Resistance-Modifying Agents. 5.[1] Synthesis and Biological Properties of Quinazolinone Inhibitors of the DNA Repair Enzyme Poly(ADP-ribose) Polymerase (PARP)

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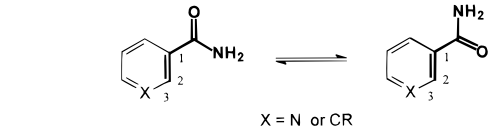
Received May 4, 1998

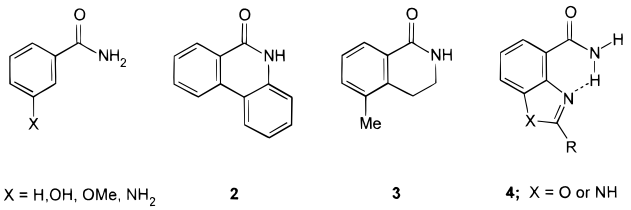
Clinical studies concerning the role of poly(ADP-ribose) polymerase (PARP) in the repair of drug- and radiation-induced DNA damage have been impeded by the poor solubility, lack of potency, and limited specificity of currently available inhibitors. A series of 2-alkyl- and 2-aryl-substituted 8-hydroxy-, 8-methoxy-, and 8-methylquinazolin-4(3*H*)-ones has been synthesized and evaluated for PARP inhibitory activity in permeabilized L1210 murine leukemia cells. 8-Methoxy- and 8-methylquinazolinones (**14**−**34**) were readily prepared by acylation of 3-substituted anthranilamides with the appropriate acid chloride, followed by base-catalyzed cyclization. The requisite 8-hydroxyquinazolinones (**6**, **35**−**39**) were synthesized by demethylation of the corresponding 8-methoxyquinazolinones with BBr_3. *N*-Methylation of 8-methoxy-2-methylquinazolinone (**15**) with MeI, followed by *O*-demethylation by BBr_3, afforded the control N^3-methylquinazolinones **42** and **43**, respectively. In general, an 8-hydroxy or 8-methyl substituent enhanced inhibitory activity in comparison with an 8-methoxy group. 2-Phenylquinazolinones were marginally less potent than the corresponding 2-methylquinazolinones, but the introduction of an electron-withdrawing or electron-donating 4′-substituent on the 2-aryl ring invariably increased potency. This was particularly evident in the 8-methylquinazolinone series (IC_{50} values 0.13−0.27 μM), which are among the most potent PARP inhibitors reported to date. N^3-Methylquinazolinones **42** and **43** were essentially devoid of activity (IC_{50} values > 100 μM). In studies with L1210 cells in vitro, a concentration of 200 μM 8-hydroxy-2-methylquinazolinone (**6**, NU1025) (IC_{50} value 0.40 μM) potentiated the cytotoxicity of the monomethylating agent 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide and γ-radiation 3.5- and 1.4-fold, respectively, at the 10% survival level.

Introduction

Enzyme-mediated repair of single- or double-strand lesions in DNA is an established mechanism of resistance to antitumor DNA-damaging drugs and radiotherapy.[2,3] Inhibition of DNA repair enzymes is thus a strategy for the potentiation of DNA-damaging anticancer therapies. Poly(ADP-ribose) polymerase (PARP, EC 2.4.2.30) is an abundant eukaryotic nuclear enzyme which comprises a 42-kDa N-terminal DNA binding domain containing two zinc fingers (FI and FII), a 22-kDa automodification domain, and a 54-kDa C-terminal catalytic domain.[4−7] Following binding of PARP to the damaged site in DNA, catalytic activation and utilization of the substrate NAD^+ occurs. Initially, the enzyme brings about the transfer of the ADP-ribose fragment of NAD^+ to target acceptor proteins including, and perhaps primarily, the automodification domain of PARP itself. Further catalytic activity results in the formation of protein-bound linear and branched homo-ADP-ribose polymers.[8]

PARP activation and subsequent poly(ADP) ribosylation are immediate cellular responses to drug- or radiation-induced DNA damage,[9] and there is evidence, from both inhibitor studies[10] and molecular genetic approaches,[11−13] that impeding PARP-mediated DNA repair may enhance the cytotoxicity of DNA-damaging therapies used in the treatment of cancer. However, extrapolation of experimental studies to the clinical setting has been hampered by the lack of potency, solubility, and limited specificity associated with currently available inhibitors, which include 3-substituted benzamides (**1**)[14] and a number of fused-ring heterocycles (e.g., **2** and **3**).[15,16]





Our initial approach to the development of novel PARP inhibitors was based on an understanding of the

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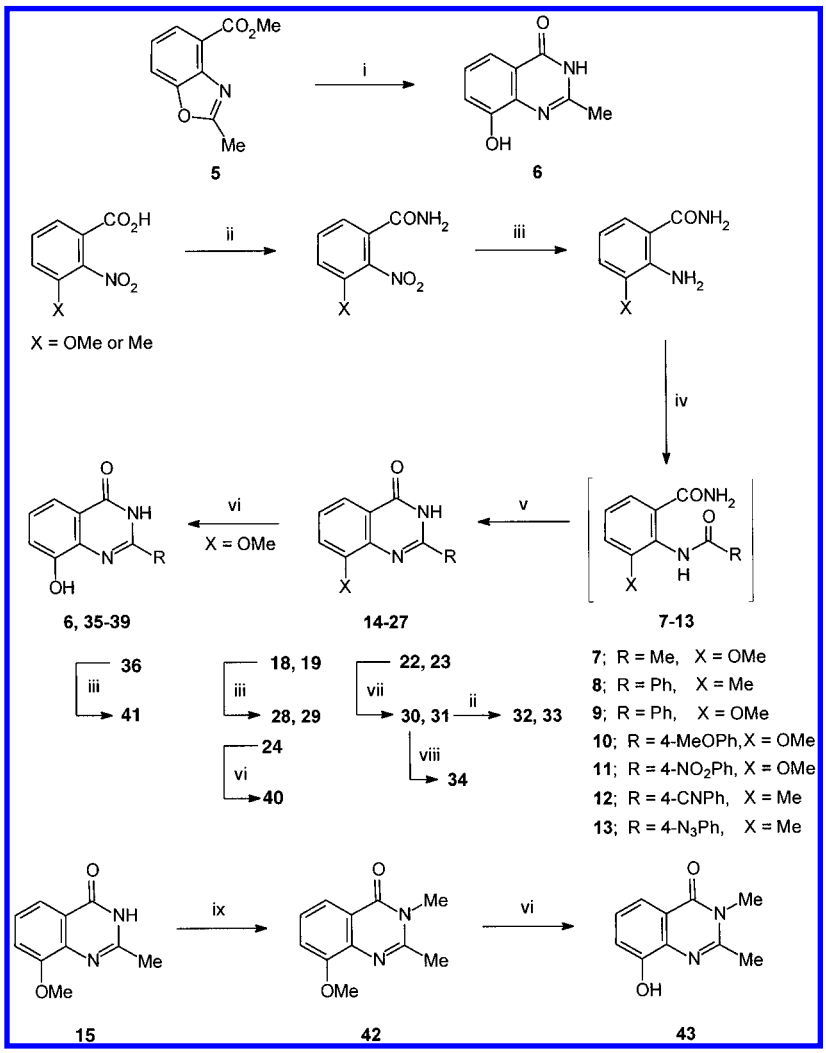
10.1021/jm980273t CCC: $15.00
Published on Web 12/01/1998

Scheme 1[a]



[a] Reagents: (i) NH_3, 55 °C, 20 bar; (ii) $SOCl_2$, DMF, THF, 25 °C, then NH_4OH; (iii) Pd/C, H_2, MeOH, 25 °C; (iv) RCOCl, pyridine, THF, 25 °C; (v) aq NaOH, reflux; (vi) BBr_3, DCM, reflux; (vii) EtOH, NaOH, reflux; (viii) $SOCl_2$, DMF, THF, 25 °C, then MeOH; (ix) MeI, K_2CO_3, MeCN, reflux.

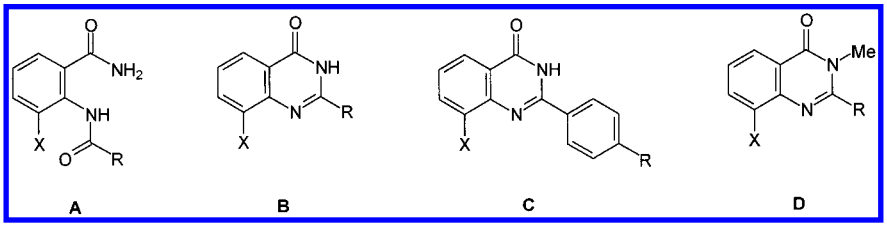
likely interaction of the nicotinamide moiety of NAD^+ with the catalytic domain of the enzyme. Ab initio calculations with nicotinamide indicated that of orientations A and B, the preferred conformation was that with the carbonyl group anti to the 1,2-bond of the ring (A).[17] Incorporation of the carbamoyl substituent into a ring system as in **2** and **3** constrains this group into the optimal binding orientation and accounts for the potent PARP-inhibitory activity of these compounds.

We have recently identified an alternative strategy for restricting the carboxamide into the active orientation, whereby an intramolecular hydrogen bond between the carbamoyl NH and an adjacent heterocyclic nitrogen, as in benzoxazole- or benzimidazole-4-carboxamides **4**, constrains the carbamoyl group within a "pseudocycle".[18] During initial synthetic studies with benzoxazole-4-carboxamides, an unexpected rearrangement occurred on treatment of the benzoxazole ester **5** with ammonia (Scheme 1) to afford the 8-hydroxyquinazolin-4(3*H*)-one NU1025 (**6**), the structure of which was subsequently confirmed by X-ray crystallography.[19] The potent PARP-inhibitory activity of **6** prompted further investigations with this class of inhibitor, and in this paper we describe the synthesis and biological evaluation of a series of quinazolin-4(3*H*)-ones varied with respect to substituents at the 2- and 8-positions. Preliminary communications describing part of this work have been published previously.[18,20]

Chemistry

The structures and properties of the compounds prepared are recorded in Table 1, and their syntheses are outlined in Scheme 1. Although, in principle, the required quinazolinones were available via rearrangement of the corresponding benzoxazole-4-carboxylates, this approach gave only modest product yields and was limited to the preparation of 8-hydroxyquinazolinones. To establish structure−activity relationships (SARs) among quinazolinone PARP inhibitors, an alternative more flexible synthetic route was sought which would enable variation at both the 2- and 8-positions. Quinazolinones are readily prepared by the acylation−cyclization of anthranilic acid derivatives,[21] and the commercially available 3-methoxy- and 3-methyl-2-nitrobenzoic acids proved convenient starting materials for the synthesis of 2-substituted quinazolinones bearing a methyl, methoxy, or hydroxyl group in the 8-position. Thus, high yields of the 3-substituted-2-nitrobenzamides

Table 1. Physical Data and PARP-Inhibitory Activity of Compounds



compd	general structure	R	X	method[a]	recryst solvents[b]	yield (%)	formula	mp (°C)	anal.[c]	PARP inhibition IC_{50} (μM)[d]
3AB										19.1
3HB[e]										8.0
QN[f]	B	H	H							15.8
3										0.44
6 (NU1025)	B	Me	OH	V	A	65	$C_9H_8N_2O_2$	253−258	CHN	0.40
7	A	Me	OMe	I	B	31	$C_{10}H_{12}N_2O_3 \cdot 0.15H_2O$	243−246	CHN	NT[g]
8	A	Ph	Me	I	B	74	$C_{15}H_{14}N_2O_2 \cdot 0.1H_2O$	190−193	CHN	NT
9	A	Ph	OMe	I	B	41	$C_{15}H_{14}N_2O_3 \cdot 0.2H_2O$	176−180	CHN	NT
10	A	4-$MeOC_6H_4$	OMe	I	B	47	$C_{16}H_{16}N_2O_4$	179−181	CHN	NT
11	A	4-$NO_2C_6H_4$	OMe	I	A	87	$C_{15}H_{13}N_3O_5$	289−290	CHN	NT
12	A	4-CNC_6H_4	Me	I	F	33	$C_{16}H_{13}N_3O_2$	299−301	CHN	NT
13	A	4-$N_3C_6H_4$	Me	I	B	89	$C_{15}H_{13}N_5O_2$	197−198	CHN	NT
14	B	Me	Me	II	B	81	$C_{10}H_{10}N_2O$	217−220	CHN	0.39
15	B	Me	OMe	II (III)	C	97 (67)	$C_{10}H_{10}N_2O_2 \cdot 0.1H_2O$	202−204	CHN	0.78
16	C	H	Me	III	B	72	$C_{15}H_{12}N_3O \cdot 0.1H_2O$	206−209	CHN	0.87
17	C	H	OMe	II (III)	B	75 (65)	$C_{15}H_{12}N_2O_2$	252−256	CHN	4.2
18	C	NO_2	Me	II	D	86	$C_{15}H_{11}N_3O_3$	317−319	CHN	0.13
19	C	NO_2	OMe	III	D	66	$C_{15}H_{11}N_3O_4 \cdot 0.3H_2O$	306−308	CHN	0.85
20	C	CF_3	Me	II	B	65	$C_{16}H_{11}N_2OF_3$	255−257	CHN	>10[h]
21	C	CF_3	OMe	II	B	64	$C_{16}H_{11}N_2O_2F_3$	287−289	CHN	39
22	C	CN	Me	II	F	21	$C_{16}H_{11}N_3O$	330−332	CHN	0.27
23	C	CN	OMe	II	F	27	$C_{16}H_{11}N_3O_2 \cdot 0.75H_2O$	306−309	CHN	1.34
24	C	OMe	Me	II	B	58	$C_{16}H_{14}N_2O_2$	227−229	CHN	0.19
25	C	OMe	OMe	II (III)	B	70 (63)	$C_{16}H_{13}N_2O_3 \cdot 0.1CH_3OH$	226−228	CHN	2.0
26	C	N_3	Me	III	B	79	$C_{15}H_{11}N_5O$	>350[i]	CHN	NT
27	C	N_3	OMe	II	B	64	$C_{15}H_{11}N_5O_2 \cdot 0.5H_2O$	176−180[i]	CHN	1.93
28	C	NH_2	Me	IV	B	52	$C_{15}H_{13}N_3O$	254−256	CHN	0.44
29	C	NH_2	OMe	IV	B	46	$C_{15}H_{13}N_3O_2 \cdot 0.4H_2O$	263−265	CHN	>0.3[h]
30	C	CO_2H	Me		B	92	$C_{16}H_{12}N_2O_3$	>350	*j*	NT
31	C	CO_2H	OMe		B	90	$C_{16}H_{12}N_2O_4$	>350	*j*	NT
32	C	$CONH_2$	Me		F	33	$C_{16}H_{13}N_3O_2$	>350	*j*	NT
33	C	$CONH_2$	OMe		F	31	$C_{16}H_{13}N_3O_3$	200	*j*	NT
34	C	CO_2Me	Me		G	15	$C_{17}H_{14}N_2O_3$	277−279	CHN	4.80
35	C	H	OH	V	A	67	$C_{14}H_{10}N_2O_2 \cdot 0.1H_2O$	280−284	CHN	1.06
36	C	NO_2	OH	V	A	38	$C_{14}H_9N_3O_4$	321−323	CHN	0.23
37	C	CF_3	OH	V	G	19	$C_{15}H_9N_2O_2F_3 \cdot 1.5H_2O$	303−306	CHN	NT
38	C	CN	OH	V	F	14	$C_{15}H_9N_3O_2$	334−337	*j*	NT
39	C	OH	OH	V	B	47	$C_{14}H_{10}N_2O_3$	288−290	CHN	0.29
40	C	OH	Me	V	B	76	$C_{15}H_{12}N_2O_4$	258−261	CHN	0.22
41	C	NH_2	OH	IV	B	57	$C_{14}H_{11}N_3O_2 \cdot 0.2H_2O$	274−276	CHN	0.52
42	D	Me	OMe		E	56	$C_{11}H_{12}N_2O_2$	133−136	CHN	>100
43	D	Me	OH		F	57	$C_{10}H_{10}N_2O_2 \cdot 0.1H_2O$	145−147	CHN	>100

[a] See the Experimental Section. [b] Recrystallization solvents: A, propan-2-ol−H_2O; B, MeOH−H_2O; C, EtOAc; D, DMF−H_2O; E, MeOH−DCM. Purified by column chromatography on silica gel: F, eluent = DCM−MeOH (95:5); G, eluent = petrol−EtOAc (8:2). [c] All elemental analyses for C, H, and N agree within ±0.4% of calculated values unless otherwise noted. [d] Concentration of inhibitor required to reduce enzyme activity to 50% of control rate. Values are the averages from at least two independent dose−response curves. For details of assay, see ref 20. [e] 3-Hydroxybenzamide. [f] Quinazolin-4(3*H*)-one. [g] Not tested. [h] Limiting solubility. [i] Decomposes. [j] No satisfactory elemental analysis obtained.

were obtained from the corresponding 2-nitrobenzoic acid derivatives, by initial conversion into the acid chloride, followed by treatment with aqueous ammonia.

Subsequent catalytic hydrogenation afforded the required 2-amino-3-methoxy- and 2-amino-3-methylbenzamides, which were invariably of sufficient purity to be used directly. Acetylation of 2-amino-3-methoxybenzamide with acetyl chloride−pyridine in THF gave the required 2-(acetylamino)benzamide **7** in excellent yield, and cyclization to the target 8-methoxy-2-methylquinazolinone **15** was effected by treatment with dilute aqueous sodium hydroxide at room temperature. Analogous reactions conducted with 2-amino-3-methoxy- or 2-amino-3-methylbenzamide and the appropriate acid chloride furnished the corresponding 2-(acylamino)-benzamides, and although several of these (**8**−**13**) were characterized, in most cases cyclization to the desired quinazolinones (**14**−**27**) could be achieved without isolation of the intermediate. Indeed, some evidence of direct quinazolinone formation was observed during the acylation reactions, presumably as a result of the pyridine or 4-(dimethylamino)pyridine employed. Preparation of the 2-(4′-nitrophenyl)quinazolinones **18** and **19** was hampered by the very poor solubility of the 2-(acylami-

no)benzamide precursors in aqueous sodium hydroxide. Consequently, more vigorous conditions (2.5 M NaOH, 100 °C) were necessary to induce cyclization, and this was achieved without detriment to the carboxamide substituent. The 2-(4-nitrophenyl)quinazolinones **18**, **19**, and **36** were smoothly reduced to the corresponding 2-(4-aminophenyl)quinazolinones **28**, **29**, and **41** by catalytic hydrogenation.

Base-catalyzed hydrolysis of the 2-(4-cyanophenyl)-quinazolinones **22** and **23** afforded the carboxylic acid derivatives **30** and **31**, from which the corresponding carboxamides **32** and **33** and the methyl ester **34** were accessible by conventional methodology. The required 8-hydroxyquinazolinones (**6**, **35**−**39**) were prepared from the appropriate 8-methoxyquinazolinones by demethylation with BBr_3 in CH_2Cl_2, with the 8-hydroxy-2-methylquinazolin-4(3*H*)-one (**6**) obtained proving identical to that synthesized via the alternative benzoxazole route.[20] However, side reactions involving the cyano group of **22** gave a poor yield of the target 2-(4-cyanophenyl)-8-hydroxyquinazolinone **38**, together with two other minor products tentatively identified (^{1}H NMR) as the corresponding carboxamide and carboxylic acid. The *N*-methylquinazolinones **42** and **43**, required as controls for PARP inhibition and in vitro potentiation studies, were readily prepared from **6** by *N*-methylation with MeI to afford **42** and subsequent 8-*O*-demethylation with BBr_3 giving the required **43**. Again, the latter compound was identical to that prepared by heating methyl 2-methylbenzoxazole-4-carboxylate with methylamine as described previously.[20]

Results and Discussion

The compounds prepared in this study were evaluated for PARP inhibitory activity in permeabilized L1210 murine leukemia cells, employing a modification of a reported method,[22] as described previously.[20] The results are summarized as IC_{50} values in Table 1. The standard PARP inhibitors 3-aminobenzamide (3AB) and 3-hydroxybenzamide (3HB), the dihydroisoquinolinone PD128763 (**3**), and the commercially available quinazolin-4(3*H*)-one (QN) were included for comparative purposes. The novel inhibitors described in this paper have allowed, for the first time, an investigation of SARs at the 8- and 2-positions of quinazolinone PARP inhibitors. Furthermore the predicted requirement for a free NH at the 3-position of quinazolinone PARP inhibitors was studied by the evaluation of two representative N^3-methylquinazolinones.

At the 8-position, in the 2-methylquinazolinone series, the 8-hydroxy (**6**) and 8-methyl (**14**) derivatives were equipotent with PD128763 (**3**) and some 20- and 50-fold more active than 3HB and 3AB, respectively. 8-Methoxy-2-methylquinazolinone **15** was approximately 2-fold less active than **6** and **14**, suggesting that the isosteric 8-hydroxyl and 8-methyl substituents are better accommodated within the active site of the enzyme than the bulkier 8-methoxy group. The greater activities of the 8-methyl and 8-hydroxy, compared to 8-methoxy, derivatives were observed throughout, regardless of the nature of the 2-substituent. This observation is also in accord with SARs for the structurally related dihydroisoquinolones, where optimal activity was also reported for a methyl or hydroxyl group at the 5-position, which corresponds to the 8-position in the quinazolinones.[16] The inhibitory activity of the 8-unsubstituted, naturally occurring 2-methylquinazolin-4(3*H*)-one has been described previously, and an IC_{50} value of 1.1 μM was reported.[23] While comparisons of IC_{50} values from different laboratories must be undertaken with caution, the authors also report an IC_{50} of 8.3 μM for benzamide under identical assay conditions, a value similar to that obtained for benzamide ($IC_{50} = 12.4 \pm 3.1$ μM; mean ± standard deviation) in our assay. This observation, taken in conjunction with the relatively weak activity of the simple quinazolinone (QN) reported here ($IC_{50} =$ 15.8 μM), suggests that substituents in the 8-position enhance PARP-inhibitory activity.

SAR studies were conducted at the quinazolinone 2-position with compounds bearing methyl, phenyl, and substituted phenyl groups, a position which was not explored in the analogous dihydroisoquinolone series.[16] Quinazolinones with a 2-phenyl substituent (**16**, **17**, **35**) exhibited reduced activity compared to their 2-methyl counterparts, regardless of the nature of the 8-substituent. Potency was again in the order 8-Me (**16**) = 8-OH (**35**) > 8-OMe (**17**). However, the introduction of either electron-withdrawing (NO_2, CN) or electron-donating (OH, OMe, NH_2) groups in the 4′-position of the 2-aryl ring markedly increased inhibitory activity, over that seen with the corresponding 2-phenylquinazolinones (**16**, **17**, **35**). The increased activity is especially evident for compounds in the 8-methylquinazolinone series (**18**, **22**, **24**, **28**, **40**), which are among the most potent PARP inhibitors reported to date. These data suggest that the electronic nature of the 4-substituent on the 2-aryl ring is not a controlling factor for PARP inhibition in this series. Surprisingly, the 2-[4-(trifluoromethyl)phenyl]- and 2-[4-(carboxymethyl)phenyl]quinazolinones (**20**, **34**) were both less active than the parent 2-phenylquinazolinone (**16**), although solubility problems hampered the biological evaluation of these compounds.

As expected, the *N*-methylquinazolinones **42** and **43** were both essentially inactive as PARP inhibitors, consistent with the observation that a free carboxamide NH is required to accept a hydrogen bond from a donor carbonyl group within the active site of the enzyme.[10,16]

For preliminary in vitro cytotoxicity potentiation studies, 2-methyl-8-hydroxyquinazolin-4(3*H*)-one (NU1025, **6**) was selected, as this compound exhibited potent PARP-inhibitory activity, was available in multigram quantities, and encompassed an 8-hydroxyl substituent amenable to conversion to a water-soluble prodrug. Chemopotentiation studies were conducted with 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC), a reactive species derived by metabolism of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) and by decomposition of the monofunctional alkylating agent Temozolomide, which is currently under clinical evaluation for the treatment of malignant melanoma and glioma.[24] MTIC is an unstable compound which decomposes, with a half-life in aqueous medium of approximately 8 min at 37 °C, to a highly reactive methyldiazonium ion.[25] As expected, MTIC elicited a concentration-dependent decrease in the survival of L1210 cells, and a concentration of 200 μM **6** potentiated MTIC cytotoxicity with an enhancement factor of 3.5 at an MTIC concentration inducing 90%

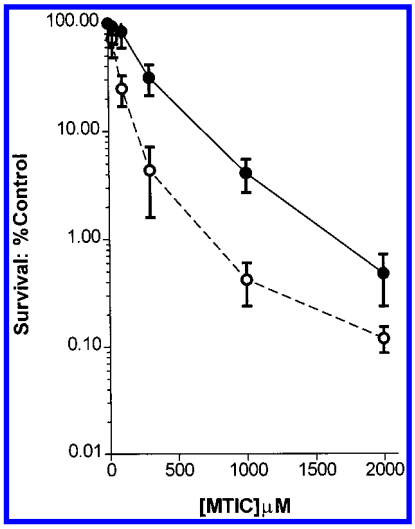


Figure 1. Cytotoxicity of MTIC ± **6**. L1210 cells were exposed to increasing concentrations of MTIC for 20 min followed by a 16-h recovery period in drug-free medium in the absence (●) or presence (○) of 200 mM **6** during both exposure and recovery periods, prior to seeding for colony formation. Each point is the mean ± standard deviation for triplicate samples from the cell populations exposed in duplicate, from a single representative experiment.

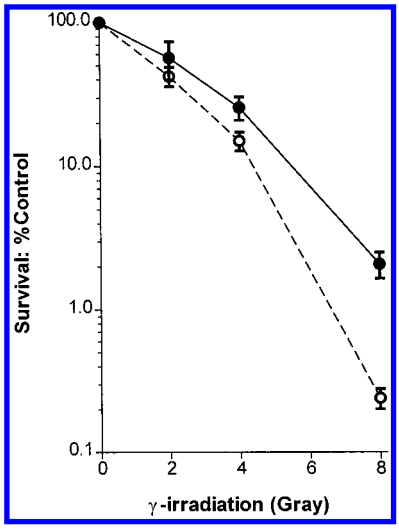


Figure 2. Cytotoxicity of γ-irradiation ± **6**. Cells were exposed to γ-irradiation and allowed to recover for 2 h in drug-free medium in the absence (●) or presence (○) of 200 mM **6**, prior to seeding for colony formation. Each point is the mean ± standard deviation for triplicate samples from the cell populations exposed in duplicate, from a single representative experiment.

Table 2. Potentiation of Cytotoxic Agents in L1210 Murine Leukemia Cells by 8-Hydroxy-2-methylquinazolin-4(3*H*)-one (**6**)[a]

cytotoxic agent	IC_{90} (units)[b]	EF_{90}[c]
MTIC[d]	811 ± 328[e] (nM)	
MTIC + **6**[f]	220 ± 68 (nM)	3.6 ± 0.5
γ-irradiation	6.36 ± 0.4 (Gy)	
γ-irradiation + **6**[f]	4.53 ± 0.5 (Gy)	1.4 ± 0.2

[a] See the Experimental Section. [b] Concentration of drug or radiation dose causing 90% cell death or a 10% relative plating efficiency. [c] Enhancement factor: $EF_{90} = IC_{90}$ or ID_{90} control/IC_{90} or ID_{90} + PARP inhibitor. [d] MTIC, 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide. [e] Standard deviation. [f] 200 μM.

cell killing (Figure 1 and Table 2). These results are consistent with our previous observation that the cytotoxicity of Temozolomide in L1210 cells was potentiated in a concentration-dependent manner by **6** and that this PARP inhibitor was some 50-fold more potent as a chemopotentiator than 3-aminobenzamide.[26] Furthermore the degree of potentiation of MTIC by **6** was of a similar magnitude to that previously reported for PD128673[27] and superior to that achieved by benzamides.[26] Quinazolinone **6** was also found to exhibit radiopotentiating activity in vitro. Thus, exposure of exponentially growing L1210 cells to γ-radiation, with a 2-h recovery period, resulted in a dose-related reduction in survival. If the cells were allowed to recover in the presence of 200 mM **6**, the enhancement factor at a level of irradiation giving 90% cell killing was 1.4, and changes to both the shoulder and the slope of the survival curve indicative of radiopotentiation were apparent (Figure 2 and Table 2). These results correlate closely to those observed with the equipotent PARP inhibitor PD128763, at a concentration of 500 μM, where a similar degree and profile of radiopotentiation were reported.[28]

The apparent discrepancy between the concentration of quinazolinone **6** required to inhibit PARP in the permeabilized cell assay (IC_{50} 0.4 μM) and that used in the chemo- and radiopotentiation experiments (200 μM) could be due to a number of factors. These include poor inhibitor uptake, differences in competing NAD^+ concentrations in the two experiments, and the need to inhibit poly(ADP-ribose) synthesis completely in order to observe potentiation in the whole cell studies. Our previously reported studies with **6** have indicated that the principal locus of action of this compound is indeed via inhibition of PARP.[26] Thus, when **6**, benzamide, 3-aminobenzamide, and PD128763 (**3**) were compared, a correlation was observed between PARP-inhibitory activity and potentiation of Temozolomide cytotoxicity. Similar correlations were also seen for the potentiation of Temozolomide-induced DNA strand breaks and restoration of cellular NAD^+ levels, consistent with PARP inhibition as the mechanism of action of these compounds.

Although not part of the current studies, the issue of the potential tumor selectivity of PARP inhibitors is an important consideration. Recent studies with PARP knockout mice demonstrate that lack of PARP activity is not associated with any major pathology,[13,29] implying that PARP inhibitors should be devoid of systemic toxicities. With respect to the likely antitumor selectivity of PARP inhibitors when used in combination with cytotoxic drugs or ionizing radiation, the lower intracellular NAD^+ concentration in tumor tissue[30−33] may confer selectivity for compounds binding at the NAD^+ site of the enzyme, as in the case of the quinazolinones described here. Furthermore, in the case of ionizing radiation, tumor selectivity is achieved by the locoregional delivery of the DNA-damaging agent. In terms of the PARP selectivity of the quinazolinones described here, the greater activity of these compounds should avoid the mechanism-unrelated effects that have been reported for the less potent nicotinamide- and benzamide-based inhibitors.[10]

Although unavailable at the outset of our studies, the crystal structures of several PARP inhibitors, including PD128763 (**3**) and NU1025 (**6**), bound to the catalytic

fragment of chicken PARP (PARP-CF) have recently been solved.[34,35] As expected for competitive inhibition, the inhibitors bind to the nicotinamide domain occupied by the substrate NAD^+. As predicted previously,[16,17] all of the inhibitors bind in essentially the same orientation, and common to all inhibitor complexes are two hydrogen bonds arising between the carbonyl and NH (or NH_2) groups and the peptide backbone of Gly-863. A third hydrogen bond occurs between the inhibitor carbonyl group and the side chain of Ser-904. Interestingly, in the case of NU1025, an additional hydrogen bond interaction between the 8-hydroxyl substituent and the hydroxyl group of Tyr-907 is also evident. This recently available information clearly offers a wide range of opportunities for improving the potency and specificity of quinazolinone-based PARP inhibitors, through crystal structure-based inhibitor design. The results of these, and related studies, will be detailed in subsequent papers in this series.

Conclusions

To date, the clinical use of PARP inhibitors to potentiate DNA-damaging anticancer therapies has not been possible owing to potency, specificity, and solubility problems encountered with available compounds. In this study we have identified a series of very potent quinazolinone-based PARP inhibitors, as determined by PARP-inhibitory activity in permeabilized L1210 cells, bearing a variety of substituents in the 2- and 8-positions. For 2-arylquinazolinones, no clear SARs have been observed, with a range of electron-donating and electron-withdrawing groups in the 4-position conferring potent inhibitory activity. At the 8-position, a methyl or hydroxyl substituent is preferred to a bulkier methoxy group. Methylation at the *N*3-position markedly reduced activity, consistent with the requirement for a donor hydrogen bond at this position. The 2-methyl-8-hydroxyquinazolinone **6** (NU1025) was shown to potentiate the cytotoxicity of both a methylating agent (MTIC) and ionizing radiation in murine L1210 leukemia cells, and the further preclinical evaluation of this PARP inhibitor is ongoing.

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. Mass spectra were determined on a Kratos MS80 spectrometer in electron impact (EI) or fast atom bombardment (FAB) mode using a *m*-nitrobenzyl alcohol matrix. Proton (^1H) and carbon (^{13}C) 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 [$^2H_6$]DMSO as solvent. NH 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). ^1H NMR spectra of para-disubstituted aromatic rings were observed as the required AA′BB′ pattern and for simplicity are quoted as doublets. Key: t = triplet, s = singlet, q = quartet, d = doublet, dd = double doublet, m = multiplet.

The TLC systems employed Merck 1.05554 aluminum sheets precoated with Kieselgel $60F_{254}$ (0.2 mm) as the adsorbent and were visualized with UV light at 254 and 365 nm. Column chromatography was conducted under medium pressure on silica gel (Kieselgel 60, 240−400 mesh). Elemental analyses were performed in house on a Carlo-Erba Instrumentazione 1106 analyzer or by Butterworth Laboratories, Middlesex, U.K., and are within ±0.4% of theory unless otherwise specified. Reagents were purchased from Aldrich Chemical Co., Gillingham, U.K., and used as received unless otherwise stated. Ethanol and methanol were dried using Mg/I_2 and stored over 4-Å molecular sieves. Diethyl ether and tetrahydrofuran were predried over $CaCl_2$ and distilled from sodium/benzophenone. Petrol (petroleum ether) refers to that fraction in the boiling range 40−60 °C.

3-Substituted-2-(*N*-acylamino)benzamides 7−13: Method I. General Procedure. To a stirred solution of 2-amino-3-methoxy- or 2-amino-3-methylbenzamide (1.0 mol equiv) in dry THF (10 mL) was added pyridine (1.1 mol equiv). A solution of the appropriate acid chloride (1.1 mol equiv) in dry THF (5 mL) was added dropwise over 30 min. The reaction mixture was stirred at ambient temperature under N_2 until TLC analysis confirmed the absence of starting material. The solvent was removed in vacuo, and the product was purified by recrystallization and/or chromatography on silica.

2-(*N*-Acetylamino)-3-methoxybenzamide (7). Compound **7** was prepared from 2-amino-3-methoxybenzamide (0.5 g, 2.8 mmol) and acetyl chloride (0.2 mL, 3.3 mmol): IR 3424, 3249, 3160, 2970, 1660 cm^{-1}; ^1H NMR δ 2.05 (s, 3H, C*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 7.14−7.18 (dd, 1H, *J* = 1.4, 7.5 Hz, 4-Ar*H*), 7.21−7.25 (dd, 1H, *J* = 1.4, 7.6 Hz, 6-Ar*H*), 7.33−7.41 (m, 2H, N*H* and 5-Ar*H*), 7.53 (br s, 1H, N*H*), 9.27 (br s, 1H, N*H*); HRMS (EI) *m*/*z* 208.0846 [M^+ calcd for $C_{10}H_{12}N_2O_3$ 208.0848]. Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

2-(*N*-Benzoylamino)-3-methylbenzamide (8). Compound **8** was prepared from 2-amino-3-methylbenzamide (0.5 g, 3.3 mmol) and benzoyl chloride (0.43 mL, 3.6 mmol): IR 3369, 3319, 3193, 1657 cm^{-1}; ^1H NMR δ 2.33 (s, 3H, C*H*$_3$), 7.36 (t, 1H, 5-Ar*H*), 7.49−7.60 (m, 3H, N*H* and 4/6-Ar*H*), 7.63−7.71 (m, 3H, 3′/4′/5′-Ph*H*), 7.84 (br s, 1H, N*H*), 8.07 (dd, 2H, 2′/6′-Ph*H*), 10.34 (br s, 1H, N*H*); HRMS (EI) *m*/*z* 254.1066 [M^+ calcd for $C_{15}H_{14}N_2O_2$ 254.1055].

2-(*N*-Benzoylamino)-3-methoxybenzamide (9). Compound **9** was prepared from 2-amino-3-methoxybenzamide (0.5 g, 2.8 mmol) and benzoyl chloride (0.4 mL, 3.3 mmol): IR 3489, 3436, 3304, 2839, 1666 cm^{-1}; ^1H NMR δ 3.88 (s, 3H, OC*H*$_3$), 7.24−7.32 (m, 2H, 4/6-Ar*H*), 7.45 (d, 1H, 5-Ar*H*), 7.49 (br s, 1H, N*H*) 7.59−7.73 (m, 4H, N*H*, 3′/4′/5′-Ph*H*) 8.06 (dd, 2H, 2′/6′-Ph*H*) 9.85 (br s, 1H, N*H*); HRMS (EI) *m*/*z* 270.1005 [M^+ calcd for $C_{15}H_{14}N_2O_3$ 270.1004]. Anal. ($C_{15}H_{14}N_2O_2 \cdot 0.1H_2O$) C, H, N.

2-[*N*-(4′-Methoxybenzoyl)amino]-3-methoxybenzamide (10). Compound **10** was prepared from 2-amino-3-methoxybenzamide (0.5 g, 2.8 mmol) and 4-methoxybenzoyl chloride (0.5 mL, 3.3 mmol), in the presence of 4-(dimethylamino)-pyridine (18.4 mg, 0.2 mmol): IR 3382, 3321, 3181, 1673 cm^{-1}; ^1H NMR δ 3.88 (s, 3H, OC*H*$_3$), 3.94 (s, 3H, OC*H*$_3$), 7.15 (d, 2H, *J* = 8.9 Hz, 3′/5′-Ar*H*), 7.23−7.31 (m, 2H, 4/5-Ar*H*), 7.41 (d, 1H, 6-Ar*H*), 7.49 (br s, 1H, N*H*), 7.62 (br s, 1H, N*H*), 8.01−8.05 (d, 2H, *J* = 8.8 Hz, 2′/6′-Ar*H*), 9.73 (br s, 1H, N*H*); HRMS (EI) *m*/*z* 300.1102 [M^+ calcd for $C_{16}H_{16}N_2O_4$ 300.1110]. Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

2-[*N*-(4′-Nitrobenzoyl)amino]-3-methoxybenzamide (11). Compound **11** was synthesized from 2-amino-3-methoxybenzamide (0.5 g, 2.8 mmol) and 4-nitrobenzoyl chloride (0.96 g, 3.0 mmol): IR 3454, 3319, 3297, 1657, 1523, 1355 cm^{-1}; ^1H NMR δ 3.89 (s, 3H, OC*H*$_3$), 7.23−7.33 (m, 2H, 4/5-Ar*H*), 7.41−7.45 (m, 2H, 6-Ar*H* and N*H*), 7.69 (br s, 1H, N*H*), 8.23 (d, 2H, *J* = 9.0 Hz, 2′/6′-Ar*H*), 8.46 (d, 2H, *J* = 8.9 Hz, 3′/5′-Ar*H*), 11.50 (br s, 1H, N*H*); HRMS (EI) *m*/*z* 315.0851 [M^+ calcd for $C_{15}H_{13}N_3O_5$ 315.0855]. Anal. ($C_{15}H_{13}N_3O_5$) C, H, N.

2-[*N*-(4′-Cyanobenzoyl)amino]-3-methylbenzamide (12). Compound **12** was synthesized from 2-amino-3-methylbenzamide (1.0 g, 6.7 mmol) and 4-cyanobenzoyl chloride (1.7 g, 10.0 mmol): IR 3431, 3191, 2226, 1666 cm^{-1}; ^1H NMR δ 2.31 (s, 3H, C*H*$_3$), 7.35 (d, 1H, 7-Ar*H*), 7.41−7.53 (m, 3H, 5/6-Ar*H* + N*H*), 7.83 (br s, 1H, N*H*), 8.07 (d, 2H, *J* = 8.5 Hz, 2′/6′-

Ar*H*), 8.20 (d, 2H, J = 8.5 Hz, 3′/5′-Ar*H*); HRMS (EI) *m/z* 279.1000 [M^+ calcd for $C_{16}H_{13}N_3O_2$ 279.1008]. Anal. ($C_{16}H_{13}N_3O_2$) C, H, N.

2-[*N*-(4′-Azidobenzoyl)amino]-3-methylbenzamide (13). Compound **13** was synthesized from 2-amino-3-methylbenzamide (2.5 g, 16.7 mmol) and 4-azidobenzoyl chloride (3.6 g, 22.9 mmol): IR 3379, 3301, 3189, 2128, 2088, 1653 cm^{-1}; ^1H NMR δ 2.31 (s, 3H, C*H*$_3$), 7.34−7.49 (m, 3H, 7-Ar*H* + 3′/5′-Ar*H*), 7.49−7.54 (m, 3H, 5/6-Ar*H* + N*H*), 7.82 (br s, 1H, N*H*), 8.11 (d, 2H, 2′/6′-Ar*H*), 10.31 (br s, 1H, N*H*); HRMS (EI) *m/z* 295.1066 [M^+ calcd for $C_{15}H_{13}N_5O_2$ 295.1069]. Anal. ($C_{15}H_{13}N_5O_2$) C, H, N.

8-Substituted-quinazolin-4(3*H*)-ones: Method II. General Procedure. The appropriate 3-substituted-2-nitrobenzamide was dissolved in dry THF (10−20 mL), and dry pyridine (1.3 mol equiv) was added. To the resulting solution stirred under N_2 at room temperature was added the requisite acid chloride in dry THF (5 mL) dropwise over 15 min. The reaction mixture was stirred at room temperature until TLC analysis indicated the absence of starting materials. Removal of solvent afforded a residue which was suspended in aqueous NaOH (0.5 M unless indicated otherwise) and stirred, with warming if necessary, until complete dissolution was observed. The solution was neutralized with concentrated aqueous HCl, and the solid which deposited was collected by filtration and washed with water. The product was purified by chromatography on silica and/or recrystallization from a suitable solvent.

8-Substituted-quinazolin-4(3*H*)-ones: Method III. General Procedure. The appropriate 3-substituted-2-(*N*-acylamino)benzamide was suspended in aqueous NaOH, and the solution was stirred, with warming if necessary, until complete dissolution was observed. After neutralization with concentrated aqueous HCl, the resulting precipitate was collected by filtration, washed with water, and dried.

2,8-Dimethylquinazolin-4(3*H*)-one (14). Compound **14** was prepared according to method II from 2-amino-3-methylbenzamide (0.5 g, 3.3 mmol) and acetyl chloride (0.36 mL, 5.0 mmol): IR 3171, 1683 cm^{-1}; ^1H NMR δ 2.44 (s, 3H, C*H*$_3$), 2.57 (s, 3H, C*H*$_3$), 7.40 (t, 1H, 6-Ar*H*), 7.68−7.72 (m, 1H, 7-Ar*H*), 7.94 (dd, 1H, Ar-5*H*), 12.25 (br s, 1H, N*H*); HRMS (EI) *m/z* 174.0790 [M^+ calcd for $C_{10}H_{10}N_2O$ 174.0793]. Anal. ($C_{10}H_{10}N_2O$) C, H, N.

8-Methoxy-2-methylquinazolin-4(3*H*)-one (15). Compound **15** was prepared according to method II from 2-amino-3-methoxybenzamide (1.5 g, 9.0 mmol) and acetyl chloride (1.4 mL, 19.9 mmol): IR 3171, 3034, 1676 cm^{-1}; ^1H NMR δ 2.43 (s, 3H, C*H*$_3$), 3.97 (s, 3H, OC*H*$_3$), 7.39 (dd, 1H, J = 1.9, 7.9 Hz, 7-Ar*H*), 7.46 (t, 1H, J = 7.8 Hz, 6-Ar*H*), 7.71 (dd, 1H, J = 1.9, 7.8 Hz, 5-Ar*H*); HRMS (EI) *m/z* 190.0740 [M^+ calcd for $C_{10}H_{10}N_2O_2$ 190.0742]. Anal. ($C_{10}H_{10}N_2O\cdot0.1H_2O$) C, H, N.

Compound **15** was also prepared by method III from 2-(*N*-acetylamino)-3-methoxybenzamide (**7**) (0.07 g, 0.34 mmol) and aqueous NaOH solution (0.5 M, 2 mL) at room temperature.

8-Methyl-2-phenylquinazolin-4(3*H*)-one (16). Compound **16** was prepared according to method III from 3-methyl-2-(*N*-benzoylamino)benzamide (**8**) (1.0 g, 0.4 mmol) and aqueous NaOH solution (0.5 M, 25 mL) at room temperature: IR 3165, 1675 cm^{-1}; ^1H NMR δ 2.72 (s, 3H, C*H*$_3$), 7.49 (t, 1H, 6-Ar*H*), 7.66 (m, 3H, 3′/4′/5′-Ph*H*), 7.79 (d, 1H, 7-Ar*H*), 8.09 (d, 1H, 5-Ar*H*), 8.30−8.35 (m, 2H, 2′/6′-Ph*H*), 12.65 (br s, 1H, N*H*); HRMS (EI) *m/z* 236.0943 [M^+ calcd for $C_{15}H_{12}N_3O$ 236.0950]. Anal. ($C_{15}H_{12}N_2O\cdot0.1H_2O$) C, H, N.

8-Methoxy-2-phenylquinazolin-4(3*H*)-one (17). Compound **17** was prepared according to method II from 2-amino-3-methoxybenzamide (1.0 g, 6 mmol) and benzoyl chloride (0.8 mL, 6.6 mmol): IR 3191, 3162, 1662 cm^{-1}; ^1H NMR δ 4.06 (s, 3H, OC*H*$_3$), 7.47−7.61 (m, 2H, 6/7-Ar*H*), 7.63−7.69 (m, 3H, 3′/4′/5′-Ph*H*), 7.83 (dd, 1H, Ar-5*H*), 8.27−8.32 (m, 2H, 2′/6′-Ph*H*), 12.70 (br s, 1H, N*H*); HRMS (EI) *m/z* 252.0895 [M^+ calcd for $C_{15}H_{12}N_2O_2$ 252.0899]. Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

Compound **17** was also prepared according to method III from 3-methoxy-2-(*N*-benzoylamino)benzamide (**9**) (0.2 g, 0.74 mmol) and aqueous NaOH (0.5 M, 2 mL) at room temperature.

8-Methyl-2-(4′-nitrophenyl)quinazolin-4(3*H*)-one (18). Compound **18** was prepared according to method II from 2-amino-3-methylbenzamide (0.5 g, 3.3 mmol) and 4-nitrobenzoyl chloride (0.93 g, 5.0 mmol), employing aqueous NaOH (2.5 M, 15 mL) at 100 °C for 2 h in the cyclization step: IR 3088, 1683, 1522, 1350 cm^{-1}; ^1H NMR δ 2.77 (s, 3H, C*H*$_3$), 7.58 (t, 1H, 6-Ar*H*), 7.86 (d, 1H, 7-Ar*H*), 8.14 (d, 1H, 5-Ar*H*), 8.51 (d, 2H, J = 9.2 Hz, 2′/6′-Ar*H*), 8.59 (d, 2H, J = 9.2 Hz, 3′/5′-Ar*H*), 12.90 (br s, 1H, N*H*); HRMS (EI) *m/z* 281.0790 [M^+ calcd for $C_{15}H_{11}N_3O_3$ 281.0800]. Anal. ($C_{15}H_{11}N_3O_3$) C, H, N.

8-Methoxy-2-(4′-nitrophenyl)quinazolin-4(3*H*)-one (19). Compound **19** was prepared according to method III by treatment of 3-methoxy-2-[*N*-(4′-nitrobenzoyl)amino]benzamide (**11**) (0.5 g, 1.6 mmol) with aqueous NaOH (2.5 M, 15 mL) at 100 °C for 2 h: IR 3037, 1686, 1522, 1348 cm^{-1}; ^1H NMR δ 4.06 (s, 3H, OC*H*$_3$), 7.54 (d, 1H, 7-Ar*H*), 7.62 (t, 1H, 6-Ar*H*) 7.84 (d, 1H, Ar-5*H*), 8.50 (s, 4H, 2′/3′/5′,6-Ar*H*), 12.95 (br s, 1H, N*H*); HRMS (EI) *m/z* 297.0766 [M^+ calcd for $C_{15}H_{11}N_3O_4$ 297.0750]. Anal. ($C_{15}H_{11}N_3O_4\cdot0.3H_2O$) C, H, N.

8-Methyl-2-[4′-(trifluoromethyl)phenyl]quinazolin-4(3*H*)-one (20). Compound **20** was prepared by method II from 2-amino-8-methylbenzamide (0.2 g, 1.3 mmol) and 4-(trifluoromethyl)benzoyl chloride (0.22 mL, 1.5 mmol): IR 3175, 1666, 1336, 1320 cm^{-1}; ^1H NMR δ 2.75 (s, 3H, C*H*$_3$), 7.56 (t, 1H, 6-Ar*H*), 7.83 (d, 1H, 7-Ar*H*), 8.08 (m, 3H, 5-Ar*H*, and 2′/6′-Ar*H*), 8.51 (d, 2H, 3′/5′-Ar*H*), 12.85 (br s, 1H, N*H*); MS (EI) *m/z* 304 (M^+). Anal. ($C_{16}H_{11}N_2OF_3$) C, H, N.

8-Methoxy-2-[4′-(trifluoromethyl)phenyl]quinazolin-4(3*H*)-one (21). Compound **21** was synthesized according to method II from 2-amino-3-methoxybenzamide (0.2 g, 1.2 mmol) and 4-(trifluoromethyl)benzoyl chloride (0.2 mL, 1.3 mmol): IR 3116, 1660 cm^{-1}; ^1H NMR δ 4.06 (s, 3H, OC*H*$_3$), 7.50−7.64 (m, 2H, 6/7-Ar*H*), 7.83 (d, 1H, Ar-5*H*), 8.04 (d, 2H, J = 8.4 Hz, 2′/6′-Ar*H*), 8.47 (d, 2H, J = 8.4 Hz, 3′/5′-Ar*H*), 12.91 (br s, 1H, N*H*); MS (EI) *m/z* 320 (M^+). Anal. ($C_{16}H_{11}N_2O_2F_3$) C, H, N.

8-Methyl-2-(4′-cyanophenyl)quinazolin-4(3*H*)-one (22). Compound **22** was prepared according to method II from 2-amino-3-methylbenzamide (0.39 g, 2.6 mmol) and 4-cyanobenzoyl chloride (0.48 g, 2.9 mmol): IR 3170, 2228, 1683 cm^{-1}; ^1H NMR δ 2.73 (s, 3H, C*H*$_3$), 7.55 (t, 1H, J = 7.6 Hz, 6-Ar*H*), 7.83 (d, 1H J = 7.6 Hz, 7-Ar*H*), 8.13−8.17 (m, 3H, 5-Ar*H* and J = 8.7 Hz, 2′/6′-Ar*H*), 8.49 (d, 2H, J = 8.7 Hz, 3′/5′-Ar*H*), 12.90 (br s, 1H, N*H*); HRMS (EI) *m/z* 261.0912 [M^+ calcd for $C_{16}H_{11}N_3O$ 261.0912]. Anal. ($C_{16}H_{11}N_3O$) C, H, N.

8-Methoxy-2-(4′-cyanophenyl)quinazolin-4(3*H*)-one (23). Compound **23** was prepared according to method II from 2-amino-3-methoxybenzamide (0.5 g, 3.0 mmol) and 4-cyanobenzoyl chloride (0.55 g, 3.3 mmol): IR 3413, 2841, 2230, 1681 cm^{-1}; ^1H NMR δ 4.05 (s, 3H, OC*H*$_3$), 7.48−7.53 (m, 1H, 7-Ar*H*), 7.59 (t, 1H, 6-Ar*H*), 7.79−7.83 (m, 1H, 5-Ar*H*), 8.14 (d, 2H, J = 8.5 Hz, 2′/6′-Ar*H*,), 8.43 (d, 2H, J = 8.5 Hz, 3′/5′-Ar*H*), 12.95 (br s, 1H, N*H*); HRMS (EI) *m/z* 277.0853 [M^+ calcd for $C_{16}H_{11}N_3O_2$ 277.0851]. Anal. ($C_{16}H_{11}N_3O_2\cdot0.75H_2O$) C, H, N.

8-Methyl-2-(4′-methoxyphenyl)quinazolin-4(3*H*)-one (24). Compound **24** was prepared as described in method II from 2-amino-3-methylbenzamide (0.2 g, 1.3 mmol) and benzoyl chloride (0.22 mL, 1.5 mmol), with the addition of 4-(dimethylamino)pyridine (8 mg, 5 mmol): IR 3177, 1674 cm^{-1}; ^1H NMR δ 2.71 (s, 3H, C*H*$_3$), 3.95 (s, 3H, OC*H*$_3$), 7.20 (d, 2H, J = 8.9 Hz, 3′/5′-ArH), 7.46 (t, 1H, 6-Ar*H*), 7.78 (d, 1H, 7-Ar*H*), 8.07 (d, 1H, 5-Ar*H*), 8.29 (d, 2H, 2′/6′-ArH), 12.63 (br s, 1H, N*H*); HRMS (EI) *m/z* 266.1046 [M^+ calcd for $C_{16}H_{14}N_2O_2$ 266.1055]. Anal. ($C_{16}H_{14}N_2O_2$) C, H, N.

8-Methoxy-2-(4′-methoxyphenyl)quinazolin-4(3*H*)-one (25). Compound **25** was prepared according to method II from 2-amino-3-methoxybenzamide (0.25 g, 0.8 mmol) and 4-methoxybenzoyl chloride (0.5 mL, 3.3 mmol) with the addition of 4-(dimethylamino)pyridine (18 mg, 0.2 mmol): IR 3174, 1667 cm^{-1}; ^1H NMR δ 3.94 (s, 3H, OC*H*$_3$), 4.04 (s, 3H, OC*H*$_3$), 7.19 (d, 2H, J = 9.0 Hz, 3′/5′-Ar*H*), 7.42−7.55 (m, 2H, 6/7-Ar*H*), 7.76−7.81 (m, 1H, 5-Ar*H*), 8.29 (d, 2H, J = 9.0 Hz, 2′/6′-Ar*H*); HRMS (EI) *m/z* 282.1008 [M^+ calcd for $C_{16}H_{13}N_2O_3$

282.1004]. Anal. ($C_{16}H_{11}N_3O \cdot 0.1CH_3OH$) C, H; N: calcd, 5.08; found, 4.50.

Compound **25** was also prepared according to method III from 3-methoxy-2-[*N*-(4′-methoxybenzoyl)amino]benzamide (**10**) (0.25 g, 0.8 mmol) with aqueous NaOH (2.5 M, 40 mL) under reflux for 2 h.

8-Methyl-2-(4′-azidophenyl)quinazolin-4(3*H*)-one (26). Compound **26** was prepared according to method III from 2-[*N*-(4′-azidobenzoyl)amino]-3-methylbenzamide (**13**) (0.5 g, 0.8 mmol) with aqueous NaOH (0.5 M, 20 mL): IR 3076, 2123, 2086, 1679 cm^{-1}; ^1H NMR δ 2.71 (s, 3H, C*H*$_3$), 7.39 (d, 2H, J = 8.7 Hz, 3′/5′-Ar*H*), 7.46−7.51 (m, 1H, 6-Ar*H*), 7.77−7.80 (m, 1H, 7-Ar*H*), 8.07−8.09 (m, 1H, 5-Ar*H*), 8.39 (d, 2H, J = 8.7 Hz, 2′/6′-Ar*H*), 12.59 (br s, 1H, NH); HRMS (EI) m/z 277.0972 [M^+ calcd for $C_{15}H_{11}N_5O$ 277.0963]. Anal. ($C_{15}H_{11}N_5O$) C, H, N.

8-Methoxy-2-(4′-azidophenyl)quinazolin-4(3*H*)-one (27). Compound **27** was prepared according to method II from 2-amino-3-methoxybenzamide (0.43 g, 2.6 mmol) and 4-azidobenzoyl chloride (0.7 g, 3.9 mmol): IR 3439, 2127, 2087, 1679 cm^{-1}; ^1H NMR δ 4.04 (s, 3H, OC*H*$_3$), 7.39 (d, 2H, J = 8.5 Hz, 3′/5′-Ar*H*), 7.45−7.58 (m, 2H, 6/7-Ar*H*), 7.80 (dd, 1H, 5-Ar*H*), 8.33 (d, 2H, J = 8.6 Hz, 2′/6′-Ar*H*), 12.65 (br s, 1H, NH); HRMS (EI) m/z 293.0914 [M^+ calcd for $C_{15}H_{11}N_5O_2$ 293.0913]. Anal. ($C_{15}H_{11}N_5O_2 \cdot 0.5H_2O$) C, H, N.

2-(4′-Aminophenyl)quinazolin-4(3*H*)-ones: Method IV. General Procedure. The appropriate 2-(4′-nitrophenyl)-quinazolinone was suspended in methanol (100 mL), palladium−carbon catalyst (50 mg) was added, and the reaction mixture was stirred under H_2 at ambient temperature and pressure until no further gas absorption was observed. After filtration through Celite the solvent was removed to afford the corresponding 2-(4′-aminophenyl)quinazolinone.

8-Methyl-2-(4′-aminophenyl)quinazolin-4(3*H*)-one (28). Compound **28** was prepared as detailed in method IV from 8-methyl-2-(4′-nitrophenyl)quinazolin-4(3*H*)-one (**18**) (0.1 g, 0.36 mmol): IR 3432, 3397, 2950, 1663 cm^{-1}; ^1H NMR δ 2.67 (s, 3H, C*H*$_3$), 5.96 (br s, 2H, N*H*$_2$), 6.75 (d, 2H, J = 8.7 Hz, 3′/5′-Ar*H*), 7.39 (t, 1H, 6-Ar*H*), 7.73 (d, 1H, 7-Ar*H*), 8.02 (d, 1H, 5-Ar*H*), 8.11 (d, 2H, J = 8.7 Hz, 2′/6′-ArH), 12.10 (br s, 1H, N*H*); HRMS (EI) m/z 251.1057 [M^+ calcd for $C_{15}H_{13}N_3O$ 251.1059]. Anal. ($C_{15}H_{13}N_3O$) C, H, N.

8-Methoxy-2-(4′-aminophenyl)quinazolin-4(3*H*)-one (29). Compound **29** was prepared according to method IV from 8-methoxy-2-(4′-nitrophenyl)quinazolin-4(3*H*)-one (**19**) (0.1 g, 0.34 mmol): IR 3446, 3337, 1671 cm^{-1}; ^1H NMR δ 4.02 (s, 3H, OC*H*$_3$), 5.93 (br s, 2H, N*H*$_2$), 6.73 (d, 2H, J = 8.7 Hz, 3′/5′-Ar*H*), 7.43−7.44 (m, 2H, 6/7-Ar*H*), 7.72−7.77 (m, 1H, 5-Ar*H*), 8.06 (d, 2H, J = 8.7 Hz, 2′/6′-Ar*H*), 12.10 (br s, 1H, N*H*); HRMS (EI) m/z 267.1013 [M^+ calcd for $C_{15}H_{13}N_3O_2$ 267.1008]. Anal. ($C_{15}H_{13}N_3O_2 \cdot 0.4H_2O$) C, H, N.

8-Hydroxy-2-(4′-aminophenyl)quinazolin-4(3*H*)-one (41). Compound **41** was prepared according to method IV from 8-hydroxy-2-(4′-nitrophenyl)quinazolin-4(3*H*)-one (**36**) (50 mg, 0.18 mmol): IR 3452, 3432, 3359, 1592 cm^{-1}; ^1H NMR δ 5.94 (br s, 2H, N*H*$_2$), 6.74 (d, 2H, J = 8.6 Hz, 3′/5′-Ar*H*), 7.25−7.38 (m, 2H, 6/7-Ar*H*), 7.63 (d, 1H, 5-Ar*H*), 8.29 (d, 2H, J = 8.5 Hz, 2′/6′-Ar*H*), 9.40 (br s, 1H, O*H*), 12.15 (br s, 1H, N*H*); HRMS (EI) m/z 204.0895 [M^+ calcd for $C_{14}H_{11}N_3O_2$ 204.0899]. Anal. ($C_{14}H_{11}N_3O_2 \cdot 0.2H_2O$) C, H, N.

8-Methyl-2-(4′-carboxyphenyl)quinazolin-4(3*H*)-one (30). To a suspension of 8-methyl-2-(4′-cyanophenyl)quinazolin-4(3*H*)-one (**22**) (0.1 g, 0.4 mmol) in ethanol (50 mL) was added aqueous NaOH solution (5 M, 20 mL), and the reaction mixture was refluxed for 4 h. The ethanol was removed in vacuo, and the pH of the residual solution was adjusted to 6.5. The resultant pale-yellow solid was collected and washed with water and a minimum volume of cold methanol: IR 3166, 3049, 1696, 1674 cm^{-1}; ^1H NMR δ 2.60 (s, 3H, C*H*$_3$), 7.39 (t, 1H, 6-Ar*H*), 7.69 (d, 1H, 7-Ar*H*), 7.97 (d, 1H, 5-Ar*H*), 8.05 (d, 2H, J = 8.5 Hz, 2′/6′-Ar*H*), 8.29 (d, 2H, J = 8.5 Hz, 3′/5′-Ar*H*), 12.70 (br s, 1H, O*H*), 13.30 (br s, 1H, N*H*); HRMS (EI) m/z 280.0844 [M^+ calcd for $C_{16}H_{12}N_2O_3$ 280.0848]. Anal. ($C_{16}H_{12}N_2O_3$) C, H; N: calcd, 10.00; found, 9.38.

8-Methoxy-2-(4′-carboxyphenyl)quinazolin-4(3*H*)-one (31). This compound was prepared in the same manner as **30** from 8-methoxy-2-(4′-cyanophenyl)quinazolin-4(3*H*)-one (**23**) (0.5 g, 1.8 mmol): ^1H NMR δ 4.05 (s, 3H, OC*H*$_3$), 7.51 (d, 1H, 7-Ar*H*), 7.59 (m, 1H, 6-Ar*H*), 7.81 (d, 1H, 5-Ar*H*), 8.18 (d, 2H, J = 8.4 Hz, 3′/5′-Ar*H*), 8.37 (d, 2H, J = 8.4 Hz, 2′/6′-Ar*H*); HRMS (EI) m/z 296.0811 [M^+ calcd for $C_{16}H_{12}N_2O_4$ 296.0797].

8-Methyl-2-(4′-carboxamidophenyl)quinazolin-4(3*H*)-one (32). To a suspension of 8-methyl-2-(4′-carboxyphenyl)-quinazolin-4(3*H*)-one (**30**) (0.2 g, 0.7 mmol) in THF (20 mL) were added thionyl chloride (0.18 mL, 2.6 mmol) and anhydrous DMF (4 drops). After stirring at room temperature for 24 h, the reaction mixture was added dropwise to concentrated NH_4OH (20 mL) and stirred at room temperature for 1 h. The volatiles were removed under reduced pressure, and the remaining solid was collected and washed with water. Chromatography on silica, employing DCM−MeOH (95:5) as eluent, afforded **32** as a cream solid: IR 3448, 3200, 1680 cm^{-1}; ^1H NMR δ 2.74 (s, 3H, C*H*$_3$), 7.52 (t, 1H, 6-Ar*H*), 7.67 (br s, 1H, N*H*), 7.82 (d, 1H, 7-Ar*H*), 8.10 (m, 3H, 5-Ar*H* and J = 8.3 Hz, 3′/5′-Ar*H*), 8.40 (d, 2H, J = 8.3 Hz, 2′/6′-Ar*H*), 12.70 (br s, 1H, N*H*); HRMS (EI) m/z 279.1021 [M^+ calcd for $C_{16}H_{13}N_3O_2$ 279.1008]. Anal. ($C_{16}H_{13}N_3O_2$) C, H, N.

8-Methoxy-2-(4′-carboxamidophenyl)quinazolin-4(3*H*)-one (33). Compound **33** was prepared from 8-methoxy-2-(4′-carboxyphenyl)quinazolin-4(3*H*)-one (**31**) (0.25 g, 0.8 mmol) in a manner analogous to **32**: IR 3396, 3201, 1672, 1597 cm^{-1}; ^1H NMR δ 4.06 (s, 3H, OC*H*$_3$), 7.47−7.60 (m, 2H, 6/7-Ar*H*), 7.66 (br s, 1H, N*H*), 7.82 (d, 1H, 5-Ar*H*), 8.13 (d, 2H, J = 8.4 Hz, 3′/5′-Ar*H*), 8.26 (br s, 1H, N*H*), 8.37 (d, 2H, J = 8.4 Hz, 2′/6′-Ar*H*), 12.8 (br s, 1H, N*H*); HRMS (EI) m/z 294.0873 [M^+ − 1 calcd for $C_{16}H_{13}N_3O_3$ 294.0879]. Anal. ($C_{16}H_{13}N_3O_3$) C, H, N.

8-Methyl-2-[4′-(carboxymethyl)phenyl]quinazolin-4(3*H*)-one (34). To a suspension of 8-methyl-2-(4′-carboxyphenyl)-quinazolin-4(3*H*)-one (**30**) (0.2 g, 0.7 mmol) in THF (20 mL) were added thionyl chloride (0.09 mL, 1.3 mmol) and 2 drops of anhydrous DMF. After the mixture was stirred at room temperature for 12 h the mixture was added dropwise to a solution of methanol (10 mL). After stirring for a further 2 h, removal of solvents furnished the product as a cream solid which was purified by chromatography on silica, employing petrol−EtOAc (8:2) as eluent: IR 3167, 1734, 1681; ^1H NMR δ 2.73 (s, 3H, C*H*$_3$), 4.00 (s, 3H, OC*H*$_3$), 7.53 (t, 1H, 6-Ar*H*), 7.82 (d, 1H, 7-Ar*H*), 8.10 (d, 1H, Ar-5*H*), 8.20 (d, 2H, J = 8.4 Hz, 3′/5′-Ar*H*), 8.45 (d, 2H, J = 8.4 Hz, 2′/6′-Ar*H*); HRMS (EI) m/z 294.1016 [M^+ calcd for $C_{17}H_{14}N_2O_3$ 294.1004]. Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

8-Hydroxyquinazolin-4(3*H*)-ones: Method V. General Procedure. The appropriate 8-methoxy-2-substituted quinazolin-4(3*H*)-one was suspended in BBr_3 (1.0 M in DCM) and stirred under reflux until no starting material was evident by TLC analysis. The solvent was removed, and the solid residue was redissolved in aqueous NaOH (2.5 M) and stirred for 3 h at room temperature. The solution was neutralized with concentrated aqueous HCl, and the resulting precipitate was collected, washed with water, and dried.

8-Hydroxy-2-methylquinazolin-4(3*H*)-one (6). Compound **6** was prepared from 8-methoxy-2-methylquinazolin-4(3*H*)-one (**15**) (0.7 g, 3.7 mmol) and BBr_3 (8.4 mL) according to method V: IR 3320, 3175, 3142, 1671 cm^{-1}; ^1H NMR δ 2.48 (s, 3H, C*H*$_3$), 7.22−7.27 (m, 2H, 7-Ar*H*), 7.30 (t, 1H, 6-Ar*H*), 7.57−7.63 (m, 1H, Ar-5*H*), 9.57 (br s, 1H, O*H*), 12.26 (br s, 1H, N*H*); HRMS (EI) m/z 176.0585 [M^+ calcd for $C_9H_8N_2O_2$ 176.0586]. Anal. ($C_9H_8N_2O_2$) C, H, N.

8-Hydroxy-2-phenylquinazolin-4(3*H*)-one (35). Compound **35** was prepared according to method V from 8-methoxy-2-phenylquinazolin-4(3*H*)-one (**17**) (0.50 g, 2 mmol) and BBr_3 (6.0 mL): IR 3380, 3152, 3125, 1642 cm^{-1}; ^1H NMR δ 7.35 (d, 1H, 7-Ar*H*), 7.46 (t, 1H, 6-Ar*H*), 7.66−7.72 (m, 4H, 5-Ar*H* and 3′/4′/5′-Ph*H*), 8.53 (dd, 2H, 2′/6′-Ph*H*), 9.75 (br s, 1H, O*H*), 12.60 (br s, 1H, N*H*); HRMS (EI) m/z 238.0745 [M^+ calcd for $C_{14}H_{10}N_2O_2$ 238.0742]. Anal. ($C_{14}H_{10}N_2O_2 \cdot 0.1H_2O$) C, H; N: calcd, 70.05; found, 69.54.

8-Hydroxy-2-(4′-nitrophenyl)quinazolin-4(3*H*)-one (36). Compound **36** was prepared according to method V from 8-methoxy-2-(4′-nitrophenyl)quinazolin-4(3*H*)-one (**19**) (0.20 g 0.7 mmol) and BBr_3 (3.0 mL): IR 3408, 3387, 1686, 1523 cm^{-1}; ^1H NMR δ 7.37 (d, 1H, 7-Ar*H*), 7.51 (t, 1H, 6-Ar*H*), 7.71 (d, 1H, 5-Ar*H*), 8.47 (d, 2H, J = 8.8 Hz, 2′/6′-Ar*H*), 8.77 (d, 2H, J = 8.8 Hz, 3′/5′Ar*H*), 9.98 (br s, 1H, O*H*), 12.85 (br s, 1H, N*H*); HRMS (EI) *m/z* 283.0591 [M^+ calcd for $C_{14}H_9N_3O_4$ 283.0593]. Anal. ($C_{14}H_9N_3O_4$) C, H, N.

8-Hydroxy-2-[4′-(trifluoromethyl)phenyl]quinazolin-4(3*H*)-one (37). Treatment of 8-methoxy-2-[4′-(trifluoromethyl)phenyl]quinazolin-4(3*H*)-one (**21**) (0.1 mg, 0.3 mmol) with BBr_3 (9.4 mL) as detailed in method V afforded compound **37**: ^1H NMR δ 7.35 (dd, 1H, J = 1.6, 7.9 Hz, 7-Ar*H*), 7.48 (t, 1H, J = 7.8 Hz, 6-Ar*H*), 7.69 (dd, 1H, J = 1.5, 7.8 Hz, 5-Ar*H*), 8.03 (d, 2H, J = 8.4 Hz, 3′/5′-Ar*H*), 8.71 (d, 2H, J = 8.3 Hz, 2′/6′-Ar*H*), 9.95 (br s, 1H, O*H*), 12.8 (br s, 1H, N*H*); HRMS (EI) *m/z* 306.0613 [M^+ calcd for $C_{15}H_9N_2O_2F_3$ 306.0616]. Anal. ($C_{15}H_9N_2O_2F_3 \cdot 1.5H_2O$) C, H, N.

8-Hydroxy-2-(4′-cyanophenyl)quinazolin-4(3*H*)-one (38). Compound **38** was prepared according to method V from 8-methoxy-2-(4′-cyanophenyl)quinazolin-4(3*H*)-one (**23**) (0.2 g, 0.72 mmol) and BBr_3 (3.6 mL): IR 3411, 2230, 1681 cm^{-1}; ^1H NMR δ 7.41 (dd, 1H, J = 1.4, 7.9 Hz, 7-Ar*H*), 7.55 (t, 1H, J = 7.9 Hz, 6-Ar*H*), 7.75 (dd, 1H, J = 1.4, 7.8 Hz, Ar-5*H*), 8.21 (d, 2H, J = 8.5 Hz, 2′/6′-Ar*H*), 8.77 (d, 2H, J = 8.5 Hz, 3′/5′-Ar*H*), 9.90 (br s, 1H, N*H*); HRMS (EI) *m/z* 263.0692 [M^+ calcd for $C_{15}H_9N_3O$ 263.0695].

8-Hydroxy-2-(4′-hydroxyphenyl)quinazolin-4(3*H*)-one (39). Compound **39** was synthesized from 8-methoxy-2-(4′-methoxyphenyl)quinazolin-4(3*H*)-one (**25**) (0.2 g, 0.71 mmol) and BBr_3 (2.2 mL) according to method V: IR 3374, 1684 cm^{-1}; ^1H NMR δ 6.98 (d, 2H, J = 8.8 Hz, 3′/5′-Ar*H*), 7.29 (dd, 1H, J = 1.6, 7.8 Hz, 7-Ar*H*), 7.38 (t, 1H, J = 7.8 Hz, 6-Ar*H*), 7.64 (dd, 1H, J = 1.7, 7.7 Hz, 5-Ar*H*), 8.40 (d, 2H, J = 8.8 Hz, 2′/6′-ArH), 9.60 (br s, 1H, O*H*), 12.30 (br s, 1H, N*H*); HRMS (EI) *m/z* 254.0685 [M^+ calcd for $C_{14}H_{10}N_2O_3$ 254.0691]. Anal. ($C_{14}H_{12}N_2O_3$) C, H, N.

8-Methyl-2-(4′-hydroxyphenyl)quinazolin-4(3*H*)-one (40). Compound **40** was prepared according to method V from 8-methyl-2-(4′-methoxyphenyl)quinazolin-4(3*H*)-one (**24**) (0.2 g, 0.75 mmol) and BBr_3 (2.3 mL): IR 3246, 3169, 1680 cm^{-1}; ^1H NMR δ 2.69 (s, 3H, C*H*$_3$), 7.00 (d, 2H, J = 6.9 Hz, 3′/5′-Ar*H*), 7.43 (t, 1H, 6-Ar*H*), 7.76 (d, 1H, 7-Ar*H*), 8.05 (m, 1H, 5-Ar*H*), 8.22 (d, 2H, J = 6.9 Hz, 2′/6′-Ar*H*), 10.30 (br s, 1H, OH), 12.40 (br s, 1H, NH); HRMS (EI) *m/z* 252.0902 [M^+ calcd for $C_{15}H_{12}N_2O_4$ 252.0899]. Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

8-Methoxy-3-*N*-methyl-2-methylquinazolin-4(3*H*)-one (42). A mixture of 8-methoxy-2-methylquinazolin-4(3*H*)-one (**15**) (0.5 g, 2.6 mmol), potassium carbonate (0.36 g, 2.6 mmol), and iodomethane (0.16 mL, 2.6 mmol) in dry acetonitrile (60 mL) was refluxed for 34 h. The solvent was removed in vacuo to leave a cream solid which was redissolved in EtOAc (30 mL), washed with water (2 × 25 mL), and dried ($MgSO_4$), and the solvent was removed to afford the product as a pale-yellow solid: IR 1683, 1659, 1599 cm^{-1}; ^1H NMR δ 2.66 (s, 3H, C*H*$_3$), 3.62 (s, 3H, N-*CH*$_3$), 3.98 (s, 3H, O*CH*$_3$), 7.40 (d, 1H, 7-Ar*H*), 7.48 (t, 1H, 6-Ar*H*), 7.71−7.75 (m, 1H, Ar-5H); HRMS (EI) *m/z* 190.0752 [M^+ calcd for $C_{11}H_{12}N_2O_2$ 190.0742]. Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

8-Hydroxy-3-*N*-methyl-2-methylquinazolin-4(3*H*)-one (43). Compound **43** was synthesized according to method V from 8-methoxy-3-*N*-methyl-2-methylquinazolin-4(3*H*)-one (**42**) (0.20 g, 0.98 mmol) and BBr_3 (2.9 mL): IR 3388, 1689, 1599; ^1H NMR δ 2.71 (s, 3H, C*H*$_3$), 3.63 (s, 3H, N*CH*$_3$), 7.25 (dd, 1H, J = 1.5, 7.8 Hz, 7-Ar*H*), 7.38 (t, 1H, J = 7.8 Hz, 6-Ar*H*), 7.62 (dd, 1H, J = 1.5, 7.8 Hz, 5-Ar*H*); HRMS (EI) *m/z* 190.0752 [M^+ calcd for $C_{10}H_{10}N_2O_2$ 190.0742]. Anal. ($C_{10}H_{10}N_2O_2 \cdot 0.1H_2O$) C, H, N.

Cytotoxicity Studies. L1210 cells were maintained as exponentially growing cultures (<8 × 10^5 cells/mL) in RPMI 1640 medium supplemented with 10% fetal calf serum (Sigma, Poole, U.K.), at 37 °C in an atmosphere of 5% CO_2 in air. The cell-doubling time was approximately 12 h. Cells were tested for mycoplasma contamination[36] every 4−8 weeks and found to be negative. Compound **6** was dissolved in DMSO at 100 mM and stored at −20 °C. MTIC (5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide) (a gift from Dr. C. Bleasdale, Department of Chemistry, The University, Newcastle upon Tyne, U.K.) was dissolved in DMSO shortly before use. Drugs were diluted in DMSO where appropriate and added to cell cultures such that the final DMSO concentration was always 1%. Unless otherwise stated, all other chemicals were obtained from Sigma (Poole, U.K.).

Duplicate samples of L1210 cells were diluted to 10^5/mL, in medium containing varying concentrations of MTIC, in the presence or absence of compound **6** (200 mM) for 20 min, centrifuged (200*g*, 25 °C), and resuspended in fresh medium containing **6** (200 mM) for a further 16 h. After the exposure period cells were centrifuged (200*g*, 25 °C) to remove the drug, resuspended in fresh medium, and counted (coulter counter Z1, Coulter Electronics, Luton, U.K.). Cell suspensions were then further diluted and dispensed in triplicate in medium (1 mL) into sterile polyurethane tubes (Falcon, Becton Dickinson, Oxford, U.K.), at a density estimated to give 10−60 colonies, and 5 mL of 0.15% (w/v) agarose (SeaKem ME agarose, Flowgen, Sittingbourne, U.K.) in medium was added. The tubes were incubated for 1−2 weeks to allow colony growth and then poured into dishes containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 0.5 mg/mL, 1 mL) to visualize the viable colonies. After 4 h colonies were counted, and the plating efficiency, relative to the appropriate control (DMSO alone or PARP inhibitor alone), was calculated. Control incubations normally gave approximately 100% plating efficiency.

For γ-irradiation studies cells were dispensed into sterile plastic bijoux bottles (Bibby Sterilin, Aldershot, U.K.), and duplicate samples were exposed to a ^{137}Cs source (Gammacell 1000 Elite, Nordian International Inc., Canada). L1210 cells were kept on ice immediately before and after irradiation. Cells were returned to 37 °C and allowed to recover, for 2 h, in the presence or absence of 200 mM **6** and then counted and seeded for colony formation in 0.15% (w/v) agarose as above.

For chemo- and radiopotentiation the degree of potentiation (enhancement factor) was calculated by comparing the IC_{90} or ID_{90} (the concentration of drug or dose of irradiation causing 90% cell death or a 10% relative plating efficiency) of the cytotoxic agent alone, with the IC_{90} or ID_{90} in the presence of the PARP inhibitor.

Acknowledgment. We thank the Cancer Research Campaign and Cancer Research Campaign Technology for generous support and the Engineering and Physical Sciences Research Council, U.K., for the award of a studentship to S.S. We also thank Dr. W. R. Leopold, Parke-Davis Pharmaceuticals, Ann Arbor, MI, for the provision of PD128763.

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JM980273T