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Discovery and SAR of 2-(1-propylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide: A potent inhibitor of poly(ADP-ribose) polymerase (PARP) for the treatment of cancer

Thomas D. Penning [a,*], Gui-Dong Zhu [a], Viraj B. Gandhi [a], Jianchun Gong [a], Sheela Thomas [a], Wilfried Lubisch [b], Roland Grandel [b], Wolfgang Wernet [b], Chang H. Park [c], Elizabeth H. Fry [c], Xuesong Liu [a], Yan Shi [a], Vered Klinghofer [a], Eric F. Johnson [a], Cherrie K. Donawho [a], David J. Frost [a], Velitchka Bontcheva-Diaz [a], Jennifer J. Bouska [a], Amanda M. Olson [a], Kennan C. Marsh [d], Yan Luo [a], Saul H. Rosenberg [a], Vincent L. Giranda [a]

[a] *Cancer Research, GPRD, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA*
[b] *Abbott Discovery Research, Neuroscience, D-67008 Ludwigshafen, Germany*
[c] *Structural Biology, GPRD, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA*
[d] *Pharmacokinetics, GPRD, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA*

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ABSTRACT

We have developed a series of cyclic amine-containing benzimidazole carboxamide poly(ADP-ribose)polymerase (PARP) inhibitors, with good PARP-1 enzyme potency, as well as cellular potency. These efforts led to the identification of a lead preclinical candidate, **10b**, 2-(1-propylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (A-620223). **10b** displayed very good potency against both the PARP-1 enzyme with a K_i of 8 nM and in a whole cell assay with an EC_{50} of 3 nM. **10b** is aqueous soluble, orally bioavailable across multiple species, and demonstrated good in vivo efficacy in a B16F10 subcutaneous murine melanoma model in combination with temozolomide (TMZ) and in an MX-1 breast xenograph model in combination with cisplatin.

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1. Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1) is a key DNA damage-sensing enzyme that facilitates the repair of DNA. All of the PARP family of enzymes share a catalytic PARP homology domain, but only PARP-1 and PARP-2 contain DNA-binding domains which localize them to the sites of DNA damage.[1] When triggered by DNA damage, PARP-1 catalyzes the transfer of ADP-ribose units from intracellular nicotinamide adenine dinucleotide (NAD^+) to nuclear acceptor proteins, leading to the formation of protein-bound ADP-ribose polymers. This cellular ADP-ribose transfer process is pivotal for the repair of DNA caused by DNA damaging chemotherapeutic agents or radiation. Thus PARP-1 and PARP-2 contribute to the resistance that often develops after cancer therapy.[2] In vivo inhibition of PARP has been shown to block this intracellular DNA repair process and increase the maximum therapeutic benefit of several cytotoxic chemotherapeutics, as well as ionizing radiation.[3–7] A significant number of potent PARP inhibitors have been described in recent years, most containing a primary amide or lactam functionality.[4–8] These inhibitors generally bind to the nicotinamide binding site of the PARP enzyme and structurally mimic the binding mode of nicotinamide.[9] Utilizing a benzimidazole carboxamide scaffold with relatively high intrinsic potency (i.e., **1**), we have developed a series of cyclic amine-substituted benzimidazole analogs leading to a lead preclinical candidate **10b** (A-620223). This compound displayed very good potency against both PARP-1 and PARP-2, along with oral efficacy in a number of preclinical murine tumor models, potentiating the efficacy of radiation and several cytotoxic agents such as temozolomide (TMZ) and cisplatin. **10b** has excellent pharmaceutical properties, with significant aqueous solubility, moderate protein binding, and good oral bioavailability in multiple species.

2. Chemistry

A nine-step large-scale synthesis of **10b** has been recently described.[10] The compounds described herein were synthesized

* Corresponding author at present address: Abbott Laboratories, Dept. R47S/AP10-3, 100 Abbott Park Road, Abbott Park, IL 60064-6101, USA. Tel.: +1 847 938 6707; fax: +1 847 935 5165.
E-mail address: thomas.penning@abbott.com (T.D. Penning).

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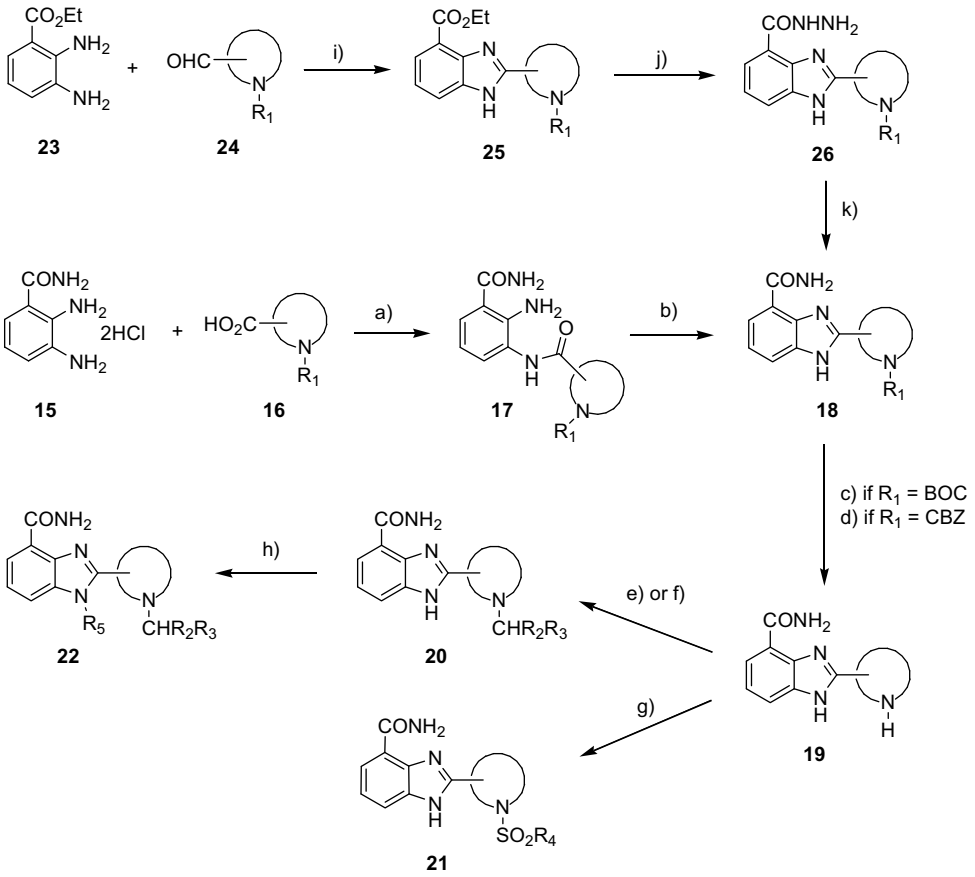


Scheme 1. General synthesis of benzimidazole carboxamide analogs. a) CDI, DMF, pyridine or EtO_2CCl, THF; b) HOAc, heat; c) TFA, CH_2Cl_2; d) 10% Pd/C, H_2, MeOH; e) $R_2R_3C(O)$, $NaBH_3CN$, MeOH; f) $R_2R_3C(O)$, $NaBH(OAc)_3$, DCE, MeOH, AcOH; g) R_4SO_2Cl; h) NaH, R_5X, DMF; i) $Cu(OAc)_2$, MeOH, H_2O, heat; j) NH_2NH_2, EtOH, heat; k) Raney Ni, DMF, H_2O, heat.

using closely-related procedures outlined in Scheme 1. 2,3-Diaminobenzamide dihydrochloride **15** was coupled to a cyclic amino acid **16**, under CDI or ethyl chloroformate conditions, to give amide **17**. Heating in acetic acid provided the corresponding substituted benzimidazole **18**. In analogs where the cyclic amine was protected with a BOC- or a CBZ-group, the protecting group was removed using trifluoroacetic acid or catalytic hydrogenation, respectively, to provide secondary amines **19**. Alkylation of **19** under sodium cyanoborohydride or sodium triacetoxyborohydride-mediated reductive amination conditions with aldehydes or ketones provided tertiary amines **20**. Alternately, amine **19** was sulfonylated using a sulfonyl chloride to provide sulfonamides **21**. Selected analogs could also be selectively alkylated at N-1 of the benzimidazole to provide alkylated compounds **22**. An alternate route has also been employed to generate benzimidazole analogs. Ethyl 2,3-diaminobenzoate **23**[11] was heated with a cyclic amino aldehyde **24** and cupric acetate in methanol and water to directly provide benzimidazole **25**. The ester was converted to the hydrazide **26** with hydrazine, followed by Raney nickel reduction to give **18**. The cupric acetate mediated benzimidazole formation with an aldehyde could also be employed with amide **15** to give **18** in a one-step process. However, overall, yields were inconsistent and purity of crude product generally not as high.

3. Results and discussion

The benzimidazole carboxamide series of PARP inhibitors is a relatively small scaffold with high intrinsic potency due to an intramolecular hydrogen-bonded conformation that closely mimics the nicotinamide binding interactions in the PARP-1 active site

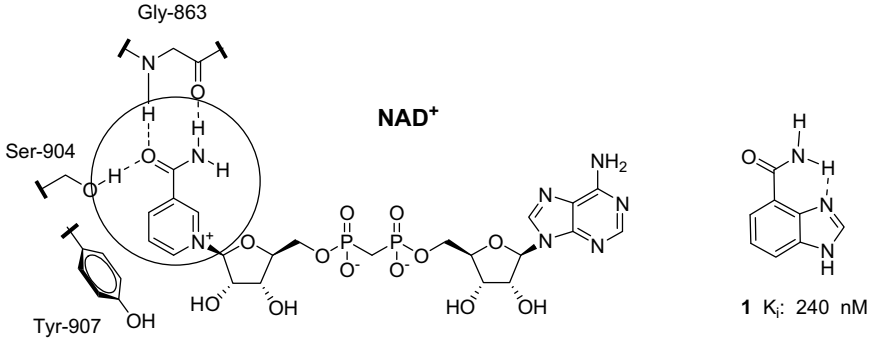
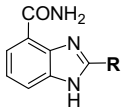
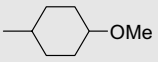
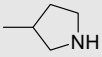
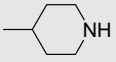
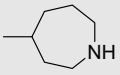


Figure 1. NAD^+ interactions with the PARP-1 enzyme.

Table 1
SAR of secondary cyclic amines



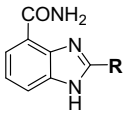
Compound	R	PARP-1 (K_i, μM)[a]	Cellular (EC_{50}, μM)[a]
1	–H	0.24	–
2a		0.044	0.21
2b		0.017	0.075
3		0.034	0.20
4		0.006	0.61
5		0.007	0.018
6		0.030	0.016
7		0.004	0.16
8a		0.037	0.029
8b		0.041	0.014
9		0.011	0.024
10		0.005	>1
11		0.005	0.031

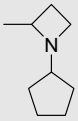
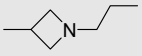
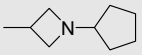
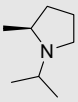
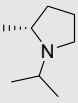
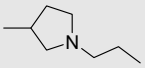
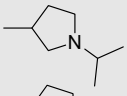
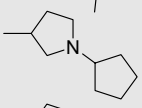
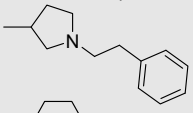
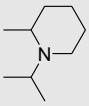
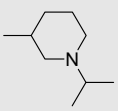
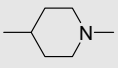
[a] Mean of two or more independent determinations.

(Fig. 1). Contributing to this scaffold's potency are key hydrogen-bond interactions between the amide and two residues in the PARP active site, Gly-863 and Ser-904, along with a π-stacking interaction with Tyr-907.[9] The benzimidazole carboxamide scaffold has been previously disclosed by the University of Newcastle[12] in which a series of phenyl-substituted analogs were described. Although these were relatively potent inhibitors of the PARP-1 enzyme, many of these analogs suffered from poor cellular potency. From our own work, most simple cycloalkyl and phenyl analogs such as **2a** and **2b** (Table 1) generally showed a profile of good enzyme potency with relatively poor cellular penetration. We found that incorporation of a basic amine into the 2-substituent of the benzimidazole ring system in many cases demonstrated a marked improvement in cellular potency, while maintaining good enzyme potency. In addition, pharmacokinetic properties also improved significantly. In this report, we describe the exploration of a series of cyclic amines, ranging from 4- to 8-membered rings. Table 1 outlines a variety of cyclic, secondary amines.

All compounds in this Table had reasonable PARP-1 enzyme potencies, with K_i values ranging from 5 to 41 nM. However, although all contained an unsubstituted cyclic amine, there were profound differences in their ability to inhibit PARP-1 in a whole cell system. Azetidines **3** and **4**, as well as 3-pyrrolidine analog **7** all showed poor cellular potency, while 2-pyrrolidines **5** and **6**

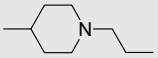
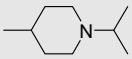
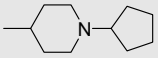
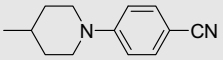
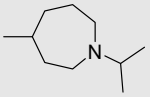
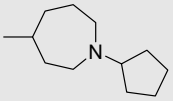
Table 2
SAR of tertiary cyclic amines



Compound	R	PARP-1 (K_i, μM)[a]	Cellular (EC_{50}, μM)[a,b]
3a		0.11	–
3b		0.18	–
4a		0.017	0.016
4b		0.015	0.018
5a		0.008	0.022
6a		0.026	0.076[b]
5b		0.021	0.012
6b		0.020	0.015
7a		0.008	0.003
7b		0.009	0.004
7c		0.005	0.002
7d		0.009	0.006
8c		0.029	0.030
8d		0.045	0.013
9a		0.030	0.015
9b		0.022	0.012
10a		0.019	0.009

(*continued on next page*)

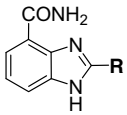
Table 2 (*continued*)

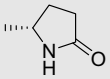
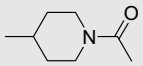
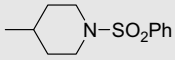
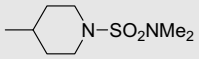
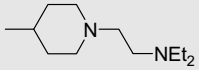
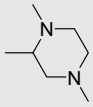
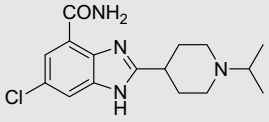
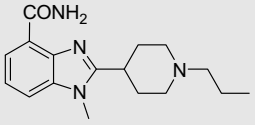
Compound	R	PARP-1 (K_i, μM)[a]	Cellular (EC_{50}, μM)[a,b]
10b		0.008	0.003
10c		0.007	0.004
10d		0.006	0.005
10e		0.003	0.021
11a		0.005	0.006
11b		0.004	0.001

[a] Mean of two or more determinations.
[b] Single determination.

demonstrated relatively good potency. Both 2- and 3-piperidine analogs **8a**, **8b** and **9**, as well as azepane analog **11** showed reasonable cellular potency, however, 4-piperidine analog **10** did not. Since none of the analogs in Table 1 met both our enzyme and cellular potency criteria (both K_i and EC_{50} of $\leqslant$10 nM), a variety of *N*-alkyl analogs were prepared and the results are highlighted in Table 2. N-Alkylated 2-azetidines showed a dramatic decrease in enzyme potency (**3a**, **3b**), while alkylated 3-azetidine analogs **4a** and **4b** maintained good enzyme potency, while showing a >30-fold increase in cellular potency. 2-Pyrrolidine analogs **5a, 5b, 6a**, and **6b** showed a similar enzyme and cellular potency profile as parent analogs **5** and **6**. Generally, little difference was noted between individual enantiomers. However, N-alkylated 3-pyrrolidine analogs **7a–7d**, showed an exceptional improvement in cellular potency, improving from a cellular EC_{50} of 160 nM for parent **7**, to $\leqslant$6 nM for all alkylated analogs. In addition, enzyme potency was maintained at <10 nM. As demonstrated by compound **7d**, significant steric bulk was tolerated on the nitrogen substituent, consistent with the presence of a large pocket in the PARP-1 active site.[13] As with the 2-pyrrolidine series, the alkylated 2- and 3-piperidine series showed very little improvement in either enzyme and cellular potencies (**8c, 8d, 9a, 9b**) relative to parent molecules **8a/b** and **9**. Alkylated 4-piperidine analogs, on the other hand, demonstrated a dramatic increase in cellular potency (**10a–10e**) relative to parent **10**, while maintaining good enzyme potency. Only analog **10e** showed cellular EC_{50} of >20 nM, possibly due to the reduced basicity of the amine. Alkylated 4-azepane analogs **11a** and **11b** also showed a very good enzyme and cellular potency profile. Analogs with somewhat more diverse modifications are highlighted in Table 3. Reduction in amine basicity by amide or sulfonamide formation (**6c, 10f–10h**) resulted in a moderate decrease in enzyme potency, but significantly decreased the cellular potency. Addition of a second basic amine (**10i, 12, 12a**) was also detrimental to potency. Small substituents such as chlorine at the 6-position of the benzimidazole ring were tolerated (i.e., **13**), but larger substituents at this position were generally detrimental to enzyme potency (data not shown). Alkylation at the 1-position of the benzimidazole ring (**14**) resulted in a moderate decrease in enzyme and cellular potency, consistent with the importance of a water-mediated hydrogen bond of the NH with Glu-988. Selected

Table 3
SAR of modified cyclic amines



Compound	R	PARP-1 (K_i, μM)[a]	Cellular (EC_{50}, μM)[a,b]
6c		0.016	0.18
10f		0.059	—
10g		0.027	>1
10h		0.028	0.17
10i		0.031	0.094[b]
12		0.026	>1
12a		0.035	0.097
13		0.018	0.009
14		0.034	0.021

[a] Mean of two or more determinations.
[b] Single determination.

compounds were also tested against the closely-related PARP-2 enzyme and all had similar K_i values (i.e., 11, 3, 5, 2 and 24 nM for **4, 6c, 10b, 10c** and **10g**, respectively). Generally, the majority of compounds within the benzimidazole class tested against both PARP-1 and PARP-2 showed similar potency against both enzymes. Although it would be expected that most PARP-1 inhibitors would also inhibit PARP-2 to some extent, there have been very limited reports detailing such selectivity data.[16]

Mouse (CD-1) pharmacokinetics for selected compounds was also assessed and the data is highlighted in Table 4. All of these analogs, with the exception of **5a**, showed excellent mouse PK, with oral bioavailabilities ranging from 26% to 100%, half-lives of 0.4 to 2 h and oral exposures from 0.8 to 3.4 μg-h/mL with a 10 mg/kg dose. Although alkylated 3-pyrrolidine, 4-piperidine and 4-azepane analogs all showed an acceptable potency profile and, in addition, several analogs showed a good mouse pharmacokinetic profile, 4-piperidine analog **10b** was selected for further characterization due to its overall profile.

An X-ray co-crystal structure of PARP-1 with **10b** is shown in Figure 2. The key interactions in the PARP-1 active site, consistent

Table 4
Mouse pharmacokinetics

Compound[a]	% *F*	$t_{1/2}$ (iv)[b]	AUC (po)[c]	C_{max} (po)[d]
5	100	1.2	3.4	1.8
5a	25	0.1	0.04	0.03
7d	89	0.8	1.3	0.7
9	26	2	1.0	0.2
9b	100	0.4	1.2	0.8
10b	63	1.2	1.2	0.4
13	100	1.4	1.7	0.3

[a] 10 mg/kg po (2.5% EtOH, 1 drop TW-80, 25% PEG400, PBS), 3 mg/kg iv (2.5% EtOH, 5% DMSO, 1 drop TW-80, 25% PEG400, PBS).
[b] h.
[c] μg h/mL.
[d] μg/mL.

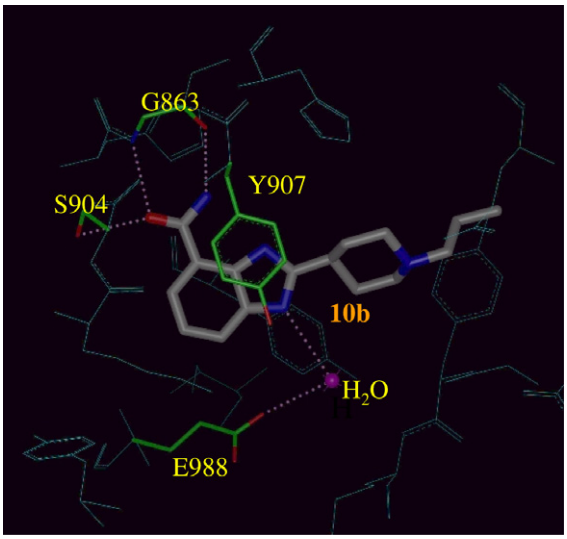


Figure 2. X-ray co-crystal structure of PARP-1 and **10b**.

with previous literature reports,[9] are highlighted. Both Ser-904 and Gly-863 are involved in key hydrogen-bond interactions with the carboxamido group of **10b**. There is also a π-stacking interaction between the benzimidazole ring and Tyr-907. In addition, Glu-988 is involved in a water-mediated hydrogen bond with the 1-NH of the benzimidazole ring system. These same interactions are also displayed in the binding of NAD^+ (Fig. 1) to the PARP-1 active site.

The pharmacokinetics of **10b** was further assessed in several other species and the results are shown in Table 5. Compound **10b** was well-absorbed in all species, with 32–82% oral bioavailability and terminal elimination half-lives in the 1.2–2.7 h range. Clearance and equilibrium volume of distribution values were relatively high. **10b** has excellent water solubility of 6 mg/mL at pH 7.8 and moderate plasma protein binding of 57–62% across various species, including human. In addition, the compound showed minimal inhibition of seven cytochrome P450s (<10%) at a concentration of 10 μg/mL.

Table 5
Multispecies pharmacokinetics for **10b**

Species	Dose[a]	% *F*	$t_{1/2}$ (iv)[b]	AUC (po)[c]	C_{max} (po)[d]	V_{ss}[e]	CL[f]
Mouse	10	63	1.2	1.2	0.4	9.1	5.3
Rat	4.1	48	2.6	0.7	0.8	10.7	2.9
Dog	4.1	82	2.7	3.7	1	3.6	0.9
Monkey	4.1	32	1.7	0.7	0.2	4.9	2

[a] mg/kg.
[b] h.
[c] μg-h/mL.
[d] μg/mL.
[e] L/kg.
[f] L/h/kg.

In vivo, **10b** showed excellent potentiation of cytotoxic agents in two subcutaneous, murine tumor models: with temozolomide in B16F10, a syngeneic melanoma model, and with cisplatin in MX-1, a human breast xenograft model. The B16F10 model (Fig. 3A), while relatively resistant to most chemotherapeutics, is moderately sensitive to TMZ and this sensitivity can be enhanced with PARP inhibitors.[5,7] Compound **10b** was administered sc via osmotic minipump (OMP) on days 1–14 at doses of 1, 12.5 and 25 mg/kg/day, while TMZ was administered at 50 mg/kg/day, po, qd, on days 3–7. **10b** significantly potentiated the efficacy of TMZ in a dose-dependent manner. Significant potentiation was observed as early as day 13 with TGI (tumor growth inhibition) values (vs vehicle control) of 83%, 79% and 74% for the 25, 12.5 and 1 mg/kg/day **10b** combination groups, respectively, compared to a 62% TGI for TMZ alone. All three dosing groups continued to differentiate from the TMZ alone group out to day 17, with TGI values (vs TMZ control) of 82%, 70% and 32% for the 25, 12.5 and 1 mg/kg/day **10b** combination groups, respectively. The **10b**/TMZ combinations were well tolerated, with maximum body weight loss for all combination groups similar to the TMZ monotherapy group (5–10%).

In the MX-1 model in female *SCID* mice (Fig. 3B), **10b** was dosed via sc OMP at doses of 5 and 25 mg/kg/day for 7 days starting on day 16 post-tumor inoculation, while cisplatin was given as a single dose on day 18 at 6 mg/kg, ip. The 25 mg/kg/day **10b**/cisplatin combination group was significantly different than cisplatin alone, with a TGI of 78% at day 48. The 5 mg/kg/day dose of **10b**, however, was not significantly different than cisplatin alone. The **10b**/cisplatin combinations were well tolerated, with maximum body weight loss for all combination groups of 2% compared to 8% loss with the cisplatin monotherapy group. Mice quickly regained weight after cessation of treatment. Although **10b** was dosed by OMP in both efficacy models, similar results were obtained when the compound was dosed by oral administration (data not shown).

4. Conclusion

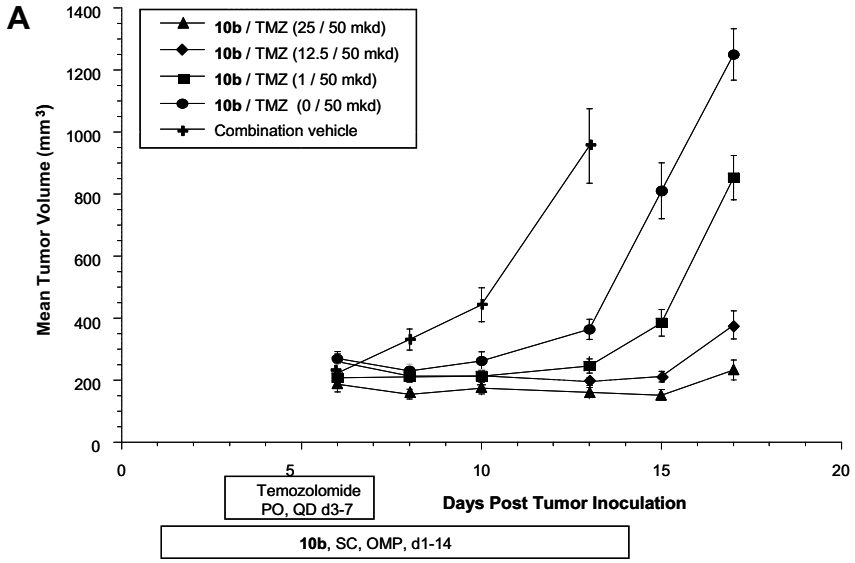
In summary, we have described a potent benzimidazole carboxamide series of PARP-1 inhibitors that, due to the presence of an appropriately substituted cyclic amino group, also demonstrated good PARP inhibition in whole cells. In addition, several compounds demonstrated a good pharmacokinetic profile in mice. Based on the overall profile, *N*-propyl-4-piperidinyl analog **10b** was identified for further pharmacological characterization. **10b** showed good pharmaceutical properties, including pharmacokinetics across multiple species. In addition, **10b** demonstrated excellent potentiation of two cytotoxic agents, temozolomide and cisplatin, in both a mouse melanoma and a breast cancer model. Compound **10b** was identified as a lead preclinical candidate for the treatment of human cancer in combination with both radiation and various cytotoxic agents.

5. Experimental

5.1. Chemistry

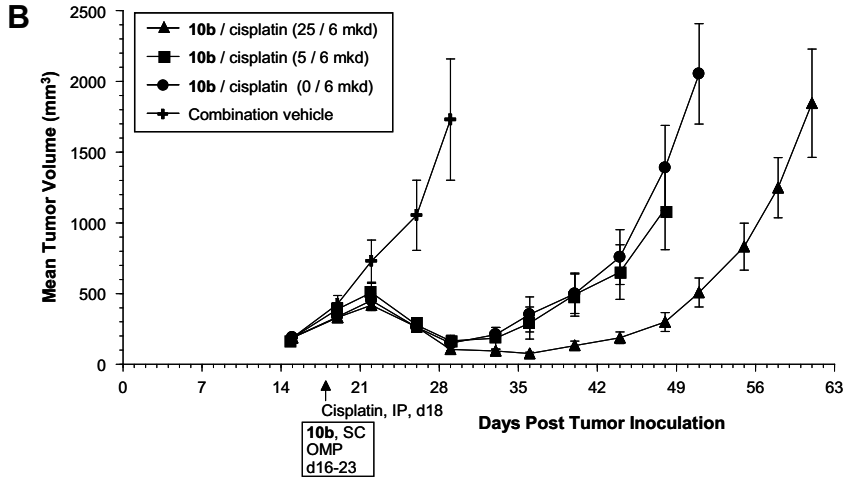
5.1.1. General

^{1}H NMR spectra were obtained on Varian M-300, Bruker AMX-400, Varian U-400, and Varian Unity Inova 500 magnetic resonance spectrometers with indicated solvent and an internal standard. Chemical shifts are given in delta (δ) values and



compd	dose (mg/kg/day)	tumor volume[a] (day 13)	%TGI vehicle[b] (day 13)	tumor volume[a] (day 17)	%TGI cytotoxic[c] (day 17)
10b/TMZ	25/50	161 ± 16	83*	233 ± 32	82*
	12.5/50	196 ± 13	79*	378 ± 45	70*
	1/50	246 ± 23	74*	853 ± 72	32*
vehicle/TMZ	0/50	364 ± 32	62*	1250 ± 83	--
combination vehicle	0/0	955 ± 120	--	--	--

* $p<0.0001$; [a] Mean (mm^3) ± SEM of 9-10 mice/group; [b] %TGI vs. vehicle control; [c] %TGI vs. TMZ control.



compd	dose (mg/kg/day)	tumor volume[a] (day 29)	%TGI[b] (day 29)	tumor volume[a] (day 48)	%TGI[b] (day 48)
10b/cisplatin	25/6	106 ± 12	32	299 ± 67	78*
	5/6	164 ± 40	0	1100 ± 289	21
vehicle/cisplatin	0/6	156 ± 28	--	1389 ± 300	--
combination vehicle	0/0	1730 ± 429	--	--	--

* $p< 0.001$; [a] Mean (mm^3) ± SEM of 9-10 mice/group; [b] %TGI vs. cisplatin control.

Figure 3. (A) In vivo efficacy of **10b** with TMZ in the B16F10 model. (B) In vivo efficacy of **10b** with cisplatin in the MX-1 model.

coupling constants (J) in Hertz (Hz). The following peak multiplicity abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broadened. Mass spectra were performed as follows: ESI (electrospray ionization) was performed on a Finnigan SSQ7000 MS run as a flow injection acquisition; DCI (desorption chemical ionization) was performed on a Finnigan SSQ7000 MS using a direct exposure probe with ammonia gas; APCI (atmospheric pressure chemical ionization) was performed on a Finnigan Navigator MS run as flow injection acquisition. Elemental analyses were performed by Quantitative Technologies Inc. Whitehouse, NJ. All manipulations were performed under nitrogen atmosphere unless otherwise indicated.

All solvents and other reagents were obtained from commercial sources and used without further purification, except where noted.

5.1.2. X-ray crystallography data

A GST fusion of h-PARP (654–1014) expressed in *Escherichia coli* was concentrated to 60 mg/mL (0.874 mM) in 50 mM pH 7.5 Tris buffer containing 150 mM NaCl and 1.5 mM DTT. To make the PARP–**10b** complex, 2 mM **10b** in DMSO was added to the protein solution and incubated for 4 h on ice. Crystals were grown at 17 °C in hanging drops after 2–5 d. The well solution contained 1.3 M ammonium sulfate and 1.3 M NaCl. X-ray diffraction data were collected at Advanced Photon Source Beamline 32-ID. The cryoprotectant solution of 1.2 M ammonium sulfate, 1.6 M NaCl and 20% ethylene glycol was used to collect the data at 110 K. The space group is P321 with cell dimensions, $a = b = 94.17$, $c = 68.38$ Å, $\alpha = 90$, $\gamma = 120$ °. There is one PARP–**10b** complex molecule per asymmetric unit. The crystal diffracted up to 2.8 Å and 8321 unique reflections were obtained using HKL2000. The overall $R_{sym}(I)$ is 0.08 and $I/\sigma(I)$ is 13.8. The overall completeness is 92%. The complex structure was solved using CCP4 molrep program with a search model of PDB entry 1UK0 and was refined with CNX 2002. The conventional and free R-factors after refinement are 0.234 and 0.317. The coordinates of the PARP–**10b** complex molecule are deposited in Protein Data Bank with the code 2RCW.

5.2. Biology

5.2.1. PARP enzyme assay[4]

Enzyme assay was conducted in buffer containing 50 mM Tris, pH 8.0, 1 mM DTT, and 4 mM $MgCl_2$. PARP reactions contained 1.5 μM [^{3}H]NAD^+ (1.6 μCi/mmol), 200 nM biotinylated histone H1, 200 nM slDNA, and 1 nM PARP-1 or 4 nM PARP-2 enzyme. Auto reactions utilizing SPA bead-based detection were carried out in 100 mL volumes in white 96-well plates. Reactions were initiated by adding 50 ml of 2× NAD^+ substrate mixture to 50 ml of 2× enzyme mixture containing PARP and DNA. These reactions were terminated by the addition of 150 ml of 1.5 mM benzamide (~1000-fold over its IC_{50}). One hundred and seventy microliters of the stopped reaction mixtures were transferred to streptavidin-coated Flash Plates, incubated for 1 h, and counted using a TopCount microplate scintillation counter. K_i data was determined from inhibition curves at various substrate concentrations.

5.2.2. Cellular PARP assay

C41 cells were treated with test compound for 30 min in a 96-well plate. PARP was activated by damaging DNA with 1 mM H_2O_2 for 10 min. Cells were washed with ice-cold PBS once and fixed with pre-chilled methanol/acetone (7:3) at −20 °C for 10 min. After air-drying, plates were rehydrated with PBS and blocked 5% non-fat dry milk in PBS-Tween (0.05%) (blocking solution) for 30 min at room temperature. Cells were incubated with anti-PAR antibody 10H (1:50) in blocking solution at room temperature for 60 min followed by washing with PBS-Tween 20 five times, and incubation with goat anti-mouse fluorescein 5(6)-isothiocyanate (FITC)-coupled antibody (1:50) and 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in blocking solution at room temperature for 60 min. After washing with PBS-Tween 20 five times, analysis was performed using an fmax Fluorescence Microplate Reader set at the excitation and emission wavelength for FITC or the excitation and emission wavelength for DAPI. PARP activity (FITC signal) was normalized with cell numbers (DAPI).

5.2.3. MX-1 tumor model

A 0.2 cc of 1:10 MX-1 tumor brei was injected subcutaneously into the flank of female *SCID* mice (Charles River Labs) on study day 0. On day 15, tumors were size matched and animals placed into study groups ($N = 10$ mice/group). PARP inhibitor therapy began on day 16, with cisplatin treatment starting on day 18. At various intervals following tumor inoculation, the individual tumor dimensions were serially measured using calibrated microcalipers and the tumor volumes calculated according to the formula $V = L \times W^2/2$ (V: volume, L: length, W: width). Mice were humanely euthanized when the tumor volumes reached a predetermined size.

5.2.4. B16F10 tumor model

For B16F10 syngeneic studies, 6×10^4 cells were mixed with 50% matrigel (BD Biosciences, Bedford, MA) and inoculated by sc injection into the flank of 6–8 week old female C57BL/6 mice, 20 g (Charles River Laboratories, Wilmington, MA). Mice were injection-order allocated to treatment groups and PARP inhibitor therapy was initiated on day 1 following inoculation, with temozolomide treatment starting on day 3. All animal studies were conducted in a specific pathogen-free environment in accordance to the Internal Institutional Animal Care and Use Committee, accredited by the American Association of Laboratory Animal Care under conditions that meet or exceed the standards set by the United States Department of Agriculture Animal Welfare Act, Public Health Service policy on humane care and use of animals, and the NIH guide on laboratory animal welfare.

5.3. Chemistry

5.3.1. Procedure A: 2-(Azetidin-2-yl)-1*H*-benzimidazole-4-carboxamide (3)

A solution of 1-(*tert*-butoxycarbonyl)azetidine-2-carboxylic acid (2.5 g, 12.4 mmol) in a mixture of pyridine (15 mL) and DMF (15 mL) was treated with 1,1′-carbonyldiimidazole (CDI, 2.2 g, 13.6 mmol) at 45 °C for 2 h. 2,3-Diaminobenzamide dihydrochloride[10] (2.8 g, 12.4 mmol) was added and the mixture stirred at ambient temperature overnight. Solvents were removed and the residue heated in 10 mL of acetic acid at 100 °C for 30 min. After concentration, the residue was partitioned between ethyl acetate and saturated sodium bicarbonate solution and the organic phase washed with water and concentrated. The residue was purified by flash chromatography using ethyl acetate to give the BOC-protected intermediate. This was treated with trifluoroacetic acid (5 mL) in dichloromethane (20 mL) for 1 h and concentrated. The residue was purified by HPLC (Zorbax, C-18, 0–100% gradient of 0.1% TFA in H_2O/0.1% TFA in CH_3CN) to afford 670 mg (12%) of the title compound as the trifluoroacetate salt. ^{1}H NMR (CD_3OD) δ 2.90–3.17 (m, 2H), 4.10–4.26 (m, 1H), 4.26–4.41 (m, 1H), 5.87 (t, $J = 8.4$ Hz, 1H), 7.42 (m, 1H), 7.69–7.83 (m, 1H), 7.89–8.09 (m, 1H). HRMS *m/z* 217.10781 (calcd for $C_{11}H_{13}N_4O$ (M+H), 217.10839).

5.3.2. 2-(Azepan-4-yl)-1*H*-benzimidazole-4-carboxamide (11)

The title compound was prepared from 1-(*tert*-butoxycarbonyl)azepane-4-carboxylic acid[14] according to procedure A. ^{1}H NMR (CD_3OD) δ 1.99–2.15 (m, 2H), 2.16–2.28 (m, 1H), 2.36–2.54 (m, 3H), 3.33–3.41 (m, 2H), 3.41–3.49 (m, 1H), 3.53–3.62 (m, 1H), 3.61–3.73 (m, 1H), 7.58 (t, $J = 7.5$ Hz, 1H), 7.91 (d, $J = 7.5$ Hz, 1H), 7.98–8.03 (d, $J = 7.4$ Hz, 1H). HRMS *m/z* 259.15500 (calcd for $C_{14}H_{19}N_4O$ (M+H), 259.15534).

5.3.3. 2-(1-(4-cyanophenyl)piperidin-4-yl)-1H-benzimidazole-4-carboxamide (10e)

The title compound was prepared from 1-(4-cyanophenyl)piperidine-4-carboxylic acid according to procedure A

(6.5% yield). ^{1}H NMR (DMSO-d_6) δ 1.89 (dd, J = 12.5, 3.1 Hz, 2H), 2.12 (s, 2H), 3.10 (t, J = 11.7 Hz, 2H), 4.05 (d, J = 13.1 Hz, 3H), 7.08 (d, J = 9.4 Hz, 3H), 7.25 (s, 1H), 7.56–7.65 (m, 4H), 7.78 (s, 1H), 9.28 (s, 1H). Anal. calcd for $C_{20}H_{19}N_5O{\cdot}0.9\ H_2O$: C: 66.43, H: 5.80, N: 19.37. Found: C: 66.67, H: 5.69, N: 19.21.

5.3.4. 6-Chloro-2-(1-isopropylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (13)

5.3.4.1. Step 1: Preparation of 2-amino-5-chloro-3-nitrobenzamide. To a solution of 2-amino-3-nitrobenzamide[10] (4.0 g, 22.08 mmol) in acetonitrile (1250 mL) was added *N*-chlorosuccinimide (3.1 g, 23.18 mmol) and the mixture stirred at 60 °C overnight. After cooling, the solid was collected by filtration, washed with acetonitrile, and dried to give 2.95 g of the title compound. The mother liquor was concentrated and the residue recrystallized from acetonitrile to give 800 mg of a second crop of solid (79% yield). ^{1}H NMR (DMSO-d_6) δ 7.75 (br s, 1H), 8.00 (d, J = 2.4 Hz, 1H), 8.19 (d, J = 2.7 Hz, 1H), 8.25 (br s, 1H), 8.47 (br s, 2H). MS (DCI/NH_3) m/z 216 $(M+H)^+$.

5.3.4.2. Step 2: Preparation of 6-Chloro-2-(1-isopropylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide. The title compound was prepared from the compound from step 1 and 1-isopropylpiperidine-4-carboxylic acid hydrochloride[15] according to procedure A (66% yield). ^{1}H NMR (DMSO-d_6) δ 1.45 (d, J = 6.7 Hz, 6H), 2.39–2.55 (m, 4H), 3.31–3.37 (m, 2H), 3.57–3.82 (m, 4H), 7.98 (d, J = 1.8 Hz, 1H), 8.10 (d, J = 1.8 Hz, 1H). Anal. calcd for $C_{16}H_{21}ClN_4O{\cdot}2.4$ HCl: C, 47.06; H, 5.78; N, 13.72. Found: C, 46.98; H, 5.50; N, 13.41.

5.3.5. Procedure B: 2-(Azetidin-3-yl)-1*H*-benzimidazole-4-carboxamide (4)

A solution of (1.2 g, 5.0 mmol) in a mixture of pyridine (10 mL) and DMF (10 mL) was treated with CDI (0.9 g, 5.5 mmol) at 45 °C for 2 h. 2,3-Diaminobenzamide dihydrochloride[10] (1.1 g, 5.0 mmol) was added and the mixture stirred at ambient temperature overnight. Solvents were removed and the residue heated in acetic acid (10 mL) at 100 °C for 30 min. After concentration, the residue was purified by flash chromatography using ethyl acetate to afford the CBZ-protected intermediate. This was dissolved in methanol (10 mL), and treated with 10% palladium on carbon (100 mg) under hydrogen for 6 h. Solid material was filtered off and the filtrate concentrated. The residue was purified by HPLC (Zorbax C-8, 0.1% trifluoroacetic acid/acetonitrile/water) to provide 820 mg (54%) of the title compound. ^{1}H NMR (CD_3OD) δ 4.51–4.58 (m, 4H), 7.42 (t, J = 7.9 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.94 (d, J = 7.6 Hz, 1H). HRMS m/z 217.10796 (calcd for $C_{11}H_{13}N_4O$ (M+H), 217.10839).

5.3.6. (*R*)-2-(Pyrrolidin-2-yl)-1*H*-benzimidazole-4-carboxamide (5)

The title compound was prepared from (*R*)-1-(benzyloxycarbonyl)pyrrolidine-2-carboxylic acid according to procedure B (57% yield). ^{1}H NMR (DMSO-d_6) δ 1.69–1.83 (m, 2H), 1.88–2.05 (m, 1H), 2.12–2.29 (m, 1H), 2.96 (t, J = 6.6 Hz, 2H), 3.31 (br s, 1H), 4.44 (dd, J = 8.0, 5.9 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.61 (d, J = 1.0 Hz, 1H), 7.64 (br s, 1H), 7.77 (dd, J = 7.8, 1.0 Hz, 1H), 9.22 (br s, 1H), 12.22 (br s, 1H). Anal. calcd for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.32; H, 5.93; N, 24.43.

5.3.7. (*S*)-2-(Pyrrolidin-2-yl)-1*H*-benzimidazole-4-carboxamide (6)

The title compound was prepared from (*S*)-1-(benzyloxycarbonyl)pyrrolidine-2-carboxylic acid according to procedure B. ^{1}H NMR (DMSO-d_6) δ 1.71–1.82 (m, 2H), 1.89–2.02 (m, 1H), 2.14–2.26 (m, 1H), 2.96 (t, J = 6.8 Hz, 2H), 4.44 (dd, J = 8.1, 6.1 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 7.61 (br s, 1H), 7.63 (dd, J = 1.4, 7.0 Hz, 1H), 7.77 (d, J = 6.4 Hz, 1H), 9.22 (br s, 1H). Anal. calcd for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.25; H, 5.99; N, 23.97.

5.3.8. 2-(Pyrrolidin-3-yl)-1*H*-benzimidazole-4-carboxamide (7)

The title compound was prepared from 1-(benzyloxycarbonyl)pyrrolidine-3-carboxylic acid according to procedure B (57% yield). ^{1}H NMR (D_2O) δ 2.45–2.55 (m, 1H), 2.73–2.81 (m, 1H), 3.54–3.61 (m, 1H), 3.69–3.74 (m, 1H), 3.74–3.80 (m, 1H), 4.01 (dd, J = 12.1, 8.4 Hz, 1H), 4.21–4.31 (m, 1H), 7.58 (t, J = 8.0 Hz, 1H), 7.87–7.93 (m, 2H). Anal. calcd for $C_{12}H_{14}N_4O{\cdot}2.4$HCl: C, 45.36; H, 5.20; N, 17.63. Found: C, 45.75; H, 4.82; N, 16.95.

5.3.9. (*R*)-2-(Piperidin-2-yl)-1*H*-benzimidazole-4-carboxamide (8a)

The title compound was prepared from (*R*)-(+)-1-(carbobenzyloxy)-2-piperidine carboxylic acid according to procedure B. ^{1}H NMR (CD_3OD) δ 1.75–1.84 (m, 2H), 1.88–1.96 (m, 2H), 1.99–2.06 (m, 1H), 2.33–2.46 (m, 1H), 3.08–3.20 (m, 1H), 3.43–3.54 (m, 1H), 4.53 (dd, J = 11.5, 3.22 Hz, 1H), 7.34–7.39 (m, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 7.7 Hz, 1H). HRMS m/z 245.13952 (calcd for $C_{13}H_{17}N_4O$ (M+H), 245.13969).

5.3.10. (*S*)-2-(Piperidin-2-yl)-1*H*-benzimidazole-4-carboxamide (8b)

The title compound was prepared from (*S*)-(−)-1-(carbobenzyloxy)-2-piperidine carboxylic acid according to procedure B. ^{1}H NMR (CD_3OD): δ 1.73–1.85 (m, 2H), 1.87–1.97 (m, 2H), 1.99–2.07 (m, 1H), 2.39 (dd, J = 12.9, 2.8 Hz, 1H), 3.09–3.19 (m, 1H), 3.48 (d, J = 11.7 Hz, 1H), 4.54 (dd, J = 11.3, 3.4 Hz, 1H), 7.35–7.41 (m, 1H), 7.75 (dd, J = 8.0, 0.9 Hz, 1H), 7.95 (dd, J = 7.5, 1.1 Hz, 1H). HRMS m/z 245.13939 (calcd for $C_{13}H_{17}N_4O$ (M+H), 245.13969).

5.3.11. 2-(Piperidin-3-yl)-1*H*-benzimidazole-4-carboxamide (9)

The title compound was prepared from 1-(benzyloxycarbonyl)piperidine-3-carboxylic acid according to procedure B. ^{1}H NMR (CD_3OD) δ 1.62–1.73 (m, 1H), 1.77–1.86 (m, 1H), 1.87–1.98 (m, 1H), 2.19–2.28 (m, 1H), 2.64–2.77 (m, 1H), 2.93–3.02 (m, 1H), 3.03–3.10 (m, 1H), 3.10–3.20 (m, 1H), 3.37 (s, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.66 (dd, J = 7.8, 1.0 Hz, 1H), 7.86 (dd, J = 7.8, 1.0 Hz, 1H). MS (DCI/NH_3) m/z 245 $(M+H)^+$.

5.3.12. 2-(Piperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10)

The title compound was prepared from 1-(benzyloxycarbonyl)piperidine-4-carboxylic acid according to procedure B. ^{1}H NMR (DMSO-d_6): δ 1.63–1.71 (m, 1H), 1.71–1.78 (m, 1H), 1.90–2.00 (m, 2H), 2.55–2.66 (m, 2H), 2.94–2.99 (m, 1H), 3.00–3.08 (m, 2H), 3.26 (br s, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.62 (d, J = 7.1 Hz, 1H), 7.64 (br s, 1H), 7.78 (d, J = 7.5 Hz, 1H), 9.34 (br s, 1H), 12.61 (br s, 1H). HRMS m/z 245.13979 (calcd for $C_{13}H_{17}N_4O$ (M+H), 245.13969).

5.3.13. (*S*)-2-(5-Oxopyrrolidin-2-yl)-1*H*-benzimidazole-4-carboxamide (6c)

The title compound was prepared from *Z*-D-pyroglutamic acid according to procedure B. ^{1}H NMR (DMSO-d_6) δ 2.14–2.19 (m, 1H), 2.27–2.36 (m, 2H), 2.53–2.60 (m, 1H), 4.99 (dd, J = 8.0, 5.3 Hz, 1H), 7.27–7.34 (m, 1H), 7.69 (d, J = 7.1 Hz, 2H), 7.82 (d, J = 7.1 Hz, 1H), 8.25 (br s, 1H), 9.20 (br s, 1H), 12.97 (brs, 1H). Anal. calcd for $C_{12}H_{12}N_4O_2$: C, 59.01; H, 4.95; N, 22.94. Found: C, 58.87; H, 4.79; N, 22.79.

5.3.14. Procedure C: 2-(*N*-Acetylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10f)

5.3.14.1. Step 1: Preparation of Methyl 2-(*N*-acetylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxylate. To a solution of ethyl 2,3-diaminobenzoate[11] (3.3 g, 19.9 mmol) in methanol (100 mL) was

added a solution of *N*-acetylpiperidine-4-carboxaldehyde (4.0 g, 25.8 mmol) in methanol (100 mL) and the mixture stirred at ambient temperature for 10 min. A solution of copper(II) acetate (5.2 g, 25.8 mmol) in water (100 mL) was added and the mixture stirred at reflux for 30 min. After cooling, concentrated hydrochloric acid (25 mL) was added and the mixture warmed to reflux. A solution of sodium sulfide nonahydrate (7.15 g, 29.8 mmol) in water (100 mL) was added and the mixture refluxed for 10 min. After cooling, the mixture was concentrated and the residue stirred in water and filtered. The filtrate was made basic with aqueous sodium bicarbonate and extracted with ethyl acetate. The organic phase was washed with water, dried and concentrated to provide 4.5 g of the title compound.

5.3.14.2. Step 2: Preparation of 2-(*N*-acetylpiperidin-4-yl)-1*H*-benzimidazole-4-carbohydrazide. A solution of the compound from step 1 (4.3 g, 14.9 mmol) and hydrazine hydrate (3.7 g, 74. 3 mmol) in ethanol (100 mL) was stirred at reflux for 2.5 h. The mixture was concentrated and the crude product used without further purification.

5.3.14.3. Step 3: Preparation of 2-(*N*-Acetylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide. A solution of the compound from step 2 in water was added to a solution of Raney nickel (5 g) in DMF (100 mL) and water (50 mL) and the mixture heated at 100 °C for 2 h. After cooling, the mixture was filtered and the filtrate concentrated. The residue was dissolved in dichloromethane and diethyl ether added to provide a precipitate. Filtration gave 3.2 g of the title compound. ^{1}H NMR (DMSO-d_6) δ 1.60–1.95 (m, 4H), 2.04 (s, 3H), 2.76 (m, 3H), 3.92 (d, J = 13.9 Hz, 1H), 4.42 (d, J = 13.1 Hz, 1H), 7.26 (t, J = 7.2 Hz, 1H), 7.64 (br s, d, J = 7.5 Hz, 2H), 7.80 (d, J = 7.2 Hz, 1H), 8.5 (br), 9.29 (br, 1H), 12.70 (br, 1H). Anal. calcd for $C_{15}H_{18}N_4O_2 \cdot 1.4H_2O$: C, 57.83; H, 6.73; N, 17.98. Found: C, 57.83; H, 6.34; N, 18.00.

5.3.15. 2-(*N*-Methylpiperidin-3-yl)-1*H*-benzimidazole-4-carboxamide (9a)

The title compound was prepared from 1-methylpiperidine-3-carboxaldehyde according to Procedure C. ^{1}H NMR (DMSO-d_6) δ 1.75 (m, 1H), 2.04 (m, 2H), 2.24 (m, 1H), 2.83 (d, J = 4.7 Hz, 3H), 3.44 (m, 2H), 3.60–3.95 (m, 3H), 7.36 (t, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 6.8 Hz, 1H), 8.80 (br, 1H), 10.62 (br, 1H). Anal. calcd for $C_{14}H_{18}N_4O \cdot 2HCl \cdot 1H_2O$: C, 48.15; H, 6.35; N, 16.04. Found: C, 47.85; H, 6.40; N, 15.87.

5.3.16. 2-(*N*-Methylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10a)

The title compound was prepared from 1-methylpiperidine-4-carboxaldehyde according to Procedure C. HRMS *m/z* 259.15584 (calcd for $C_{14}H_{19}N_4O$ (M+H), 259.15534).

5.3.17. 2-(1-(2-(Diethylamino)ethyl)piperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10i)

The title compound was prepared from 1-(2-diethylaminoethyl)piperidine-4-carboxaldehyde according to Procedure C. ^{1}H NMR (CD_3OD) δ 1.37 (t, J = 7.1 Hz, 6H), 1.82–2.38 (m, 6H), 2.65–3.54 (m, 11H), 7.39 (t, J = 7.9 Hz, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 7.5 Hz, 1H). Anal. calcd for $C_{19}H_{29}N_5O \cdot 2.3HCl \cdot 1H_2O$: C, 51.24; H, 7.54; N, 15.73. Found: C, 51.35; H, 7.61; N, 15.49.

5.3.18. 2-(1,4-Dimethylpiperazin-2-yl)-1*H*-benzimidazole-4-carboxamide (12a)

The title compound was prepared from 1,4-dimethylpiperazine-2-carboxaldehyde according to Procedure C. ^{1}H NMR (CD_3OD) δ 2.38 (s, 3H), 2.94 (m, 2H), 3.01 (s, 3H), 3.39 (m, 2H), 3.68 (m, 1H), 3.82 (m, 1H), 4.44 (m, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.95 (dd, J = 8.1, 0.9 Hz, 1H), 8.06 (dd, J = 7.6, 0.9 Hz, 1H). Anal. calcd for $C_{14}H_{19}N_5O \cdot 2.5HCl \cdot 2H_2O$: C, 41.98; H, 6.42; N, 17.49. Found: C, 42.21; H, 6.53; N, 17.46.

5.3.19. 2-Piperazin-2-yl-1*H*-benzimidazole-4-carboxamide (12)

5.3.19.1. Step 1: Preparation of 2-(1,4-dibenzylpiperazin-2-yl)-1*H*-benzimidazole-4-carboxamide. The title compound was prepared from 1,4-dibenzylpiperazine-2-carboxaldehyde according to Procedure C. ^{1}H NMR (DMSO-d_6) δ 2.95–3.7 (7H), 3.8–4.9 (4H), 7.1–7.55 (8H), 7.65 (2H), 7.85 (2H), 7.94 (1H), 8.7 (br), 12.2 (br).

5.3.19.2. Step 2: Preparation of 2-Piperazin-2-yl-1*H*-benzimidazole-4-carboxamide. A solution of the compound from step 1 (1.83 g, 3.67 mmol) and 10% palladium on carbon (1 g) in methanol (250 mL) was stirred under hydrogen until starting material was consumed. The catalyst was filtered off and the filtrate concentrated. To a solution of the residue in isopropanol (20 mL) was added 20% hydrogen chloride in isopropanol (50 mL) and the precipitate filtered to obtain 1.1 g of the title compound. ^{1}H NMR (CD_3OD) δ 3.39–3.72 (m, 5H), 3.97 (dd, J = 13.6, 3.4 Hz, 1H), 5.08 (dd, J = 10.2, 3.7 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.86 (dd, J = 8.1, 1.0 Hz, 1H), 7.99 (dd, J = 7.5, 1.0 Hz, 1H). HRMS *m/z* 246.13497 (calcd for $C_{12}H_{16}N_5O$ (M+H), 246.13494).

5.3.20. Procedure D: 2-(1-Isopropylazetidin-2-yl)-1*H*-benzimidazole-4-carboxamide (3a)

A solution of **3** (100 mg, 0.22 mmol) in methanol (10 mL) was stirred with formaldehyde (37 wt% in water, 163 μL, 2.2 mmol) at ambient temperature overnight. Sodium cyanoborohydride (117 mg, 2.2 mmol) was added and the solution stirred at ambient temperature for 3 h. After concentration, the residue was purified by HPLC (Zorbax C-8, 0.1% trifluoroacetic acid/acetonitrile/water) to give the title compound (28 mg, 23%). ^{1}H NMR (CD_3OD) δ 1.25 (d, J = 6.4 Hz, 6H), 2.92 (d, J = 8.3 Hz, 1H), 3.00–3.14 (m, 1H), 3.66–3.79 (m, 1H), 4.19–4.29 (m, 2H), 5.84 (t, J = 9.0 Hz, 1H), 7.40–7.47 (m, 1H), 7.80–7.84 (m, 1H), 8.00 (dd, J = 7.7, 1.2 Hz, 1H). HRMS *m/z* 259.15523 (calcd for $C_{14}H_{19}N_4O$ (M+H), 259.15534).

5.3.21. 2-(1-Cyclopentylazetidin-2-yl)-1*H*-benzimidazole-4-carboxamide (3b)

The title compound was prepared from **3** and cyclopentanone according to procedure D (37% yield). ^{1}H NMR (CD_3OD) δ 1.50–1.85 (m, 6H), 1.98–2.21 (m, 2H), 2.88–3.02 (m, 1H), 3.02–3.19 (m, 1H), 4.03 (s, 1H), 4.16–4.35 (m, 2H), 5.82 (t, J = 8.9 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.95–8.03 (m, 1H). HRMS *m/z* 285.17066 (calcd for $C_{16}H_{21}N_4O$ (M+H), 285.17099).

5.3.22. 2-(1-Propylazetidin-3-yl)-1*H*-benzimidazole-4-carboxamide (4a)

The title compound was prepared from **4** and propionaldehyde according to procedure D (28% yield). ^{1}H NMR (CD_3OD) δ 1.03 (t, J = 7.5 Hz, 3H), 1.62–1.71 (m, 2H), 3.31–3.35 (m, 2H), 4.42–4.73 (m, 4H), 7.39 (t, J = 7.9 Hz, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H). HRMS *m/z* 259.15507 (calcd for $C_{14}H_{19}N_4O$ (M+H), 259.15534).

5.3.23. 2-(1-Cyclopentylazetidin-3-yl)-1*H*-benzimidazole-4-carboxamide (4b)

The title compound was prepared from **4** and cyclopentanone according to procedure D (27% yield). ^{1}H NMR (CD_3OD) δ 1.58–1.72 (m, 2H), 1.72–1.88 (m, 4H), 2.03–2.20 (m, 2H), 3.92–4.07 (m, 1H), 4.38–4.51 (m, 1H), 4.58–4.64 (m, 4H), 7.39 (t, J = 6.8 Hz, 1H), 7.77 (d, J = 6.8 Hz, 1H), 7.93 (d, J = 6.44 Hz, 1H). HRMS *m/z* 285.17078 (calcd for $C_{16}H_{21}N_4O$ (M+H), 285.17099).

5.3.24. (*R*)-2-(1-methylpyrrolidin-2-yl)-1*H*-benzimidazole-4-carboxamide (5a)

The title compound was prepared from **5** and formaldehyde according to procedure D (67% yield). ^{1}H NMR (CD_3OD) δ 2.30–2.46 (m, 2H), 2.46–2.61 (m, 1H), 2.78–2.88 (m, 1H), 3.09 (s, 3H), 3.45–3.57 (m, 1H), 3.87–4.00 (m, 1H), 5.00–5.13 (m, 1H), 7.53 (t, J = 7.8 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 7.5 Hz, 1H). MS (DCI/NH_3) m/z 245 $(M+H)^+$.

5.3.25. (*S*)-2-(1-Methylpyrrolidin-2-yl)-1*H*-benzimidazole-4-carboxamide (6a)

The title compound was prepared from **6** and formaldehyde according to procedure D (67% yield). ^{1}H NMR (CD_3OD) δ 2.34–2.41 (m, 2H), 2.52 (m, 1H), 2.83 (m, 1H), 3.09 (s, 3H), 3.49 (m, 1H), 3.94 (m, 1H), 5.06 (br s, 1H), 7.53 (t, J = 8.0 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H). MS (APCI) m/z 245 $(M+H)^+$.

5.3.26. (*R*)-2-(1-Isopropylpyrrolidin-2-yl)-1*H*-benzimidazole-4-carboxamide (5b)

The title compound was prepared from **5** and acetone according to procedure D (60% yield). ^{1}H NMR (CD_3OD) δ 1.37 (d, J = 6.4 Hz, 3H), 1.39 (d, J = 6.4 Hz, 3H), 2.23–2.32 (m, 2H), 2.33–2.44 (m, 1H), 2.66–2.77 (m, 1H), 3.46–3.58 (m, 1H), 3.75–3.90 (m, 2H), 5.08–5.19 (m, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 7.4 Hz, 1H). Anal. calcd for $C_{15}H_{20}N_4O$·1.6TFA: C, 48.07; H, 4.79; N, 12.32. Found: C, 48.26; H, 4.85; N, 12.55.

5.3.27. (*S*)-2-(1-Isopropylpyrrolidin-2-yl)-1*H*-benzimidazole-4-carboxamide (6b)

The title compound was prepared from **6** and acetone according to procedure D. ^{1}H NMR (CD_3OD) δ 1.42 (d, J = 5.9 Hz, 6H), 2.33 (m, 2H), 2.43–2.50 (m, 1H), 2.72–2.83 (m, 1H), 3.57 (m, 1H), 3.81–3.89 (m, 2H), 5.26 (m, 1H), 7.51 (t, J = 7.8 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 8.02 (d, J = 7.5 Hz, 1H). Anal. calcd for $C_{15}H_{20}N_4O$·1.7 HCl: C, 53.89; H, 6.54; N, 16.76. Found: C, 53.76; H, 6.59; N, 16.67.

5.3.28. 2-(1-Isopropylazepan-4-yl)-1*H*-benzimidazole-4-carboxamide (11a)

The title compound was prepared from **11** and acetone according to procedure D (23% yield). ^{1}H NMR (CD_3OD) δ 1.41 (d, J = 6.9 Hz, 6H), 2.06–2.22 (m, 2H), 2.20–2.31 (m, 1H), 2.34–2.43 (m, 1H), 2.53 (d, J = 5.3 Hz, 2H), 3.34–3.56 (m, 2H), 3.56–3.65 (m, 2H), 3.65–3.79 (m, 2H), 7.59 (t, J = 8.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 8.00 (d, J = 7.5 Hz, 1H). HRMS m/z 301.20161 (calcd for $C_{17}H_{25}N_4O$ (M+H), 301.20229).

5.3.29. 2-(1-Cyclopentylazepan-4-yl)-1*H*-benzimidazole-4-carboxamide (11b)

The title compound was prepared from **11** and cyclopentanone according to procedure D (25% yield). ^{1}H NMR (CD_3OD) δ 1.63–1.93 (m, 6H), 2.03-2.28 2.03–2.28 (m, 5H), 2.33–2.41 (m, 1H), 2.42–2.52 (m, 2H), 3.36–3.49 (m, 1H), 3.55–3.73 (m, 3H), 3.72–3.83 (m, 2H), 7.53 (t, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.4 Hz, 1H). HRMS m/z 327.21744 (calcd for $C_{19}H_{27}N_4O$ (M+H), 327.21794).

5.3.30. 2-(1-Methylpiperidin-2-yl)-1*H*-benzimidazole-4-carboxamide (8c)

The title compound was prepared from (±)-1-(benzyloxycarbonyl)-2-piperidine carboxylic acid according to procedure B, followed by procedure D using formaldehyde. ^{1}H NMR (CD_3OD) δ 1.71–1.87 (m, 1H), 1.90–2.12 (m, 3H), 2.14–2.26 (m, 1H), 2.29–2.39 (m, 1H), 2.81 (s, 3H), 3.32–3.36 (m, 1H), 3.73 (d, J = 13.2 Hz, 1H) 4.52–4.67 (m, 1H), 7.41–7.45 (m, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 7.7 Hz, 1H). Anal. calcd for $C_{14}H_{18}N_4O$·1.5 TFA: C, 47.56; H, 4.58; N, 13.05. Found: C, 47.84; H, 4.52; N, 13.23.

5.3.31. 2-(1-Isopropylpiperidin-2-yl)-1*H*-benzimidazole-4-carboxamide (8d)

The title compound was prepared as described for **8c** using acetone in place of formaldehyde. ^{1}H NMR (CD_3OD) δ 1.30 (d, J = 6.6 Hz, 3H), 1.36 (d, J = 6.9 Hz, 3H), 1.74–1.88 (m, 1H), 1.98–2.06 (m, 2H), 2.09–2.15 (m, 1H), 2.28–2.35 (m, 2H), 3.17–3.24 (m, 1H), 3.36–3.46 (m, 1H), 3.67 (d, J = 11.8 Hz, 1H), 4.74–4.80 (m, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.82 (d, J = 9.0 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H). Anal. calcd for $C_{16}H_{22}N_4O$·1.9TFA: C, 47.28; H, 4.79; N, 11.14. Found: C, 47.49; H, 4.54; N, 11.24.

5.3.32. 2-(1-Isopropylpiperidin-3-yl)-1*H*-benzimidazole-4-carboxamide (9b)

The title compound was prepared from **9** and acetone according to procedure D. ^{1}H NMR (CD_3OD) δ 1.43 (d, J = 6.5 Hz, 6H), 1.89–1.99 (m, 1H), 2.00–2.10 (m, 1H), 2.21 (d, J = 14.3 Hz, 1H), 2.33–2.41 (m, 1H), 3.11–3.20 (m, 1H), 3.46 (t, J = 12.2 Hz, 1H), 3.57 (d, J = 11.8 Hz, 1H), 3.61–3.72 (m, 2H), 3.89 (d, J = 11.5 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H). Anal. calcd for $C_{16}H_{22}N_4O$·2.2TFA: C, 45.61; H, 4.54; N, 10.43. Found: C, 45.43; H, 4.85; N, 10.53.

5.3.33. 2-(1-Isopropylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10c)

The title compound was prepared from **10** and acetone according to procedure D. ^{1}H NMR (D_2O) δ 1.30 (d, J = 6.8 Hz, 6H), 2.08–2.23 (m, 2H), 2.45 (br d, J = 14.2 Hz, 2H), 3.11–3.26 (m, 2H), 3.47–3.66 (m, 4H), 7.48 (t, J = 8.0 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.81 (d, J = 8.1 Hz, 1H). Anal. calcd for $C_{16}H_{22}N_4O$·1.8 HCl·1.3 H_2O: C, 51.19; H, 7.09; N, 14.92, Cl, 17.00. Found: C, 51.03; H, 7.15; N, 14.69, Cl, 16.88.

5.3.34. 2-(1-Cyclopentylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10d)

The title compound was prepared from **10** and cyclopentanone according to procedure D. ^{1}H NMR (CD_3OD) δ 1.67–1.81 (m, 4H), 1.81–1.91 (m, 2H), 2.20–2.26 (m, 3H), 2.26–2.32 (m, 1H), 2.49 (d, J = 14.0 Hz, 2H), 3.21 (t, J = 12.0 Hz, 2H), 3.44–3.52 (m, 1H), 3.55–3.65 (m, 1H), 3.81 (d, J = 12.8 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H). HRMS m/z 313.20172 (calcd for $C_{18}H_{25}N_4O$ (M+H), 313.20229).

5.3.35. Procedure E: 2-(1-Propylpyrrolidin-3-yl)-1*H*-benzimidazole-4-carboxamide (7a)

To a solution of **7** (200 mg, 0.87 mmol) in dichloroethane (8 mL) and methanol (1 mL) was added propionaldehyde (127 μL, 1.74 mmol) and the solution stirred at ambient temperature for 1 h. Sodium triacetoxyborohydride (369 mg, 1.74 mmol) and acetic acid (100 μL, 1.74 mmol) were added and the mixture stirred at ambient temperature overnight. After concentration, the residue was purified by HPLC (Zorbax, C-18, 0.1% TFA/CH_3CN/H_2O). The trifluoroacetate salt was dissolved in dichloromethane/methanol, treated with 1N HCl in ether and concentrated to provide 159 mg (48%) of the hydrochloride salt. ^{1}H NMR (D_2O) δ 1.03 (t, J = 7.5 Hz, 3H), 1.78–1.87 (m, 2H), 2.50–2.56 (m, 0.5H), 2.66–2.71 (m, 0.5H), 2.83 (dd, J = 14.0, 7.6 Hz, 0.5H), 2.90–2.96 (m, 0.5H), 3.33–3.47 (m, 2H), 3.54–3.68 (m, 1H), 3.91 (t, J = 11.3 Hz, 0.5H), 3.96–4.01 (m, 1H), 4.02–4.07 (m, 0.5H), 4.16 (dd, J = 12.5, 7.0 Hz, 0.5H), 4.31–4.37 (m, 1H), 4.50–4.55 (m, 0.5H), 7.65 (t, J = 7.9 Hz, 1H), 7.95 (d, J = 8.1 Hz, 1H), 7.97 (d, J = 8.1 Hz, 1H). MS (APCI) m/z 273 $(M+H)^+$.

5.3.36. 2-(1-Isopropylpyrrolidin-3-yl)-1*H*-benzimidazole-4-carboxamide (7b)

The title compound was prepared from **7** and acetone according to procedure E (54% yield). ^{1}H NMR (D_2O) δ 1.45 (d, J = 6.4 Hz, 6H),

2.44–2.51 (m, 0.5H), 2.57–2.64 (m, 0.5H), 2.73–2.80 (m, 0.5H), 2.83–2.91 (m, 0.5H), 3.42–3.48 (m, 0.5H), 3.56–3.62 (m, 0.5H), 3.62–3.70 (m, 1H), 3.88–3.96 (m, 1H), 4.09 (dd, J = 11.9, 6.4 Hz, 0.5H), 4.23 (m, 1H), 4.38 (m, 0.5H), 7.57 (t, J = 8.1 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H). Anal. calcd for $C_{15}H_{20}N_4O\cdot$ 2.7 HCl: C, 48.59; H, 6.17; N, 15.11. Found: C, 48.37; H, 6.24; N, 14.79.

5.3.37. 2-(1-Cyclopentylpyrrolidin-3-yl)-1*H*-benzimidazole-4-carboxamide (7c)

The title compound was prepared from **7** and cyclopentanone according to procedure E (54% yield). ^{1}H NMR (D_2O) δ 1.67–1.87 (m, 6H), 2.18–2.26 (m, 2H), 2.46–2.55 (m, 0.5H), 2.63–2.69 (m, 0.5H), 2.80 (dd, J = 14.5, 7.8 Hz, 0.5H), 2.88–2.94 (m, 0.5H), 3.42–3.50 (m, 0.5H), 3.55–3.62 (m, 0.5H), 3.64–3.70 (m, 0.5H), 3.77–3.84 (m, 1H), 3.91–3.97 (m, 1H), 4.01 (dd, J = 10.4, 7.9 Hz, 0.5H), 4.13 (dd, J = 12.7, 6.9 Hz, 0.5H), 4.30 (q, J = 7.1 Hz, 1H), 4.45–4.53 (m, 0.5H), 7.63 (t, J = 8.1 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.95 (d, J = 7.6 Hz, 1H). Anal. calcd for $C_{17}H_{22}N_4O\cdot$2.9 HCl: C, 50.53; H, 6.21; N, 13.86. Found: C, 50.83; H, 6.20; N, 13.49.

5.3.38. 2-(1-Phenethylpyrrolidin-3-yl)-1*H*-benzimidazole-4-carboxamide (7d)

The title compound was prepared from **7** and phenylacetaldehyde according to procedure E (45% yield). ^{1}H NMR (CD_3OD) δ 2.48–2.56 (m, 1H), 2.73 (m, 1H), 3.11–3.15 (m, 2H), 3.59–3.64 (m, 2H), 3.66 (m, 2H), 3.93–4.04 (m, 2H), 4.13–4.20 (m, 1H), 7.26–7.38 (m, 5H), 7.40 (t, J = 8.0 Hz, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H). Anal. calcd for $C_{20}H_{22}FN_4O\cdot$2.25 TFA: C, 49.79; H, 4.14; N, 9.48. Found: C, 49.96; H, 4.19; N, 9.54.

5.3.39. 2-(1-(Phenylsulfonyl)piperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10g)

To a solution of **10** (100 mg, 0.41 mmol) in pyridine (5 mL) was added benzenesulfonyl chloride (109 mg, 0.62 mmol) and the mixture stirred at ambient temperature for 16 h. The mixture was concentrated, stirred in water and methanol for 30 min and filtered. The solid was washed with water and methanol and dried to give 90 mg (57%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 1.78–1.95 (m, 2H), 2.06–2.19 (m, 2H), 2.53–2.59 (m, 2H), 2.93–3.06 (m, 1H), 3.74 (d, J = 11.9 Hz, 2H), 7.26 (t, J = 7.6 Hz, 1H), 7.60–7.63 (m, 1H), 7.63–7.66 (m, 1H), 7.67 (t, J = 1.9 Hz, 1H), 7.69–7.70 (m, 1H), 7.72–7.75 (m, 1H), 7.76–7.78 (m, 1H), 7.78 (br s, 1H), 7.79–7.82 (m, 1H), 9.18 (br s, 1H), 12.68 (br s, 1H). Anal. calcd for $C_{19}H_{20}N_4O_3S$: C, 59.36; H, 5.24; N, 14.57. Found: C, 59.23; H, 5.20; N, 14.64.

5.3.40. 2-(1-(*N*,*N*-dimethylsulfamoyl)piperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (10h)

To a solution of **10** (150 mg, 0.614 mmol) in dichloromethane (8 mL) and methanol (1 mL) was added triethylamine (171 μL, 1.23 mmol) and dimethylsulfamoylchloride (79 μL, 0.737 mmol) and the mixture stirred at ambient temperature overnight. The solution was purified by flash chromatography on silica gel using methanol in dichloromethane to afford 165 mg (77%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 1.81–1.93 (m, 2H), 2.12 (t, J = 12.6 Hz, 2H), 2.79 (s, 6H), 3.03 (t, J = 11.5 Hz, 2H), 3.15 (t, J = 11.2 Hz, 1H), 3.65–3.70 (m, 2H), 7.29 (t, J = 7.5 Hz, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.67 (s, 1H), 7.83 (d, J = 7.1 Hz, 1H), 9.31 (br s, 1H), 12.74 (br s, 1H). HRMS m/z 352.14350 (calcd for $C_{15}H_{22}N_5O_3S$ (M+H), 352.14379).

5.3.41. 1-Methyl-2-(1-propylpiperidin-4-yl)-1*H*-benzimidazole-4-carboxamide (14)

To a solution of **10b** (100 mg, 0.35 mmol) in DMF (5 mL) was added sodium hydride (60% in mineral oil, 0.35 mmol, 14 mg) and the mixture stirred at ambient temperature for 1 h. Iodomethane (52 mg, 0.37 mmol) was added and the mixture stirred at ambient temperature for 16 h and concentrated. The residue was partitioned between ethyl acetate and brine and the organic phase was washed with brine and concentrated. Purification by flash chromatography on silica gel using 20% methanol in dichloromethane afforded 70 mg (67%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 0.95 (t, J = 7.5 Hz, 3H), 1.65–1.77 (m, 2H), 2.10–2.26 (m, 4H), 3.01–3.17 (m, 4H), 3.18–3.25 (m, 1H), 3.29 (s, 3H), 3.38–3.49 (m, 1H), 3.57–3.69 (m, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.77 (dd, J = 8.1, 0.9 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 9.07 (br s, 1H), 9.22 (br s, 1H). HRMS m/z 301.20211 (calcd for $C_{17}H_{25}N_4O$ (M+H), 301.20229).

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