = 8.3, 2H), 7.71 (bd, J = 7.5, 1H), 3.84 (s, 2H), 2.69 (br s, 4H), 1.87 (br s, 4H). ^{13}C NMR (67 MHz, CD_3OD): δ 68.45, 57.61, 55.03, 24.10, 22.13. HRMS (ESI) calculated for $C_{24}H_{23}N_5O$ (M+H)$^+$: 398.1980, found 398.1989.

4.2.5.24. 2-(4′-(3-(Piperidin-1-yl)propoxy)-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (11d). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.67 (s, $CONH_2$), 9.39 (br s, NH), 8.33 (d, J = 8.3, 1H), 7.96 (d, J = 8.0, 2H), 7.85 (br s, 1H), 7.72 (d, J = 8.0, 2H), 7.66 (d, J = 8.3, 2H), 7.33 (br s, 1H), 7.03 (d, J = 8.5, 2H), 4.09 (br s, 2H), 2.92 (br s, 2H), 2.11 (br s, 2H), 1.70 (br s, 4H), 1.48 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.35, 158.64, 143.68, 131.43, 129.95, 129.35, 128.12, 126.06, 115.05, 65.44, 53.83, 52.57, 24.12, 23.27, 22.21. HRMS (ESI) calculated for $C_{28}H_{30}N_4O_2$ (M+H)$^+$: 455.2446, found 455.2441.

4.2.5.25. 2-(4′-(2-(Piperidin-1-yl)ethoxy)-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (11e). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.61 (br s, $CONH_2$), 9.42 (s, NH), 8.28 (d, J = 7.5, 2H), 7.87 (d, J = 6.8, 1H), 7.77 (d, J = 8.3, 2H), 7.73 (d, J = 7.5, 1H), 7.63 (d, J = 8.3, 2H), 7.31 (t, J = 7.5, 1H), 6.98 (d, J = 8.3, 2H), 4.06 (t, J = 5.3, 2H), 2.68 (t, J = 5.3, 2H), 1.46 (t, J = 5.3, 4H), 1.31 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.88, 159.01, 152.30, 142.30, 135.97, 131.92, 130.43, 129.52, 128.37, 128.01, 127.82, 127.38, 127.08, 126.86, 123.44, 122.78, 115.54, 65.75, 57.51, 54.67, 25.66, 24.08, 21.74. HRMS (ESI) calculated for $C_{27}H_{28}N_4O_2$ (M+H)$^+$: 441.2290, found 441.2291.

4.2.5.26. 2-(4′-(Cyclopropanecarboxamido)-2′,3-difluoro-[1,1′-biphenyl]-4-yl)-6-fluoro-1*H*-benzimidazole-4-carboxamide (11f). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.60 (s, NH), 9.26 (s, NH), 8.35 (t, J = 7.8, 1H), 7.99–7.60 (m, 5H), 7.43 (s, 1H), 7.41 (s, 1H), 1.80–1.75 (m, 1H), 0.84–0.81 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.46, 165.10, 160.45, 160.42, 158.00, 157.97, 147.99, 141.44, 141.32, 138.44, 130.82, 130.50, 125.30, 124.05, 120.11, 116.39, 115.77, 115.27, 110.78, 106.43, 102.10, 14.84, 7.75. HRMS (ESI) calculated for $C_{24}H_{17}N_4F_3O_2$ (M+H)$^+$: 451.1381, found 451.1378.

4.2.5.27. 6-Fluoro-2-(4′-propionamido-[1,1′-biphenyl]-4-yl)-1*H*-benzimidazole-4-carboxamide (11g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, NH), 9.35 (s, NH), 8.27 (d, J = 8.0, 2H), 7.86 (d, J = 8.3, 2H), 7.24 (br s, 4H), 7.59 (s, 1H), 7.57 (s, 1H), 2.34 (q, J = 7.6, 2H), 1.08 (t, J = 7.6, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.38, 165.20, 158.47, 152.47, 141.81, 139.54, 133.45, 128.27, 127.58, 127.36, 127.16, 126.82, 125.98, 123.31, 119.53, 110.30, 101.68, 29.68, 9.75. HRMS (ESI) calculated for $C_{23}H_{19}N_4F_1O_2$ (M+H)$^+$: 403.1570, found 403.1575.

4.2.5.28. 2-(3′-(Cyclopropanecarboxamido)-3-fluoro-[1,1′-biphenyl]-4-yl)-6-fluoro-1*H*-benzimidazole-4-carboxamide (11h). ^{1}H NMR (270 MHz, DMSO-d_6): δ 10.38 (s, NH), 9.27 (s, NH), 8.36 (t, J = 8.1, 1H), 8.04 (s, 1H), 7.71–7.60 (m, 5H), 7.43 (s, 1H), 7.41 (s, 1H), 1.80 (m, 1H), 0.82 (m, 4H). ^{13}C NMR (67 MHz, DMSO-d_6): δ 174.42, 165.36, 160.90, 158.47, 148.31, 144.92, 140.54, 138.71, 137.95, 136.25, 131.24, 130.03, 124.00, 123.64, 122.00, 119.73, 117.77, 116.12, 114.85, 111.14, 102.37, 15.08, 7.74.

HRMS (ESI) calculated for $C_{24}H_{18}N_4F_2O_2$ $(M+H)^+$: 433.1475, found 433.1480.

4.2.5.29. 2-(4′-(Cyclopropanecarboxamido)-3-fluoro-3′-methoxy-[1,1′-biphenyl]-4-yl)-6-fluoro-1*H*-benzimidazole-4-carboxamide (11i). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.53 (s, NH), 9.27 (s, NH), 8.33 (t, J = 8.0, 1H), 8.10 (d, J = 8.3, 1H), 7.87 (d, J = 12.9, 1H), 7.78 (dd, J = 1.7, 8.3, 1H), 7.60 (bm, 2H), 7.44 (s, 1H), 7.37 (dd, J = 1.9, 8.5, 1H), 3.98 (s, 3H), 2.12 (m, 1H), 0.80–0.76 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.08, 164.84, 160.48, 158.06, 149.56, 148.05, 144.20, 137.53, 135.84, 133.15, 130.55, 128.27, 123.45, 122.98, 121.81, 118.87, 115.17, 114.18, 110.60, 109.48, 101.92, 55.97, 14.26, 7.44. HRMS (ESI) calculated for $C_{25}H_{20}N_4F_2O_3$ $(M+H)^+$: 463.1581, found 463.1572.

4.2.5.30. 2-(4′-(Cyclopropanecarboxamido)-3′-methoxy-[1,1′-biphenyl]-4-yl)-6-fluoro-1*H*-benzimidazole-4-carboxamide (11j). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.51 (s, NH), 9.33 (s, NH), 8.30 (d, J = 8.3, 2H), 8.07 (d, J = 7.5, 1H), 7.92 (d, J = 7.5, 2H), 7.60 (s, 1H), 7.57 (s, 1H), 7.40 (s, 1H), 7.31 (d, J = 8.3, 2H), 3.98 (s, 3H), 2.12 (m, 1H), 0.81 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.99, 164.97, 159.55, 152.79, 149.62, 141.92, 138.28, 135.81, 134.68, 131.53, 127.38, 127.06, 121.97, 118.66, 109.36, 101.31, 55.88, 14.22, 7.81. HRMS (ESI) calculated for $C_{25}H_{21}N_4F_1O_3$ $(M+H)^+$: 445.1675, found 445.1680.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.05.051.

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