Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2020, 12(4): 8-15
(http://www.derpharmachemica.com/archive.html)

Visible Light Photoredox-Catalyzed Synthesis of Quinazolinone Derivatives and their cytotoxicity

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ABSTRACT

Visible light promoted efficient and eco-friendly photocatalytic method for the synthesis of quinazolinones. This protocol involves commencing readily available substituted isatins and 2-aminobenzamide by using Rose Bengal as an efficient recyclable photocatalyst. This method is operationally simpler and selective, carried out in shorter reaction time with visible light in higher yields. Using this protocol, a series of twenty compounds has been synthesized, all the synthesized compounds were evaluated for their cytotoxic potential on three human cancer cell lines and most of the compounds exhibited moderate to good cytotoxic activity, while some of them showed promising cytotoxicity with IC_{50} values ranging between 1.13 μM-1.77 μM.

Keywords: Cytotoxicity, Quinazolinones, Rose Bengal, Photocatalyst, Visible light.

INTRODUCTION

Quinazolinone derivatives are important bioactive nitrogen-containing heterocycles and are present in several natural products such as vitamins, alkaloids, etc. Due to their diverse pharmacological and biological activities [1] quinazolinone scaffolds are very important in the synthesis of various biologically active compounds. Recently, these compounds are reported to exhibit as gene associated peptide and vasopressin receptor antagonists [2,3]. Febrifugine (I), a quinazolinone alkaloid was first isolated from the Chinese herb Dichoria febrifuges. It has important biological properties such as antimalarial [4], anticancer [5]and anti-inflammatory [6,7]. DPC-961 (II) and SM-15811 (III) (Figure 1) are compounds having quinazolinone ring and are known to exhibit 2nd generation anti-HIV activity, ion exchanger properties and also used to treat heart diseases and luotonine A showed excellent cytotoxicity towards the murine leukemia and rutaecarpine used extensively as a remedy for headache, cholera, and dysentery. Considering the significance of quinazolinones from an application perspective, although a number of methods have been made to construct the quinazolinones [8], these routes mainly rely on using anthranilic acid or its derivatives as starting materials. Generally, metal catalysed reductive cyclization of anthranilamide with ketones, aldehydes and isatins by the alternative approaches used for the synthesis of substituted quinazolinones [9].

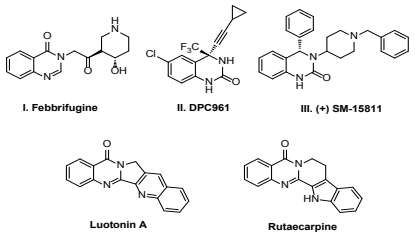


Figure 1: Biologically important quinazolinones

However, these methods have some eco-friendly aspects but these methods are associated with several drawbacks such as usage of hazardous organic solvents, reagents, metal catalysts, harsh reaction conditions and low yields. So, there is a need for the development of green, sustainable, efficient and safety and alternative method for the synthesis of quinazolinones via visible light promoted metal free photocatalyst in environmental aspect was highly desirable.

In the present scenario, visible light photo redox catalysis has fascinated the chemists in the forefront of the organic synthesis due to its attractive features like mild reaction conditions, safe, cheap, abundant and renewable energy source [10]. It is a dominant tool to complete novel organic chemical transformations through single-electron-transfer pathway. Continuing our efforts towards the synthesis of heterocyclic scaffolds [11] herein, we developed a visible light driven environmentally benign process for the synthesis of quinazolinones from readily available 2-aminobenzamide and isatins at room temperature under air by using organic dye such as Rose Bengal as a recyclable photocatalyst.

MATERIALS AND METHODS

All chemicals, reagents were purchased from the commercial sources and were used without further purification. Reactions were monitored by TLC on silica gel glass plate containing 60 GF-254, and visualization was done by UV light and iodine vapour. ^{1}H and ^{13}C NMR spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) or Innova Varian-VXR-unity (400 MHz, 500 MHz) instruments. Chemical shifts were expressed in parts per million (in ppm) downfield from TMS expressed as internal standard and coupling constants are expressed in Hz. ^{1}H NMR spectral data were reported in the following order: multiplicity (s: singlet; brs: broad singlet; d: doublet; dd: doublet of doublets; t: triplet; m: multiplet), coupling constants in Hz, and number of protons. ESI mass spectra were recorded on a Micromass Quattro LC using ESI+ software with capillary voltage 3.98 kV and an ESI mode positive ion trap detector. High resolution mass spectra were recorded on a QSTAR XL Hybrid MS-MS mass spectrometer. Melting points were determined with an electro thermal digital melting point apparatus IA9100 and are uncorrected.

General reaction procedure for the preparation of compounds (3a-3t)

All the reactions were performed using the following procedure.

To the solution of 2-aminobenzamide (1) (1 mmol) in ethanol add substituted isatin (2) (1 mmol) and Rose Bengal (2 mol%), under the visible light (15 W) setup at room temperature for 2 h, appearance of precipitate indicates the product formation and which was also monitored by the TLC. The solvent was removed by the rota vacuum then purified by the column chromatography to offered quinazolinones in good to excellent yields.

1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3a)

Dirty white solid; 87% yield; mp 290°C-295°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.76 (s, 1H), 7.80 (d, *J*=7.9 Hz, 1H), 7.52 (d, *J*=7.4 Hz, 1H), 7.29-7.21 (m, 2H), 7.03 (t, *J*=7.4 Hz, 2H), 6.85 (d, *J*=8.2 Hz, 1H), 6.76 (t, *J*=7.6 Hz, 1H), 6.67 (d, *J*=8.1 Hz, 1H), 6.15 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.79, 164.14, 146.53, 141.88, 132.98, 130.38, 129.18, 126.73, 124.94, 121.93117.05, 114.07, 113.77, 109.96, 70.84; IR (KBr pellets) υ: 3363, 3339, 3179, 3050, 1730, 1706, 1663, 1620, 1509, 1483, 1470, 1359, 1324, 1185, 748 cm^{-1}; ESI-MS : *m/z*=266 $(M+H)^{+}$; HRMS (ESI) *m/z* for $C_{15}H_{12}O_2N_3$ calculated *m/z*: 266.0924, found *m/z*: 266.0922 $(M+H)^{+}$.

5-chloro-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3b)

White solid; 80% yield; mp 289°C-294°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.98 (s, 1H), 7.76 (t, *J*=7.6 Hz, 2H), 7.23 (s, 2H), 6.96 (t, *J*=7.2 Hz, 1H), 6.82-6.58 (m, 4H) ; ^{13}C NMR (75 MHz, DMSO-d_6): ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.80, 164.10, 145.72, 141.31, 139.09, 133.55, 133.28, 130.99, 127.05, 117.82, 113.84, 112.42, 83.96, 70.76; IR (KBr pellets) υ: 3252, 1731, 1660, 1651, 1613, 1514, 1504, 1483, 1440, 1359, 1268, 1190, 753, 694 cm^{-1}; ESI-MS: *m/z*=300 $(M+H)^{+}$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2N_3Cl$ calculated *m/z*: 300.0534, found *m/z*: 300.0534 $(M+H)^{+}$.

5-bromo-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3c)

Light red solid; 77% yield; mp 280°C-285°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.02 (s, 1H), 7.79 (d, *J*=7.55 Hz, 1H), 7.61 (d, *J*=1.70 Hz, 1H), 7.48-7.51 (m, 1H), 7.40 (dd, *J*=1.88 & 8.30 Hz, 1H), 7.24 (dt, *J*=1.51 & 8.30 Hz, 1H), 6.74-6.78 (m, 2H), 6.68 (d, *J*=7.93 Hz, 1H), 6.43 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.28, 163.92, 146.04, 140.91, 133.01, 132.89, 131.17, 127.81, 126.74, 117.32, 113.88, 113.74, 111.67, 70.83; IR (KBr pellets) υ:1731, 1660, 1614, 1513, 1482, 1434, 1358, 1302, 1268, 1189, 1145, 1122, 817, 752, 628, 566, 538 cm^{-1}; ESI-MS: *m/z*=343 $(M)^{+}$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2N_3Br$ calculated *m/z*: 343.0029, found *m/z*: 343.0030 $(M)^{+}$.

5-flouoro-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3d)

White solid; 79% yield; mp 285-287°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.98 (s, 1H), 7.78 (m, 2H), 7.23 (bs, 2H), 7.01 (t, *J* =7.36 Hz, 1H), 6.81-6.66 (m, 3H), 6.58 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.80, 164.10, 146.14, 137.63, 133.09, 130.43, 126.80, 117.40, 116.80, 113.94, 113.83, 112.67, 110.70, 71.12; IR (KBr pellets) υ: 3282, 1741, 1661, 1632, 1613, 1520, 1185, 1146, 1124, 1042, 817, 758, 710, 693, 636, 616 cm^{-1}; ESI-MS: *m/z*=284 $(M+H)^{+}$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2N_3F$ calculated *m/z*: 284.0829, found *m/z*: 284.0831 $(M+H)^{+}$.

5-iodo-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3e)

White solid; 80%yield; mp 240-245°C; ^{1}HNMR(300MHz, DMSO-d_6): 10.04 (s, 1H), 7.77 (d, *J*=8.6 Hz, 3H), 7.58 (d, *J*=7.9 Hz, 1H), 7.25 (t, *J*=7.55 Hz, 1H), 6.76-6.64 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.02, 164.18, 145.72, 141.31, 139.09, 133.55, 133.28, 130.99, 127.05, 117.82, 113.84, 112.42, 83.96, 70.76; IR (KBr pellets) υ: 3282, 1741, 1661, 1613, 1520, 1484, 1425, 1371, 1335, 1299, 1265, 1185, 1146, 1124, 1042, 817, 758, 636, 616 cm-1; ESI-MS: *m/z*=390 $(M)^{+}$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2N_3I$ calculated *m/z*: 391.9890, found *m/z*: 391.9892 $(M)^{+}$.

5-methyl-1H-spiro[indoline-3,2-quinazoline]-2,4'(3'H)-dione (3f)

White solid; 85% yield; mp 150-155°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.81 (s, 1H), 7.75 (d, *J*=7.17 Hz, 1H), 7.40 (brs, 1H), 7.34 (s, 1H), 7.22 (t, *J*=8.30 Hz, 1H), 7.07 (d, *J*=7.55 Hz, 1H), 6.65-6.80 (m, 3H), 6.49 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.77, 164.19, 146.40, 139.20, 132.94, 131.16, 130.62, 129.00, 126.75, 125.49, 117.06, 113.97, 113.72, 109.73, 70.92, 20.48; IR (KBr pellets) υ: 3314, 2922, 2853, 1706, 1655, 1613, 1513, 1489, 1357, 1329, 1270, 1207, 1154, 811, 751, 698 cm^{-1}; ESI-MS: *m/z*=280 (M+H); HRMS (ESI) *m/z* for $C_{16}H_{14}O_2N_3$ calculated *m/z*: 280.1080, found *m/z*: 280.1081 (M+H)$^+$.

5-nitro-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3g)

White solid; 84% yield; mp 230-235°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.85 (s, 1H), 8.19-8.40 (m, 3H), 7.71 (d, *J*=7.17 Hz, 1H), 7.14 (s, 1H), 7.24 (t, *J*=8.49 Hz, 1H), 7.00 (d, *J*=8.68 Hz, 1H), 6.75 (t, *J*=7.55 Hz, 1H), 6.67 (d, *J*=7.93 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.04, 163.82, 148.22, 145.90, 142.41, 133.19, 130.02, 127.10, 126.81, 120.62, 117.62, 113.90, 109.99, 70.56; IR (KBr pellets) υ: 3342, 3021, 2860, 1727, 1649, 1616, 1522, 1482, 1405, 1339, 1296, 1252, 1229, 1189, 1143, 1121, 1085, 837, 746, 733, 690, 635, 550 cm^{-1}; ESI-MS: *m/z*=311 (M+H)$^+$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_4N_4$ calculated *m/z*: 311.0774, found *m/z*: 311.0775 (M+H)$^+$.

1-benzyl-5-chloro-1-H-spiro[indoline-3,2-quinazoline]-2,4(3H)-dione (3h)

White solid; 79% yield; mp 150°C-155°C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.1 (s, 1H), 7.8 (d, *J*=7.9 Hz, 1H), 7.6 (s, 1H), 7.55 (t, *J*=8.1 Hz, 2H), 6.9 (s, 1H), 6.77-6.68 (m, 4H), 6.5 (bs, 1H), 6.0 (bs, 1H), 4.9 (s, 1H), 4.8 (s, 2H,), 3.8 (d, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.16, 141.26, 133.8, 133.4, 130.75, 129.4, 126.09, 123.8, 121.6, 116.4, 115.41, 113.70, 107.65, 69.27, 41.89; IR (KBr pellets, υ: 3758, 3713, 3707, 3685, 3644, 3625, 3583, 3270, 2923, 2357, 1737, 1731, 1715, 1666, 1660, 1650, 1644, 1633, 1613, 1514, 1504, 1494, 1485 1434, 1336 cm^{-1}; ESI-MS *m/z*: 389 (M+); HRMS (ESI) *m/z* for $C_{22}H_{17}ClN_3O_2$ calculated *m/z*: 390.1003, found *m/z*: 390.1004 (M+H)$^+$.

5-methoxy-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3i)

White solid; 85% yield; mp 270°C-275°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.74 (s, 1H), 7.79 (d, *J*=7.8 Hz, 1H), 7.5 (s, 1H), 7.45 (bs, 1H), 7.1 (s, 1H), 6.81-6.45 (m, 4H), 6.45 (s, 1H), 3.75 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.8, 163.94, 155.10, 154.46, 146.58, 134.95, 132.87, 130.34, 128.28, 127.81, 126.64, 123.55, 116.93, 116.58, 115.37, 144.08, 113.69, 111.36, 110.39, 108.35, 71.18, 55.29; IR (KBr pellets) υ: 3314, 3215, 2924, 1719, 1648, 1609, 1513, 1485, 1440, 1357, 1296, 1270, 1234, 1162, 1043, 1024, 824, 794, 756, 715, 698, 672, 621, 578 cm^{-1}; ESI-MS : *m/z*=296 (M+H)$^+$; HRMS (ESI) *m/z* for $C_{16}H_{14}O_3N_3$ calculated *m/z* : 296.1029, found *m/z*: 296.1030 (M+H)$^+$.

5,6-dimethyl-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3j)

White solid; 87% yield; mp 320°C-325°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.81 (s, 1H), 7.75 (d, *J*=7.2 Hz, 1H), 7.40 (bs, 1H), 7.34 (s, 1H), 7.22 (t, *J*=8.30 1H), 7.07 (d, *J*=7.55 Hz, 1H), 6.80-6.65 (m, 2H), 6.49 (s, 1H), 2.39 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.78, 164.20, 146.41, 139.20, 132.94, 131.16, 130.62, 129.00, 126.75, 125.49, 117.06, 113.97, 113.72, 109.73, 70.92, 20.48; IR (KBr pellets) υ: 3311, 3301, 2902, 2833, 1706, 1685, 1623, 1513, 1489, 1357, 1329, 1270, 1207, 1154, 811, 751, 698 cm^{-1}; ESI-MS : *m/z*=293 (M+H)$^+$; HRMS (ESI) *m/z* for $C_{17}H_{16}O_2N_3$ calculated *m/z*: 294.1237, found *m/z*: 294.1239 (M+H)$^+$.

1-benzyl-1'H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3k)

White solid; 82% yield; mp 240°C-245°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (s, 1H), 7.79 (d, *J*=7.4 Hz, 1H), 7.68 (s, 1H), 7.59 (d, *J*=8.2 Hz, 1H), 7.12 (t, *J*=7.2 Hz, 1H), 6.93-6.84 (m, 3H), 6.77-6.68 (m, 5H), 5.94 (s, 2H), 4.72 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.05, 171.39.164.31, 149.33, 146.18, 142.32, 134.91, 131.78, 130.43, 128.21, 127.11, 126.86, 124.83, 120.30, 117.44, 114.89, 113.88, 109.01, 107.78, 100.53, 70.65, 42.92; IR (KBr pellets) υ: 3329, 3179, 3054, 2918, 1705, 1667, 1615, 1511, 1489, 1466, 1454, 1362, 1314, 1264, 1175, 1142, 993, 749, 696, 678, 630 cm^{-1}; ESI-MS : *m/z*=356 (M+H)$^+$; HRMS (ESI) *m/z* for $C_{22}H_{18}O_2N_3$ calculated *m/z*: 356.1393, found *m/z*: 356.1395 (M+H)$^+$.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-1H-spiro[indoline-3,2-quinazoline]2,4(3H)-dione (3l)

White solid; 84% yield; mp 260°C-265°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 (s, 1H), 7.79 (d, *J*=7.6 Hz, 1H), 7.66 (s, 2H), 7.57 (d, *J*=7.2 Hz, 1H), 7.48 (d, *J*=7.3 Hz, 1H), 7.31-6.68 (m, 7H), 5.94 (s, 2H), 4.72 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.05, 164.22, 147.44, 146.47, 146.28, 142.27, 133.03, 130.38, 128.64, 126.79, 124.75, 122.64, 120.31, 117.31, 114.05, 113.84, 109.16, 107.80, 107.41, 100.57, 70.67, 42.67; IR (KBr pellets) υ: 3193, 3059, 1658, 1670, 1615, 1487, 1467, 1444, 1356, 1272, 1244, 1190, 1174, 1143, 1100, 1036, 743, 695 cm^{-1}; ESI-MS: *m/z*=400 (M+H)$^+$; HRMS (ESI) *m/z* for $C_{23}H_{18}O_4N_3$ calculated *m/z*: 400.1291, found *m/z*: 400.1293 (M+H)$^+$.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-5-chloro-1H-spiro[indoline-3,2-quinazoline]2,4(3H)-dione (3m)

White solid; 79% yield; mp 195°C-200°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.11 (d, *J*=7.17 Hz, 1H), 7.68 (d, *J*=9.63 Hz, 1H), 7.52 (t, *J*=7.93 Hz, 1H), 7.41-7.25 (m, 3H), 6.99 (t, *J*=6.42 Hz, 2H), 6.85-674 (m, 4H), 6.50 (s, 1H), 5.92 (d, *J*=7.36, 2H), 4.81 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.57, 166.34, 145.08, 136.68, 133.49, 131.48, 131.20, 130.37, 127.83, 125.66, 124.99, 123.36, 120.15, 116.69, 116.12, 114.68, 111.76, 111.06, 107.59, 107.05, 100.35, 42.59; IR (KBr pellets) υ: 3272, 1670, 1659, 1651, 1647, 1632, 1614, 1573, 1562, 1551, 1536, 1532, 1513, 1492, 1483, 1470, 1453, 1432, 1415, 1391, 1370, 1335, 1171, 1252, 1123, 1080, 1028, 817, 753 cm^{-1}; ESI-MS: *m/z*=434 (M+H)$^+$; HRMS (ESI) *m/z* for $C_{23}H_{17}O_4N_3Cl$ calculated *m/z*: 434.0903, found *m/z*: 434.0905 (M+H)$^+$.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-5-bromo-1H-spiro[indoline-3,2-quinazoline]2,4 (3H)-dione (3n)

White solid; 76% yield; mp 160°C-165°C; ^{1}H NMR (DMSO-D6, 300 MHz, ppm) δ: 7.53, (t, *J*=8.3 Hz, 3H), 7.3 (bs, 1H), 7.20 (t, *J*=8.3 Hz, 3H), 6.7 (d, *J*=7.9 Hz, 3H), 6.62 (t, *J*=7.7 Hz, 2H), 5.9 (bs, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.47, 164.31, 149.33, 146.18, 142.32, 134.91,

133.07, 131.78, 128.26, 127.11, 126.86, 124.83, 122.67, 120.30, 117.44, 116.44, 114.89, 113.88, 133.64, 109.01, 70.65, 42.92; IR (KBr pellets) υ: 3411, 2923, 2853, 1731, 1650, 1585, 1546, 1487, 1452, 1402, 1315, 1257, 1151, 743 cm^{-1}; ESI-MS *m/z*: 433 (M+). HRMS (ESI) *m/z* for $C_{22}H_{17}BrO_2N_3$ calculated *m/z*: 434.0498, found *m/z*: 434.0499 $(M+H)^+$.

7-fluoro-1H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3o)

light white solid; 79% yield; mp 310°C-315°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.96 (s,1H), 7.78 (d, *J*=7.5 Hz, 2H), 7.70 (s, 1H), 7.23 (s, 1H) 7.09, 6.99 (t, *J*=7.36 Hz, 1H), 6.81-6.78 (m, 2H), 6.68 (s, *J*=7.9 Hz, 1H) 6.58 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.80, 164.10, 146.14, 137.63, 133.09, 130.43, 126.80, 117.40, 116.80, 113.94, 113.83, 112.67, 110.70, 71.12; IR (KBr pellets) υ: 3326, 3177, 1732, 1614, 1588, 1516, 1486, 1370, 1358, 1314, 1272, 1254, 1203, 1152, 1083, 1033, 902, 736, 686, 661, 603, 578, 527 cm^{-1}; ESI-MS: *m/z*=284 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2N_3F$ calculated *m/z*: 284.0829, found *m/z*: 284.0831 $(M+H)^+$.

1'H-spiro[cyclohexane-1,2'-quinazoline]-4'(3'H)-one (3p)

White solid; 86% yield; mp 235°C-239°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.25, (s, 1H), 6.85 (d, *J*=7.93 Hz, 2H), 5.24 (s, 1H), 2.77 (bs, 1H), 2.17 (s, 2H), 1.82-1.45 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.69, 145.95, 132.90, 127.12, 116.85, 114.25, 67.67, 36.95, 24.17, 21.00; IR(KBr pellets) υ: 3365, 3169, 3023, 2926, 2852, 1647, 1504, 1482, 1416, 1381, 1323, 1268, 1143, 756 cm^{-1}; ESI-MS: *m/z*=217 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{13}H_{17}ON_2$ calculated *m/z*: 217.1335, found *m/z*: 217.1336 $(M+H)^+$.

6-chloro-1'H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3q)

White solid; 81% yield; mp 284°C-289°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (s, 1H), 6.51 (d, *J*=7.82 Hz, 3H), 6.33 (d, *J*=7.93 Hz, 1H), 5.97 (t, *J*=8.23 Hz, 1H), 5.51-5.39 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.46, 163.62, 145.16, 140.78, 138.53, 132.72, 130.42, 126.49, 117.26, 111.86, 83.40, 70.20, 59.20, 19.43, 13.11; IR (KBr pellets) υ: 3256, 1734, 1661, 1651, 1612, 1516, 1505, 1483, 1442, 1358, 1267, 1191, 754, 695 cm^{-1}; ESI-MS: *m/z*=300 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2N_3Cl$ calculated *m/z*: 300.0532, found *m/z*: 300.0534 $(M+H)^+$.

6-bromo-1'H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3r)

Brown solid; 73% yield; mp 275°C-280°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.77 (s, 1H), 6.47 (t, *J*=8.68 Hz, 3H), 6.30 (t, *J*=7.93 Hz, 1H), 5.95 (t, *J*=7.55 Hz, 1H), 5.49-5.37 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.85, 165.02, 146.56, 142.15, 139.92, 134.38, 131.82, 127.88, 118.68, 114.67, 113.25, 84.79, 71.59, 60.59, 14.50; IR (KBr pellets) υ: 1735, 1661, 1617, 1514, 1483, 1435, 1358, 1301, 1267, 1187, 1146, 1121, 816, 754, 628, 564, 539 cm^{-1}; ESI-MS: *m/z*=343 $(M)^+$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2N_3Br$ calculated *m/z*: 344.0029, found *m/z*: 343.0031 $(M)^+$.

4-methyl-1'H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3s)

White solid; 76% yield; mp 152-154°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.98 (s, 2H), 7.57 (t, *J*=7.55 Hz, 5H), 7.56 (s, 1H), 6.99 (s, 2H), 2.03 (d, *J*=7.36 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.24, 163.54, 155.97, 145.59, 137.08, 132.54, 126.25, 116.85, 116.25, 115.94, 113.39, 112.11, 111.79, 110.15, 70.57; IR (KBr pellets) υ: 3317, 2921, 2851, 1708, 1653, 1613, 1514, 1486, 1357, 1328, 1270, 1205, 1151, 817, 755, 698 cm^{-1}; ESI-MS: *m/z*=280 (M+H); HRMS (ESI) *m/z* for $C_{16}H_{14}O_2N_3$ calculated *m/z*: 280.1081, found *m/z*: 280.1083 $(M+H)^+$.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-6-chloro-1'H-spiro[indoline-3,2'-quinazoline]-2,4'(3'H)-dione (3t)

White solid; 79% yield; mp 195°C-200°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.18 (d, *J*=7.18 Hz, 1H), 7.78 (s, 1H), 7.61(s, 1H), 7.50-7.35 (m, 3H), 7.11-7.06 (m, 1H), 76.93-6.83 (m, 4H), 6.60 (s, 1H), 6.02 (d, *J*=7.17 Hz, 2H), 4.96 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.06, 152.48, 147.05, 144.80, 136.40, 133.22, 130.09, 127.55, 124.72, 119.87, 116.41, 114.40, 110.78, 106.77, 100.07, 42.65; IR (KBr pellets) υ: 3272, 1670, 1659, 1651, 1647, 1632, 1614, 1573, 1562, 1551, 1536, 1532, 1513, 1492, 1483, 1470, 1453, 1432, 1415, 1391, 1370, 1335, 1171, 1252, 1123, 1080, 1028, 817, 753 cm^{-1}; ESI-MS: *m/z*=434 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{23}H_{17}O_4N_3Cl$ calculated *m/z*: 434.0903, found *m/z*: 434.0905 $(M+H)^+$.

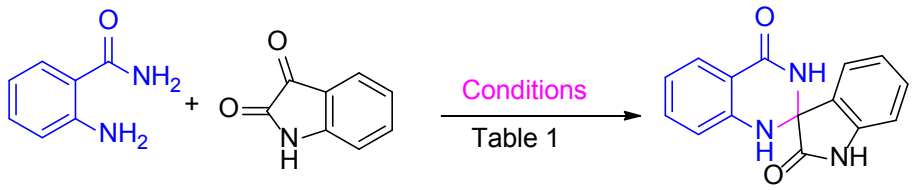
MTT ASSAY

The cytotoxicity of these compounds was determined using MTT assay.13 Cancer cells (DU-145, MCF-7, HeLa and A549) were used in this assay. 1×104 cells/well were seeded in 200 µl Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% FBS in each well of 96-well micro culture plates and incubated for 24 h at 37°C in a CO_2 incubator. All the derivatives diluted to the desired concentrations (500 nM, 1 µM, 5 µM, 10 µM, 25 µM, 50 µM, 75 µM, 100 µM and 150 µM) in culture medium, were added to the wells with respective vehicle control. Doxorubicin treated cells, in the same concentration range were used as standards. After 48 h of incubation, 10 µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) (5 mg/ml) was added to each well and the plates were further incubated for 4 h. Then the supernatant from each well was carefully removed, Formosan crystals were dissolved in 100 µl of DMSO and absorbance at 570 nm wavelength was recorded at a wavelength of 540 nm using an ELx800 micro plate reader (BioTek, USA) [12,13].

RESULTS AND DISCUSSION

Chemistry

Table 1: Reaction optimization conditions[a]



1a **2a** **3a**

Entry	Photocatalyst (mol%)	T (^{O}C)/ t (h)	Solvent	Yield (3a) (%)[b]
1[c]	_	rt/6	Methanol	10
2[c]	_	rt /6	Water	12
3[c]	_	rt /6	Acetonitrile	15
4	Rhodamine B (1 mol%)	rt /3	Ethanol	55
5	Rhodamine B (2 mol%)	rt /3	Methanol	50
6	Rose Bengal (2 mol%)	rt /3	Water	40
7	Rose Bengal (2 mol%)	rt /3	DCM	45
8	Rose Bengal (2 mol%)	rt /3	2-propanol	50
9	Rose Bengal (2 mol%)	rt /3	Acetonitrile	65
10	Rose Bengal (2 mol%)	rt /3	DCE	42
11	Rose Bengal (2 mol%)	rt /3	acetone	46
12	Rose Bengal (1 mol%)	rt /3	ethanol	60
13	Rose Bengal (2 mol%)	rt /2	ethanol	90
14	Rose Bengal (3 mol%)	rt /2	ethanol	91
15	Rose Bengal (2 mol%)	rt /6	methanol	84
16	Ru $(bpy)_3Cl_2.6H_2O$ (2 mol%)	rt /5	methanol	80
17	Ir $(ppy)_3$(2 mol%)	rt /5	methanol	78
18	Eosin-y (2 mol%)	rt /4	methanol	76
19[d]	Rose Bengal (2 mol%)	rt /6	methanol	ND

[a] Reaction conditions: 1a (1 mmol), 2a (1 mmol), solvent (10 mL) and a 15 W white LED bulb kept at a distance of 10 cm (approx.) from the reaction vessel.
[b] Yields of the isolated products after column chromatography.
[c] Absence of photocatalyst.
[d] the reaction was run in dark. ND=the desired product was not detected on TLC.

Initially, to understand our idea and optimize the reaction conditions the key reaction was performed by using 2-aminobenzamide (1a) and isatin (2a) without any catalyst at atmospheric temperature (entry 1-3, Table 1) for 6 h, here the reaction was not preceded good, confirmed by the TLC monitoring. Further, a solution of 1a and 2a in the presence of 1 mol% of Rhodamine B was illuminated with a 15 W white LED bulb at room temperature for 3 h and quinazolinone was obtained in 50%-55% yield (Table 1, entries 4, 5). We increased the amount of Rhodamine B from 1 mol% to 2 mol% then we observed that there is no much increase in the yield of the product. Next, the control experiments were observed in the absence of a photocatalyst or light (entries 1-3) and switch on-off trials were also shown that the light was necessary. This result encouraged us to optimize the reaction conditions further, a various photocatalysts were screened. The choice of photocatalysts showed Rose Bengal was the most efficient catalyst (Table 1, entries 6-15). Moreover, other photocatalyst such as eosin-y, Ru and Ir photocatalysts has been less efficient while compared to Rose Bengal (Table 1, entries 16-18). Once Rose Bengal has been identified as the best photocatalyst for promoting this reaction (scheme 1), on the way to optimization of the amount of photocatalyst necessary for obtaining high yield of quinazolinone 3. A sequence of trails has been conducted by diverse quantities of Rose Bengal wherein it was observed that use of 2 mol% catalyst gave the finest result. Dropping the

quantity of catalyst resulted in poor yield. As we increased the amount of Rose Bengal from 2 mol% to 3 mol%, there is no much increment in the yield of the desired product. Moreover, the advantage of the Rose Bengal is its reusability,[12] the catalyst can be reused up to three cycles without loss of catalytic property as shown in Figure 2.

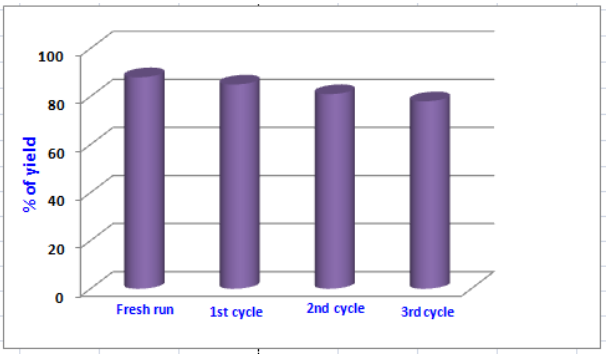
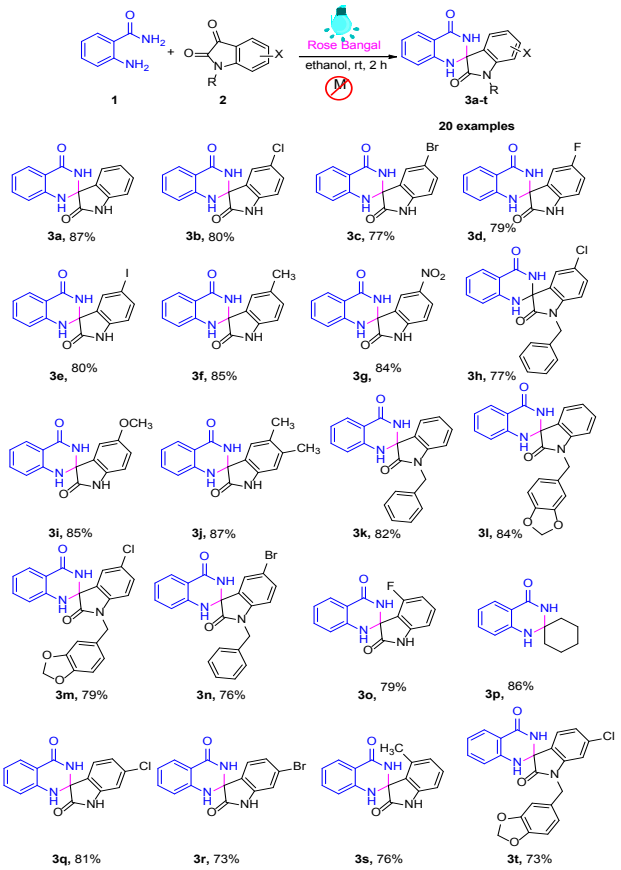


Figure 2: Graph representing reusability of the catalyst

In addition, all the favoured solvents were screened for the reaction by using 2 mol% of Rose Bengal as a photocatalyst in the presence of white LED bulb (15 W). Among all these solvents tested ethanol was found to be the best given 90% yield of compound 3a in a reaction time of 2 h (Table 1, entry 13). In other solvents such as water, acetone, dichloroethane (DCE), ethanol, 2-propanol, acetonitrile and dichloromethane (DCM) the yield of the reaction was moderate (Table 1, entries 6-12).

Having conventional best reaction conditions in hand, we extended the scope of the present photocatalytic protocol using various substituted isatins and 2-Aminobenzamide for the synthesis of corresponding products.

Table 2: Synthesis of quinazolinone derivatives



Interestingly, it was successful with various Isatins (e.g. H, Cl, Br, Me, F, NO2, I, di-metheyl, benzyl, 5-Cl-benzyl, 5-Br-benzyl and di-Chloro) and 2-amino benzamide in excellent yields (Table 2, 70%-90%). Furthermore, the optimized condition has been efficiently constructive to synthesize quinazolinones.

Plausible Mechanism

The plausible mechanism for the formation of quinazolinone ring from isatin and 2-aminobenzamide is outlined in Figure 3. The reaction is hypothetical to proceed with the formation of a keto imine from 2-aminobenzimide with isatin. Hence, this step was promoted by the photocatalyst Rose Bengal and it reacted with the keto group to form an imine derivative (a) with the elimination of water. The excited state of Rose Bengal (RB)

was generated by visible light irradiation and converted to RB* and it abstract an electron from the imine via SET process followed by the formation of RB radical anion which is converted to ground state RB by dioxygen resulted in the formation of desired product (3a).

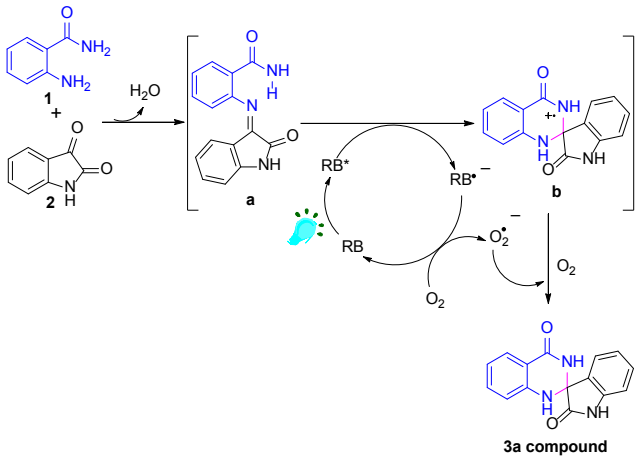


Figure 3: Plausible mechanism

Cytotoxic activity

To estimate the cytotoxicity of quinazolinone derivatives (3a-t) MTT assay was carried out on the selected human cancer cell lines, such as lung cancer (A549), breast cancer (MCF-7) and prostate cancer (DU-145). The cytotoxicity results in comparison to the positive controls like doxorubicin are expressed in IC50 values and are summarized in Table 3. The results revealed that the all the synthesized compounds 3a-t exhibited good to moderate cytotoxic activities with IC50 values ranging from 1.13 µM to 29.6 µM. Among them the compounds 3h and 3p showed promising cytotoxicity with IC50 values 1.77 µM and 1.13 µM respectively against DU-145 cell line.

Table 3: Cytotoxicity of the compounds 3a-t (aIC50 in µM)

Compound	A549[b]	MCF-7[c]	DU145[d]
3a	14.2	8.72	12.9
3b	14.8	11.6	13.2
3c	12.2	18.3	14.8
3d	15.1	14.7	11.6
3e	27.9	21.9	10.3
3f	17.3	25.1	11.9
3g	15.6	29.6	10.6
3h	4.72	2.36	1.77
3i	7.91	4.61	3.34
3j	8.47	7.32	9.12
3k	8.95	3.99	7.23
3l	8.15	3.12	2.83
3m	12.1	5.35	8.15
3n	9.13	4.23	12.8
3o	8.24	10.9	2.98
3p	7.19	2.98	1.13
3q	18.1	5.22	8.97
3r	9.61	9.31	13.2
3s	19.8	11.4	9.17
3t	13.4	4.16	3.97
Doxorubicin	7.22	2.31	0.59

[a]50% Inhibitory concentration after 48 h of drug treatment and the values are average of three individual experiments
[b]Lung cancer
[c]Breast cancer

[d]rostate cancer.

CONCLUSION

In conclusion, a simple, efficient and environmentally benign visible light mediated method for the synthesis of quinazolinone derivatives has been developed by employing Rose Bengal as a recyclable metal free photocatalyst in ethanol. The advantages of this method include its simplicity in operation, use of renewable visible light as an energy source, eco-friendly reactions, higher yields and absence of side products. Using this protocol, a series of twenty compounds has been synthesized and this is a practical and economical method for the development of functionalized quinazolinones. The synthesized compounds were evaluated for their cytotoxic potential on selected human cancer cell lines. Some of these compounds showed IC50 values ranging between 1.13 μM to 1.77 μM against DU-145 cell line.

ACKNOWLEDGEMENTS

J.K thanks to Vardhaman College of Engineering for providing support.

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