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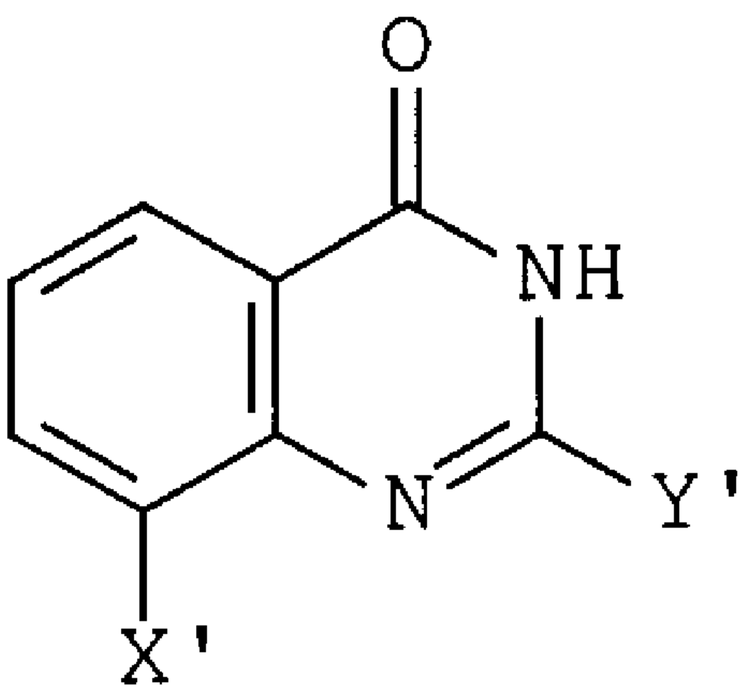


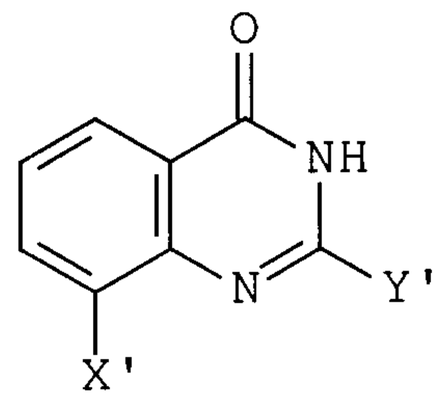
II

(57) Abrégé/Abstract:
A range of quinazolinone compounds are disclosed which can act as potent inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase or PARP enzyme (EC 2.4.2.30), and which thereby can provide useful therapeutic compounds for use in conjunction with DNA-damaging cytotoxic drugs or radiotherapy to potentiate the effects of the latter. The compounds disclosed comprise certain quinazolinone compounds having the general structural formula II (see formula II) or pharmaceutically acceptable salt thereof, characterised in that X' represents hydroxyl, alkyl, alkoxy or an optionally substituted aryl (e.g. phenyl) or aralkyl (e.g. benzyl) group, and Y' represents hydrogen, alkyl or an optionally substituted aryl (e.g. phenyl) or aralkyl (e.g. benzyl) group.

ABSTRACT

PARP DNA Repair Enzyme Inhibitors

A range of quinazolinone compounds are disclosed which can act as potent inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase or PARP enzyme (EC 2.4.2.30), and which thereby can provide useful therapeutic compounds for use in conjunction with DNA-damaging cytotoxic drugs or radiotherapy to potentiate the effects of the latter. The compounds disclosed comprise certain quinazolinone compounds having the general structural formula II



II

or pharmaceutically acceptable salt thereof,

characterised in that

X' represents hydroxyl, alkyl, alkoxy or an optionally substituted aryl (e.g. phenyl) or aralkyl (e.g. benzyl) group,

and

Y' represents hydrogen, alkyl or an optionally substituted aryl (e.g. phenyl) or aralkyl (e.g. benzyl) group.

QUINAZOLINONE COMPOUNDS

This application is a division of PCT International Application No. PCT/GB95/00513 bearing Canadian Application Serial No. 2,184,747 with the International Filing Date of March 9, 1995.

The present invention relates to certain quinazolinone compounds that are of interest as being at least potentially useful chemotherapeutic agents by virtue of an ability to inhibit the activity of the enzyme poly ADP-ribosyltransferase (EC 2.4.2.30), also known as poly(ADP-ribose) polymerase, commonly referred to as ADPRT or PARP. In general, the latter abbreviation, PARP, will be used throughout the present specification.

BACKGROUND

At least in higher organisms, the enzyme poly ADP-ribosyltransferase is known to catalyse a transfer of the ADP-ribose moiety from the oxidized form NAD^+ of nicotinamide adenine dinucleotide to nuclear acceptor proteins so as to form homo ADP-ribose polymers, and this process has been implicated in a number of cellular events such as, for example, repair of DNA damage, development of cellular differentiation, transformation of cells by oncogenes, and gene expression. A common feature in a number of these processes is the formation and repair of DNA strand breaks and the stage which involves the PARP enzyme appears to be that of DNA ligase II-mediated strand rejoining. In the majority of cases a role for poly ADP-ribosylation has been implicated by the use of inhibitors of the PARP enzyme, and this has led to suggestions that such inhibitors, by interfering with the intracellular DNA repair mechanism, may have a useful chemotherapeutic role insofar as they should be able to modify treatment resistance characteristics and potentiate or enhance the effectiveness of cytotoxic drugs in chemotherapy or of radiation in radiotherapy where a primary effect of the treatment is that of

causing DNA damage in target cells, as for example in many forms of antitumour therapy.

In this connection, several classes of PARP inhibitors are already known, including benzamide itself and various nicotinamide and benzamide analogues, especially 3-substituted benzamides with small substituent groups such as 3-amino, 3-hydroxy and 3-methoxy. PARP inhibitory activity of certain N-substituted benzamides has also been reported in EP-A-0305008 wherein it has also been proposed to use these compounds in medicine for increasing the cytotoxicity of radiation or of chemotherapeutic drugs.

Regarding this use of benzamides as chemotherapeutic agents, a number of studies on such compounds that are known to exhibit PARP inhibitory activity have confIRmed that they can potentiate the cytoxicity of a range of antitumour agents *in vitro*, for example, bleomycin and methylating drugs. More limited data has further indicated that such benzamides can also potentiate the activity of cytotoxic drugs *in vivo*, although the dose requIRements have appeared to be rather high (e.g. in the region of 0.5g kg^{-1} per dose for 3-aminobenzamide) and there may be associated problems in preparing satisfactory pharmaceutical formulations and in avoiding toxicity limitations. Furthermore, a number of the known benzamides have also been shown clearly to have potential as radiosensitizers, increasing for example ionising radiation-induced tumour cell kill both *in vitro* and *in vivo*, and it is believed that in many cases this effect is related to these compounds acting as PARP inhibitors and interfering with DNA repair.

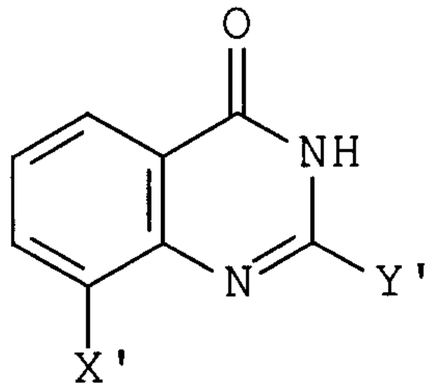
However, notwithstanding the existing data from *in vitro* and *in vivo* studies suggesting that PARP inhibitors have considerable potential as useful chemotherapeutic agents which merit further clinical evaluation, for instance in connection with cancer therapy, currently available known PARP inhibitors are not considered as yet to be entirely

suitable to represent candidate drugs. Accordingly, there is a need to find and develop a greater range of compounds having potentially useful PARP inhibitory properties.

DISCLOSURE OF THE INVENTION

The present invention identifies a new range or ranges of compounds of interest as PARP inhibitors that can be useful in medicine, especially when administered in conjunction with at least certain cytotoxic drugs or with radiotherapy for increasing the cytotoxic effectiveness thereof. In general, the compounds of this invention as hereinbelow defined include certain quinazolinones of which at least some may be formed by molecular rearrangement of related benzamide compounds. By virtue of their structure in general such compounds are adapted to act as an alternative substrate to NAD^+ for the PARP enzyme.

More specifically, from one aspect, the invention resides in the use of a compound as herein defined for the manufacture of a medical or veterinary preparation for use in therapy for inhibiting activity of the enzyme poly(ADP-ribose)polymerase or PARP (also known as ADP-ribosyl transferase or ADPRT), such enzyme inhibition constituting an element of a therapeutic treatment, said compound providing an active PARP enzyme inhibiting agent and being a quinazolinone compound having the general structural formula II



II

or a pharmaceutically acceptable salt thereof,

characterised in that

X' represents hydroxyl, alkyl, alkoxy, or an optionally substituted aryl (e.g. phenyl) or aralkyl (e.g. benzyl) group,

and

Y' represents hydrogen, alkyl or an optionally substituted aryl (e.g. phenyl) or aralkyl (e.g. benzyl) group.

The invention also provides for use in therapy as active pharmaceutical substances quinazolinone compounds having the general structural formula II (or a pharmaceutically acceptable salt thereof) in which

X' represents hydroxyl, alkyl or alkoxy

and

Y' represents alkyl or an optionally substituted aralkyl (e.g. benzyl) group or an optionally substituted phenyl group other than a phenyl group having a 4-propoxy substituent or a 2-alkoxy substituent,

subject to a proviso that

if X' is methyl, Y' is not butyl,

if X' is methoxy, Y' is not methyl or 4-hydroxyphenyl, and

if X' is hydroxy, Y' is not methyl or ethyl or phenyl.

The invention further provides novel quinazolinone compounds having the general structural formula II (or a pharmaceutically acceptable salt thereof) in which:

X' represents hydroxyl, alkyl or alkoxy

and

Y' represents alkyl or an optionally substituted aralkyl (e.g. benzyl) group or an optionally substituted phenyl group other than a phenyl group

having a 4-propoxy substituent or a 2-alkoxy substituent,

subject to a proviso that

if X' is methyl, Y' is not butyl, isopropyl, phenyl or 2-aminophenyl,

if X' is ethyl, Y' is not 4-hydroxyphenyl,

if X' is methoxy, Y' is not methyl, isopropyl, 4-methylphenyl, 4-hydroxyphenyl or 4-methoxyphenyl,

if X' is ethoxy, Y' is not isopropyl,

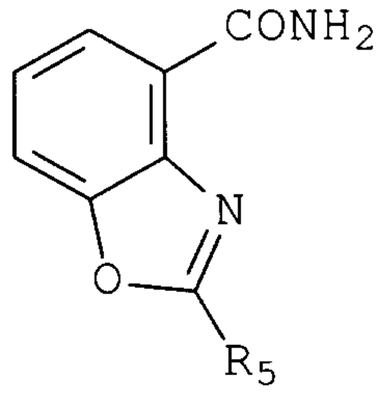
if X' is propoxy, Y' is not a halogen substituted phenyl group, and

if X' is hydroxy, Y' is not methyl or ethyl or phenyl.

Alkyl groups when present as such or as a moiety in other groups such as alkoxy will generally be composed of 1-8 carbon atoms, preferably 1-6 carbon atoms, and more usually 1-4 carbon atoms.

In a preferred embodiment, Y' is phenyl or a phenyl group having a substituent selected from the group consisting of $-NO_2$, $-NH_2$, -OH and alkyl.

It has been found that in attempting to prepare benzoxazole-4-carboxamide compounds having a structural formula IV



IV

wherein R^5 is H, alkyl or an optionally substituted aralkyl or aryl group, in some methods of preparation which could be expected to yield the desired compound the product is liable to undergo a molecular rearrangement

(especially if liquid ammonia is used to form the carboxamide) and an 8-hydroxy quinazolinone derivative is obtained instead of the expected benzoxazole. Unexpectedly, it has been found that at least some such quinazolinone derivatives, which may of course be prepared by various other methods, possess a potentially very useful biological activity as PARP inhibitors of high activity. Examples of such compounds which are of particular interest include:

(a) 8-hydroxy-2-methylquinazolin-4-[3*H*]one;

(b) 8-hydroxyquinazolin-4-[3*H*]one;

(c) 8-hydroxy-2-(4-nitrophenyl)-quinazolin-4-one;

(d) 8-methoxy-2-methylquinazolin-4[3*H*]-one;

(e) 8-methoxy-2-phenylquinazolin-4[3*H*]-one;

(f) 8-hydroxy-2-phenylquinazolin-4[3*H*]-one;

(g) 2,8-dimethylquinazolin-4[3*H*]-one.

The invention also embraces or extends to methods of preparing compounds as hereinbefore defined (including intermediates in some cases) and to the therapeutic use of such compounds. This includes their use for making medical or veterinary preparations or pharmaceutical formulations containing an effective PARP inhibitory amount of the active compound for administration to a patient in conjunction with a cytotoxic drug or radiotherapy in order to increase the cytotoxic effectiveness of the latter. Such preparations or formulations may be made up in accordance with any of the methods well known in the art of pharmacy for administration in any suitable manner, for example orally, parenterally (including subcutaneously, intramuscularly or intravenously), or topically, the mode of administration, type of preparations or formulation and the dosage being generally determined by the details of the associated cytotoxic drug chemotherapy or radiotherapy that is to be enhanced.

As indicated, the compounds according to this invention have at least potential as PARP inhibitors, and *in vitro* tests hereinafter described have demonstrated positive pharmacological activity which it is believed reflects the activity to be found *in vivo* in the course of therapeutic clinical use.

It will be understood that where reference is made in this specification to compounds of formula II such reference should be construed as extending also to their pharmaceutically acceptable salts where relevant. Also, where any of the compounds referred to can exist in more than one enantiomeric form, all such forms, mixtures thereof, and their preparation and uses are within the scope of the invention.

DESCRIPTION OF EXAMPLES OF PREFERRED EMBODIMENTS

The following examples and descriptions of stages in synthetic routes of preparation of various preferred compounds of interest serve to further illustrate the present invention, but should not be construed in any way as a limitation thereof.

In the first example (EXAMPLE 1), a method of preparing an intermediate compound used in EXAMPLE 2 is described.

EXAMPLE 1

Methyl 2-(4-Nitrophenyl)benzoxazole-4-carboxylate

(a) 1st stage - Preparation of Methyl 3-hydroxy-2-(N-4-nitrobenzoyl)aminobenzoate

Methyl 2-amino-3-hydroxybenzoate (0.5g; 2.99mmol) was dissolved in m-xylene (40ml) with warming to 60°C. 4-Nitrobenzoyl chloride (0.556ml; 2.99mmol) was added dropwise, and this was left to stir for 4 hours. The solution was cooled to ambient temperature and the m-xylene removed under reduced pressure. The solid was dissolved in water (100ml) and the organics extracted into ethyl acetate (3 x 50ml). The organic fractions

were pooled, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure.

The title product was purified via column chromatography (1:4 ethyl acetate:petrol as eluent) to yield an orange solid (49%).

IR : cm^{-1}: 3443 (OH), 2953, 1697, 1649, 1404. **M/Z;** 316 (15% M^+).

^1H: $CDCl_3$: δ = 3.95 (3*H*; s; O**C**H_3), 7.25 (1H; t; H_5 J=8Hz), 7.31 (1H; dd; H_4,; J=6,& 2Hz), 7.67 (1H; dd; H_6,); 8.26 (2H; dd; H_2'; H_6' J=2.3Hz); 8.30 (2H; dd; H_3'; H_5' J=2.2Hz); 9.81 (1H; s; OH); 12.30 (1H; s; NH).

(b) 2nd Stage - Preparation of Methyl 2-(4-nitrophenyl)-benzoxazole-4-carboxylate

Methyl 3-hydroxy-2-(N-4-nitrobenzoyl)aminobenzoate (0.1g; 0.34 mmol) was dissolved in m-xylene (20ml), and to this was added triethylamine (0.033ml; 0.45mmol) and pyridinium-4-toluene sulphonate (0.070g; 0.28mmol). This was refluxed for 32 hours. The m-xylene was removed under reduced pressure and the remaining solid dissolved in water. The organics were extracted into ethyl acetate (3 x 30ml), dried, filtered and the solvent removed under reduced pressure to yield a brick red solid (74%).

IR : cm^{-1}:, 1726; 1522; 1556, **M/Z;** 298 (84%, M^+) 267 (100%, -OCH_3), 240 (-CO) 1H: $CDCl_3$: δ = 4.01 (3*H*; s; OCH_3), 7.44 (1H; t; H_6 J=8.1Hz), 7.76 (1H; dd; H_7,; J=7.2,& 1Hz), 8.04 (1H; dd; H_5,), 8.35 (2H; dd; H_2'; H_6' J=2.2Hz); 8.46 (2H; d; H_3'; H_5' J=2.2Hz).

EXAMPLE 2

8-Hydroxy-2-(4-nitrophenyl)quinazolin-4-one

(Compound NU1057)

Methyl 2-(4-nitrophenyl)benzoxazole-4-carboxylate (0.20g) obtained as described in Example 1 (2nd stage)

was dissolved in liquid ammonia (30ml) and sealed in an autoclave. The reaction mixture was left at 55°C, 20bar for 20 hours. Under these conditions, the expected 2-(4-nitrophenyl)benzoxazole derivative apparently rearranged to give the corresponding quinazolinone derivative. Once the reaction was complete the ammonia was removed and the resulting solid recrystallised from boiling ethyl acetate and petrol (84%).

IR cm^{-1}:, **M/Z**; 283 (38%, M^+) 267 (100%, $-NH_2$), 240 (-CO) ^1H: $CDCl_3$: δ = 7.35 (1H; dd; H_5), 7.5 (1H; t; H_6;), 7.7 (1H; dd; H_7); 8.45 (2H; d; H_2'; H_6'); 8.79 (2H; d; H_3'; H_5'), 9.98 (1H; br s; NH), 12.8 (1H; br s; NH).

Similarly, in attempting to prepare benzoxazole-4-carboxamide, the product underwent a molecular rearrangement yielding 8-hydroxy-quinazolin-4-[3*H*]one (Compound NU1026) which had quite strong PARP inhibitory activity.

Further examples now follow of the preparation of more quinazolinone compounds of particular interest.

EXAMPLE 3

8-Methoxy-2-methylquinazolin-4-[3H]-one (Compound NU1063)

Method A

(a) 1st Stage - Preparation of 3-Methoxy-2-nitrobenzamide

A solution of 3-methoxy-2-nitrobenzoic acid (0.1g, 5.1 mmol), thionyl chloride (0.55ml, 7.6 mmol) and dimethylformamide (0.2ml), in THF (10ml) was stirred for 12 hours at 25°C under nitrogen. Aqueous ammonia (6ml) was cautiously added and the mixture was stirred for a further 15 minutes, the solvent was removed under reduced pressure and the remaining solid was washed with ice-cold water and collected (0.74g, 75%) m.p. 219-222°C

δH (200MHz, d6-DMSO) 4.01 (s, 3*H*, OCH_3); 7.41-7.46 (dd, 1H, Ar-4H); 7.55-7.60 (dd, 1H, Ar-6H); 7.69 7.77 (m, 1H, Ar-5H); 7.84 (br s, 1H, -NH); 8.31 (br s, 1H, -NH); **M/Z** 196 (34.3%, M^+)

ν_{max}/cm^{-1} 3350 (br), 3180 (br), 3000, 2970, 2920, 2820, 1675.

Elemental analysis: found C 49.03, H 3.93, N 13.97, $C_8H_8N_2O_4$ requires C 48.98, H 4.11, N 14.28%.

(b) 2nd Stage - Preparation of 2-Amino-3-methoxybenzamide

3-Methoxy-2-nitrobenzamide (0.5g, 2.5 mmol) was dissolved in dry methanol (40ml) and hydrogenated using palladium-carbon catalyst (80mg). The catalyst was removed by filtration through Celite, and the residual product (0.35g) was collected and dried (83%) m.p. 145-147°C

δH(200MHz, d_6-DMSO) 3.88 (s, 3*H*, $-OCH_3$); 6.40 (br s, 2H, $-NH_2$); 6.54-6.62 (t, 1H, Ar-5H); 6.96-6.99 (dd, 1H, Ar-4H); 7.23 (br s, 1H, -NH); 7.29-7.33 (dd, 1H, Ar-6H); 7.85 (br s, 1H, -NH); **M/Z** 166 (43.8%, M^+) ν_{max}/cm^{-1} 3480, 3370, 3330, 3150, 2970, 2850, 1680, 1620.

Elemental analysis: found C 57.54, H 5.99, N 16.61, $C_8H_{10}N_2O_2$ requires C 57.82, H 6.07, N 16.86%.

(c) 3rd Stage - Preparation of 2-N-Acetylamino-3-methoxybenzamide

To a solution of 2-amino-3-methoxybenzamide (0.5g, 3 mmol) in dry THF (15ml), containing pyridine (0.3ml; 3.9 mmol), was added acetyl chloride (0.2ml, 3.3. mmol) in THF (2ml) dropwise, and the reaction mixture was stirred overnight under nitrogen. The solvent was removed under vacuum and the remaining white slurry washed with aqueous sodium bicarbonate solution, filtered and washed with water. The white product (0.19g, 31 %) was collected and dried.

m.p. 243-246°C

δH (200MHz, d_6-DMSO) 2.05 (s, 3*H*, $-CH_3$); 3.88 (s, 3*H*, $-OCH_3$); 7.14-7.18 (dd, 1H, Ar-4H); 7.21-7.25 (dd, 1H, Ar-6H); 7.33-7.41 (m, 2H, -NH and Ar-5H; 7.53 (br s, 1H, -NH); 9.27 (br s, 1H,-NH); **M/Z** 208 (16.6, M^+)

ν_{max}/cm^{-1} 3420, 3240 (br), 3160, 3020, 2980, 2870, 1660.

Elemental analysis: found C 56.98, H 5.38, N 12.78, $C_{10}H_{12}N_2O_3$ requires C 57.68, H 5.81, N 13.46%.

(d) Final Stage - Preparation of 8-Methoxy-2-methylquinazolin-4-[3*H*]-one

2-N-Acetylamino-3-methoxybenzamide (0.07g, 0.34 mmol) from 3rd stage was dissolved in aqueous sodium hydroxide solution (2% w/v, 2ml) and the solution was stirred for 12 hours at 25°C. The reaction mixture was neutralised with dilute aqueous hydrochloric acid and the resulting white precipitate that was deposited was collected by filtration and washed thoroughly with water. The title compound was recrystallised from ethyl acetate (0.043g, 67%)

m.p. 202-204°C (sublimes).

EXAMPLE 3a

8-Methoxy-2-methylquinazolin-4-[3*H*]-one (Compound NU1063)

Alternative Method B

To a mixture of 2-amino-3-methoxybenzamide (1.0g, 6 mmol) from the 2nd stage of Example 3 and pyridine (0.6ml, 7.8 mmol) in dry THF (25ml), was added a solution of acetyl chloride (0.9ml, 13 mmol) in THF (2ml) dropwise, and the mixture was stirred overnight under nitrogen. The solvent was removed under vacuum and the remaining white slurry was resuspended in 2% aqueous sodium hydroxide solution and neutralised with aqueous hydrochloric acid, whereupon a white precipitate formed. The product was collected by filtration and recrystallised from methanol-water (0.915g, 80%) m.p. 202-204°C (sublimes)

δH (200MHz, d_6-DMSO) 2.43 (s, 3*H*, $-CH_3$); 3.97 (s, 3*H*, $-OCH_3$); 7.37-7.50 (m, 2H, Ar-6/7H); 7.68-7.73 (dd, 1H, Ar-5H); δ C (d_6-DMSO); 21.83 ($-CH_3$); 56.05 ($-OCH_3$); 114.96, 116.99 (Ar-67C); 121.95 (C-CH_3); 126.5 (Ar-5C); 140.0 (Ar-8AC); 153.26 (Ar-8C); 154.33 (Ar-4aC); 162.04 (C=O); **M/Z**

190 (96.6%, M^+) ν_{max}/cm^{-1} 3171, 3034, 2903, 1676, 1620, 1574, 1483.

Elemental analysis: found C 62.14, 62.36, H 5.18, 5.29, N 14.23, 14.36; $C_{10}H_{10}N_2O_2$ requires C 63.15, H 5.30, N 14.73%.

EXAMPLE 4

8-Hydroxy-2-methylquinazolin-4-[3*H*]-one (Compound NU1025)

A solution of 8-methoxy-2-methylquinazolin-4-[3*H*]-one (0.7g, 3.7 mmol) from Example 3 in BBr3 (1.0 M in CH_2Cl2) 8.4ml, 8.4 mmol) was heated under reflux for 24 hours under nitrogen. Solvents were removed by distillation under vacuum and the remaining residue was hydrolysed with sodium hydroxide solution (10% w/v). Acidification with aqueous hydrochloric acid afforded a white precipitate, which was removed. The filtrate was extracted with ethyl acetate (3 x 30ml), dried ($MgSO_4$) and the solvent was removed under vacuum. Recrystallisation from propan-2-ol-water afforded the target compound (65%) m.p. 253-258°C

δH (200MHz, d_6-DMSO) 2.48 (s, 3*H*, $-CH_3$); 7.22-7.41 (m, 2H, Ar=6/7H); 7.57-7.62 (dd, 1H, Ar-5H); 9.57 (s, 1H, -OH); 12.26 (s, 1H, -NH); δC (d_6-DMSO); 21.72 ($-CH_3$); 115.78, 118.42 (Ar-6/7C); 121.76 (C-CH_3); 126.54 (Ar-5C); 138.27 (Ar-8aC) 152.58 (Ar-8C); 152.87 (Ar-4aC); 162.05 (C=0); **M/Z** 176 (100%, M^+); ν_{max}/cm^{-1} 3320, 3175, 3030, 2900, 2800, 1670.

Elemental analysis: found C 61.39, H 4.54, N 15.88, $C_9H_8N_2O_2$ requires C 61.36, H 4.58, N15.94%.

EXAMPLE 5

8-Methoxy-2-phenylquinazolin-4-[3*H*]-one (Compound NU1065)

Method A

(a) 1st Stage - Preparation of 2-N-Benzoylamino-3-methoxybenzamide

To a stirred solution of 2-amino-3-methoxybenzamide (0.5g, 3 mmol) from the 2nd stage of Example 5 in dry THF (15ml), containing pyridine (0.3ml, 3.0 mmol), was added benzoyl chloride (0.4ml, 3.3 mmol) in THF (2ml) dropwise. The reaction mixture was stirred under nitrogen at 25°C. The solvent was removed under vacuum to afford a white slurry which was washed with sodium bicarbonate solution, filtered and washed with water. Recrystallisation from methanol-water afforded the title compound (0.2g, 41%)

m.p. 176-180°C;

δH (200MHz, d_6-DMSO) 3.88 (s, 3*H*, $-OCH_3$); 7.24-7.32 (m, 2H, Ar-4/6H); 7.41-7.49 (m, 2H, -NH, Ar-5H); 7.59-7.73 (m, 4H, -NH, Ph-3'/4'H); 8.04-8.08 (dd, 2H, Ph-2'H); 9.85 (s, 1H, -NH); mlz 270 (74.6%, M^+).

(b) 2nd Stage - Preparation of 8-Methoxy-2-phenyl quinazolin-4-[3*H*]-one

2-N-Benzoylamino-3-methoxybenzamide (0.2g, 0.74 mmol) was dissolved in aqueous sodium hydroxide solution (2% w/v, 2ml) and the solution was stirred at room temperature for 12 hours. The reaction mixture was neutralised with hydrochloric acid, and the resulting white precipitate that formed was collected by filtration and recrystallised from methanol/water (0.12g, 65%) m.p. 252-256°C.

EXAMPLE 5a

8-Methoxy-2-phenylquinazolin-4-[3*H*]-one (Compound NU1065)

Alternative Method B

To a solution of 2-amino-3-methoxybenzamide (1.0g, 6 mmol) (from the 2nd stage of Example 3) and pyridine (0.6ml, 7.8 mmol) in dry THF (25ml), was added a solution of benzoyl chloride (0.8ml, 6.6 mmol) in THF (2ml) dropwise, and the mixture was stirred overnight under nitrogen. The solvent was removed under vacuum and the remaining white slurry was resuspended in 2% aqueous sodium hydroxide solution and neutralised with aqueous

hydrochloric acid, whereupon a white precipitate formed. The product was collected by filtration and recrystallised from methanol-water (1.1g, 75%) m.p. 252-256°C;

δH (200MHz, d_6-DMSO) 4.06 (s, 3*H*, $-OCH_3$); 7.47-7.61 (m, 2H, Ar-4/6H); 7.63-7.69 (m, 3*H*, Ph-3'/4'H); 7.80-7.85 (dd, 1H, Ar-5H); 8.27-8.32 (m, 2H, Ph-2'H); 12.70 (s, 1H, -NH); **M/Z** 252 (100%, M^+); ν_{max}/cm^{-1} 3330, 3190, 3170, 3120, 3070, 2950, 2890, 2830, 1660.

Elemental analysis: found C 71.38, H 4.39, N 11.17,

$C_{15}H_{12}N_2O_2$ requires C 71.42, H 4.79, N 11.10%.

EXAMPLE 6

8-Hydroxy-2-phenylquinazolin-4-[3*H*]-one (Compound NU1068)

A solution of 8-methoxy-2-phenylquinazolin-4-[3*H*]-one (0.5g, 2 mmol) from Example 5 or 5a in BBr3 (1.0 M in CH_2Cl_2) (6ml, 6 mmol) was heated under reflux for 24 hours under nitrogen. Solvents were removed by distillation under vacuum and the remaining residue was hydrolysed with sodium hydroxide solution (10% w/v). Acidification with aqueous hydrochloric acid afforded a white precipitate, which was removed. The filtrate was extracted with ethyl acetate (3 x 30ml), dried ($MgSO_4$) and the solvent was removed under vacuum. Recrystallisation from propan-2-ol afforded the target compound (0.187mg, 67%)

m.p. 280-284°C;

δH (200MHz), d_6-DMSO) 7.73-7.50 (m, 2H, Ar-6/7H); 7.66-7.72 (m, 4H, Ar-5H, Ph-3'/4'H); 8.51-8.54 (dd, 2H, Ph-2H); 9.75 (bs, 1H, -OH); 12.60 (bs, 1H, -NH); δC (d_6-DMSO); 116.01, 118.68 (Ar-6/7C); 122.03 (C-Ph); 127.43-128.76 (Ph-1'/2'/3'/4'C); 137.98 (Ar 8aC); 150.72 (Ar-8C); 153.31 (Ar-4aC); 162.62 (C=O); **M/Z** 238 (100%, M^+); ν_{max}/cm^{-1} (approx. values) 3380 (br), 3180, 3120, 3050, 2940, 1640.

Elemental analysis: found C 69.54, H 4.05, N 11.46, C14H10N2O2 requires C 70.58, H 4.23, N 1176%.

EXAMPLE 7

2,8-Dimethylquinazolin-4-[3*H*]-one (Compound NU1069)

To a solution of 2-amino-3-methylbenzamide (0.5g, 3.3 mmol) (prepared by conventional methods) and pyridine (0.35ml, 4.3 mmol) in dry THF (15ml), was added a solution of acetyl chloride (0.36ml, 5.0 mmol) in THF (2ml) dropwise, and the mixture was stirred overnight under nitrogen. The solvent was removed under vacuum and the remaining white slurry was resuspended in 2% aqueous sodium hydroxide solution and neutralised with aqueous hydrochloric acid, whereupon a white precipitate formed. The solid was collected and recrystallised from methanol-water to furnish the required quinazolinone (0.47g, 81%) m.p. 217-220°C;

δH (200MHz, d_6-DMSO) 2.44 (s, 3*H*, $-CH_3$); 2.57 (s, 3*H*, $-CH_3$); 7.36-7.44 (t, 1H, Ar-6H); 7.68-7.72 (dd, 1H, Ar-7H); 7.97-8.01 (dd, 1H, Ar-5H); 12.25 (br s, 1H, -NH); **M/Z** 174 (100%, M^+); ν_{max}/cm^{-1} 3325, 3180, 3040, 2990, 2910, 2880, 2795, 1680, 1620.

Elemental analysis: found C 68.76, H 5.57, N 15.90,

$C_{10}H_{10}N_2O$ requires C 68.94, H 5.76, N 16.08%.

ASSAY FOR PARP INHIBITORY ACTIVITY

Compounds of the present invention, particularly those detailed in the preceding Examples, have been tested *in vitro* for activity as PARP inhibitors using the following methods and materials.

In principle, the PARP assay used relies upon activating endogenous PARP (as hereinafter described) in cells containing exogenous [^{32}P]-NAD^+ introduced therein by suspending the cells in a solution of [^{32}P]-NAD^+ to which they have been rendered permeable in an initial pre-treatment step. The poly(ADP-ribose) which is then

synthesised by the enzyme can be precipitated by trichloracetic acid (TCA) and the amount of radio-labelled ^{32}P incorporated therein measured, e.g. using a scintillation counter, to give a measure of the activity of the PARP under the particular conditions of the experiment. By repeating the experiment following the same procedure, and under the same conditions, in the presence of each compound to be tested the reduction in enzyme activity, representative of the inhibitory effect of the test compound, can then be ascertained from the reduction, if any, of the amount of [^{32}P] measured in the TCA precipitated poly(ADP-ribose).

The results of this assay may be expressed in terms of percentage inhibition or reduction in activity for one or more different concentrations of each compound tested, or it may be expressed in terms of that concentration of the tested compound which reduces the enzyme activity by 50%, i.e. the IC_{50} value. Thus, with a range of different compounds a set of comparative values for inhibitory activity can be obtained.

In practice, L1210 murine leukaemia cells were used as the source of the PARP enzyme after being rendered permeable to exogenous [^{32}P]NAD by exposure to hypotonic buffer and cold shock. In the preferred technique developed, which has been found to give exact and reproducible results, a defined amount of a small synthetic oligonucleotide, in particular a single strand oligo-nucleotide having the palindromic sequence CGGAATTCCG, is introduced into the cell suspension for activating the PARP enzyme. This oligonucleotide sequence snaps back on itself to form a double-stranded molecule with a single blunt end and provides an effective substrate for activation of PARP. Its behaviour as a potent activator of the enzyme was confirmed in the tests carried out.

The experimental protocol adopted, in which a synthetic oligonucleotide as mentioned above is

introduced as a specific activator of PARP, discriminates between PARP and other mono-ADP-ribosyltransferases in the cells. Thus, introduction of such synthetic oligonucleotides causes a 5 to 6 fold stimulation in the radioactive label incorporated and this is attributable solely to PARP activity.

Further details of the assay are given below.

Materials

The materials used included the following:

DTT (Dithiothreitol)

A 100mM (15.4mg/ml) solution (for use as an anti-oxidant) was made up, divided into 500µl aliquots and stored at -20°C.

Hypotonic buffer:

9mM Hepes	(214mg/100ml)
4.5% Dextran	(4.5g/100ml)
4.5mM $MgCl_2$	(92mg/100ml)

The above ingredients were dissolved in about 80ml distilled water, pH was adjusted to 7.8 (NaOH/HC1), the solution was then made up to 100ml with distilled water, and stored in a refrigerator. DTT was added to 5mM just before use (50µl/ml).

Isotonic buffer:

40mM Hepes	(1.9g/200ml)
130mM KCl	(1.94g/200ml)
4% Dextran	(8g/200ml)
2mM EGTA	(152mg/200ml)
2.3mM $MgC1_2$	(94mg/200ml)
225mM Sucrose	(15.39g/200ml)

The above ingredients were dissolved in about 150ml distilled water, pH was adjusted to 7.8 (NaOH/HCl), the solution was then made up to 200ml

with distilled water and stored in a refrigerator. DTT was added to 2.5mM just before use (25µl/ml).

NAD

NAD was stored as a solid in pre-weighed aliquots at -20°C. From these, solutions of a concentration of approximately 6mM (4-4.5mg/ml) were freshly made up shortly before performing an assay, and the molarity was checked by measuring the optical density (O.D.) at 260nm. The stock solution was then diluted with water to give a concentration of 600µM and a small amount of ^{32}P labelled NAD was added (e.g. 2-5µl/ml).

Oligonucleotide

The oligonucleotide having the palindromic sequence CGGAATTCCG, synthesised by conventional means, was vacuum dried and stored as pellets in a freezer. Before use, it was made up to 200µg/ml in 10mM Tris/HCl, pH 7.8, with each pellet being dissolved completely in 50ml of buffer. The solution was then heated to 60°C in a water bath for 15 minutes, and allowed to cool slowly to ensure correct reannealing. After adding 9.5ml of buffer, the concentration was checked by measuring the optical density of a diluted sample at 260nm. The main solution was then diluted to a concentration of 200µg/ml and stored in 500µl aliquots in a freezer, ready for use.

TCA

Solutions of TCA (Trichloroacetic acid) were prepared at two concentrations. 10% TCA + 10% sodium pyrophosphate, and 1% TCA + 1% sodium pyrophosphate.

Cells

The L1210 cells used as the source of the PARP enzyme were maintained as a suspension culture in RPMI medium + 10% foetal bovine serum + glutamine

and antibiotics (penicillin and streptomycin). HEPES and sodium bicarbonate were also added, and the cells were seeded in 100ml of medium such that there would be a concentration of approximately 8 x 105/ml at the time of carrying out an assay.

Method

The compounds being tested were generally made up as a concentrated solution in DMSO (Dimethyl sulphoxide). The solubility of the compound was then checked by adding a quantity of the DMSO solution to a quantity of the isotonic buffer, in the required final proportions that were to be used in carrying out the assay, and after an interval the solution was examined under a microscope for any signs of crystals forming.

A desired quantity of the cells, ascertained by counting with a haemocytometer, was then centrifuged (1500rpm in a Europa™ model 24M centrifuge for 5 minutes), the supernatant removed, and the pellets obtained were resuspended in 20ml Dul A at 4°C before centrifuging again at 1500rpm and 4°C. After again removing the supernatant, the cells were resuspended at a concentration of 3 x 107 cells/ml in ice cold hypotonic buffer and left for 30 minutes on ice. Nine volumes were then added of ice cold isotonic buffer, and the cells, now rendered permeable to exogenous NAD^+, were then used within the next hour for carrying out an assay. The permeablisation of the cells may be checked at this stage by adding duplicate aliquots of cells to an equal volume of trypan blue, leaving for 5 minutes and then counting on a haemocytometer.

The assay was then carried out using for convenience plastic 15ml conical bottomed assay tubes set up in a shaking water bath at 26°C which is the optimum temperature for this enzyme. In a typical assay using the oligonucleotide solution at a concentration of 5µg/ml

and the test compound/DMSO solution at a concentration of 2%, and carrying out the assay in quadruplicate, there would then be placed in each assay tube 5µl of the oligonucleotide solution, 50µl of the 600µm NAD + [^{32}P]-NAD solution, 8µl of the test compound/DMSO solution, and 37µl of water. Prior to the start of the experiment this "cocktail" would be pre-warmed for 7 minutes at 26°C, as would be also the cell suspension. The reaction would then be started by adding 300µl of the cell suspension. The reaction would be stopped by adding 2ml of the 10% TCA + 10% sodium pyrophosphate solution.

In addition to the above, six assay tubes would usually be set up as blanks, these containing the same ingredients as above but, before adding the cell suspension, TCA solution is added to prevent any reaction from taking place. This enables corrections to be applied for any non-specific binding of the labelled material to the filter used (see below).

After adding the cell suspension at timed intervals to each of the assay tubes, the 10% TCA + 10% sodium pyrophosphate at 4°C was added to each assay tube exactly 5 minutes after addition of the cell suspension to that tube. Then, after leaving the tubes on ice for a minimum time of one hour, the contents of each individual tube were filtered through an individual filter funnel of a suction filter apparatus using GF/C filter elements (rough side up) wetted with 10% TCA. After filtering the contents of each tube and rinsing the filters several times with 1% TCA + 1% sodium pyrophosphate solution, the filters were carefully removed and dried before being placed in individual scintillation vials. Four additional scintillation vials were also set up as reference standards containing 10µl of the 600µM NAD + [^{32}P]-NAD solution, 10ml scintillant then being added to each vial. Counting was carried out for 2 minutes on a β counter to obtain measures of the ^{32}P present, and thus

the amount of the poly(ADP-ribose) and activity of the PARP enzyme.

RESULTS OF *IN VITRO* PARP INHIBITION STUDIES

Apart from applying the PARP enzyme assay in accordance with the standard procedure outlined above to a range of compounds which have been made in accordance with the present invention, for comparison purposes it was also applied to certain benzamide compounds, in particular 3-hydroxybenzamide, 3-methoxybenzamide and 3-aminobenzamide, that are already known to exhibit certain PARP inhibitory activity. A full tabulated list of the compounds which have been made and/or studied is hereinafter presented in TABLE III, together with the PARP inhibition assay results obtained in different experiments for different concentrations of the compounds when tested using the assay hereinabove described.

In reviewing this list, the known PARP inhibitors 3-aminobenzamide, 3-methoxybenzamide and 3-hydroxybenzamide may be regarded as reference compounds. Although there is considerable variation in activity, and in some cases at least the higher concentrations for aqueous solutions of the test compounds could not be achieved because of low solubility, in general the compounds of the present invention which were tested showed a useful degree of activity, particularly compounds NU1025, NU1057, NU1063, NU1068 and NU1069.

In contrast to the results obtained for the compounds of the present invention, which have in many cases showed PARP enzyme inhibitory properties that are well above average and at least comparable with, if not considerably better than, those of other known benzamide PARP inhibitors, various analogous nicotinamide compounds studied showed no, or very poor, inhibitory activity when tested in the same manner at similar concentrations.

FURTHER BIOLOGICAL ACTIVITY STUDIES

Again using cultures of the murine leukaemia L1210 cell line, growth inhibition experiments were carried out to assess the cytostatic effects of the compounds and clonogenic survival assays were performed to assess cytotoxicity, especially in relation to use of the compounds in conjunction with DNA damaging cytotoxic agents such as cytotoxic antitumour drugs or high energy radiation. DNA damage and the effect of the PARP inhibitors on the process of DNA strand break formation and repair has also been assessed by carrying out DNA strand break assays and monitoring by alkaline elution in accordance with published techniques.

By way of example some further details are given below of studies carried out using the quinazolinone compounds identified by the reference numbers NU1025 and NU1057 (derivable by molecular rearrangement of corresponding benzoxazole compounds) as representative examples of the PARP inhbiting compounds of the present invention, and also using for comparison the known PARP inhibitors 3-aminobenzamide (3AB) and benzamide (BZ) itself. Results of experiments using the alkylating agent temozolomide (TM) are also reported, taking this as a illustrative example of a cytotoxic DNA damaging antitumour drug, and in some of the studies carried out gamma ray IRradiation was used to damage the cells.

In the growth inhibition assays, typically the L1210 cells would be seeded at 1 x 10^4/ml in triplicate in 24 well multidishes, and 24 hours later the compounds or drugs being tested would be added in selected combinations and concentrations. At this time one set of replicates would be counted using a Coulter counter (N_0), and 48 hours later the remaining samples would be counted (N_1). The percentage (%) growth inhibition of drug-treated samples could then be estimated. In drug combination experiments, where evidence of synergistic effects on cell growth or clonogenicity was being sought,

a single, fixed concentration of a cytotoxic drug sample, e.g. temozolomide, would be taken as the control value.

As an illustration of the results obtained, there is shown in TABLE I at the end of this description the IC_{50} values of the above-mentioned PARP inhibitors when used alone and in conjunction with a fixed concentration (100μM) of temozolomide, as estimated from the growth inhibition experiments. Although not shown, it may be noted that exposure of the cells to TM alone caused inhibition of cell growth with an IC_{50} value of 361±25μM. Also, it was established that co-exposure of the cells to 100μM TM with increasing concentrations of the PARP inhibitors caused a synergistic increase in growth inhibition throughout a range of concentrations.

It will be seen from Table I that 10-20 fold higher concentrations of the compound NU1025 used alone were required to inhibit cell growth than were required when the compound was used in conjunction with 100μM TM. For example, the IC_{50} of NU1025 alone was 0.41mM, and this was reduced to 0.04mM in the presence of TM. In comparison, only 2-3 fold differences were obtained with 3AB and BZ, where there was considerable overlap between the growth inhibitory effects of the compounds *per se* and their effects in conjunction with TM. An identical rank order was obtained when comparing the effectiveness of the compounds as PARP inhibitors and their ability to inhibit cell growth which at least suggests that PARP function is essential for cell growth.

In the clonogenic survival assays, typically the L1210 cells were exposed to varying concentrations of TM ± a fixed concentration of PARP inhibitor for a fixed time of 16 hours, prior to counting and seeding for colony formation in 0.12-0.15% agarose in drug-free medium. After 7-10 days colonies were stained with 0.5mg/ml MTT and counted by eye on a gridded light box. Survival curves were plotted and typical DEF10 values obtained are hereinafter given in Table II (DEF10 being

defined as the ratio of the concentration of TM that reduces survival to 10% divided by the concentration of TM that reduces survival to 10% in the presence of a fixed concentration of PARP inhibitor). Each DEF10 value in Table II represents the average ratio ± S.E. (standard error) derived from the averaged 10% survival for TM alone (675 ± 31μM from 22 independent survival curves) divided by individual 10% survival values from at least 3 independent survival curves performed in the presence of a fixed concentration of inhibitor.

A reasonable correlation was found between growth inhibitory effects and cytotoxic effects for TM alone with an IC_{50} value of 361μM ± 25μM and a LD50 value of 251 ± 13μM respectively, despite differing exposure times (48 hours for growth inhibition and 16 hours for cytotoxicity). TM has a half life in culture of about 40 minutes, and therefore will exert its full effects well before the minimum duration of exposure of either experiment. All compounds potentiated TM cytotoxicity, but NU1025 produced about the same DEF10 values at very much lower concentrations than 3AB and BZ respectively. For example, 50μM NU1025 and 5mM 3AB gave equivalent DEF10 values of about 4. For NU1025 maximal potentiation of cytotoxicity was obtained by a concentration in the range of 50-100μM, and was significant at doses as low as 10μM.

In other clonogenic survival assays gamma ray irradiation was used to damage the cells. Typically, L1210 cells (3ml, 4 x 103/ml in plastic bijoux bottles) were irradiated at 4°C with varying doses of gamma rays in the presence or absence of 10mM 3AB or 200μM NU1057 and a final concentration of 2% DMSO. The cells were then incubated at 37°C for 2 hours in the continued presence or absence of PARP inhibitor prior to seeding for colony formation. A significant potentiation of gamma ray cytotoxicity by NU1057 was observed, with a DEF10 of 1.1.

Repair of potentially lethal damage (PLD) occurs when cells are held in stationary-phase following initiation of PLD prior to allowing cell division to take place. In further typical experiments, L1210 cells were allowed to repair gamma ray PLD in the presence or absence of 3AB or NU1025 as follows. L1210 cells were maintained in culture until they had attained stationary phase (>106cells/ml). They were diluted to 1.5 x 105/ml in conditioned medium from stationary-phase cultures to prevent further cell division. Replicate 2ml samples of cells in plastic bijoux were held on ice prior to and immediately following 8 Gray gamma ray irradiation. 1ml of 3x final concentration of compounds 3AB or NU1025 made up in conditioned medium from stationary cultures was added (to give final concentrations of 106cells/ml in 1% DMSO ± 10mM 3AB or 200μM NU1025) and the cells were incubated at 37°C for 0, 2 or 4 hours prior to resuspending in drug-free medium and seeding for colony formation. Unirradiated stationary phase cultures incubated at 37°C for 0, 2 or 4 hours with 1% DMSO ± 10mM 3AB or 200μM NU1025 were used as appropriate controls for determining relative cell survival. In the absence of PARP inhibitor cell survival increased with time allowed for PLD repair to take place. For example, when seeded immediately after irradiation (no repair) only about 0.2% of the cells survived, but after a 4 hour repair period this had increased to 0.7%. It was observed that both 3AB and NU1025 blocked this repair.

The cytotoxic effects of the PARP inhibiting compounds alone has also been investigated. In one set of experiments, the LD50 values for a 24 hours exposure of L1210 cells were 14 ± 1.0mM (3AB); 6.0 ± 1.5mM (BZ) and 1.6 ± 0.1Mm (NU1025). The LD50 values differed by $\leq$3-fold from the IC_{50} values but maintained the same rank order with respect to their potency as PARP inhibitors. In agreement with the growth inhibition data there was $\geq$10-fold difference between the concentration of NU1025 needed to produce maximal potentiation of TM cytotoxicity

and the concentration needed to produce cytotoxicity *per se*.

In respect of DNA strand break assays carried out, it was found that a 1 hour treatment with TM resulted in a concentration-dependent increase in the rate of elution which provides a measure of the extent of DNA strand breakage. Changes in DNA strand break levels were detectable at levels of TM as low as 150μM, a concentration which reduced survival by about 30%. All the compounds were tested for their ability to produce strand breaks when used alone. A 24 hour incubation of cells with 1mM NU1025, and 20mM 3AB or BZ had no significant effect on DNA strand break levels compared to untreated cells. However, coincubation for 1 hour of a fixed concentration of TM (150μM) with increasing concentrations of all PARP inhibitors tested caused a progressive increase in the rate of elution (extent of strand breakage) compared to TM alone.

The results for all the 3 representative compounds mentioned have been summarised by plotting values of a parameter related to extent of strand breakage versus inhibitor concentration. For all the compounds, the strand breakage increased linearly with increasing concentration, but values started increasing significantly for NU1025 at about 100μM, whereas concentrations above 3mM and 5mM were required to significantly increase values for BZ and 3AB respectively. Again, the rank order and potency of the compounds in the DNA strand break assay demonstrated an excellent correlation with *in vitro* PARP inhibitory potency.

Overall, it is believed that the studies carried out give clear evidence that the PARP inhibitory characteristics of compounds of this invention reflects an ability of these compounds to potentiate the cytotoxicity of DNA damaging agents such as certain cytotoxic antitumour drugs and radiation used in radiotherapy. Accordingly,

such compounds should be especially useful for administration in conjunction with such cytotoxic drugs or radiotherapy to potentiate their effect in the course of medical treatment as hereinbefore indicated.

Summary

Although the present invention should be regarded overall as comprising each and every novel feature or combination of features disclosed herein, the main aspects of the invention comprise, principally but not exclusively, broadly the following:-

(i) Novel compounds of formula (II) as defined herein;

(ii) Compounds of formula (II) with substituents as hereinbefore defined (including salts thereof) for therapy or for use in medicine and in the manufacture of medical preparations, useful for example as PARP inhibitors to be administered in conjunction with cytotoxic drugs or with radiotherapy to potentiate the effectiveness of the latter in treatment of cancer;

(iii) Processes for the preparation of novel compounds of formula (II) as defined herein, including any novel intermediate compounds produced in carrying out such processes;

(iv) Pharmaceutical formulations comprising a compound of formula (II) as defined herein together with a pharmaceutically acceptable carrier therein;

(v) Processes for the preparation of a pharmaceutical formulation as defined in (iv) above, e.g. by methods referred to herein;

(vi) Quinazolinone compounds of formula (II), possibly representing molecularly rearranged compounds of formula (IV), as herein disclosed, for therapy or for use in medicine and in the manufacture of medical preparations, useful for example as PARP

inhibitors to be administered in conjunction with cytotoxic drugs or with radiotherapy to potentiate the effectiveness of the latter in treatment of cancer, and pharmaceutical formulations comprising said quinazolinone compounds.

TABLE I

INHIBITOR	IC_{50} (mM) ± SE INHIBITOR ALONE	IC_{50} (mM) ± SE INHIBITOR + 100μM TM
3-AMINOBENZAMIDE	6.7 ± 0.2	2.5 ± 0.1
BENZAMIDE	2.5 ± 0.3	0.84 ± 0.12
NU1025	0.41 ± 0.06	0.04 ± 0.003

TABLE II

INHIBITOR	CONCENTRATION	$DEF_{10}^{\bullet}$
3-AMINOBENZAMIDE	1mM 5mM	2.4 ± 0.3 4.1 ± 0.4
BENZAMIDE	1mM 3mM	4.0 ± 0.7 6.9 ± 0.2
NU1025	10μM 50μM 100μM	2.0 ± 0.2 4.0 ± 0.5 5.1 ± 0.7

TABLE III

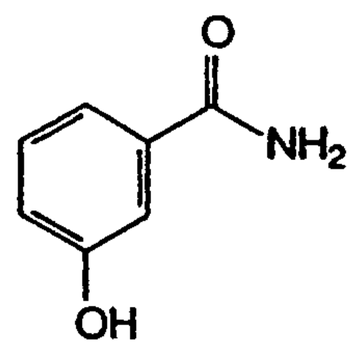
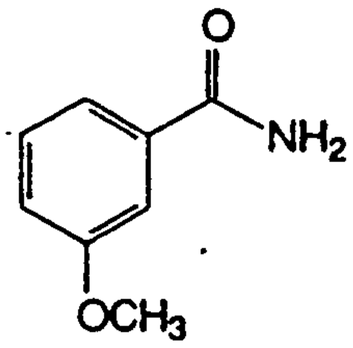
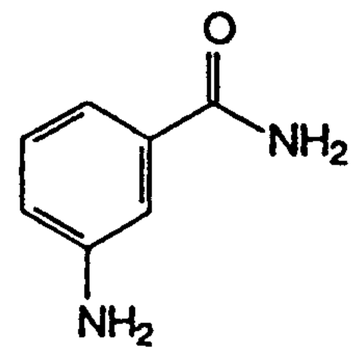
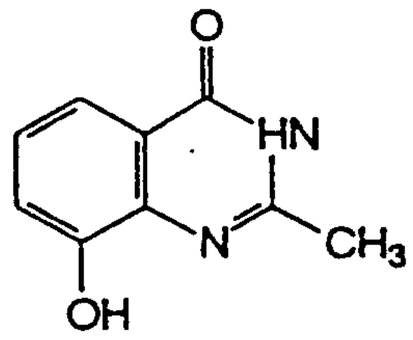
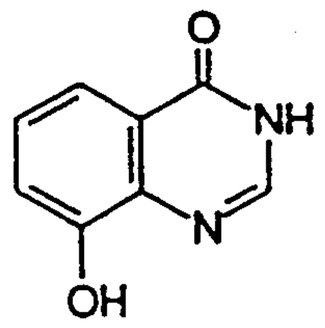
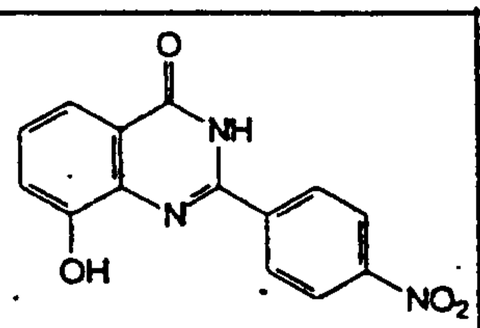
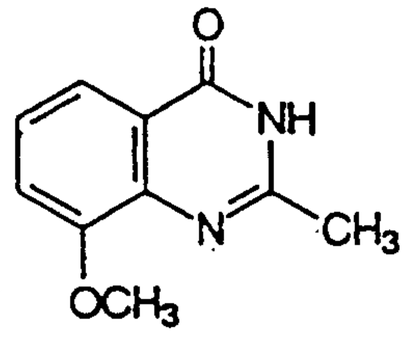
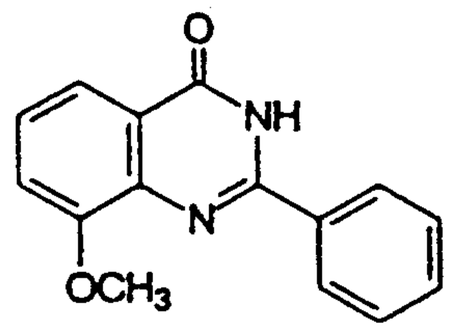
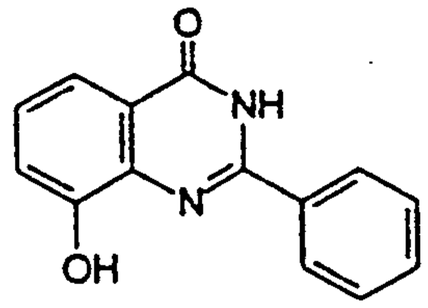
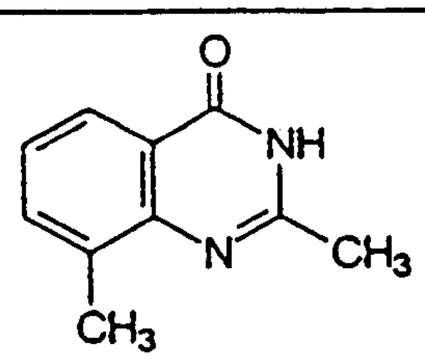
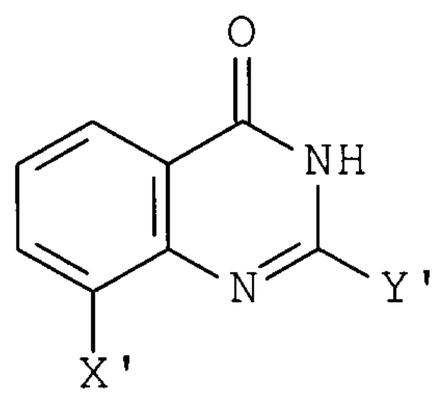
House Number	Name	Structure	% Inhibition		
			10μM	30μM	100μM
Ref	3-hydroxybenzamide $C_7H_7NO_2$ MW = 137		35	59	81
Ref	3-methoxybenzamide $C_8H_9NO_2$ MW = 151		55	78	89
Ref	3-aminobenzamide $C_7H_8N_2O_2$ MW = 136		36	63	79
NU1025	8-hydroxy-2-methyl-quinazolin-4-[*3H*]one $C_9H_8N_2O_2$ MW = 176		92 1μM = 63 0.1μM = 18 0.5μM = 59 1.0μM = 68 IC_{50} = 0.4 μM	92	96.
NU1026	8-hydroxyquinazolin-4-[*3H*]one $C_8H_6N_2O_2$ MW = 162		78 0.5μM = 18 1.0μM = 38 2.0μM = 54 IC_{50} = 2 μM	87	95

TABLE III (contd.)

House Number	Name	Structure	% Inhibition		
			10μM	30μM	100μM
NU1057	8-hydroxy-2-(4-nitrophenyl)--quinazolin-4-one $C_{14}H_9N_3O_4$ MW = 283.2		92 IC_{50} = 0.23μM		
NU1063	8-methoxy-2-methylquinazolin-4[*3H*]--one $C_{10}H_{10}N_2O_2$ MW = 190.2		IC_{50} = 0.78μM		
NU1065	8-methoxy-2-phenylquinazolin-4[*3H*]--one $C_{15}H_{12}N_2O_2$ MW = 252.27		IC_{50} = 4.2μM		
NU1068	8-hydroxy-2-phenylquinazolin-4[*3H*]--one $C_{14}H_{10}N_2O_2$ 238.24		IC_{50} = 0.53μM		
NU1069	2,8-dimethylquinazolin-4[*3H*]--one $C_{10}H_{10}N_2O_2$ 174.2		IC_{50} = 0.2μM		

CLAIMS:

1. Use of a compound for inhibiting activity of the enzyme poly(ADP-ribose)polymerase or PARP (also known as ADP-ribosyl transferase or ADPRT), such enzyme inhibition constituting an element of a therapeutic treatment, said compound providing the active PARP enzyme inhibiting agent and being a quinazolinone compound having the general structural formula II



II

or a pharmaceutically acceptable salt thereof,

wherein

X' represents hydroxyl, C_{1-6} alkyl, C_{1-6} alkoxy or an optionally substituted phenyl or benzyl group,

and

Y' represents hydrogen, C_{1-6} alkyl or an optionally substituted phenyl or benzyl group.

2. Use of a compound as claimed in Claim 1 wherein the compound is a quinazolinone having the general structural formula II wherein Y' is phenyl or a phenyl group having a substituent selected from $-NO_2$, $-NH_2$, -OH and alkyl.

3. Use of a compound as claimed in Claim 2 wherein X' is hydroxyl.

4. Use of a compound as claimed in Claim 1 wherein the compound is a quinazolinone having the general structural formula II wherein X' is hydroxyl and Y' is C_{1-6} alkyl.

5. Use of a compound as claimed in any one of Claims 2 to 4 wherein the compound is produced by a molecular rearrangement of a benzoxazole-4-carboxamide compound.

6. Use of a compound as claimed in Claim 1 wherein the compound is one of the following:

(a) 8-hydroxy-2-methylquinazolin-4-[3*H*]one;

(b) 8-hydroxyquinazolin-4-[3*H*]one;

(c) 8-hydroxy-2-(4-nitrophenyl)-quinazolin-4-one;

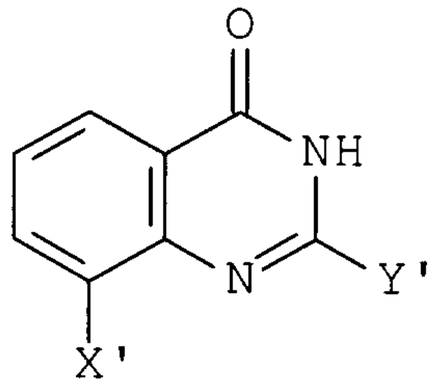
(d) 8-methoxy-2-methylquinazolin-4[3*H*]-one;

(e) 8-methoxy-2-phenylquinazolin-4[3*H*]-one;

(f) 8-hydroxy-2-phenylquinazolin-4[3*H*]-one;

(g) 2,8-dimethylquinazolin-4[3*H*]-one.

7. A quinazolinone compound having the general structural formula II



II

or a pharmaceutically acceptable salt thereof,

wherein

X' represents hydroxyl, C_{1-6} alkyl or C_{1-6} alkoxy

and

Y' represents C_{1-6} alkyl or an optionally substituted phenyl group other than a phenyl group

having a 4-propoxy substituent or a 2-alkoxy substituent,

subject to a proviso that

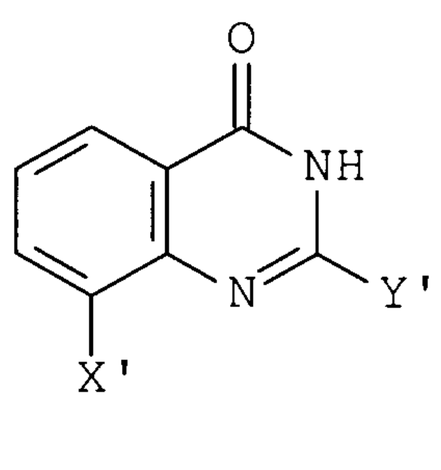
if X' is methyl, Y' is not butyl,

if X' is methoxy, Y' is not methyl or 4-hydroxy-phenyl, and

if X' is hydroxy, Y' is not methyl, ethyl or phenyl

said compound being for use as a PARP inhibitor to potentiate cytotoxic effects of a cytotoxic drug or radiotherapy.

8. A quinazolinone compound having the general structural formula II



II

or a pharmaceutically acceptable salt thereof,

wherein

X' represents hydroxyl, C_{1-6} alkyl or C_{1-6} alkoxy

and

Y' represents C_{1-6} alkyl or an optionally substituted phenyl group other than a phenyl group having a 4-propoxy substituent or a 2-alkoxy substituent,

subject to a proviso that

if X' is methyl, Y' is not butyl, isopropyl, phenyl or 2-aminophenyl,

if X' is ethyl, Y' is not 4-hydroxyphenyl,

if X' is methoxy, Y' is not methyl, isopropyl, 4-methylphenyl, 4-hydroxyphenyl or 4-methoxyphenyl,

if X' is ethoxy, Y' is not isopropyl,

if X' is propoxy, Y' is not a halogen substituted phenyl group, and

if X' is hydroxy, Y' is not methyl, ethyl or phenyl.

9. A compound as claimed in Claim 7 or 8 wherein Y' is a phenyl group having a substituent selected from $-NO_2$, $-NH_2$, -OH and alkyl.

10. A compound as claimed in Claim 9 wherein X' is hydroxyl.

11. A compound as claimed in Claim 7 or 8 wherein X' is hydroxyl and Y' is C_{1-6} alkyl.

12. A compound as claimed in any one of Claims 9 to 11 produced by a molecular rearrangement of a benzoxazole-4-carboxamide compound.

13. A quinazolinone compound as claimed in Claim 7 which is one of the following:

(a) 8-hydroxy-2-(4-nitrophenyl)-quinazolin-4-one; and

(b) 8-methoxy-2-phenylquinazolin-4[3*H*]-one.

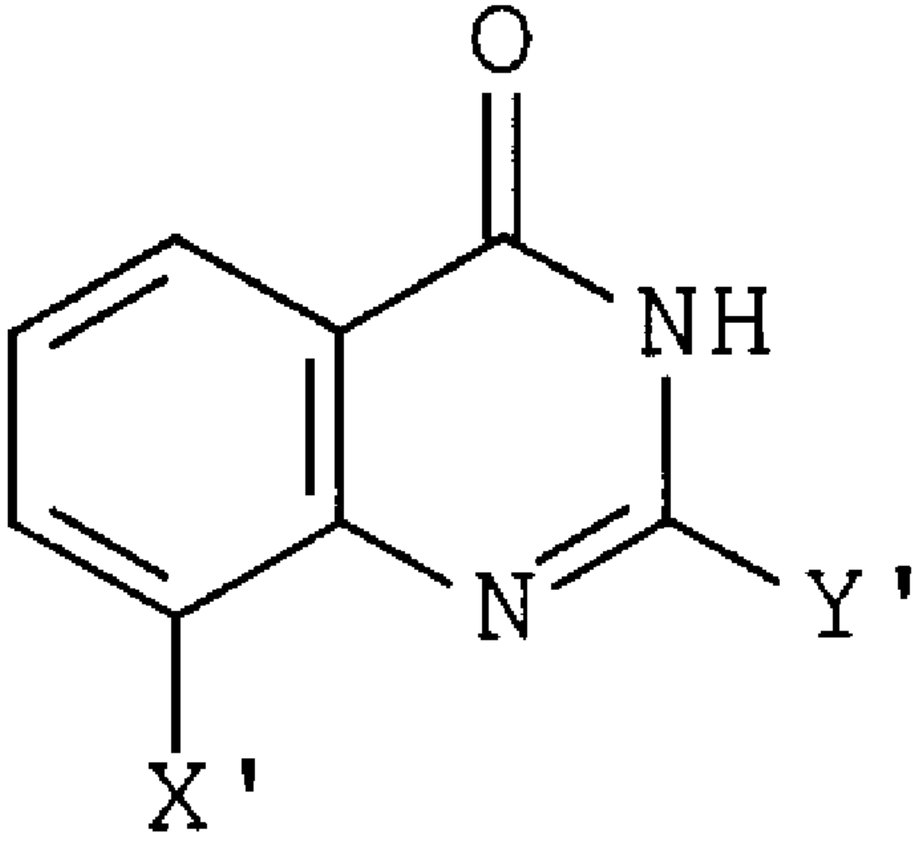
14. A compound as claimed in claim 7 which is 2,8-dimethylquinazolin-4[3*H*]-one.

15. A compound as claimed in any one of Claims 7 to 14 for use as an active PARP-inhibiting substance.

16. A pharmaceutical composition containing a compound as claimed in Claim 15 in unit dosage form and a pharmaceutically acceptable carrier, for treating a mammal likely to benefit from treatment with a PARP-inhibiting agent in the course of therapy.

17. A pharmaceutical composition for medical use comprising an effective PARP-inhibiting amount of a compound as claimed in any one of Claims 7 to 14 together with a pharmaceutically acceptable carrier.

18. A pharmaceutical composition as claimed in Claim 16 or 17 for use in conjunction with cytotoxic agents in antitumour therapy.



II