A facile synthesis of 2-[3-aRYL-4-oxo-3, 4-dihydro-quinazolin-2-ylsulfanyl]-propionic acid ARYLIDINE hydrazides as POTENTIAL PARP inhibitory agents.

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Abstract:

PARP is an important factor and hence a novel drug target for the therapy of retinopathy, nephropathy, neuropathy and accelerated atherosclerosis etc. Inhibitors of PARP prevent these effects and might therefore be useful in the treatment of diseases like myocardial infarction or organ transplantation. We describe here the synthesis of 2-[3-(3-chloro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-ylsulfanyl]-propionic acid (2-chloro-benzylidene)-hydrazide which is found to be moderately active for the PARP inhibition activity.

Introduction:

Poly (ADP-ribose) polymerase (PARP) is a nuclear protein involved in the response to DNA damage, where it catalyses the polymerization of NAD (nicotinamide adenine diphosphate) into chains of poly (ADP-ribose) polymers(Satoh and Lindahl 1994).The polymerization occurs on several nuclear proteins and on the PARP itself. Following activation by DNA strand breaks, PARP hydrolyses NAD and catalyses the formation of PARP onto itself and other nuclear proteins, with the release of nicotinamide. PARP binds to single and double stranded DNA breaks; Thus PARP is involved in the maintainance of chomatin integrity through repair of mild DNA damage.

In contrast, when cells experience massive levels of DNA damage and DNA strand breaks, activation of PARP can lead to depletion of NAD and ATP, resulting in a marked decrease in energy- dependent processes in DNA repair. In this situation, activation of PARP by massive DNA damage processes may actually be suicide response (Yamamoto et.al 1981; Sims et.al.1983; Schraufstatter et.al.1986; Szabo and Dawson 1998; Pieper et.al 1999; Ha and Snyder 2000), since it causes rapid NAD and ATP depletion and leads to cell death, before the cell can repair the DNA damage.

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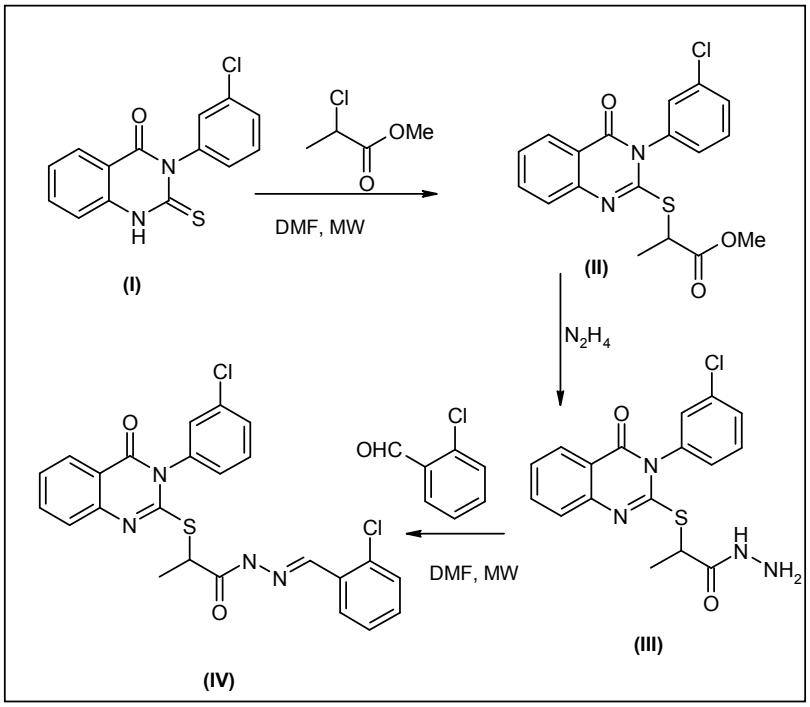
The dysfunctioning of PARP leads to ischemic injury in the brain, increased apoptosis in cancer cells, prevention of infiltration of neutrophils and subsequent inflammation. Diabetic patients frequently suffer from retinopathy, nephropathy, neuropathy and accelerated atherosclerosis, due to loss of endothelial function. PARP is an important factor and hence a novel drug target for the therapy of these dysfunctions.

Inhibitors of PARP prevent these effects and might therefore be useful in the treatment of diseases like myocardial infarction or organ transplantation. Benzamide, 6(5H)-phenanthridinone (PND) and3,4-dihydro-5-[4-1-(1-piperdinyl) buthoxy]-1(2H)-isoquinolinone (DPQ) are three PARP inhibitors with increasing degrees of potency and selectivity (Suto et.al. 1991).

Quinazoline derivatives are of special importance because of their versatile biological & pharmacological activities. The title quinazolinone derivative was synthesized as below.

Scheme



2-(3-(3-Chloro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-ylsulfanyl)- propionic acid methyl ester (II)

The mixture of 3-(3-chloro-phenyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-one **(I)** 7.2 g (25 mmol), methyl 2-chloropropionate 3.06 g (25mmol), 2.5 ml DMF and 1 g powdered NaOH was irradiated with microwaves (900 W /2450 MHz frequency) for 4 minutes on 50% power using a scientific microwave oven. The mixture was acidified and the solid was filtered, dried and recrystallized from Ethanol to get 7.9 g of **(II)**. Yield 75%, M.P.138^{o}C, Mol. Wt.374, Molecular formula $C_{18}H_{15}ClN_2O_3S$, Anal. C, 57.5 (57.68%); H, 4.0 (4.03%); N, 7.4 (7.47%). IR(KBr): ν_{max}, 1740(>C=O),1690(cyclic amido >C=O), 1625(C=N), 1060cm^{-1}(-O-), PMR (DMSO-d_6): δ, 1.5(3H,d J=7Hz. ,CH_3), 3.75(3H, s,OCH_3), 4.3(2H,q J=7Hz.,CH), 7.2-8.1(8H,m,Ar-H) ppm.

2-[3-(3-Chloro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-ylsulfanyl]-propionic acid hydrazide (III)

3.74 g of **(II)** (0.1 mol) mixed with 0.1 mol of hydrazine hydrate in 5ml methanol was refluxed on a steam bath for three hours. The separated solid was filtered and recrystalized from ethanol to get 3.18 g of **(III).** Yield 85%, M. P. 184^{o}C. , Molar Mass: 374, Mol. Formula: $C_{17}H_{15}ClN_4O_2S$, Anal.: C, 54.4(54.47%); H, 4.0(4.03%); N, 15.00 (14.95%), IR (KBr): ν_{max}, 3180-3060($NHNH_2$), 1675(cyclic >C=O), 1660(acyclic>C=O), 1620 (C=N), 760 cm^{-1} (C-Cl); PMR(DMSO-d_6):δ, 1.50 (3H,d,J=7.5Hz.,CH_3), 4.3(1H, q, J=7.5Hz, CH), 4.9(2H, s, br, exchangeable with D_2O, NH_2), 7.2-8.1(8H,m,Ar-H), 9.8(1H, s, br, CONH) ppm.

2-[3-(3-Chloro-phenyl)-4-oxo-3,4- dihydro-quinazolin-2-ylsulfanyl]-propionic acid [1-(2-chloro-benzylidene)hydrazide (IV)

Mixture of Compound **(III)** 1.87 g (5 mmol) and 2-chloro benzaldehyde 0.7 g (5 mmol) in 0.5 ml DMF and 2-3 drops of acetic acid was irradiated with microwaves (900 W /2450 MHz frequency) for 5 minutes on 50% power using a scientific microwave oven. When the irradiation was stopped, the mixture was added to ice-water mixture and the separated solid was filtered, conveniently dried and recrystallized from ethanol to get 1.94 g of **(IV).** Yield 78%, M.P. 332^{o}C., Molar Mass: 496, Mol. Formula : $C_{24}H_{18}Cl_2N_4O_3S$, Anal.: C,58.00(57.95%); H,3.68(3.65%); N,11.30(11.26 %) ,IR (KBr): ν_{max}, 1720(cyclic amido >C=O), 1680(acyclic >C=O),

1620(C=N), 760 cm^{-1} (C-Cl); PMR(DMSO-d_6): δ,1.45(3H,d, J=7.5Hz.,CH_3), 4.35(1H,q, J=7.5Hz.,CH),7.3-8.35(12H,m,Ar-H), 8.5(3H,s,=C-CH),9.5(1H,s,CONH) ppm.

PARP inhibition was optimized using PARP activity assay like DELFIA assay.

The DELFIA time resolved fluorescence assay for inhibition of poly (ADP-ribose) polymerase (PARP) is a 96-well microplate assay that measures the inhibition of PARP-activated incorporation of biotinylated nicotinamide adenine diphosphate (NAD). The incorporation was detected using Europium labeled streptovidine. The method was employed to screen test compound for PARP inhibitory activity in high throughput primary screening and secondary screening.

The inhibitor 3-amino benzamide was used as a standard for inhibition of PARP activity. The reaction was performed in 96-well microplate format. 15 μM NAD /Bio-NAD and 0.5U PARP enzyme reacted in the presence of 205 μg histones and 1 μg sheared DNA . The incorporation of biotin labeled NAD was detected using Eu-labelled streptavidin and the fluorescence was measured. The compound **(IV)** has shown 14% inhibition of PARP. in the DELFIA assay.

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