Synthesis of some sulfonamide incorporating enaminone, quinolone moieties and thiazoloquinazoline derivative induce the cytoprotective enzyme NAD(P)H: Quinone Oxidoreductase 1.

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Abstract

Sulfonamide resonant biologically active enaminone derivatives 3, 5, and quinolone derivative 8 have been created. Correspondingly, the thiazoloquinazoline derivative 13 was acquired in decent yield via reaction of 2-isothiocyanate derivative 9 with the L-nor ephedrine 10. The assemblies of the prepared amalgams stood established by microanalysis, IR, 1H-NMR, 13C-NMR and mass spectral information. Furthermore amalgams 3 was proved by X-ray crystallographic analysis. The NQO1 inducer activity of the synthesized compounds was assessed by means of a measurable bioassay in Hepa1c1c7 murine hepatoma cells. The thiazoloquinazoline (13) exhibited remarkable activity. Besides, incorporating the thiazol moiety within a heterocyclic ring system (quinazoline) increases the inducer potency. On the other hand, the enaminone derivatives (3 and 5) and quinoline (8) showed weak activity.

Keywords: NQO1, Electrophilicity cytoprotection, Enaminones, Quinolone, Quinazoline.

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Introduction

Chemotherapy is the mainstay for cancer conduct, the custom of obtainable chemotherapeutics is recurrently inadequate in arrears to obnoxious sideways possessions [1]. Midst the heterocyclic orderings, particularly persons encompassing enaminone and pyridine ring are allied with diverse pharmacological assets for example cytoprotection [2,3], antibacterial [4-6], anticonvulsant [7], antiviral [8], anti-HIV [9], antifungal and antimiycobacterial events [10]. Freshly substantial courtesy has been keen to the construction of new derivatives of quinoline on the justification of their stated biological activities [11-18]. From the prose review, numerous methods have been pronounced for the elaboration of substituted quinolones [19-21], which as a period have been testified to have anticancer and anti- leukaemic activity. Quinolines were institute to own numerous pharmacological belongings, counting uncontaminated [22-24] besides antitumor [25-31] deeds. Likewise, the chemistry of quinazoline and fused quinazoline spinoffs has been of cumulative curiosity, subsequently numerous of these compounds demonstrated quite a few biotic accomplishments too beneficial request as per antitumor [32-40], besides antiseptic go-betweens [41]. Alternatively, amongst the extensive assortment of compounds experienced by way of antitumor go-betweens, sulfonamides have fascinated prodigious courtesy, as countless sulfonamide offshoots were testified to have thought-provoking antitumor bustle [42-46]. Quite a lot of devices have been stated for the anticancer movement of the sulfonamide composites then the maximum protuberant of these mechanisms was concluded the reserve of the carbonic anhydrase isozymes [47-50]. The appliance of lump reserve by sulfonamide Carbonic Anhydrase (CA) inhibitor was optional by Boyle and Chegwidden [51], that these compounds possibly will diminish the endowment of bicarbonate for the amalgamation of nucleotides and erstwhile cell components for instance membrane lipids. In persistence of our exertion it give the impression of curiosity to manufacture roughly innovative 4-(quinoline-1-yl)-benzenesulfonamide and pyrimido[4,5-b] (quinoline-10-yl) benzenesulfonamide spinoffs, manner hypothetically vigorous lateral manacles, for instance cyano [32], ureido, thioureido [34] and thione [39] as per equivalents of multifarious E7070 [50] (Figure 1), to be assessed as probable cytoprotective mediators. In this effort we synthesized the enaminone, quinolone and the fused tricyclic thiazoloquinazoline derivatives brilliant with improved electron kinship for superior biological interactions by mono, bicyclic and tricyclic-fused heterocyclic systems to create compounds 3, 5, 8 and 13 to study their conceivable role in persuading the cytoprotective enzyme NQO1.
Figure 1. Compound E7070, a sulfonamide compound in progressive quantifiable hearings by way of anticancer negotiator.

**Experimental**

Melting points (°C, uncorrected) were strong-minded in uncluttered capillaries on a Gallenkemp melting point gadget (SanyoGallenkemp, Southborough, UK). Pre-caked silica gel saucers (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were castoff for thin layer chromatography, dichloromethane/methanol (9:5:0.5 mL) blend was cast-off as a emergent solvent system. IR spectra were logged in KBr discs via IR-Shimadzu spectrometer (Shimadzu, Tokyo, Japan). NMR spectra in (DMSO-d6) were chronicled on Bruker Ac-500 ultra-shield NMR spectrometer (Bruker, Flawil, Switzerland, δppm) at 500 MHz, consuming TMS as internal orthodox. Elemental analyses were achieved on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). Entirely compounds were within ±0.4 % of the hypothetical morals.

**Results**

4-(5,5-Dimethyl-3-oxocyclohex-1-enylamino)benzenesulfonamide (3)

A mixture of 5,5-dimethyl-cyclohexane-1,3-dione 1 (1.40 g, 0.01 mol) and sulfanilamide 2 (1.72 g, 0.01 mol) in absolute ethanol (15 ml) was refluxed for 3 h. The reaction mixture was cooled and then poured onto cold water, the obtained solid was recrystallized from ethanol to give 3 [52]. Yield, 81%; m.p. 235-237°C; IR, cm⁻¹: 3478, 3314, (NH, NH₂), 3055 (CH arom.), 2956, 2840 (CH aliph.), 1630 (C=O), 1324, 1150 (SO₂). ¹H-NMR (DMSO-d6) δ: 1.1 [s, 6H, 3CH₃], 2.1-2.4 [2s, 4H, 2CH₂], 5.3 [s, 2H, NH₂], 5.5 [s, 1H, CH], 7.2, 7.7 [2d, 4H, AB-system Ar-H], 11.1 [s, 1H, NH, D₂O-exchangeable]. MS, m/z (%): 294 [M⁺] (26.11%), 130 (100%). Anal. Calcd. For C₁₄H₁₈N₂O₃S (294): C, 57.12; H, 6.16; N, 9.52. Found: C, 57.50; H, 6.45; N, 9.18.

Oxo cyclohexenylamino)benzenesulfonamide (5)

A mixture of 1,3-cyclohexanedicarboxyl-amino)benzenesulfonamide 4 (1.12 g, 0.01 mol) and sulfanilamide 2 (1.72 g, 0.01 mol) in ethanol (30 mL) was refluxed for 5 h. The reaction mixture was cooled and then poured onto cold water, the obtained solid was crystallized from ethanol to give 5 [53]: Yield, 88%; m.p. 236-238°C; IR, cm⁻¹: 3354, 3263, 2210 (NH, NH₂), 3032 (CH arom.), 2940, 2870 (CH aliph.), 1611 (C=O), 1360, 1184 (SO₂). ¹H-NMR (DMSO-d6) δ: 1.0-2.2 [m, 6H, 3CH₂], 5.5 [s, 1H, CH], 7.0-7.8 [m, 6H, Ar-H SO₂NH₂], 9.0 [s, 1H, NH, D₂O-exchangeable]. Anal. Calcd. For C₁₂H₁₄N₂O₃S: C, 54.12; H, 5.30; N, 10.52. Found: C, 53.81; H, 5.62; N, 10.23.

4-[2-Amino-3-cyano-4-(2, 4-dichlorophenyl)-5-oxo-5, 6, 7, 8-tetrahydro quinolin-1(4H)-yl]benzenesulfonamide (8)

**Method A:** A mixture of enamino 5 (2.66 g, 0.01 mol) and 2-(2,4- dichlorobenzylidene) malononitrile 6 (2.23 g, 0.01 mol) in ethanol (20 mL) containing 3 drops of triethylamine was refluxed for 5 h. The reaction mixture was filtered while hot and the solid obtained was crystallized from dioxane to give 8 [53].

**Scheme 1.** Formation of enamiones and quinolone derivative 3, 5 and 8.

**Method B:** A solution of enamino 5 (2.66 g, 0.01 mol), 2,4-dichlorobenzaldehyde (1.75 g, 0.01 mol), and malononitrile (0.66 g, 0.01 mol) in absolute ethanol (20 mL) containing three drops of trimethylamine was refluxed for 6 h. The obtained solid after concentration was filtered and crystallized from ethanol to give 8. Yield, 90%; m.p. 291-293 °C;IR, cm⁻¹: 3464, 3347 (NH₂), 3064 (CH arom.), 2957, 2860 (CHaliph.), 2171
(CN), 1634 (C=O), 1374, 1189 (SO₂), 706 (C-Cl). 1H- NMR (DMSO-d⁶) δ: 1.8-2.2 [m, 6H, 3CH₂], 4.9 [s, 1H, CH], 5.4 [s, 2H, NH₂, D₂O-exchangable], 7.1-8.0 [m, 9H, Ar-H SO₂NH₂].

13C- NMR(DMSO-d⁶) δ: 21.9, 27.8, 34.8, 37.6, 58.6, 113.4, 116.5, 118.3 (CN), 128.2, 129.6, 130.9, 131.7, 132.8, 133.6, 136.7, 142.6, 145.9, 155.6, 167.8, 197.5 (C=O). MS, m/z (%): 489 [M⁺] (1.8), 73 (100). Anal. Calcd. For C₂₂H₁₈Cl₂N₄O₃S: C, 53.99; H, 3.71; N, 11.45. Found: C, 54.33; H, 3.49; N, 11.10.

**Scheme 2.** Formation of thiazoloquinazoline 13.

**Table 1.** NQO1 inducer activity of enaminone derivatives 3, 5, quinoline derivative 8 and thiazoloquinazoline 13.

<table>
<thead>
<tr>
<th>Compds No.</th>
<th>Induction Magnitude (Fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4-(5, 5-Dimethyl-3-oxocyclohex-1-enyla-mino)benzenesulfonamide) (3).</td>
<td>0.97</td>
</tr>
<tr>
<td>(Oxocyclohexenylamino)benzenesulfonamide) (5).</td>
<td>0.87</td>
</tr>
<tr>
<td>(4-[2-Amino-3-cyano-4-(2,4-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydro quinolin-1(4H)-yl]benzenesulfonamide) (8).</td>
<td>0.95</td>
</tr>
<tr>
<td>(1S, 2S)-3-methyl-2-phenyl-2, 3-dihydrothiazolo-[2, 3-b] quinazolin-5-one (13).</td>
<td>1.34</td>
</tr>
</tbody>
</table>

**Biological assay**

The NQO1 inducer motion was robust-minded by earnings of a measurable microtiter plate assay [55]. Hepa1c1c7 cells were full-fledged in αMEM supplemented with 10% (v/v) fetal bovine serum that had been heat- and charcoal- inactivated. Cells were habitually upheld in a humidified atmosphere at 37°C, 5% CO₂. For apiece experiment, cells (10⁴ per well) were plated in 96-well plates. Subsequently 24 h, the cell culture medium was traded with fresh medium comprising enaminones, and the cells were grown for a further 48 h.

Eight repeats of 8 serial dilutions of each compound were rummage-sale. Compounds were primed as stock solutions in DMSO, and then diluted in the cell culture medium 1:1000. The final concentration of DMSO in the medium was maintained at 0.1% (v/v). At the end of the 48 h exposure
period, cells were lysed for 30 min at 25°C in digitonin (0.1 g/L, pH 7.8). The precise activity of NQO1 was appraised in cell lysates by revenue of menadione as a substrate. Protein concentrations were strong-minded in apiece well by the BCA protein assay (Thermo Scientific).

![Figure 4](image)

**Discussion**

In this inquiry a string of enaminone results 3 and 5 and quinolone 7 attitude sulfonamide moiety and thiazoloquinazoline 13 were synthesized (Schemes 1 and 2) and biologically assessed for their in vitro cytoprotective activity. Enaminone 3 was obtained by condensation of 5, 5-dimethyl-1, 3-cyclohexandione 1 with sulfanilamide 2 [52].

The assembly of compound 3 was verified by microanalysis and spectral data. Moreover compound 3 was recognized by X-ray crystallographic analysis [56]. Alternatively, condensation of 1, 3-cyclohex-andione 4 with sulfanilamide 2 presented the corresponding enaminone 5, which upon reaction with 2-(2,4-dichlorobenzylidene) malononitrile 6, in ethanol containing a catalytic amount of triethylamine, bore 2-aminoquinoline-3-carbonitrile derivative 8 passing through the creation of the intermediate Michael type product 7, followed by intramolecular cyclization (Scheme 1).

Compound 8 was decidedly synthesized by alternative course linking one-pot condensation of the 2,4-dichlorobenzaldehyde, malononitrile, and enaminone 5 in a molar ratio (1:1:1) in refluxing ethanol comprising trimethylamine as catalyst. In this circumstance, formation of compound 8 exemplified in terms of early condensation of the aldehyde with malononitrile meet the expense of the triggered arylidenemalononitrile 6, followed by totaling of the enaminone 5 to the arylidenemalononitrile 6.

The consistent thiazoloquinoline 13 was gained in decent yield through reaction of 2-isothiocyanato derivative 9 with L-norephedrine (2-amino-1-phenylpropan-1-ol) 10 in dimethylformamide encompassing a catalytic quantity of trimethylamine. This reaction was ensue settled the creation of the intermediate 11 and 12 followed by intramolecular cyclization to bestowed the thiazoloquinazoline derivative 13 (Scheme 2).

**Biological activity**

NAD(P)H: quinone acceptor oxidoreductase 1 (NQO1) is a cytoprotective enzyme which is triggered by electrophilic compounds via the Keap1/Nrf2 pathway [29]. Plentiful compounds which have been publicized to induce NQO1 have been successively found to the broadly cytoprotective and to meritoriously inhibit tumor formation in animal models [30,31]. We found that the quinazoline ring carrying a biologically active thiazole moiety (thiazoloquinazoline) 13 bare notable activity. While, the enaminone derivatives 3, 5 and quinolone 8 revealed weak NQO1 inducer activity (Table 1 and Figure 4).

**Conclusion**

We testimony here the synthesis of exactly enaminone derivatives 3, 5, quinoline derivative 8 comprehending a biologically active sulfonamide moiety and thiazoloquinoline 13, it was evidently pragmatic that the fused three cyclic rings thiazoloquinazoline 13 is extra potent than enaminone derivatives 3, 5 and quinoline derivative 8. Still, joining the thiazolo moiety within a heterocyclic ring system (quinazoline) proliferations the inducer potency.

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