Resistance-Modifying Agents. 9.[1] Synthesis and Biological Properties of Benzimidazole Inhibitors of the DNA Repair Enzyme Poly(ADP-ribose) Polymerase

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The nuclear enzyme poly(ADP-ribose) polymerase (PARP) facilitates the repair of DNA strand breaks and is implicated in the resistance of cancer cells to certain DNA-damaging agents. Inhibitors of PARP have clinical potential as resistance-modifying agents capable of potentiating radiotherapy and the cytotoxicity of some forms of cancer chemotherapy. The preclinical development of 2-aryl-1*H*-benzimidazole-4-carboxamides as resistance-modifying agents in cancer chemotherapy is described. 1*H*-Benzimidazole-4-carboxamides, particularly 2-aryl derivatives, are identified as a class of potent PARP inhibitors. Derivatives of 2-phenyl-1*H*-benzimidazole-4-carboxamide (**23**, $K_i = 15$ nM), in which the phenyl ring contains substituents, have been synthesized. Many of these derivatives exhibit K_i values for PARP inhibition < 10 nM, with 2-(4-hydroxymethylphenyl)-1*H*-benzimidazole-4-carboxamide (**78**, $K_i = 1.6$ nM) being one of the most potent. Insight into structure−activity relationships (SAR) for 2-aryl-1*H*-benzimidazole-4-carboxamides has been enhanced by studying the complex formed between 2-(3-methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (**44**, $K_i = 6$ nM) and the catalytic domain of chicken PARP. Important hydrogen-bonding and hydrophobic interactions with the protein have been identified for this inhibitor. 2-(4-Hydroxyphenyl)-1*H*-benzimidazole-4-carboxamide (**45**, $K_i = 6$ nM) potentiates the cytotoxicity of both temozolomide and topotecan against A2780 cells in vitro (by 2.8- and 2.9-fold, respectively).

Introduction

Poly(ADP-ribose) polymerase (PARP, EC 2.4.2.30) is a nuclear enzyme with an important role in the process of genomic repair.[2,3] Inhibition of PARP in tumor cells may potentiate radiotherapy and certain kinds of cancer chemotherapy targeted to DNA.[4] Three structural domains control the activity of PARP: the N-terminal DNA binding domain (containing two zinc fingers), a central automodification domain, and the highly conserved C-terminal catalytic region.[5] The enzyme initially recognizes and binds to a site of DNA damage. Binding to DNA stimulates PARP to catalyze the synthesis of linear and branched ADP-ribose polymers from substrate nicotinamide adenine dinucleotide (NAD^+), with concomitant release of nicotinamide.[6] The mechanism of polymer formation has recently been probed using the techniques of X-ray crystallography and site-directed mutagenesis, which have shown the significance of the strictly conserved active site residue Glu 988.[7] The polymer can be anchored either to the automodification domain of PARP or to an adjacent histone. Poly(ADP-ribose) is in a dynamic state, its rapid synthesis being followed by a degradation catalyzed by the enzyme poly(ADP) glycohydrolase, before resynthesis occurs.[8,9] The binding of PARP to a DNA nick may cause a transient halt to cellular activity, allowing access of DNA repair enzymes to the damage.[10] Alternatively, formation of poly(ADP-ribose) or the fragments from metabolism of this polymer may signal to a cell that its DNA is damaged.[8]

The ability of tumor cells to recognize and repair damage to DNA inflicted by cancer therapy is an important mechanism of resistance to treatment. However, in the absence of DNA damage, PARP does not appear to be necessary for cell survival. PARP 'knockout' mice are viable and appear to be largely unaffected by the lack of the enzyme.[11] If PARP has no involvement in critical physiological processes, then inhibiting the enzyme is unlikely to induce major toxicity. PARP is therefore an attractive target for drug design in the context of cancer treatment.

Early studies identified nicotinamide (**1**) and various 3-substituted benzamides [e.g. **2**, R = NH_2 (3AB)] as inhibitors of PARP.[12−14] However, these compounds lack potency and specificity, and some are poorly soluble in water. A significant advance was the synthesis of the dihydroisoquinolines **3** and **4** in which the carboxamide is constrained within a ring. Compound **3** is much more active than **4** as an inhibitor of PARP (IC_{50} values of 0.41 and 8 μM, respectively).[15] These results suggest that the conformation of NAD^+ that binds to PARP has the carboxamide carbonyl *anti* to the C2−C3 bond. This supposition was confirmed by ab initio molecular orbital

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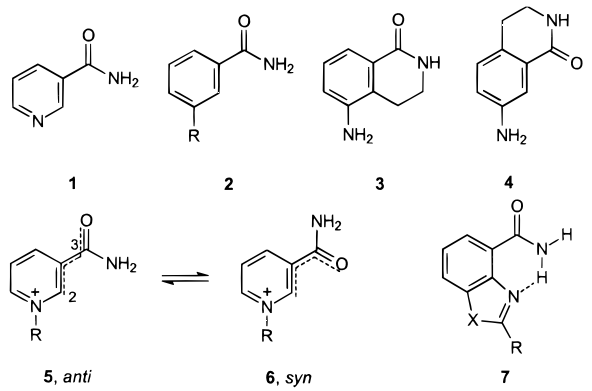
calculations on the nicotinamide moiety of NAD^+, which revealed that the conformation with the carbonyl positioned *anti* to the 2,3-bond (**5**) is preferred over that in **6**.[16] The carboxamide group can also be restricted via an intramolecular hydrogen bond, as demonstrated for benzoxazole-4-carboxamides **7** (X = O, R = Me)[17a] and for benzimidazole-4-carboxamides[17b] (see also below).



In this paper, we report the synthesis of a series of 2-arylbenzimidazole-4-carboxamides **7** (X = NH), which are potent inhibitors of PARP and are able to act as resistance-modifying agents[18] for radiotherapy and certain types of chemotherapy.[19] These compounds contain an intramolecular hydrogen bond, which helps to impose the conformation of the molecule necessary for inhibitory activity against PARP. We have previously reported on the ability of quinazolinone inhibitors of PARP to augment DNA-damaging drugs.[18−20]

Results and Discussion

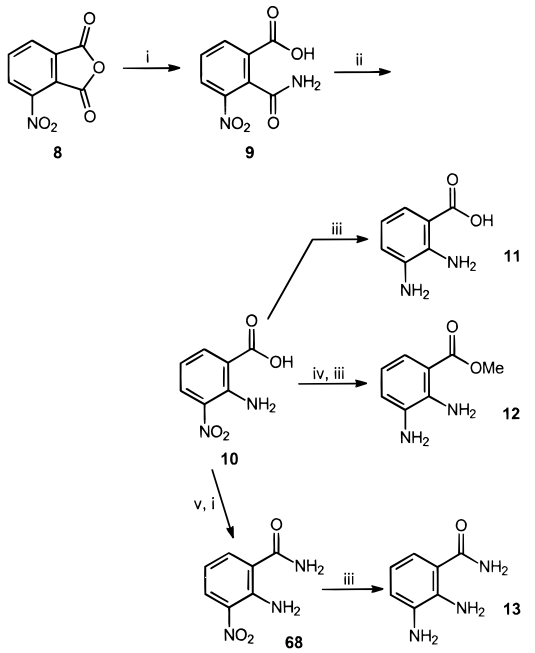
Chemistry. A variety of methods have been employed to synthesize the benzimidazole-4-carboxamides described herein. The 2-substituted benzimidazole-4-carboxylic acids **18**, **20**, and **22** were obtained by heating 2,3-diaminobenzoic acid (**11**) with a carboxylic acid in the presence of hydrochloric or polyphosphoric acid (Phillips synthesis; cf. Scheme 2).[21,22] The required 2,3-diaminobenzoic acid (**11**) was readily obtained in multigram quantities from 3-nitrophthalic anhydride (**8**) by a literature procedure (Scheme 1).[23] Thus, regioselective ring opening of **8** with ammonia yielded amide **9**, a substrate for a Hofmann rearrangement that afforded 2-amino-3-nitrobenzoic acid (**10**). Reduction of **10** gave 2,3-diaminobenzoic acid (**11**), while other standard reactions gave the useful intermediates **12**−**14** and **68** (Scheme 1). The benzimidazole-4-carboxylic acids **18**, **20**, and **22** were each converted into the corresponding amides **19**, **21**, and **23**, respectively, via the acid chloride (Scheme 2).

Methyl 2,3-diaminobenzoate (**12**) was used to prepare 2-arylbenzimidazole-4-carboxamides **24**−**30**. The more nucleophilic 3-amino group of **12** was selectively acylated with an aryl acid chloride. The resulting intermediate amide was cyclized under acidic conditions to give the methyl ester of a 2-arylbenzimidazole-4-carboxylic acid, which was converted to the required amide by treatment with ammonia under pressure.

Both of the methods described above were inefficient and not generally applicable. A modified version of a procedure first reported by Weidenhagen[24] was developed for the direct preparation of numerous 2-arylbenzimidazole-4-carboxylic acids and amides. In this method either 2,3-diaminobenzoic acid (**11**) or its amide (**13**) was treated with an aryl aldehyde and copper(II) acetate[25] to give the desired 2-arylbenzimidazole-4-carboxylic acid or amide (Tables 2 and 3). Of all the methods utilized to synthesize the benzimidazole inhibitors described herein, the route starting with amide **13** (Scheme 2) was the most efficient.

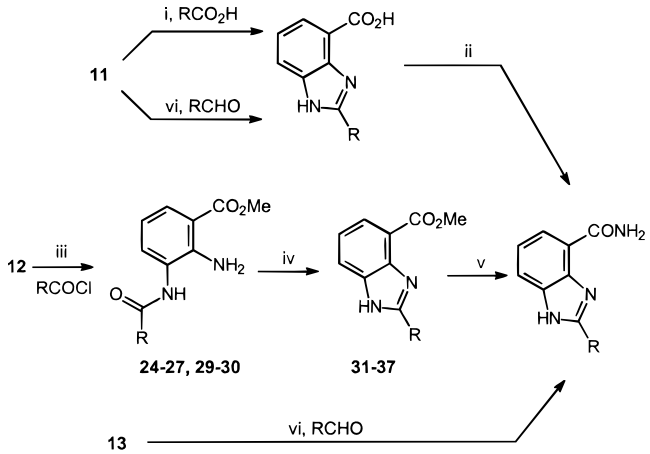
For the preparation of 2-trifluoromethylbenzimidazole (**17**; Scheme 3), methyl 2-amino-3-nitrobenzoate (**14**) was acylated with trifluoroacetic anhydride to yield **15**. Hydrogenation (see introduction to the Experimental Section) of **15** yielded methyl 2-trifluoromethylbenzimidazole-4-carboxylate (**16**) directly, the cyclization step occurring in situ. The activating effect of the trifluoromethyl group enabled the conversion of **16** into amide

Scheme 1[a]



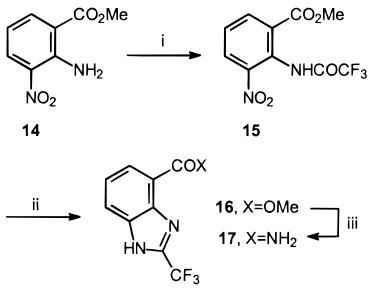
[a] Reagents: (i) NH_3(aq); (ii) KOH, Br_2, H_2O; (iii) 10% Pd/C, H_2, MeOH; (iv) HCl, MeOH; (v) $SOCl_2$/DMF, THF.

Scheme 2[a]



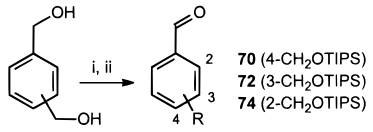
[a] Reagents: (i) RCO_2H, 4 M HCl or PPA, heat; (ii) $SOCl_2$ then NH_3(aq); (iii) RCOCl, Et_3N, DMAP, THF, 0 °C; (iv) AcOH, heat; (v) NH_3(l), 80 °C, 40 atm; (vi) RCHO, $Cu(OAc)_2$, MeOH, heat, then H_2S.

Scheme 3[a]



[a] Reagents: (i) $(CF_3CO)_2O$, Et_3N, THF; (ii) 10% Pd/C, H_2, MeOH; (iii) NH_3(aq).

Scheme 4[a]



[a] Reagents: (i) NaH, TIPSCl, THF; (ii) MnO_2, THF; TIPS = trisopropylsilyl.

17 by treatment with aqueous ammonia. 2-(4-Hydroxyphenyl)benzimidazole-4-carboxamide (**45**) was prepared from the corresponding 4-methoxy compound **38** by treatment with BBr_3 in DCM. 4-Carboxyphenylbenzimidazole (**46**) was obtained by basic hydrolysis of 2-(4-cyanophenyl)benzimidazole-4-carboxamide (**41**). Benzimidazole *N*-alkylation and *N*-acylation (**47**−**49**) was achieved by treatment of the appropriate benzimidazole with an equivalent of potassium hydroxide in acetone before addition of the alkylating (MeI) or acylating agent (BzCl and CbzCl).[26]

2-Arylbenzimidazole-4-carboxamides containing a hydroxymethyl group at the 2-, 3-, and 4-positions (**78**−**80**) were prepared by the Weidenhagen method from the corresponding aldehyde precursors (**70**, **72**, **74**). These were prepared by monosilylation of the appropriate benzene-dimethanol with triisopropylsilyl chloride (Scheme 4), with benzene-1,2-dimethanol giving the highest yield (85%) because further silylation is inhibited by the adjacent bulky *ortho*-silyl group. Oxidation of each monosilylated benzene-dimethanol with manganese dioxide afforded the required aldehyde.

Compounds expected to exhibit low activity against PARP were synthesized for comparative biological studies. The *N*-methylamides **88** and **90** were obtained by reaction of the corresponding methyl ester with methylamine. In these amides a critical hydrogen-bonding site is blocked by *N*-methylation. Likewise, 2-arylbenzimidazole-4-carboxylic acids (e.g. **91** synthesized from 2,3-diaminobenzoic acid (**11**) and 4-hydroxybenzaldehyde via the Weidenhagen method) lack the amide functionally required for binding to PARP.

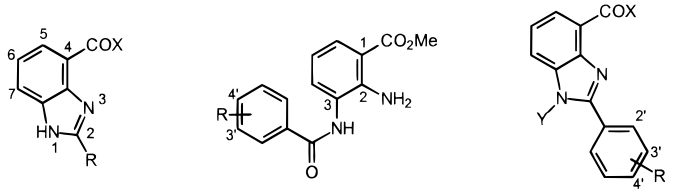
Inhibitory Activities of 2-Substituted Benzimidazole-4-carboxamides Against PARP. In this study, structure−activity relationships (SAR) for a series of 2-substituted benzimidazole-4-carboxamides have been probed by assaying for in vitro activity against recombinant human PARP. The simple benzimidazole-4-carboxamides (**17**, **19**, **21**) showed sub-micromolar activity against PARP and are considerably more active than the classical PARP inhibitor 3-aminobenzamide (**2**, R = NH_2, K_i = 3100 nM). However, placing a phenyl group at the 2-position gave a significant increase in activity (**23**, K_i = 15 nM). A series of 2-arylbenzimidazole-4-carboxamides was therefore synthesized to enable the SAR for substituents on the phenyl ring to be explored. A variety of substituents, with differing electronic properties, were introduced at the 3- and 4-positions (**38**−**46**). Inhibition data showed that PARP tolerates a variety of 3- and 4-substituents on the 2-phenyl group well and does not discriminate significantly between electronically differing groups. 2-(4-Cyanophenyl)benzimidazole-4-carboxamide (**41**, K_i = 4 nM) was the most active compound among these inhibitors. Comparison of 2-(3-methoxyphenyl)benzimidazole-4-carboxamide (**44**) with 2-(3-hydroxyphenyl)benzimidazole-4-carboxamide (**60**) showed little difference in activity (K_i = 6 nM for both compounds), indicating that there is no favorable hydrogen-bonding interaction with the protein for the 3-hydroxy group. Similarly, 2-(4-methoxyphenyl)benzimidazole-4-carboxamide (**38**) and 2-(4-hydroxyphenyl)benzimidazole-4-carboxamide (**45**) were equipotent (K_i = 6.8 and 6 nM, respectively). The position of the substituent (3 versus 4) is also relatively unimportant for the aforementioned examples. However, 2-(4-hydroxymethylphenyl)benzimidazole-4-carboxamide (**78**) was significantly more active (K_i = 1.6 nM) than its 3-hydroxymethyl isomer **79** (6.8 nM). As expected, the precursor (**75**) of **78**, containing a bulky trialkylsilyl group, exhibited much reduced activity (K_i = 85 nM).

Both 2-(2-methoxyphenyl)benzimidazole-4-carboxamide (**61**, K_i = 126 nM) and 2-(2-trifluoromethylphenyl)benzimidazole-4-carboxamide (**81**, K_i = 190 nM) were considerably less active than their 3- and 4-substituted counterparts. However, comparison of the data for the 2-methoxy group (**61**) with the isosteric 2-hydroxymethyl group (**80**, K_i = 10.6 nM) suggests that the hydroxymethyl group of **80** interacts with the enzyme via a hydrogen bond or solvent molecule. Furthermore, this compound is nearly as potent as its 3-isomer **79** (see above). It is important to note that 2-(2-chlorophenyl)benzimidazole-4-carboxamide (**82**) and 2-(2-fluorophenyl)benzimidazole-4-carboxamide (**84**) are both well-tolerated by the enzyme (K_i = 9.4 and 9 nM, respectively) and are comparable in activity to their 3-analogues (e.g. **83**). It appears that relatively small groups and substituents containing a hydroxyl group can be accommodated at the 2-position.

Benzimidazole-4-carboxamides containing a disubstituted (**62**, **63**, **67**, **86**, **87**) and trisubstituted (**64**) 2-phenyl group were synthesized to explore further the SAR. These compounds showed improved aqueous solubility compared to monosubstituted analogues. The disubstituted inhibitors were generally well-accepted by the enzyme, with the benzimidazole-4-carboxamide (**86**) being particularly active (K_i = 2.0 nM). The isomeric benzimidazole **62** was 6-fold less active (K_i = 13 nM). The 2-(3,4,5-trimethoxyphenyl)benzimidazole-4-carboxamide (**64**) exhibited a sharp decrease in activity (K_i = 136 nM) indicating that while one *meta*-substituent is well-tolerated, an additional *meta*-substituent is not.

For compounds **47** and **49** there is a decrease in inhibitory activity as the size of the N(1) substituent increases (K_i = 32 and 530 nM for methyl and benzyl-

Table 1. Physical and Biological Activity Data

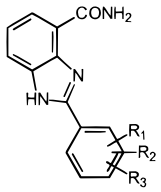


I II III

compd	structure	X	Y	R	method[a]	yield (%)	molecular formula	anal.[b]	K_i (nM)[c]
3-AB[d]									3100[e]
17	I	NH_2		CF_3		75	$C_9H_6F_3N_3O_2$	C, H, N	350
18	I	OH		H	A	77	$C_8H_6N_2O_2$	C, H, N	NT[f]
19	I	NH_2		H	B	13	$C_8H_7N_3O$	C, H, N[g]	95 ± 11
20	I	OH		Me	A	72	$C_9H_8N_2O_2$	*h*	NT
21	I	NH_2		Me	B	14	$C_9H_9N_3O$	C, H, N[i]	99 ± 6
22	I	OH		Ph	A	20	$C_{14}H_{10}N_2O_2$	*h*	NT
23	I	NH_2		Ph	C	62	$C_{14}H_{11}N_3O{\cdot}0.1EtOH$	C, H, N	15
24	II			4′-OMe	D	62	$C_{16}H_{16}N_2O_4$	C, H, N	NT
25	II			4′-CF_3	D	14	$C_{16}H_{13}F_3N_2O_3$	C, H, N	NT
26	II			4′-NO_2	D	45	$C_{15}H_{13}N_3O_5$	C, H, N	NT
27	II			4′-CN	D	37	$C_{16}H_{13}N_3O_3$	C, H, N	NT
28	II			4′-NH_2	*j*	92	$C_{15}H_{15}N_3O_3$	C, H, N	NT
29	II			3′-CF_3	D	26	$C_{16}H_{13}F_3N_2O_3$	C, H, N	NT
30	II			3′-OMe	D	23	$C_{16}H_{16}N_2O_4$	C, H, N	NT
31	III	OMe		4′-OMe	E	75	$C_{18}H_{18}N_2O_5$	*h*	NT
32	III	OMe		4′-CF_3	E	70	$C_{18}H_{15}F_3N_2O_4$	C, H, N	NT
33	III	OMe		4′-NO_2	E	65	$C_{15}H_{11}N_3O_4$	C, H, N	NT
34	III	OMe		4′-CN	E	72	$C_{16}H_{11}N_2O_5$	*h*	NT
35	III	OMe		4′-NH_2	E	91	$C_{17}H_{17}N_3O_4$	C, H, N	NT
36	III	OMe		3′-CF_3	E	96	$C_{18}H_{15}F_3N_2O_4$	C, H, N	NT
37	III	OMe		3′-OMe	E	58	$C_{18}H_{18}N_2O_5$	*h*	NT
38	III	NH_2		4′-OMe	F	80	$C_{15}H_{13}N_3O_2$	C, H, N	6.8 ± 3.9
39	III	NH_2		4′-CF_3	F	48	$C_{15}H_{10}F_3N_3O{\cdot}0.1H_2O$	C, H, N	1.2
40	III	NH_2		4′-NO_2	F	74	$C_{14}H_{10}N_4O_3$	C, H, N	8 ± 0[e]
41	III	NH_2		4′-CN	F	73	$C_{15}H_{10}N_4O$	C, H, N	4
42	III	NH_2		4′-NH_2	F	25	$C_{14}H_{12}N_4O{\cdot}0.2MeOH$	C, H, N	11
43	III	NH_2		3′-CF_3	F	72	$C_{15}H_{10}F_3N_3O$	C, H, N	8 ± 0[e]
44	III	NH_2		3′-OMe	F	46	$C_{15}H_{13}N_3O_2$	C, H, N	6
45	III	NH_2		4′-OH	F	57	$C_{14}H_{11}N_3O_2$	C, H, N	6
46	III	NH_2		4′-CO_2H	F	47	$C_{15}H_{11}N_3O_3$	*h*	290
47	III	NH_2	Me	4′-OMe	G	30	$C_{16}H_{15}N_3O_2$	C, H, N	32[e]
48	III	NH_2	Bz	4′-OMe	G	15	$C_{22}H_{17}N_3O_3$	C, H, N	NT (see text)
49	III	NH_2	Cbz	4′-OMe	G	61	$C_{23}H_{19}N_3O_4$	C, H, N	530

[a] See Experimental Section. [b] Elemental analyses are ±0.4% of calculated values. [c] Assay against PARP full length protein unless otherwise stated. [d] 3-Aminobenzamide. [e] Assayed against PARP L713F protein. [f] Not tested. [g] Anal. C, H; N: found 24.59, expected 26.09. [h] Satisfactory analysis not obtained. [i] Anal. C, H; N: found 23.39, expected 24.0. [j] Prepared by hydrogenation of **26**.

Table 2. Physical and Biological Activity Data

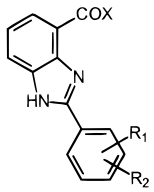


compd	R_1	R_2	R_3	method[a]	yield (%)	molecular formula	anal.[b]	K_i (nM)[c]
59	4′-Cl			H, I	38	$C_{14}H_{10}ClN_3O{\cdot}0.1MeOH$	C, H, N	3 ± 0.4
60	3′-OH			H, I	62	$C_{14}H_{11}N_3O_2$	C, H, N	6.3 ± 2.1
61	2′-OMe			H, I	38	$C_{15}H_{13}N_3O_2$	C, H, N	126 ± 3
62	3′-OMe	4′-OH		H, I	20	$C_{15}H_{13}N_3O_3{\cdot}0.1MeOH$	C, H, N	13
63	3′-OMe	4′-OMe		H, I	14	$C_{16}H_{15}N_3O_3{\cdot}0.5HCl$	C, H, N	5.4 ± 1.2
64	3′-OMe	4′-OMe	5′-OMe	H, I	12	$C_{17}H_{17}N_3O_4$	C, H, N	136 ± 12
65	4′-NMe_2			H, I	17	$C_{16}H_{16}N_4O$	C, H, N	5.8 ± 0.9
66	4′-CO_2Et			H, I	20	$C_{17}H_{15}N_3O_3{\cdot}0.1EtOH$	C, H, N	8.1 ± 4
67	3′,4′ (OCH_2O)			H, I	15	$C_{15}H_{11}N_3O_3$	C, H, N[d]	5.9 ± 0.4

[a] See Experimental Section. [b] Elemental analyses are ±0.4% of calculated values. [c] Assay against PARP full length protein unless otherwise stated. [d] Anal. H, N; C: found 64.58, expected 64.05.

oxycarbonyl, respectively). An accurate value could not be determined for **48** because of the instability of this compound in the assay medium. These results indicate a tight pocket in the active site region near the imidazole N(1)H. An unsubstituted imidazole is optimum, either because the free N(1)H interacts with a water molecule within the active site or because an N(1) substituent interacts unfavorably with the protein.

Table 3. Physical and Biological Data



compd	X	R_1	R_2	method[a]	yield (%)	molecular formula	anal.[b]	K_i (nM)[c]
75	NH_2	4′-CH_2OTIPS		I	85	$C_{24}H_{33}N_3O_2Si$	NT[d]	85
76	NH_2	3′-CH_2OTIPS		I	48	$C_{24}H_{33}N_3O_2Si$	NT	NT
77	NH_2	2′-CH_2OTIPS		I	43	$C_{24}H_{33}N_3O_2Si$	NT	NT
78	NH_2	4′-CH_2OH			65	$C_{15}H_{13}N_3O_2$	C, H, N[e]	1.6 ± 0.4
79	NH_2	3′-CH_2OH			47	$C_{15}H_{13}N_3O_2$	C, H, N	6.8 ± 0.3
80	NH_2	2′-CH_2OH			51	$C_{15}H_{13}N_3O_2$	C, H, N	10.6 ± 0.5
81	NH_2	2′-CF_3		I	43	$C_{15}H_{10}F_3N_3O{\cdot}0.2H_2O$	C, H, N	190
82	NH_2	2′-Cl		I	24	$C_{14}H_{10}ClN_3O$	C, H, N	9.4 ± 0.9
83	NH_2	3′-Cl		I	24	$C_{14}H_{10}ClN_3O{\cdot}0.33MeOH$	C, H, N	8.4 ± 0.5
84	NH_2	2′-F		I	57	$C_{14}H_{10}FN_3O$	*f*	9
86	NH_2	3′-OH	4′-OMe	H, C	87	$C_{15}H_{13}N_3O_2{\cdot}0.75H_2O$	C, H, N[g]	2.0 ± 0.2
87	NH_2	3′-O-allyl	4′-OMe	I	40	$C_{18}H_{17}N_3O_3$	*f*	9.4
88	NHMe	4′-OH			94	$C_{15}H_{13}N_3O_2{\cdot}H_2O$	C, H, N	inactive[h]
90	NHMe	4′-OMe			69	$C_{16}H_{15}N_3O_2{\cdot}0.6EtOH{\cdot}0.1H_2O$	C, H, N	inactive[i]
91	OH	4′-OH			15	$C_{14}H_{10}N_2O_3$	*f*	10^4

[a] See Experimental Section. [b] Elemental analyses are ±0.4% of calculated values. [c] Assay against PARP full length protein unless otherwise stated. [d] Not tested. [e] Anal. C, H; N: found 14.42, expected 15.72. [f] Satisfactory analysis not obtained. [g] Anal. C, H; N: found 13.62, expected 14.15. [h] No inhibition at 100 μM. [i] No inhibition at 10 μM.

As expected, replacement of the primary carboxamide of 2-arylbenzimidazole-4-carboxamides with a secondary amide (**88**, **90**) or carboxylic acid (**91**) removed all activity. Two amide protons are an absolute requirement for inhibitory activity: one proton being involved in the essential intramolecular hydrogen bond, while the other is required as a hydrogen-bonding donor to a backbone amide carbonyl group of the enzyme, as discussed below.

The data presented in this section indicate that a variety of substituents at either the 3- or 4-position or at both positions enhance the potency of 2-phenylbenzimidazole-4-carboxamide as an inhibitor of PARP. The data can be understood with reference to the crystal structure of 2-(3-methoxyphenyl)benzimidazole-4-carboxamide (**44**) (see below), which shows that the 3-methoxyphenyl ring occupies a large, well-defined pocket in the protein structure but with space around the ring edge that permits a variety of further substituents.

Potentiation of Topotecan and Temozolomide by 2-(4-Hydroxyphenyl)benzimidazole-4-carboxamide (45). The ability of PARP inhibitors, including 2-methylbenzimidazole (**21**) to act as radio- and chemopotentiators in vitro has been demonstrated.[20,27] Good potentiation, as well as enzymatic inhibition, is vital if these compounds are to function as therapeutically useful resistance-modifying agents. Growth inhibition assays demonstrated that 2-(4-hydroxyphenyl)benzimidazole-4-carboxamide (NU1085, **45**) potentiates topotecan (TP), a topoisomerase I inhibitor, and temozolomide (TM), a monofunctional alkylating agent recently approved for the treatment of glioma.

A2780 human ovarian carcinoma cells were grown in the presence of varying concentrations of TM with or without 10 μM NU1085 (**45**). Cells exposed to TM alone showed reduced cell survival compared to those treated in combination with a PARP inhibitor (see Figure 1). Likewise, cells exposed to TP and NU1085 (**45**) responded in a similar manner, the presence of the PARP inhibitor leading to an increase in cell growth inhibition (see Figure 2). Pooled data from two independent experiments are given in Table 4. Calculation of the IC_{50} values for TM and TP with and without NU1085 allowed the determination of the potentiation factor, PF_{50}, for NU1085 against each drug. The benzimidazole PARP inhibitor potentiated the cytotoxicity of temozolomide and topotecan by 2−3-fold. A more detailed discussion of related potentiation experiments has been reported previously.[20]

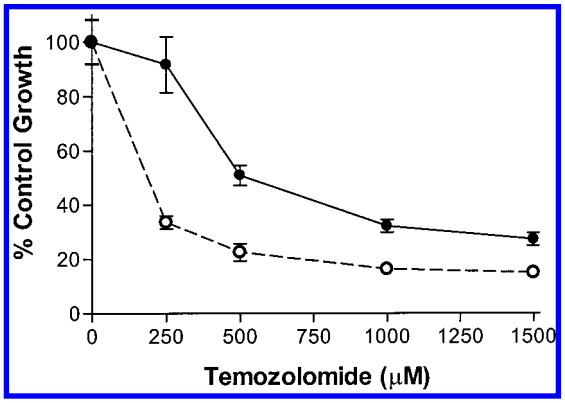


Figure 1. Inhibition of A2780 cell growth by temozolomide: potentiation by NU1085 (**45**). Cell growth was determined by sulforhodamine B assay after 72 h of continuous exposure to increasing concentrations of temozolomide with or without co-exposure to 10 μM NU1085. Data are the mean of 5 replicates: temozolomide alone, ●; temozolomide + 10 μM NU1085, ○.

Structure Determination of the Complex Between PARP and 2-(3-Methoxyphenyl)benzimidazole-4-carboxamide (44) by X-ray Crystallography. The above conclusions from 'traditional' analysis of the SAR data have been validated by an X-ray crystallographic study of a complex between a representative inhibitor, 2-(3-methoxyphenyl)benzimidazole-4-carboxamide (**44**), and the catalytic domain of chicken PARP (see Figure 3). The carboxamide group of **44** forms

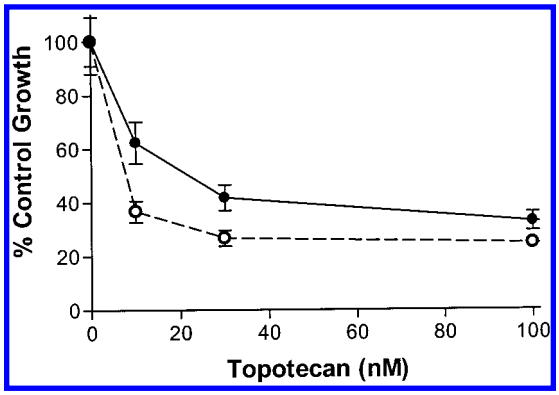


Figure 2. Inhibition of A2780 cell growth by topotecan: potentiation by NU1085 (**45**). Cell growth was determined by sulforhodamine B after 72 h of continuous exposure to increasing concentrations of topotecan with or without co-exposure to 10 μM NU1085 assay. Data are the mean of 5 replicates: topotecan alone, ●; topotecan + 10 μM NU1085, ○.

Table 4. Summary of Potentiation of Temozolomide and Topotecan Growth Inhibition by 10 μM NU1085 (**45**)[a]

	IC_{50}	
	temozolomide (μM)	topotecan (nM)
drug alone	525, 368	22.6, 22
drug + 10 μM NU1085	188, 183	7.8, 8.7
PF_{50}[b]	2.8, 2.0	2.9, 2.5

[a] Data are from two independent experiments of the type shown in Figures 1 and 2. [b] PF_{50} is the potentiation factor at 50% growth inhibition, i.e., IC_{50} for temozolomide (or topotecan) alone/IC_{50} for temozolomide (or topotecan) +PARP inhibitor.

three important hydrogen bonds, similar to the hydrogen bonding observed with other inhibitors bound to PARP.[28,29] In this case the inhibitor carbonyl oxygen accepts two hydrogen bonds: one from the side chain oxygen of Ser 904 (3.3 Å) and the other from a Gly 863 polypeptide amide NH (2.9 Å). The amide NH of **44** *syn* to the carbonyl group is a hydrogen bond donor to the Gly 863 backbone carbonyl oxygen (3.2 Å). The conformation observed for the carboxamide group of **44** was also seen in the crystal structure (i.e. measured for the protein-free compound) of the analogous benzimidazole **38** and may be that intrinsically preferred by all of 2-arylbenzimidazole-4-carboxamides described herein.[17b]

The benzimidazole of **44** lies in a deep pocket formed by residues Phe 897, Ala 898, Lys 903, and Glu 988 and is buried between two tyrosine residues (Tyr 896 and 907). The residue Tyr 907 is approximately coplanar with the benzimidazole, while Tyr 896 is rotated approximately 37° from coplanarity. The 2-(3-methoxyphenyl) group is located above a large, solvent-containing cavity of the enzyme. The 2-phenyl ring is rotated approximately 23° relative to the benzimidazole plane. Both faces of the 2-phenyl group are exposed to solvent, as is the 3-methoxy group, although it is partially shielded from solvent by a mobile Gln 763 side chain. The 3-methoxy group is on the same side of the inhibitor as the 4-carboxamide and is centered over the large enzyme cavity. The unsubstituted 4- and 5-edge, on the other side of the inhibitor, is oriented toward the surface of the enzyme cavity, which is composed of the Gln 763 side chain (near 4) and main chain atoms from the carbonyl of 888 through the amide of 890 (near 5). This edge of the 2-phenyl ring is shielded from solvent, being from 3.7 to 4.9 Å away from the protein. Comparing the

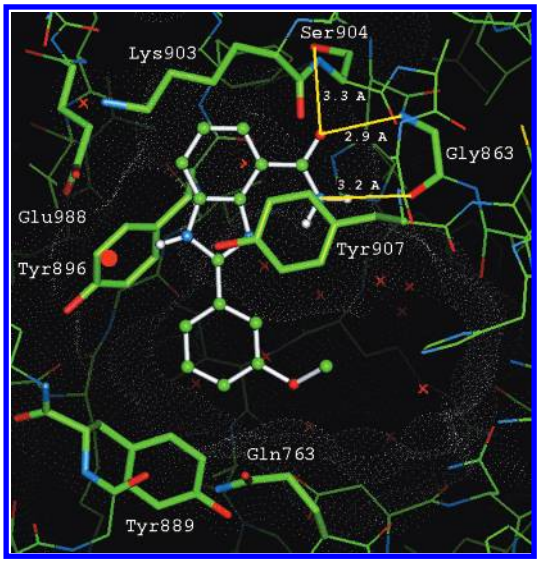


Figure 3. Compound **44** bound to the PARP active site. The inhibitor is shown with white bonds, green spheres for carbon, red spheres for oxygen, and blue spheres for nitrogen. The protein is shown with bonds, also color-coded by elemental type. Important residues surrounding the inhibitor are labeled and shown with thick bonds. Three important hydrogen bonds between the inhibitor and protein are shown as yellow lines. Water molecules are shown as red crosses except for the one water molecule that is hydrogen-bonded to the inhibitor and is shown as a red sphere. The protein molecular surface is shown with white dots.

3-, 4-, and 5-positions, the most available space is at the exposed position where the 3-methoxy is found. Taking into consideration the mobility of the Gln 763 side chain, significant space is also available at the 4-position, whereas less space for substituents is found extending from the 5-position. The information from the crystal structure is in accord with the findings regarding inhibitory potencies of inhibitors (see above), in particular for 2-(3,4,5-trimethoxyphenyl)benzimidazole-4-carboxamide (**64**, K_i = 136 nM).

An ordered water molecule is located 3.0 Å from the benzimidazole N(1) nitrogen and is a hydrogen bond acceptor. This water molecule is also 2.8 Å from the catalytically important carboxylate of Glu 988. Another water is found 3.5 Å from the 4-carboxamide nitrogen, which is also 3.1 Å from the Gly 863 carbonyl oxygen, 3.6 Å from the side chain oxygen of Ser 864, and 3.5 Å from a third water molecule.

Conclusions

Structural modifications around a benzimidazole-4-carboxamide core have identified 2-phenyl-1*H*-benzimidazoles as potent PARP inhibitors with clearly defined structure−activity requirements.[30] 2-(4-Hydroxyphenyl)benzimidazole-4-carboxamide (NU1085, **45**) has been adopted as a benchmark inhibitor of PARP due to its potency, ease of synthesis, and relatively good solubility in water. Potentiation experiments have shown that NU1085 (**45**) can increase the potency of the cytotoxic agents topotecan and temozolomide by up to 3-fold. Clinically, a drug combination that could result in reduced dosage levels and a decreased possibility of drug resistance is extremely attractive. Consideration of K_i, PF_{50}, and crystallographic data is guiding the

synthesis of potent, selective, and bioavailable compounds, which may be clinically useful PARP inhibitors.

Experimental Section

Melting points were obtained on a Kofler hot stage apparatus and are uncorrected. Infrared spectra (IR) were recorded as KBr disks on a Nicolet 20 PC Fourier transform spectrometer. Proton and carbon-13 nuclear magnetic resonance (NMR) spectra were recorded at 200 and 50 MHz, respectively, on a Bruker WP 200 spectrometer employing the deuterated solvent as internal standard. Unless indicated otherwise, spectra were recorded in DMSO-d_6 as solvent. NH and OH signals appeared as broad singlets (br s) exchangeable with D_2O. Chemical shift values are quoted in parts per million (ppm) and coupling constants (J) in hertz (Hz). ^{1}H NMR spectra of *para*-disubstituted aromatic rings were observed as an AA′BB′ pattern but for simplicity are quoted as doublets; key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were determined on a Kratos MS80 spectrometer in electron impact (EI) mode or fast atom bombardment (FAB mode using a *m*-nitrobenzyl alcohol matrix). The TLC systems employed Merck 1.05554 aluminum sheets precoated with Kieselgel 60F_{254} (0.2 mm) and were visualized with UV light at 254 or 365 nm. Column chromatography was conducted under medium pressure on silica (Kieselgel 60, 240−400 mesh). Elemental analyses were performed either in-house on a Carlo-Erba Instrumentazione 1106 analyzer or by Butterworth Laboratories, Middlesex, U.K., and are within ±0.4% of theoretical value unless otherwise specified. Reagents for organic synthesis were purchased from Aldrich Chemical Co. (Gillingham, U.K.) and Lancaster Synthesis (Morecambe, U.K.) and were used as received unless otherwise stated. Routine and tissue culture chemicals for growth inhibition assays were obtained from Sigma Chemical Co. (Poole, U.K.) unless stated otherwise. Ethanol and methanol were dried using Mg/I_2 and stored over molecular sieves. Diethyl ether and tetrahydrofuran were predried over $CaCl_2$ and distilled from sodium/benzophenone. Petroleum ether refers to that fraction in the boiling range 40−60 °C. Solvents were removed under reduced pressure using a rotary evaporator. Concentrated '880' ammonia (aqueous ammonia solution) was used for amide formation from acid chlorides.

Unless otherwise stated, catalytic hydrogenations were performed for solutions in methanol using 10% Pd/C catalyst (10 mol % w/w) stirred under hydrogen at atmospheric pressure until gas absorption ceased. After filtration through Celite and removal of the solvent, the resulting material was used directly or purification was performed as described below.

2-Amino-3-Nitrobenzoic Acid (10).[22] Potassium hydroxide pellets (22.6 g, 0.40 mol) in water (110 mL) were cooled to 0 °C. Bromine (2.3 mL, 0.045 mol), followed by 3-nitrophthalamic acid (**9**)[22] (9.0 g, 0.047 mol), was added. The reaction mixture was warmed at 60 °C for 3 h, allowed to cool to room temperature, and stirred overnight. The orange precipitate was filtered off and taken up in the minimum quantity of water to give a red solution, which was adjusted to pH 4 with concentrated HCl, precipitating a yellow solid. Filtration, followed by recrystallization from water, yielded a bright yellow solid (7.27 g, 93%): mp 208−209 °C (lit.[22] 208−209 °C).

2,3-Diaminobenzoic Acid (11). 2-Amino-3-nitrobenzoic acid (**10**) (2.44 g, 13 mmol) was hydrogenated (see introduction to the Experimental Section). The crude product was purified by column chromatography (DCM−15% MeOH) to give a deep red solid that was used directly (1.34 g, 66%): mp 204−206 °C (lit.[24] 201 °C).

Methyl 2-Amino-3-nitrobenzoate (14). A solution of 2-amino-3-nitrobenzoic acid (**10**) (5.0 g, 0.027 mol) in methanol (80 mL) was cooled to 0 °C. Dry hydrogen chloride gas was bubbled through the solution for 20 min, after which it was heated at reflux for 6 h. After cooling and standing the reaction mixture overnight, the precipitate was collected and recrystallized (petroleum ether−EtOAc) to yield bright yellow crystals (3.6 g, 60%): mp 95−96 °C; IR 3452, 3317, 1702, 1254 cm^{-1}; ^{1}H NMR δ 3.96 (3 H, s), 6.80−6.88 (1 H, t), 8.29−8.34 (1 H, d), 8.41−8.46 (1 H, d), 8.46 (2 H, br s); ^{13}C NMR δ 52.7, 114.1, 114.4, 132.3, 132.9, 139.6, 146.6, 167.1; HRMS (EI) *m*/*z* 196.0479 [M$^+$ calcd 196.0484 for $C_8H_8N_2O_4$].

Methyl 2,3-Diaminobenzoate (12). Methyl 2-amino-3-nitrobenzoate (**14**) (1.12 g, 5.73 mmol) was hydrogenated (see introduction to the Experimental Section) to give a brown solid that was used directly (0.9 g, 94%): mp 63−64 °C; IR 3428, 3368, 3347, 1693 cm^{-1}; ^{1}H NMR δ 3.86 (3 H, s), 4.8−5.0 (2 H, br s), 6.2−6.4 (2 H, br s), 6.45−6.53 (1 H, t), 6.78−6.84 (1 H, d), 7.17−7.23 (1 H, d); ^{13}C NMR δ 51.6, 109.3, 115.7, 117.7, 119.1, 136.2, 134.0, 169.0; HRMS (EI) *m*/*z* 166.0744 [M$^+$ calcd 166.0742 for $C_8H_{10}N_2O_2$].

Method A. Benzimidazole Synthesis (Phillips Method).[21,22] A solution of 2,3-diaminobenzoic acid (**11**) (0.66−3.29 mmol) in 4 M HCl or polyphosphoric acid (PPA) (approximately 3 mL/mmol) containing an appropriate carboxylic acid (3 equiv) was heated at reflux for 1 h. After cooling the reaction mixture to room temperature, the precipitate was collected and redissolved in hot methanol to which activated charcoal was added. The suspension was filtered and the solvents removed to yield the product.

1*H*-Benzimidazole-4-carboxylic Acid (18). Method A. 2,3-Diaminobenzoic acid **11** and formic acid in 4 M HCl yielded the product, 77%: mp >300 °C; IR 3125, 1715 cm^{-1}; ^{1}H NMR δ 7.7−7.8 (1H, t), 8.2−8.3 (2H, dd), 9.65 (1H, s); HRMS (EI) *m*/*z* 162.0445 [M$^+$ calcd 162.0429 for $C_8H_6N_2O_2$]. Anal. ($C_8H_6N_2O_2$) C, H, N.

Method B. Amide Formation Using Thionyl Chloride. A solution of a benzimidazole-4-carboxylic acid (400−500 mg) in thionyl chloride (10 mL) was boiled under reflux for 2−3 h. Removal of the excess of thionyl chloride yielded a solid that was suspended in dry THF (10 mL). The suspension was added to stirred concentrated ammonia solution (50 mL). The ammonia solution was evaporated and the residue was taken up in water (20 mL). The resulting solution was extracted with EtOAc and the combined extracts were dried ($MgSO_4$). After removal of the organic solvent the residual solid was purified as appropriate.

1*H*-Benzimidazole-4-carboxamide (19). Method B. The residual solid was redissolved in 0.1 M HCl (20 mL). After filtration and adjusting the pH of the solution to pH 7, it was progressively basified to pH 12 (1 M NaOH). Extractions (EtOAc) were taken at each pH unit change. The extracts were combined and dried ($MgSO_4$) and the solvent was removed. Recrystallization (EtOAc) yielded off-white crystals (50 mg, 13%): mp 214−218 °C; IR 3322, 3150, 1680 cm^{-1}; ^{1}H NMR δ 7.34−7.42 (1 H, t), 7.75 (1 H, s), 7.81−7.85 (1 H, d), 7.89−7.93 (1 H, d), 8.46 (1 H, br s), 9.4 (1 H, br s), 13.1 (1 H, br s); HRMS (EI) *m*/*z* 161.0590 [M$^+$ calcd 161.0589 for $C_8H_7N_3O$]. Anal. ($C_8H_7N_3O$) C, H; N: found 24.59, expected 26.09.

2-Methyl-1*H*-benzimidazole-4-carboxylic acid (20). Method A. 2,3-Diaminobenzoic acid (**11**) and acetic acid in 4 M HCl yielded the product (72%): mp 188−191 °C; IR 2853, 1720 cm^{-1}; ^{1}H NMR δ 2.95 (3 H, s), 7.66−7.74 (1 H, t), 8.10−8.14 (2 H, dd); HRMS (EI) *m*/*z* 176.0582 [M$^+$ calcd 176.0586 for $C_9H_8N_2O_2$].

2-Methyl-1*H*-benzimidazole-4-carboxamide (21). Method B. Recrystallization (EtOAc) yielded a white solid (70 mg, 14%): mp 233−238 °C; IR 3297, 3071, 1684 cm^{-1}; ^{1}H NMR δ 2.68 (3 H, s), 7.30−7.38 (1 H, t), 7.72−7.76 (1 H, d), 7.86,7.90 (1 H, d), 7.72−7.90 (1 H, br s), 9.4 (1 H, br s), 12.8 (1 H, br s); HRMS (EI) *m*/*z* 175.0749 [M$^+$ calcd 175.0746 for $C_9H_9N_3O$]. Anal. ($C_9H_9N_3O$) C, H; N: found 23.39, expected 24.00.

2-Phenyl-1*H*-benzimidazole-4-carboxylic Acid (22). Method A. 2,3-Diaminobenzoic acid (**11**) and benzoic acid were heated to 150−160 °C in PPA. After cooling to room temperature, ice was added and the resulting solution was neutralized with sodium hydroxide (10 M) and extracted (EtOAc). The crude product was purified by column chromatography (DCM−15% MeOH), 20%: mp 287−288 °C; IR 3433, 3336, 1680 cm^{-1}; ^{1}H NMR δ 7.35−7.43 (1 H, t), 7.62−7.68 (3 H, m), 7.87−7.91

(1 H, d), 7.95−7.99 (1 H, d), 8.38−8.44 (2 H, d), 12.5 (1 H, br s); HRMS (EI) *m/z* 238.0750 [M^+ calcd 238.0742 for $C_{14}H_{10}N_2O_2$].

Method C. Amide Formation Using Thionyl Chloride/DMF. A benzimidazole-4-carboxylic acid (0.2−1.8 mmol) was suspended in THF. Thionyl chloride (1.5 equiv) and DMF (2 drops) were added. The resulting mixture was stirred at room temperature until the carboxylic acid had been consumed (monitoring by TLC). The mixture was added dropwise to concentrated ammonia solution. Removal of the ammonia allowed recovery of the amide by filtration or extraction (EtOAc). Further purification was performed as appropriate.

2-Phenyl-1*H*-benzimidazole-4-carboxamide (23). Method C. A white precipitate was obtained (31 mg, 62%): mp 239−241 °C; IR 3184, 1669 cm^{-1}; ^1H NMR δ 7.43−7.51 (1 H, t), 7.69−7.72 (3 H, m), 7.86−7.90 (1 H, d), 7.97 (1 H, br s), 7.98−8.01 (1 H, d), 8.37−8.41 (2 H, d), 9.5 (1 H, br s); ^{13}C NMR δ 115.4, 122.7, 123.3, 127.2, 129.4, 130.9, 135.7, 141.8, 152.3, 166.6; HRMS (EI) *m/z* 237.0907 [M^+ calcd 237.0902 for $C_{14}H_{11}N_3O$]. Anal. ($C_{14}H_{11}N_3O$·0.1EtOH) C, H, N.

Methyl 2-*N*-(Trifluoroacetyl)amino-3-nitrobenzoate (15). To methyl 2-amino-3-nitrobenzoate (**14**) (100 mg, 0.51 mmol) and triethylamine (234 μL, 1.68 mmol) in THF (10 mL) was added a solution of trifluoroacetic anhydride (238 μL, 1.68 mmol) in THF (5 mL) dropwise, with stirring at room temperature (12 h). Water (0.5 mL) was added and the solvents were removed to yield a yellow oil. Ice water (0.5 mL) was added to the oil to give a white crystalline precipitate, which was collected, washed with ice water and dried (95 mg, 64%): mp 126−129 °C; IR 3295, 1726 (br) cm^{-1}; ^1H NMR δ 3.94 (3H, s), 7.82−7.90 (1 H, t), 8.28−8.33 (1 H, d), 8.36−8.42 (1 H, d), 11.8 (1 H, br s); HRMS (EI) *m/z* 292.0316 [M^+ calcd 292.0307 for $C_{10}H_7F_3N_2O_5$].

Methyl 2-Trifluoromethyl-1*H*-benzimidazole-4-carboxylate (16). Hydrogenation (see introduction to the Experimental Section) of **15** (51 mg, 0.21 mmol) gave a clear oil, which crystallized under petroleum ether (38 mg, 75%): mp 86−88 °C; IR 3586, 3337, 3247, 3015, 1712, 1200, 1148 cm^{-1}; ^1H NMR δ 4.07 (3 H, s), 7.56−7.63 (1 H, t), 8.11−8.15 (1 H, d), 8.19−8.23 (1 H, d), 13.8 (1 H, br s); HRMS (EI) *m/z* 244.0462 [M^+ calcd 244.0460 for $C_{10}H_7F_3N_3O_2$].

2-Trifluoromethyl-1*H*-benzimidazole-4-carboxamide (17). A solution of methyl 2-trifluoromethyl-1*H*-benzimidazole-4-carboxylate (**16**) (26 mg, 0.1 mmol) in aqueous ammonia solution (5 mL) was stirred at room temperature for 12 h. Ammonia was removed under reduced pressure and the aqueous residue was washed with EtOAc (3 × 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed, to give the product (18 mg, 75%): mp 228−230 °C; IR 3351, 3187, 1669, 1157, 1144 cm^{-1}; ^1H NMR δ 7.57−7.65 (1 H, t), 7.97−8.01 (2 H, d), 8.06−8.10 (1 H, d), 8.70 (1 H, br s), 14.4 (1 H, br s); HRMS (EI) *m/z* 229.0460 [M^+ calcd 229.0463 for $C_9H_6F_3N_3O$]. Anal. ($C_9H_6F_3N_3O$) C, H, N.

Method D. Acylation of Methyl 2,3-Diaminobenzoate. A solution of methyl 2,3-diaminobenzoate (**12**) (0.78−4.04 mmol, 1 equiv), triethylamine (1.5 equiv) and 4-(dimethylamino)pyridine (DMAP, 5 mol %) in half the required volume of THF (approximately 10 mL/100 mg **12**) was cooled to 0 °C. The appropriate acid chloride (1 equiv) was dissolved in the remaining THF and added to the cooled solution over 30 min. The reaction mixture was allowed to warm to room temperature. The precipitate was collected, redissolved in EtOAc and the resulting solution was washed twice with water followed by saturated brine. The organic layer was dried ($MgSO_4$), and the solvents removed to leave a solid residue that was purified as appropriate.

Methyl 2-Amino-3-*N*-(4-methoxybenzoyl)aminobenzoate (24). Method D. Recrystallization from MeOH−water yielded the product, 62%: mp 179−180 °C; IR 3426, 3278, 1699, 1632 cm^{-1}; ^1H NMR δ 3.92 (3 H, s), 3.94 (3 H, s), 6.59 (2 H, br s), 6.68−6.75 (1 H, t), 7.13−7.17 (2 H, d), 7.43−7.46 (1 H, d), 7.79−7.83 (1 H, d), 8.07−8.12 (2 H, d), 9.7 (1 H, br s); ^{13}C NMR δ 52.0, 55.8, 110.6, 113.8, 114.7, 125.0, 126.8, 129.1, 130.1, 133.2, 147.4, 162.2, 165.7, 168.3; HRMS (EI) *m/z* 300.1118 [M^+ calcd 300.1110 for $C_{16}H_{16}N_2O_4$]. Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

Methyl 2-Amino-3-*N*-(4-trifluoromethylbenzoyl)aminobenzoate (25). Method D. Recrystallization from aqueous MeOH yielded a white solid, 14%: mp 180−181 °C; IR 3389, 3292, 1701, 1653, 1330 cm^{-1}; ^1H NMR δ 3.93 (3 H, s), 6.70−6.76 (3 H, m), 7.46−7.49 (1 H, d), 7.81−7.85 (1 H, d), 7.99−8.03 (2 H, d), 8.29−8.33 (2 H, d), 10.05 (1 H, br s); HRMS (EI) *m/z* 338.0886 [M^+ calcd 338.0878 for $C_{16}H_{13}F_3N_2O_3$]. Anal. ($C_{16}H_{13}F_3N_2O_3$) C, H, N.

Methyl 2-Amino-3-*N*-(4-nitrobenzoyl)aminobenzoate (26). Method D. Column chromatography (DCM−1% MeOH) and recrystallization (MeOH) yielded the product, 45%: mp 196−197 °C; IR 3382, 3293, 3257, 1702, 1658, 1525 cm^{-1}; ^1H NMR δ 3.94 (3 H, s), 6.70−6.78 (1 H, t), 6.66 (2H, br s), 7.48−7.51 (1 H, d), 7.83−7.87 (1 H, d), 8.33−8.38 (2 H, d, J = 8.8), 8.46−8.51 (2 H, d, J = 8.8), 10.15 (1 H, br s); (EI) *m/z* 315.0844 [M^+ calcd 315.0855 for $C_{15}H_{13}N_3O_5$]. Anal. ($C_{15}H_{13}N_3O_5$) C, H, N.

Methyl 2-Amino-3-*N*-(4-cyanobenzoyl)aminobenzoate (27). Method D. Column chromatography (DCM−1% MeOH) and recrystallization (MeOH) yielded the product, 37%: mp 198−202 °C; IR 3486, 3374, 3246, 2231, 1688, 1647 cm^{-1}; ^1H NMR δ 3.93 (3 H, s), 6.68−6.76 (1 H, t), 6.72 (2 H, br s), 7.45−7.49 (1 H, d), 7.81−7.86 (1 H, d), 8.11−8.15 (2 H, d, J = 8.4), 8.25−8.29 (2 H, d, J = 8.4), 10.1 (1 H, br s); HRMS (EI) *m/z* 295.0963 [M^+ calcd 295.0957 for $C_{16}H_{13}N_3O_3$]. Anal. ($C_{16}H_{13}N_3O_3$) C, H, N.

Methyl 2-Amino-3-*N*-(4-aminobenzoyl)aminobenzoate (28). Methyl 2-amino-3-*N*-(4-nitrobenzoyl)aminobenzoate (**26**) (246 mg, 0.78 mmol) was hydrogenated (see introduction to the Experimental Section) to give a white solid (204 mg, 92%): mp 197−200 °C; IR 3473, 3375, 3349, 3283, 1695, 1635 cm^{-1}; ^1H NMR δ 3.94 (3 H, s), 5.87 (2 H, s), 6.54 (2 H, s), 6.68−6.73 (2 H, d), 6.73−6.76 (1 H, t), 7.42−7.47 (1 H, d), 7.78−7.82 (1 H, d), 7.83−7.87 (2 H, d), 9.4 (1 H, br s); HRMS (EI) *m/z* 285.1104 [M^+ calcd 285.1113 for $C_{15}H_{15}N_3O_3$]. Anal. ($C_{15}H_{15}N_3O_3$) C, H, N.

Methyl 2-Amino-3-*N*-(3-trifluoromethylbenzoyl)aminobenzoate (29). Method D. Column chromatography (DCM−10% MeOH) and recrystallization (MeOH) yielded a white solid, 26%: mp 157−159 °C; IR 3368, 3284, 1706, 1651, 1250 cm^{-1}; ^1H NMR δ 3.93 (3 H, s), 6.69−6.77 (1 H, t), 6.73 (2 H, s), 7.45−7.49 (1 H, d), 7.82−7.92 (2 H, m), 8.06−8.10 (1 H, d), 8.40−8.44 (1 H, d), 8.48 (1 H, s), 10.1 (1 H, br s); HRMS (EI) *m/z* 338.0877 [M^+ calcd 338.0878 for $C_{16}H_{13}F_3N_2O_3$]. Anal. ($C_{16}H_{13}F_3N_2O_3$) C, H, N.

Methyl 2-Amino-3-*N*-(3-methoxybenzoyl)aminobenzoate (30). Method D. Column chromatography (DCM−10% MeOH) and recrystallization (petroleum ether−EtOAc) yielded the product, 23%: mp 124−125 °C; IR 3386, 3292, 1698, 1646 cm^{-1}; ^1H NMR δ 3.92 (3 H, s), 3.93 (3 H, s), 6.61 (2 H, s), 6.68−6.76 (1 H, t), 7.22−7.27 (1 H, d), 7.44−7.47 (1 H, d), 7.49−7.57 (1 H, t), 7.66 (1 H, s), 7.67−7.71 (1 H, d), 7.79−7.84 (1 H, d), 9.8 (1 H, br s); HRMS (EI) *m/z* 300.1117 [M^+ calcd 300.1110 for $C_{16}H_{16}N_2O_4$]. Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

Method E. Benzimidazole Preparation Catalyzed by Glacial Acetic Acid. The *N*-acyl starting material (**24**−**30**, 0.2−1.6 mmol) was dissolved in glacial acetic acid (10 mL/mmol), and heated at 120 °C until the reaction was complete (monitoring by TLC). The solvent was removed and the solid residue purified as appropriate.

Methyl 2-(4-Methoxyphenyl)-1*H*-benzimidazole-4-carboxylate Acetate Salt (31). Method E. Recrystallization (petroleum ether−EtOAc) yielded a white crystalline solid, 75%: mp 141−142 °C; IR 3375, 1718, 1697 cm^{-1}; ^1H NMR δ 2.02 (3 H, s), 3.97 (3H, s), 4.09 (3 H, s), 7.21−7.25 (2 H, d, J = 8.6), 7.39−7.46 (1 H, t), 7.90−7.93 (1 H, d), 8.00−8.04 (1 H, d), 8.36−8.40 (2 H, d, J = 8.6), 12.1 (1 H, br s), 12.3−12.4 (1 H, br s); ^{13}C NMR δ 21.4, 52.4, 55.6, 114.4, 121.7, 122.4, 124.3, 129.6, 153.6, 161.3, 166.1, 172.4; HRMS (EI) *m/z* 282.0991 [M^+ − AcOH, calcd 282.1004 for $C_{16}H_{14}N_2O_3$]. Anal. ($C_{18}H_{18}N_2O_5$) C, H, N.

Methyl 2-(4-Trifluoromethylphenyl)-1*H*-benzimidazole-4-carboxylate Acetate Salt (32). Method E. Recrystallization from petroleum ether−EtOAc yielded the product, 70%: mp 138−140 °C; IR 3329, 1709, 1694 cm^{-1}; ^1H NMR δ 2.01 (3 H, s), 7.44−7.52 (1H, t), 7.97−8.14 (4H, m), 8.62−8.66 (2H, d), 12.1 (br s), 12.7−12.8 (1 H, br s); HRMS (EI) *m/z* 320.0402 [M^+ − AcOH, calcd 320.0773 for $C_{16}H_{11}F_3N_2O_2$]. Anal. ($C_{18}H_{15}F_3N_2O_4$) C, H, N.

Methyl 2-(4-Nitrophenyl)-1*H*-benzimidazole-4-carboxylate (33). Method E. Recrystallization from MeOH yielded the product, 65%: mp 208−210 °C; IR 1720, 1513 cm^{-1}; ^1H NMR δ 4.21 (3 H, s), 7.57−7.65 (1 H, t), 8.10−8.12 (1 H, d), 8.23−8.27 (1 H, d), 8.60−8.64 (2 H, d, J = 8.8), 8.78−8.82 (2 H, d, J = 8.8), 13.04 (1 H, br s); HRMS (EI) *m/z* 297.0743 [M^+ calcd 297.0750 for $C_{15}H_{11}N_3O_4$]. Anal. ($C_{15}H_{11}N_3O_4$) C, H, N.

Methyl 2-(4-Cyanophenyl)-1*H*-benzimidazole-4-carboxylate (34). Method E. Recrystallization from petroleum ether−EtOAc yielded the product, 72%: mp 195−198 °C; IR 3448, 2229, 1692 cm^{-1}; ^1H NMR δ 4.09 (3 H, s), 7.44−7.53 (1 H, t), 7.97−8.01 (1 H, d), 8.10−8.13 (1 H, d), 8.13−8.17 (2 H, d, J = 8.4), 8.58−8.62 (2 H, d, J = 8.4), 12.8 (1 H, br s); HRMS (EI) *m/z* 277.0851 [M^+ calcd 277.0851 for $C_{16}H_{11}N_2O_5$].

Methyl 2-(4-Aminophenyl)-1*H*-benzimidazole-4-carboxylate Acetate Salt (35). Method E. Recrystallization from petroleum ether−EtOAc yielded the product, 91%: mp 162−164 °C; IR 3451, 3369, 1692, 1648 cm^{-1}; ^1H NMR δ 2.02 (3 H, s), 4.08 (3 H, s), 5.81 (2 H, s), 6.75−6.80 (2 H, d, J = 8.6), 7.32−7.40 (1 H, t), 7.83−7.86 (1 H, d), 7.93−7.97 (1 H, d), 8.08−8.13 (2 H, d, J = 8.6), 11.9 (1 H, br s), 12.1 (1 H, br s); HRMS (EI) *m/z* 267.1016 [M^+ − AcOH, calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

Methyl 2-(3-Trifluoromethylphenyl)-1*H*-benzimidazole-4-carboxylate Acetate Salt (36). Method E. The product was obtained as a white solid, 96%: mp 105−107 °C; IR 3339, 1708, 1328 cm^{-1}; ^1H NMR δ 2.01 (3 H, s), 4.09 (3 H, s), 7.44−7.51 (1 H, t), 7.79−8.13 (4 H, m), 8.71−8.75 (1 H, d), 8.82 (1 H, s), 11.8−12.2 (1 H, br s), 12.8−13.0 (1 H, br s); HRMS (EI) *m/z* 320 [M^+ − AcOH], 288.0529 [−CH_3OH, calcd 288.0510 for $C_{15}H_7F_3N_2O$]. Anal. ($C_{18}H_{15}F_3N_2O_4$) C, H, N.

Methyl 2-(3-Methoxyphenyl)-1*H*-benzimidazole-4-carboxylate Acetate Salt (37). Method E. Recrystallization from petroleum ether−EtOAc yielded the product, 58%: mp 93−94 °C; IR 3453, 3375, 1707 cm^{-1}; ^1H NMR δ 1.99 (3 H, s), 3.96 (3 H, s), 4.06 (3 H, s), 7.15−7.21 (1 H, d), 7.38−7.46 (1 H, t), 7.51−7.59 (1 H, t), 7.91−8.00 (3 H, m), 8.04−8.08 (1 H, d), 12.0 (1 H, br s), 12.5 (1 H, br s); HRMS (EI) *m/z* 282.1003 [M^+ − AcOH, calcd 282.1004 for $C_{16}H_{14}N_2O_3$]. Anal. ($C_{18}H_{18}N_2O_5$) C, H, N.

Method F. Amide Preparation Under High Pressure. A benzimidazole methyl ester (**38**−**44**, 0.1−1.06 mmol) was dissolved in an excess of liquid ammonia (20−40 mL), placed in a sealed reaction vessel (a glass-lined stainless steel bomb), and heated to 80 °C for 24 h generating an internal pressure of ca. 40 atm. The ammonia was removed and the solid residue washed with ice-cold water and purified appropriately.

2-(4-Methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (38). Method F. Recrystallization from MeOH−water yielded the product, 80%: mp 261−263 °C; IR 3321, 3141, 1656 cm^{-1}; ^1H NMR δ 3.96 (3 H, s), 7.23−7.27 (2 H, d, J = 8.6), 7.37−7.45 (1 H, t), 7.78−7.82 (1 H, d), 7.87 (1 H, br s), 7.93−7.96 (1 H, d), 8.27−8.31 (2 H, d, J = 8.6), 9.4−9.5 (1 H, br s), 13.3−13.4 (1 H, br s); ^{13}C NMR δ 60.6, 119.8, 119.9, 126.8, 127.2, 127.4, 128.0, 133.8, 140.6, 146.9, 157.3, 166.4, 171.6; HRMS (EI) *m/z* 267.1016 [M^+ calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

2-(4-Trifluoromethyl)-1*H*-benzimidazole-4-carboxamide (39). Method F. Recrystallization from MeOH−water yielded the product, 48%: mp 301−305 °C; IR 3155, 1668, 1318 cm^{-1}; ^1H NMR δ 7.45 (1 H, t), 7.88−7,92 (1 H, d), 7.99 (1 H, br s), 8.03 (1 H, d), 8.06−8.10 (2 H, d, J = 8.1), 8.55−8.59 (2 H, d, J = 8.1), 9.3−9.4 (1 H, br s), 13.7−13.8 (1 H, br s); HRMS (EI) *m/z* 288.0535 [M^+ − NH_3, calcd 288.0510 for $C_{15}H_7F_3N_2O$]. Anal. ($C_{15}H_{10}F_3N_3O\cdot 0.1H_2O$) C, H, N.

2-(4-Nitrophenyl)-1*H*-benzimidazole-4-carboxamide (40). Method F. Column chromatography (DCM−1% MeOH) and recrystallization from MeOH yielded the product, 74%: mp >310 °C; IR 3436, 3073, 1661 cm^{-1}; ^1H NMR δ 7.48−7.56 (1 H, t), 7.90−7.94 (1 H, d), 8.00 (1 H, s), 8.00−8.04 (1 H, d), 8.52−8.56 (2 H, d, J = 8.8), 8.60−8.64 (2 H, d, J = 8.8), 9.3−9.4 (1 H, br s), 13.8−14.0 (1 H, br s); HRMS (EI) *m/z* 282.0750 [M^+ calcd 282.0753 for $C_{14}H_{10}N_4O_3$]. Anal. ($C_{14}H_{10}N_4O_3$) C, H, N.

2-(4-Cyanophenyl)-1*H*-benzimidazole-4-carboxamide (41). Method F. Recrystallization from MeOH yielded the product, 73%: mp >310 °C; IR 3275, 3178, 2231, 1659 cm^{-1}; ^1H NMR δ 7.45−7.49 (1 H, t); 7.87−7.91 (1 H, d), 7.91 (1 H, br s), 7.98−8.02 (1 H, d); 8.13−8.17 (2 H, d, J = 8.3), 8.50−8.54 (2 H, d, J = 8.3), 9.2−9.4 (1 H, br s), 13.6−13.8 (1 H, br s); ^{13}C NMR δ 112.8, 115.8, 118.8, 123.4, 123.8, 127.8, 133.3, 133.6, 141.6, 141.8, 150.3, 166.4; HRMS (EI) *m/z* 262.0849 [M^+ calcd 262.0855 for $C_{15}H_{10}N_4O$]. Anal. ($C_{15}H_{10}N_4O$) C, H, N.

2-(4-Aminophenyl)-1*H*-benzimidazole-4-carboxamide (42). Method F. Column chromatography (DCM−10% MeOH) yielded the product, 25%: mp 237−240 °C; IR 3338, 3214, 1651 cm^{-1}; ^1H NMR δ 5.90 (2 H, s), 6.79−6.83 (2 H, d, J = 8.3), 7.31−7.39 (1 H, t), 7.71−7.75 (1 H, d), 7.84 (1 H, s), 7.88−7.92 (1 H, d), 8.00−8.04 (2 H, d, J = 8.3), 9.55 (1 H, br s), 13.0 (1 H, br s); HRMS (EI) *m/z* 252.1002 [M^+ calcd 252.1011 for $C_{14}H_{12}N_4O$]. Anal. ($C_{14}H_{12}N_4O\cdot 0.2MeOH$) C, H, N.

2-(3-Trifluoromethylphenyl)-1*H*-benzimidazole-4-carboxamide (43). Method F. Recrystallization from MeOH yielded the product, 72%: mp 268−270 °C; IR 3349, 3176, 1668, 1330 cm^{-1}; ^1H NMR δ 7.44−7.52 (1 H, t), 7.88−8.04 (5 H, m), 8.66−8.70 (1 H, d), 8.70 (1 H, s), 9.3 (1 H, br s), 13.6 (1 H, br s); ^{13}C NMR δ 48.9, 115.6, 121.9, 123.1, 123.6, 127.2, 129.9, 130.6, 130.7, 131.2, 135.7, 136.0, 150.7, 166.5; HRMS (EI) *m/z* 305 [M^+], 288 [−CH_3OH for $C_{15}H_7F_3N_2O$]. Anal. ($C_{15}H_{10}F_3N_3O$) C, H, N.

2-(3-Methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (44). Method F. Recrystallization from MeOH yielded the product, 46%: mp 223−225 °C; IR 3409, 3169, 1662 cm^{-1}; ^1H NMR δ 3.99 (3 H, s), 7.22−7.27 (1 H, d), 7.43−7.51 (1 H, t), 7.58−7.66 (1 H, t). 7.85−8.01 (5 H, m), 9.4−9.5 (1 H, br s), 13.5 (1 H, br s); HRMS (EI) *m/z* 267.1005 [M^+ calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

2-(4-Hydroxyphenyl)-1*H*-benzimidazole-4-carboxamide (45). Boron tribromide (1 M/DCM) (3.8 mL, 3.8 mmol) was transferred to a flask containing 2-(4-methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (**38**) (202.4 mg, 0.76 mmol). The mixture was refluxed for 24 h and distilled to dryness. The solid residue was treated with 10% NaOH (10 mL), followed by the dropwise addition of concentrated hydrochloric acid to neutralization. The white precipitate was collected and dissolved in EtOAc (10 mL). The resulting solution was washed with water (2 × 3 mL) and dried ($MgSO_4$). Removal of the solvent gave the product, 110 mg, 57%: mp 266−267 °C; IR 3424, 3309, 3156, 1642 cm^{-1}; ^1H NMR δ 7.03−7.07 (2 H, d, J = 8.5), 7.34−7.42 (1 H, t), 7.75−7.79 (1 H, d), 7.85 (1 H, br s), 7.90−7.94 (1 H, d), 8.15−8.19 (2 H, d, J = 8.5), 9.4−9.6 (1 H, br s), 10.0−10.4 (1 H, br s), 13.0−13.4 (1 H, br s); HRMS (EI) *m/z* 253.0859 [M^+ calcd 253.0851 for $C_{14}H_{11}N_3O_2$]. Anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2-(4-Carboxyphenyl)-1*H*-benzimidazole-4-carboxamide (46). A solution of **41** (150 mg, 0.57 mmol) in aqueous sodium hydroxide (20%, 2 mL) and ethanol (5 mL) was boiled under reflux overnight. The solvent was removed, to yield a brown oil to which water (2 mL) was added followed by concentrated HCl to pH 7. The precipitated white solid was collected, washed with water and recrystallized from MeOH to yield the product (75 mg, 47%): mp > 300 °C; IR 3342, 3152, 1711, 1649 cm^{-1}; ^1H NMR δ 7.45−7.52 (1 H, t), 7.87−7.91 (1 H, d), 7.95 (1 H, s), 7.98−8.02 (1 H, d), 8.22−8.26 (2 H, d, J = 8.2), 8.46−8.50 (2 H, d, J = 8.2), 9.35 (1 H, br s), 13.39 (1 H, br s); HRMS (EI) *m/z* 281.0793 [M^+ calcd 281.0800 for $C_{15}H_{11}N_3O_3$].

Method G. Benzimidazole 1-*N*-Alkylation/Acylation.[26] The benzimidazole amide was suspended in acetone (100 mg/4

mL), powdered potasssium hydroxide (1 equiv) was added and the reaction stirred for 1 h when all solids had dissolved. The alkylating or acylating agent (1 equiv) was added and the reaction mixture stirred for a further 4 h. The solvent was removed to give a solid residue that was washed with water and purified appropriately.

2-(4-Methoxyphenyl)-1-*N*-methylbenzimidazole-4-carboxamide (47). Method G. Benzimidazole **38** (105 mg, 0.4 mmol) and KOH (22 mg, 0.4 mmol) were treated with methyl iodide (25 μL, 0.40 mmol). Column chromatography (DCM−5% MeOH) gave fine white crystals (33 mg, 30%): mp 289−292 °C; IR 3309, 3141, 1671 cm^{-1}; ^{1}H NMR δ 3.95 (3 H, s), 4.02 (3 H, s), 7.22−7.27 (2 H, d), 7.44−7.52 (1 H, t), 7.86−8.00 (5 H, m), 9.4 (1 H, br s); ^{13}C NMR δ 37.3, 60.7, 119.5, 119.5, 126.6, 127.1, 127.5, 128.3, 136.3, 142.2, 145.4, 158.9, 166.0, 171.3; HRMS (EI) *m/z* 281.1158 [M$^+$ calcd 281.1164 for $C_{16}H_{15}N_3O_2$]. Anal. ($C_{16}H_{15}N_3O_2$) C, H, N.

2-(4-Methoxyphenyl)-1-*N*-benzoylbenzimidazole-4-carboxamide (48). Method G. Benzimidazole **38** (366 mg, 1.37 mmol) and KOH (77 mg, 1.37 mmol) were treated with benzoyl chloride (175 μL, 1.51 mmol). Column chromatography (DCM−2% MeOH) and recrystallization (acetone−petroleum ether) yielded white prisms (163 mg, 32%): mp 207−210 °C; IR 3446, 1690, 1666 cm^{-1}; ^{1}H NMR δ 3.86 (3 H, s), 7.02−7.06 (2 H, d), 7.50−7.65 (4 H, m), 7.72−7.82 (3 H, m), 7.88−7.92 (2 H, d), 8.08 (1 H, s), 8.10−8.14 (1 H, d), 9.1−9.2 (1 H, br s); HRMS (EI) *m/z* 371.1279 [M$^+$ calcd 371.1270 for $C_{22}H_{17}N_3O_3$]. Anal. ($C_{22}H_{17}N_3O_3\cdot 0.1H_2O$) C, H, N.

2-(4-Methoxyphenyl)-1-*N*-benzyloxycarbonylbenzimidazole-4-carboxamide (49). Method G. Benzimidazole **38** (437 mg, 1.64 mmol) and KOH (92 mg, 1.64 mmol) were treated with benzyl chloroformate (234 μL, 1.64 mmol). Column chromatography (DCM−4% MeOH) and recrystallization (MeOH) afforded a white solid (398 mg, 61%): mp 202−204 °C; IR 3379, 3164, 1746, 1687 cm^{-1}; ^{1}H NMR δ 3.92 (3 H, s), 5.51 (2 H, s), 7.05−7.10 (2 H, d, J = 8.8), 7.37−7.48 (5 H, m), 7.57−7.65 (1 H, t), 7.68−7.86 (2 H, d, J = 8.8), 8.01 (1 H, br s), 8.08−8.13 (1 H, d), 8.23−8.27 (1 H, d), 9.02 (1 H, br s); HRMS (EI) *m/z* 401.1394 [M$^+$ calcd 401.1376 for $C_{23}H_{19}N_3O_4$]. Anal. ($C_{23}H_{19}N_3O_4$) C, H, N.

Method H. Benzimidazole Preparation from 2-Amino-3-nitrobenzoic Acid (10).[24] A solution of 2-amino-3-nitrobenzoic acid (**10**; 4.1−5.5 mmol) and sodium hydroxide (1.1 equiv) in water was hydrogenated (see introduction to the Experimental Section). Following acidification to pH 3−4 (glacial acetic acid), a solution of the appropriate aldehyde (1.4 equiv) in methanol and copper(II) acetate (1.4 equiv) in water was added. The mixture was stirred vigorously, heated briefly to boiling and filtered hot. The precipitate was washed with water and dissolved in ethanol containing concentrated HCl (1 mL HCl, 20 mL EtOH). A solution of sodium sulfide (1.4 equiv) in water was added. The resulting solution was warmed and filtered through Celite. After adjusting the pH of the filtrate to 5−6 (concentrated HCl) it was diluted with water. The ethanol was removed and the pH of the remaining aqueous solution adjusted to 4−5 (concentrated HCl). The crude product was collected by filtration, washed with water, and dissolved in 0.5 M sodium hydroxide. The aqueous layer was washed with EtOAc and the pH adjusted to 4−5 (concentrated HCl). The carboxylic acid was recovered by filtration and purified further as necessary.

2-(4-Chlorophenyl)-1*H*-benzimidazole-4-carboxylic Acid (50). Method H. Product obtained in 80% yield: mp 306−308 °C;[24] IR 3395, 3092, 1715, 755 cm^{-1}; ^{1}H NMR δ 7.53−7.60 (1 H, t), 7.77−7.81 (2 H, d), 8.01−8.05 (1 H, d), 8.09−8.13 (1 H, d), 8.44−8.48 (2 H, d); HRMS (EI) *m/z* 272.0364 [M$^+$ calcd 272.0353 for $C_{14}H_9ClN_2O_2$]. Anal. ($C_{14}H_9ClN_2O_2$) C, H, N.

2-(3-Hydroxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (51). Method H. Product obtained in 67% yield: mp 329−330 °C; IR 3369, 1632 1596, 1491 cm^{-1}; ^{1}H NMR δ 7.01−7.04 (1 H, d), 7.42−7.44 (2 H, m), 7.81−8.01 (4 H, m); HRMS (EI) *m/z* 236.0583 [M$^+$ − H_2O, calcd 236.0586 for $C_{14}H_8N_2O_2$].

2-(2-Methoxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (52). Method H. Column chromatography (DCM−10% MeOH) and recrystallization from MeOH yielded the product, 50%: mp 278−279 °C; IR 3419, 2838, 1677 cm^{-1}; ^{1}H NMR δ 4.19 (3 H, s), 7.30−7.33 (1 H, t), 7.42−7.50 (2 H, m), 7.63−7.66 (1 H, m), 7.91−7.95 (1 H, d), 8.05−8.09 (1 H, d), 8.48−8.52 (1 H, d), 11.9 (1 H, br s), 13.6 (1 H, br s); HRMS (EI) *m/z* 268.0847 [M$^+$ calcd 268.0848 for $C_{15}H_{12}N_2O_3$]. Anal. ($C_{15}H_{12}N_2O_3$) C, H, N.

2-(3-Methoxy-4′-hydroxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (53). Method H. Product obtained in 44% yield: mp 299−302 °C; IR 3519, 3075, 2847, 1600 cm^{-1}; ^{1}H NMR δ 4.00 (3 H, s), 6.99−7.03 (1 H, d), 7.34−7.42 (1 H, t), 7.85−7.97 (5 H, m,), 9.8 (1 H, br s); HRMS (EI) *m/z* 284.0800 [M$^+$ calcd 284.0797 for $C_{15}H_{12}N_2O_4$]. Anal. ($C_{15}H_{12}N_2O_4$) C, H, N.

2-(3,4-Dimethoxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (54). Method H. Product obtained in 46% yield: IR 3079, 2839, 1604 cm^{-1}; ^{1}H NMR δ 3.95−4.01 (6H, d), 7.21−7.25 (1 H, d), 7.41 (1 H, t), 7.88−8.02 (4 H, m); HRMS (EI) *m/z* 298.0946 [M$^+$ calcd 298.0954 for $C_{16}H_{14}N_2O_4$]. Anal. ($C_{16}H_{14}N_2O_4\cdot$HCl) C, H, N.

2-(3,4,5-Trimethoxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (55). Method H. Product obtained in 40% yield: mp 293−296 °C; IR 3260, 2937, 2833, 1593 cm^{-1}; ^{1}H NMR δ 3.84 (3 H, s), 4.02 (6 H, s), 7.50 (1 H, t), 7.84 (2 H, s), 7.95−7.99 (1 H, d), 8.04−8.08 (1 H, d); HRMS (EI) *m/z* 328.1064 [M$^+$ calcd 328.1059 for $C_{17}H_{16}N_2O_5$]. Anal. ($C_{17}H_{16}N_2O_5\cdot$ 0.1MeOH) C, H, N.

2-(4-*N*,*N*-Dimethylaminophenyl)-1*H*-benzimidazole-4-carboxylic Acid (56). Method H. Product obtained in 48% yield: mp > 360 °C; IR 3436, 3258, 3179, 2927, 2843, 1646, 1603 cm^{-1}; ^{1}H NMR δ 3.12 (6 H, s), 6.91−6.96 (2 H, d, J = 8.9), 7.37−7.41 (1 H, t), 7.82−7.86 (1 H, d), 7.88−7.92 (1 H, d), 8.21−8.25 (2 H, d, J = 8.8); HRMS (EI) *m/z* 281.1154 [M$^+$ calcd 281.1164 for $C_{16}H_{15}N_3O_2$]. Anal. ($C_{16}H_{15}N_3O_2\cdot$0.65MeOH) C, H, N.

2-(4-Ethoxycarbonylphenyl)-1*H*-benzimidazole-4-carboxylic Acid (57). Method H. Product obtained in 31% yield: mp 278 °C; IR 3297, 1721 cm^{-1}; ^{1}H NMR δ 1.41−1.48 (3 H, t), 4.40−4.50 (2 H, q), 5.49−7.58 (2 H, m), 7.93−7.96 (1 H, d), 8.02−8.06 (1 H, d), 8.17−8.24 (2 H, d, J = 8.2), 8.55−8.59 (2 H, d, J = 8.2); HRMS (EI) *m/z* 310.0955 [M$^+$ calcd 310.0954 for $C_{17}H_{14}N_2O_4$].

2-(3,4-Methylenedioxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (58). Method H. Column chromatography (DCM−10% MeOH) yielded the product, 33%: mp 304−306 °C; IR 3270, 2786, 1503, 1480, 1249 cm^{-1}; ^{1}H NMR δ 6.22 (2 H, s), 7.15−7.19 (1 H, d), 7.34−7.41 (1 H, t), 7.86−7.99 (4 H, m); HRMS (EI) *m/z* 282.0628 [M$^+$ calcd 282.0641 for $C_{15}H_{10}N_2O_4$]. Anal. ($C_{15}H_{10}N_2O_4\cdot 0.5H_2O$) C, H, N.

2-(4-Chlorophenyl)-1*H*-benzimidazole-4-carboxamide (59). Method C. Recrystallization from MeOH yielded the product, 47%: mp 262−264 °C; IR 3389, 3173, 1657, 1596, 758 cm^{-1}; ^{1}H NMR δ 7.43−7.50 (1 H, t), 7.76−7.80 (2 H, d, J = 8.4), 7.84−7.88 (1 H, d), 7.93 (1 H, br s), 7.96−8.00 (1 H, d), 8.35−8.40 (2 H, d, J = 8.5), 9.6 (1 H, br s), 13.6 (1 H, br s); HRMS (EI) *m/z* 271.0648 [M$^+$ calcd 271.0512 for $C_{14}H_{10}ClN_3O$]. Anal. ($C_{14}H_{10}ClN_3O\cdot$0.1MeOH) C, H, N.

2-(3-Hydroxyphenyl)-1*H*-benzimidazole-4-carboxamide (60). Method C. Column chromatography (DCM−10% MeOH) and recrystallization from MeOH yielded the product, 92%: mp 294−296 °C; IR 3411, 3332, 3190, 1661, 1600 cm^{-1}; ^{1}H NMR δ 7.02−7.07 (1 H, dd), 7.40−7.53 (2 H, m), 7.73−7.84 (3 H, m), 7.95−7.99 (2 H, d), 9.50 (1 H, s), 9.95 (1 H, br s), 13.45 (1 H, br s); HRMS (EI) *m/z* 253.0863 [M$^+$ calcd 253.0851 for $C_{14}H_{11}N_3O_2$]. Anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2-(2-Methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (61). Method C. Recrystallization from MeOH−water yielded the product, 75%: mp 224−226 °C; IR 3421, 3321, 3154, 2849, 1673 cm^{-1}; ^{1}H NMR δ 4.15 (3 H, s), 7.23−7.31 (1 H, t), 7.36−7.47 (2 H, m), 7.61−7.69 (1 H, t), 7.89−7.97 (3 H, m), 8.47−8.51 (1 H, d), 9.5 (1 H, br s), 12.6 (1 H, br s); HRMS (EI) *m/z* 267.1003 [M$^+$ calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

2-(3-Methoxy-4′-hydroxyphenyl)-1*H*-benzimidazole-4-carboxamide (62). Method C. Recrystallization from MeOH

yielded the product, 46%: mp 244−245 °C; IR 3379, 3195 3001, 2838, 1659, 1598 cm^{-1}; ^1H NMR δ 4.01 (3 H, s), 7.04−7.08 (1 H, d), 7.35−7.39 (1 H, t), 7.76−7.81 (4 H, m), 7.91−7.95 (1 H, d), 9.5 (1 H, br s), 9.8 (1 H, br s), 13.2 (1 H, br s); HRMS (EI) *m/z* 283.0961 [M^+ calcd 283.0957 for $C_{15}H_{13}N_3O_3$]. Anal. ($C_{15}H_{13}N_3O_3$·0.1MeOH) C, H, N.

2-(3,4-Dimethoxyphenyl)-1*H*-benzimidazole-4-carboxamide (63). Method C. Recrystallization from MeOH yielded the product, 31%: mp 267−269 °C; IR 3422, 2834, 2777, 1648, 1603 cm^{-1}; ^1H NMR δ 3.96−4.03 (6 H, d), 7.26−7.30 (1 H, d), 7.39−7.46 (1 H, t), 7.80−7.98 (5 H, m), 9.5 (1 H, br s), 13.3 (1 H, br s); HRMS (EI) *m/z* 297.1110 [M^+ calcd 297.1113 for $C_{16}H_{15}N_3O_3$]. Anal. ($C_{16}H_{15}N_3O_3$·0.5HCl) C, H, N.

2-(3,4,5-Trimethoxyphenyl)-1*H*-benzimidazole-4-carboxamide (64). Method C. Recrystallization from MeOH−water yielded the product, 30%: mp 275−276 °C; IR 3436, 3258, 3179, 2927, 2843, 1646 cm^{-1}; ^1H NMR δ 3.84 (3 H, s), 4.02 (6 H, s), 7.40−7.86 (1 H, t), 7.82 7.86 (2 H, s), 7.82−7.86 (2H, d), 7.95−7.91 (1 H, d), 9.6 (1 H, br s), 13.4 (1 H, br s); HRMS (EI) *m/z* 327.1212 [M^+ calcd 327.1219 for $C_{17}H_{17}N_3O_4$]. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

2-(4-*N*,*N*-Dimethylaminophenyl)-1*H*-benzimidazole-4-carboxamide (65). Method C. Column chromatography (DCM−10% MeOH) and recrystallization from MeOH yielded the product, 36%: mp 281−283 °C; IR 3302, 3167, 2817, 1660, 1610 cm^{-1}; ^1H NMR δ 3.12 (6 H, s), 6.94−6.98 (2 H, d), 7.31−7.39 (1 H, t), 7.72−7.76 (1 H, d), 7.82−7.83 (1 H, br s), 7.88−7.92 (1 H, d), 8.13−8.17 (2H, d); HRMS (EI) *m/z* 280.1326 [M^+ calcd 280.1324 for $C_{16}H_{16}N_4O$]. Anal. ($C_{16}H_{16}N_4O$) C, H, N.

2-(4-Ethoxycarbonylphenyl)-1*H*-benzimidazole-4-carboxamide (66). Method C. Column chromatography (DCM−10% MeOH) and recrystallization from EtOH yielded the product, 66%: mp 110−112 °C; IR 3380, 1691, 1654 cm^{-1}; ^1H NMR δ 1.42−1.49 (3 H, t), 4.41−4.51 (2 H, q), 7.45−7.53 (1 H, t), 7.86−8.02 (3 H, m), 8.23−8.27 (2 H, d, J = 8.4), 8.47−8.51 (2 H, d, J = 8.3), 9.40 (1 H, br s), 13.74 (1 H br s); HRMS (EI) *m/z* 309.1114 [M^+ calcd 309.1113 for $C_{17}H_{15}N_3O_3$]. Anal. ($C_{17}H_{15}N_3O_3$·0.1EtOH) C, H, N.

2-(3,4-Methylenedioxyphenyl)-1*H*-benzimidazole-4-carboxamide (67). Method C. Column chromatography (DCM−20% MeCN) yielded the product, 46%: mp 286−288 °C; IR 3345, 3144, 2788, 1650, 1602, 1245 cm^{-1}; ^1H NMR δ 6.25 (2 H, s), 7.21−7.26 (1 H, d), 7.37−7.45 (1 H, t), 7.78−7.97 (5 H, m); HRMS (EI) *m/z* 281.0803 [M^+ calcd 281.0800 for $C_{15}H_{11}N_3O_3$]. Anal. ($C_{15}H_{11}N_3O_3$) H, N; C: found 64.58, expected 64.05.

Method I. From 2-Amino-3-nitrobenzamide (68).[23,24] 2-Amino-3-nitrobenzamide (1.35−5.56 mmol) in methanol (20 mL/mmol) was hydrogenated (see introduction to the Experimental Section). Following filtration through Celite, the alcoholic filtrate was acidified with glacial acetic acid (5 equiv). The resulting solution was stirred during the addition of the appropriate aldehyde (1.05 equiv) dissolved in methanol (10−20 mL) followed by copper acetate (1.2 equiv) dissolved in water (10−20 mL). The colored suspension was briefly heated to boiling, allowed to cool to room temperature and stirred vigorously for 1 h. Hydrogen sulfide gas was bubbled through the cooled (0 °C) solution for approximately 10 min, and the resulting brown precipitate removed by filtration through Celite. The filtrate was evaporated and purification was performed as appropriate.

2-Amino-3-nitrobenzamide (68). Method C. 2-Amino-3-nitrobenzoic acid (5 g, 0.027 mol) yielded an orange precipitate that was recovered by filtration. Recrystallization from MeOH (500 mL) yielded the product (3.79 g, 76%): mp 234−235 °C; IR 3430, 3297, 3206, 1695, 1686 cm^{-1}; ^1H NMR δ 6.74−6.82 (1 H, t), 7.74 (1 H, br s), 8.04−8.08 (1 H, d), 8.27 (1 H, br s), 8.27−8.31 (1 H, d), 8.6 (2 H, br s); ^{13}C NMR δ 114.0, 119.2, 129.8, 132.5, 136.8, 146.4, 170.3; HRMS (EI) *m/z* 181.0495 [M^+ calcd 181.0487 for $C_7H_7N_3O_3$].

Method J. Silylation Procedure. The appropriate benzenedimethanol isomer (7.46−45 mmol) and sodium hydride (1 equiv) were suspended in THF (1 g/10 mL) under nitrogen and stirred for 1 h. Triisopropylsilyl (TIPS) chloride (1 equiv) was added dropwise and the reaction mixture was stirred overnight. Water (1 mL/5 mL reaction mixture) was added and the product was extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and the solvents were removed to give the product which was purified as appropriate.

Method K. Manganese Dioxide Oxidation. Monosilyl-protected benzene-dimethanol (5−17 mmol) was dissolved in tetrahydrofuran (1 g/20 mL), activated manganese dioxide (5 equiv) was added, and the reaction mixture stirred overnight. Filtration through Celite and removal of solvents yielded the product that was purified as appropriate.

(4-Triisopropylsilyloxymethylphenyl)methanol (69). Method J. Column chromatography (petroleum ether−20% EtOAc) gave a clear oil, 64%: ^1H NMR ($CDCl_3$) δ 1.06−1.09 (18 H, d), 1.09−1.14 (3 H, m), 1.77−1.83 (1 H, t), 4.63−4.66 (2 H, d), 4.82 (2 H, s), 7.32 (4 H, s).

4-(Triisopropylsilyloxymethyl)benzaldehyde (70). Method K. Alcohol **69** yielded an oil, purified by column chromatography (petroleum ether−5% EtOAc), 78%: IR 2944, 2891, 2866, 2728, 1705, 1609, 1579 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04−1.22 (21 H, m), 4.90 (2 H, s), 7.48−7.52 (2 H, d), 7.82−7.86 (2 H, d), 10.0 (1 H, s); ^{13}C NMR δ 12.0, 18.0, 64.7, 126.0, 129.8, 135.3, 148.9, 192.1; HRMS (EI) *m/z* 292.1873 [M^+ calcd 292.1859 for $C_{17}H_{28}O_2Si$].

(3-Triisopropylsilyloxymethylphenyl)methanol (71). Method J. Column chromatography (petroleum ether−20% EtOAc) yielded a clear oil, 38%: ^1H NMR ($CDCl_3$) δ 1.07−1.15 (21 H, m), 1.75 (1 H, t), 4.66−4.68 (2 H, d), 4.83 (2 H, s), 7.21−7.33 (4 H, m).

3-(Triisopropylsilyloxymethyl)benzaldehyde (72). Method K. Alcohol **71** afforded a clear oil purified by column chromatography (petroleum ether−5% EtOAc), 81%: IR 2944, 2866, 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04−1.22 (21 H, m), 4.89 (2 H, s), 7.45−7.53 (1 H, t), 7.62−7.66 (1 H, d), 7.73−7.77 (1 H, d), 7.85 (1 H, s), 10.0 (1 H, s); ^{13}C NMR δ 12.0, 18.1, 64.4, 127.0, 128.2, 128.9, 131.8, 136.5, 142.9, 192.5.

(2-Triisopropylsilyloxymethylphenyl)methanol (73). Method J. Pure oil obtained directly, 85%: ^1H NMR ($CDCl_3$) δ 1.03−1.22 (21 H, m), 4.67 (2 H, s), 4.86 (2 H, s), 7.24−7.38 (4 H, m).

2-(Triisopropylsilyloxymethyl)benzaldehyde (74). Method K. Alcohol **73** afforded a clear oil, purified by column chromatography (petroleum ether−5% EtOAc), 61%: IR 2943, 2866, 2729, 1696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99−1.15 (21 H, m), 5.17 (2 H, s), 7.34−7.41 (1 H, t), 7.52−7.60 (1 H, t), 7.72−7.77 (1 H, d), 7.78−7.82 (1 H, d), 10.1 (1 H, s); HRMS (EI) *m/z* 249.1321 [M^+ − isopropyl, calcd 249.1312 for $C_{14}H_{21}O_2Si$].

2-(4-Triisopropylsilyloxymethylphenyl)-1*H*-benzimidazole-4-carboxamide (75). Method I. Reaction with aldehyde **70**. Column chromatography (DCM−8% MeOH) yielded a yellow solid, 85%: mp 171−176 °C; ^1H NMR δ 1.15−1.31 (21 H, m), 5.00 (2 H, s), 7.40−7.48 (1 H, t), 7.63−7.67 (2 H, d, J = 8.2), 7.81−7.85 (1 H, d), 7.91 (1 H, br s), 7.96−7.99 (1 H, d), 8.30−8.34 (2 H, d, J = 8.2), 9.5 (1 H, br s), 13.5 (1 H, br s); HRMS (EI) *m/z* 423.2349 [M^+ calcd 423.2342 for $C_{24}H_{33}N_3O_2Si$].

2-(3-Triisopropylsilyloxymethylphenyl)-1*H*-benzimidazole-4-carboxamide (76). Method I. Reaction with aldehyde **72**. Column chromatography (DCM−8% MeOH) yielded an orange solid, 48%: ^1H NMR δ 1.18−1.49 (21 H, m), 5.04 (2 H, s), 7.43−7.47 (1 H, t), 7.60−7.73 (2 H, m), 7.84−7.88 (1 H, d), 7.98−8.02 (2 H, d), 8.18−8.22 (1 H, d), 8.43 (1 H, s), 9.45 (1 H, br s), 13.5 (1 H, br s).

2-(2-Triisopropylsilyloxymethylphenyl)-1*H*-benzimidazole-4-carboxamide (77). Method I. Reaction with aldehyde **74**. Column chromatography (DCM−8% MeOH) yielded an oily solid, 43%: IR 3186, 2943, 2865, 1661, 1598 cm^{-1}; ^1H NMR δ 0.88−1.09 (21 H, m), 5.25 (2 H, s), 7.25−7.33 (1 H, t), 7.39−7.46 (1 H, t), 7.50−7.57 (1 H, t), 7.64−7.68 (1 H, d), 7.79−7.83 (4 H, m), 9.06 (1 H, br s), 13.2 (1H, br s); HRMS (EI) *m/z* 423.2348 [M^+ calcd 423.2342 for $C_{24}H_{33}N_3O_2Si$].

Method L. Silyl Deprotection. Silyl-protected benzimidazole (1.6−3.05 mmol) was dissolved in tetrahydrofuran (1

g/20 mL), 1 M tetrabutylammonium fluoride (TBAF; 1.05 equiv) in THF was added, and the reaction mixture stirred for 4 h at room temperature. Water (15 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4) and purified as appropriate.

2-(4-Hydroxymethylphenyl)-1*H*-benzimidazole-4-carboxamide (78). Method L. Column chromatography (DCM−10% MeOH) yielded a white solid, 65%: mp 257−258 °C; IR 3332, 3295, 3170, 1658, 1600 cm^{-1}; ^1H NMR δ 4.69−4.72 (2 H, d), 5.47 (1 H, t), 7.40−7.48 (1 H, t), 7.61−7.65 (2 H, d, J = 8.1), 7.81−7.85 (1 H, d), 7.91 (1 H, s), 7.95−7.99 (1 H, d), 8.29−8.33 (2 H, d, J = 8.1), 9.5 (1 H, br s), 13.4 (1 H, br s); ^{13}C NMR δ 62.5, 114.9, 122.2, 122.3, 122.9, 126.7, 126.9, 127.5, 135.3, 141.5, 145.4, 152.0, 166.2; HRMS (EI) *m/z* 267.1006 [M^+ calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{15}H_{13}N_3O_2$) C, H; N: found 14.42, expected 15.72.

2-(3-Hydroxymethylphenyl)-1*H*-benzimidazole-4-carboxamide (79). Method L. Column chromatography (DCM−10% MeOH) and recrystallization from EtOH yielded the product, 47%: mp 249−250 °C; IR 3341, 3280, 3173, 1658, 1635, 1602 cm^{-1}; ^1H NMR δ 4.72−4.74 (2 H, d), 5.48−5.54 (1 H, t), 7.41−7.48 (1 H, t), 7.56−7.60 (1 H, d), 7.61−7.69 (1 H, t), 7.81−7.85 (1 H, d), 7.92 (1 H, s), 7.96−7.99 (1 H, d), 8.19−8.22 (1 H, d), 8.33 (1 H, s), 9.5 (1 H, br s), 13.55 (1 H, br s); ^{13}C NMR δ 62.8, 115.2, 122.5, 123.1, 125.0, 125.4, 126.0, 127.5, 128.8, 129.0, 135.5, 141.6, 143.8, 152.2, 166.4; HRMS *m/z* (EI) 267.1012 [M^+ calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{15}H_{13}N_3O_2$·0.2EtOH) C, H, N.

2-(2-Hydroxymethylphenyl)-1*H*-benzimidazole-4-carboxamide (80). Method L. Column chromatography (DCM−5% MeOH) and recrystallization from EtOH yielded the product, 51%: mp 264−268 °C; IR 3402, 3179, 1650, 1604 cm^{-1}; ^1H NMR δ 5.05 (2 H, s), 5.65 (1 H, br s), 7.43−7.51 (1 H, t), 7.56−7.71 (2 H. m), 7.87−8.01 (5 H, m), 9.4 (1 H, br s), 13.3 (1 H, br s); HRMS (EI) *m/z* 267.0998 [M^+ calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{15}H_{13}N_3O_2$·0.2EtOH) C, H, N.

2-(2-Trifluoromethylphenyl)-1*H*-benzimidazole-4-carboxamide (81). Method I. Column chromatography (DCM−10% MeOH) and recrystallization from EtOH−petroleum ether yielded the product (43%): mp 265−268 °C; IR 3309, 3163, 1670, 1658; ^1H NMR δ 7.48−7.56 (1 H, t), 7.88−7.92 (3 H, m), 8.00−8.12 (4 H, m), 9.4 (1 H, br s), 13.6 (1 H, br s); ^{13}C NMR δ 115.5, 121.2, 123.0, 126.7, 127.2, 127.7, 128.3, 129.1, 130.9, 132.2, 132.8, 134.9, 141.3, 150.0, 166.2; HRMS (EI) *m/z* 305.0764 [M^+ calcd 305.0776 for $C_{15}H_{10}F_3N_3O$]. Anal. ($C_{15}H_{10}F_3N_3O$·0.2H_2O) C, H, N.

2-(2-Chlorophenyl)-1*H*-benzimidazole-4-carboxamide (82). Method I. Column chromatography (1:1 petroleum ether−EtOAc) and recrystallization from MeOH yielded the product, 24%: mp 234−235 °C; IR 3385, 3326, 3182, 1663 cm^{-1}; ^1H NMR δ 7.47−7.54 (1 H, t), 7.67−7.92 (4 H, m), 7.99−8.12 (2 H, 2 d), 9.38 (1 H, br s), 13.39 (1 H, br s); HRMS (EI) *m/z* 271.0521 [M^+ calcd 271.0512 for $C_{14}H_{10}ClN_3O$]. Anal. ($C_{14}H_{10}ClN_3O$) C, H, N.

2-(3-Chlorophenyl)-1*H*-benzimidazole-4-carboxamide (83). Method I. Column chromatography (EtOAc) and recrystallization from MeOH yielded the product, 24%: mp 260−261 °C; IR 3349, 3175, 3156, 1657, 1635, 1602, 1578 cm^{-1}; ^1H NMR δ 7.44−7.52 (1 H, t), 7.72−7.74 (2 H, d), 7.85−7.89 (1 H, d), 7.91 (1 H, s), 7.98−8.02 (1 H, d), 8.30−8.35 (1 H, t), 8.41 (1 H, s), 9.4 (1 H, br s), 13.6 (1 H, br s); HRMS (EI) *m/z* 271.0507 [M^+ calcd 271.0512 for $C_{14}H_{10}ClN_3O$]. Anal. ($C_{14}H_{10}ClN_3O$·0.33MeOH) C, H, N.

2-(2-Fluorophenyl)-1*H*-benzimidazole-4-carboxamide (84). Method I. Column chromatography (EtOAc−30% petroleum ether) and recrystallization from MeOH yielded the product, 57%: mp 207−210 °C; IR 3436, 3354, 3189, 1664, 1603, 1585 cm^{-1}; ^1H NMR δ 7.36−7.54 (3 H, m), 7.64−7.69 (1 H, m), 7.80−7.85 (1 H, d), 7.87 (1 H, s), 7.91−7.97 (1 H, d), 8.34−8.38 (1 H, t), 9.35 (1 H, br s), 13.13 (1 H, br s); ^{13}C NMR δ 115.6, 116.6, 117.2, 122.7, 123.2, 125.3, 130.5, 132.7, 135.3, 140.7, 147.1, 158.6, 160.6, 166.1; HRMS (EI) *m/z* 255.0803 [M^+ calcd 255.0808 for $C_{14}H_{10}FN_3O$].

3-*O*-Allyl-4-methoxybenzaldehyde (85). To 3-hydroxy-4-methoxybenzaldehyde (1.0 g, 6.6 mmol) and powdered anhydrous potassium carbonate (1.0 g, 7.2 mmol) in acetonitrile (30 mL) was added allyl bromide (0.63 mL, 7.2 mmol) in acetonitrile (15 mL). The reaction was stirred for 16 h at room temperature after which the solvent was removed. The resulting yellow solid was suspended between water (20 mL) and EtOAc (20 mL). The aqueous layer was washed with EtOAc (2 × 20 mL). The combined organic extracts were dried ($MgSO_4$), and the solvent removed to yield an oil. This was purified by column chromatography (petroleum ether−20% EtOAc) to yield a clear oil, 1.12 g, 89%: IR 2935, 2841, 1685, 1595, 1585, 1510 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.94 (3 H, s), 4.62−4.66 (2 H, d), 5.27−5.33 (1 H, d, J = 1.3, J = 10.4), 5.37−5.46 (1 H, d, J = 1.4, J = 17.3), 6.05−6.16 (1 H, m), 6.94−6.98 (1 H, d), 7.38 (1 H, s), 7.41−7.46 (1 H, d), 9.8 (1 H, s); ^{13}C NMR δ ($CDCl_3$) 56.1, 69.7, 110.7, 110.9, 118.5, 126.8, 130.0, 132.5, 148.5, 154.9, 190.8; HRMS (EI) *m/z* 192.0794 [M^+ calcd 192.0786 for $C_{11}H_{12}O_3$].

2-(3-Hydroxy-4-methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (86). Methods H, C. 2-Amino-3-nitrobenzoic acid (**10**) (1 g, 5.5 mmol) and isovanillin (1.2 g, 6 mmol) yielded the product, 87%: mp 155−159 °C; IR 3379, 3169, 1648, 1600, 1490 cm^{-1}; ^1H NMR δ 3.96 (3 H, s), 7.20−7.24 (1 H, d), 7.36−7.43 (1 H, t), 7.73−7.80 (3 H, m), 7.91−7.95 (2 H, d), 9.5 (2 H, br s). 13.2 (1 H, br s); HRMS (EI) *m/z* 283.0949 [M^+ calcd 283.0957 for $C_{15}H_{13}N_3O_3$]. Anal. ($C_{15}H_{13}N_3O_3$·0.75H_2O) C, H; N: found 14.19, expected 14.73.

2-(3-*O*-Allyl-4-methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (87). Method I. Reaction with aldehyde **85**. Column chromatography (DCM−10% MeOH) and recrystallization from EtOH yielded the product, 40%: ^1H NMR δ 3.79 (3 H, s), 4.79−4.82 (2 H, d), 5.40−5.45 (1 H, d, J = 10.3), 5.54−5.63 (1 H, d, J = 17.2), 6.14−6.33 (1 H, m), 7.27−7.31 (1 H, d), 7.38−7.46 (1 H, t), 7.79−7.83 (1 H, d), 7.87 (1 H, s), 7.94−7.97 (3 H, m), 9.45 (1 H, br s), 13.3 (1 H, br s); HRMS (EI) *m/z* 323.1271 [M^+ calcd 323.1270 for $C_{18}H_{17}N_3O_3$].

2-(4-Hydroxyphenyl)-1*H*-benzimidazole-4-*N*-methylcarboxamide (88). Methyl 2-(4′-hydroxyphenyl)-1*H*-benzimidazole-4-carboxylate (200 mg, 0.75 mmol) was dissolved in ethanol containing 33% methylamine (10 mL) and stirred overnight. Removal of solvents, followed by column chromatography (DCM−7.5% MeOH) yielded an off-white solid, 187 mg, 94% (recrystallized from DCM−MeOH): mp 289−290 °C; IR 3108, 1614, 1479 cm^{-1}; ^1H NMR δ 3.11−3.13 (3 H, d, J = 4.65), 4.22 (1 H, br s), 7.04−7.08 (2 H, d, J = 8.6), 7.35−7.43 (1 H, t), 7.75−7.79 (1 H, d), 7.93−7.97 (1 H, d), 8.21−8.26 (2 H, d, J = 8.6), 9.95−9.97 (1 H, d, J = 4.65), 10.22 (1 H, br s), 13.25 (1 H, br s); HRMS (EI) *m/z* 267.1001 [M^+ calcd 267.1008 for $C_{15}H_{13}N_3O_2$]. Anal. ($C_{15}H_{13}N_3O_2$·H_2O) C, H, N.

Methyl 2-(4-Methoxyphenyl)-1*H*-benzimidazole-4-carboxylate (89). Method I. Methyl 2-amino-3-nitrobenzoate (**14**) was used. Column chromatography (1:1 petroleum ether−EtOAc) gave a brown solid, 62%: mp 142−144 °C; IR 3374, 1696 cm^{-1}; ^1H NMR δ 3.97 (3 H, s), 4.09 (3 H, s), 7.20−7.24 (2 H, d, J = 8.8), 7.38−7.46 (1 H, t), 7.90−7.94 (1 H, d), 8.02−8.06 (1 H, d), 8.37−8.41 (2 H, d, J = 8.8), 12.38 (1 H, br s); HRMS (EI) *m/z* 282.1018 [M^+ calcd 282.1004 for $C_{16}H_{14}N_2O_3$].

2-(4-Methoxyphenyl)-1*H*-benzimidazole-4-*N*-methylcarboxamide (90). A solution of methyl 2-(4′-methoxyphenyl)-1*H*-benzimidazole-4-carboxylate (**89**) (209 mg, 0.74 mmol) in ethanol containing 33% methylamine (20 mL) was stirred overnight. Removal of solvents and recrystallization from EtOH yielded off-white needles, 144 mg, 69%: mp 252−256 °C; IR 3187, 1646 cm^{-1}; ^1H NMR δ 3.12 (3 H, d), 3.98 (3 H, s), 7.25−7.29 (2 H, d, J = 8.4), 7.39−7.46 (1 H, t), 7.78−7.82 (1 H, d), 7.95−7.99 (1 H, d), 8.33−8.38 (2 H, d, J = 8.4), 9.93 (1 H, br s), 13.3 (1 H, br s); ^{13}C NMR δ 26.3, 55.7, 114.8, 121.8, 122.3, 122.7, 129.0, 135.5, 141.5, 152.4, 161.5, 165.7; HRMS (EI) *m/z* 281.1152 [M^+ calcd 281.1164 for $C_{16}H_{15}N_3O_2$]. Anal. ($C_{16}H_{15}N_3O_2$·0.6EtOH·0.1H_2O) C, H, N.

2-(4-Hydroxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (91). Method I. 2-Amino-3-nitrobenzoic acid (**10**) was used. Column chromatography (DCM−MeOH 15%) gave an

orange solid that was recrystallized from EtOH, 15%: mp 346−347 °C; IR 3429, 3217, 1615 cm^{-1}; ^1H NMR δ 4.47 (1 H, br s) 6.99−7.03 (2 H, d, J = 8.5), 7.34−7.42 (1 H, t), 7.84−7.88 (1 H, d), 7.92−7.96 (1 H, d), 8.22−8.26 (2 H, d, J = 8.5), 10.1−10.2 (1 H, br s); HRMS (EI) m/z 254.0687 [M^+ calcd 254.0691 for $C_{14}H_{10}N_2O_3$].

Crystallography. Purified protein and cocrystals of the C-terminal catalytic domain of chicken PARP were obtained using procedures similar to those previously described.[31] In this study, 70 mM Tris, pH 8.5, 19% PEG-3400, and 8% isopropyl alcohol were used as precipitant. X-ray diffraction data were collected from this $P2_12_12_1$ crystal form: a = 59.4 Å, b = 64.3 Å, c = 97.0 Å, at SSRL beamline 7-1.[32] Data resolution is from 20.0−2.2 Å; 95006 observations were scaled and merged into 19029 unique reflections using DENZO and SCALEPACK.[33] The overall R-merge is 6.1%, the ratio $I/\sigma(I)$ is 28.3, and the data is 97.9% complete. Corresponding values for the high-resolution data shell (2.28−2.20 Å) are 22.2%, 9.8, and 95.5%, respectively. The structure of **44** complexed to PARP was solved and refined using this data, the program X-PLOR,[34] and the protein and solvent starting model 1PAX from the Protein Data Bank.[28] The conventional and free R-factors after refinement are 19.4% and 27.4%. The rms deviations between model and ideal bond distances, bond angles, dihedral angles, and improper angles are 0.007 Å, 1.3°, 23.6°, and 1.1°. The model coordinates have been deposited in the Protein Data Bank with the code 1EFY.

PARP Expression and Purification. Human full length PARP protein was expressed using a baculovirus expression system and purified to homogeneity as described by Giner and co-workers.[35]

Assay of Inhibitors with PARP. The enzymatic assay was performed as previously described[36,37] with minor modifications. Samples (50 μL) containing 20 nM purified PARP protein, varying concentrations of inhibitor, 2% (v/v) DMSO, saturating amounts of DNase I-activated calf thymus DNA (10 μg/mL), and NAD^+ (500 μM containing 0.5 μCi [^{32}P]NAD^+) were incubated in sample buffer [50 mM Tris, pH 8.0, 10 mM $MgCl_2$, 1 mM tris(carboxyethyl)phosphine·hydrochloride] at 25 °C for 5 min in 96-well plates. Under these conditions, the reaction rate was linear with time up to 8 min. The reaction was stopped by the addition of an equal volume of ice-cold 40% (v/v) trichloroacetic acid and incubation at 0 °C for 30 min. The samples were transferred to a Bio-Dot microfiltration apparatus (BioRad), filtered through Whatman GF/C glass fiber filter paper and washed 3× with 150 μL of wash buffer [5% (v/v) trichloroacetic acid, 1% (v/v) inorganic pyrophosphate]. The filter was removed from the filtration apparatus, washed once with 50 mL of wash buffer and twice with 50 mL ethanol, and dried. Incorporation of [^{32}P]ADP-ribose into the acid insoluble material was quantified using a PhosphorImager (Molecular Dynamics) and ImageQuant software. Inhibition constants (K_i) were calculated by nonlinear regression analysis (KaleidaGraph, Synergy Software) using the velocity equation for competitive inhibition.[38] For tight-binding inhibitors, K_i values were calculated using the equation described.[39]

Growth Inhibition Assays. Solutions of temozolomide (temodal; a gift from the Cancer Research Campaign, London, U.K.), topotecan (hycamptin; Smith Kline Beecham) and NU-1085 (**45**) in DMSO at 150, 2 and 100 mM, respectively, were stored at −20 °C. A2780 human ovarian carcinoma cells were grown in HEPES-supplemented RPMI 1640 medium + 10% foetal calf serum in an atmosphere of 5% CO_2 in air at 37 °C. Cells were verified as being free of mycoplasma by fluorescence microscopy, following fixation and staining with Hoechst 33258 at monthly intervals.

Cells were seeded at 2.0−2.5 × 10^3 cells/well in 100 μL of medium in replicate 96-well plates (leaving the outer wells with 100 μL of medium alone to minimize 'edge effect') and allowed to attach overnight. After 16−24 h the medium was replaced with that containing varying concentrations of temozolomide or topotecan with or without 10 μM NU1085 (**45**) in a final DMSO concentration of 1%. For each drug concentration 5 replicate wells were used. A replicate plate was fixed as described below to obtain an estimate of the cell density at the start of the drug incubation. The plates were incubated for 72 h at 37 °C before assaying (together with the preincubation plate) for cell growth as previously described.[40] The optical density of the wells was read on a computer-interfaced MR700 microtiter-plate reader (Dynex, Billingshurst, U.K.) relative to an air blank using a 570-nm filter. Cell growth was expressed as percent (%) of an appropriate control, i.e. 1% DMSO or 10 μM NU1085 (**45**). The IC_{50} values (concentration of drug required to reduce growth by 50%) were determined by nonlinear regression curve-fitting using GraphPad PRISM (San Diego, CA) software. The potentiation factor, PF_{50}, was calculated from IC_{50} of topotecan or temozolomide alone ÷ IC_{50} of topotecan or temozolomide + NU1085.

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