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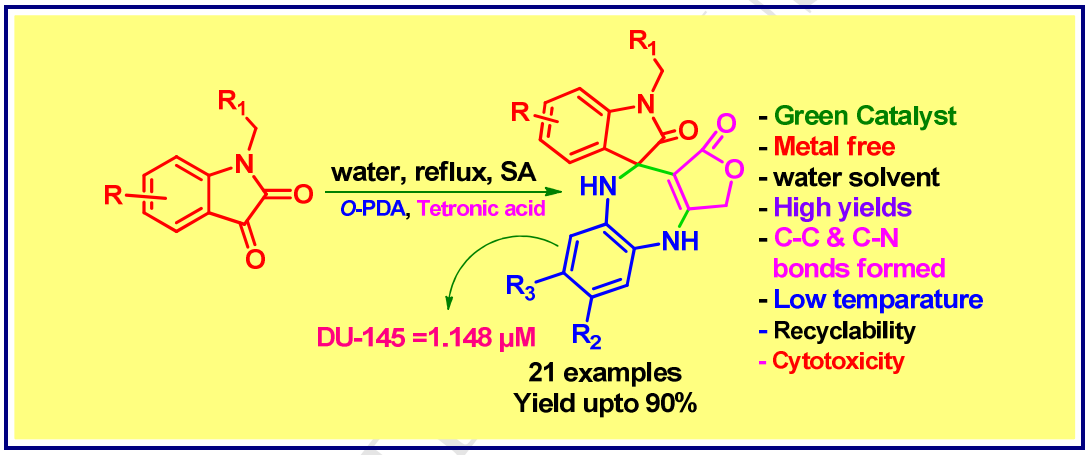

A facile one pot C-C and C-N bond formation for the synthesis of spiro-benzodiazepines and their cytotoxicity

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Abstract

An efficient, multicomponent and environmentally benign protocol has been developed for the synthesis of spiro-benzodiazepines through C-C and C-N bond formations in a single step. This one-pot protocol proceeds *via* three component reaction of *o*-phenylenediamines, tetronic acid and isatins by using mild and inexpensive catalyst like sulphamic acid in water. A variety of spiro-benzodiazepine derivatives has been synthesized in excellent yields by using this protocol in a shorter reaction time. All the synthesized compounds were evaluated for their cytotoxic potential on different human cancer cell lines and most of the compounds exhibited moderate to good cytotoxic activity, while some of them like **4f**, **4h**, **4i**, **4j** and **4q** showed promising cytotoxicity with IC_{50} values ranging between 1.14-1.69 µM.

Keywords: Cytotoxicity, Spiro-benzodiazepines, Multicomponent reactions, C-C and C-N bonds, Sulphamic acid.

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Introduction

Nitrogen containing seven membered heterocycles like benzodiazepines display a diverse range of pharmacological properties[1] such as analgesic, sedative, anti-depressive, anti-convulsant, anti-anxiety, anti-cancer[2,3] and play a significant role in the treatment of central nerves system disorders.[4,5] Benzodiazepines exhibit excellent binding ability because of conformational enantiomeric property.[6-8] On the other hand, spiro-heterocycles are found in numerous biologically important natural products as well as synthetic congeners.[9] Moreover, the derivatives of tetronic acid (tetrahydrofuran-2, 4-dione) are also play an essential role in medicinal chemistry.[10,11]

<Figure-1>

Owing to their extensive applications (**Figure 1**) in medicinal chemistry wangt.al[12] reported microwave assisted synthesis of indoline-spiro benzofurodiazepines in the presence of acetic acid (**Scheme 1**), though the reported method is efficient, it have some limitations like use of unstable, flammable acetic acid, high energy source (200 W), pertinent only to small scale production due to microwave synthesis and fail to report isatin-N substituted products. Therefore, a mild, greener and more efficient protocol for the synthesis of spiro benzodiazepines is highly desirable.

< Scheme 1>

Multicomponent reactions allow the formation of several bonds in a single operation and provide certain advantages such as simplicity in extraction, purification processes and high degree of atom economy. The design of new multicomponent reactions with greener procedures for C-C and C-N bond formation has attracted great attention, particularly in the areas of drug discovery and organic chemistry.

In view of the special properties of water like high dielectric constant, cohesive energy, extraordinary effect on reaction rates, neutral, natural and non-toxic in nature Furthermore, besides having selectivity and unique reactivity that cannot be obtained in many other organic solvents, water also offers an easy approach for the separation of heavy metals as well as by-products, organic reagents and catalysts from the aqueous phase. Conversely, heterogeneous solid acids are useful over homogeneous acid catalysts as they can be simply recovered from the reaction mixture by filtration and can be reused after activation or without activation, making it economically more feasible. During the last few years, sulphamic acid (SA) has been used as a cost-effective, stable, less corrosive, non-hygroscopic and highly efficient greener heterogeneous acid catalyst for acid-catalyzed reactions. It has been used as an efficient catalyst for acetylation of alcohols and phenols,[13-15] esterification,[16] tetrahydropyranylation,[17] Beckmann rearrangement,[18] Biginelli condensation,[19] Pechmann condensation,[20] Hantzsch reaction,[21] Michael,[22] and Mannich reactions.[23]

Considering these advantages and in the path of our efforts to develop environmentally benign methods for the synthesis of bioactive compounds,[24] herein we report a facile protocol for the synthesis of spiro-benzodiazepine derivatives in excellent yields under milder reaction conditions by using an efficient and recyclable catalyst like sulphamic acid. Moreover these compounds have been evaluated for their cytotoxic potential to know the general sense of how the anticancer activity was affected by electronic character of the diamines and isatins.

Results and discussion

Chemistry

<Table 1>

Initially to optimize the reaction procedure a model reaction was performed using isatin (1 mmol), *o*-phenylenediamine (1 mmol) and tetronic acid (1 mmol) without any

catalyst under various conditions. We observed that the reaction did not proceed until 12 h (entry 1-10, **Table 1**). The same reaction was performed in the presence of different catalysts in water under refluxing conditions. The catalysts such as HCl, $ZnCl_2$, CH_3SO_3H, *p*-TSA and Amberlite IR-120 can catalyze this reaction with moderate yields (entries 11-19). The best result was obtained with sulphamic acid, according to the yield and time (entries 20 and 21). In addition, $CHCl_3$, CH_3CN, THF and methanol (entries 23-26, **Table 1**) were also tested in various solvents under refluxing conditions and in this product **4a** was formed in slightly lower yields, even with prolonged time (entry 27).

<Table 2>

After obtaining the desired product, the amount of sulfamic acid required for this reaction was optimized. It was found that when the amount of sulfamic acid was increased from 2 mole % to 5, 10, 15, 20 and 25 mole %, the yields were also enhanced from 46 % to 60 %, 72 %, 84 %, 87 % and 87 %, respectively (entries 1-6, **Table 2**). Therefore, 20 mole % of sulfamic acid is most favourable to proceed this reaction in water.

< Scheme 2>

<Table 3>

The scope and generality of the reaction was performed with different set of substituents under the optimized conditions (**Scheme 2**). All these reactions proceeded efficiently to offer the products in excellent yields (75–90%) (**4a–4u**, **Table 3**), moreover the reaction promotes the formation of two C-N and one C-C bond. The isatins used possess both electron-withdrawing as well as electron-donating substituents and the diamines used were *o*-phenylenediamine, 4-methyl-, 4-nitro-, 3-methyl- and 4, 5-dichloro-*o* phenylenediamines. The results summarized are in **Table 3**. It is observed from the results, that isatin with electron withdrawing group such as a nitro substituents (entries **4g** and **4n**, **Table 3**) reacted easily providing good yields of the corresponding product. Similarly, very good yields of

products were obtained in the absence of substituents on isatin (entry **4a**, **Table 3**). The presence of weakly electron withdrawing groups such as halogen substituents on isatin was unfavourable to the efficiency of the reaction (entries **4b-4e, 4l** and **4m**, **Table 3**), the reaction with benzyl groups on isatin (entries **4h-4j**, **Table 3**) progressed straight forwardly and the products were obtained in good yields. In the case of diamines, absence of substituent on diamines did not show much result on the yields or reaction rates. The presence of electron donating substituents such as methyl on diamine provided products in good yields. Overall very good yields of the desired spiro-benzodiazepine derivatives were obtained with this method.

To know the feasibility of the reaction, a gram scale synthesis of **4a** was tried. **4g** of isatin, *o*-phenylenediamine (2.94g) and tetronic acid (2.72g), was dissolved in water, were stirred under the optimized reaction conditions and achieved the desired product **4a** in 86 % yield.

Plausible mechanism

The reaction proceeds with the formation of enamine (**I**) by the reaction of *o*-phenylenediamine and tetronic acid. The enamine readily attacks on keto group of isatin to form (**II**) which undergoes dehydration to form (**III**), which is then converted into the final product **4** through intramolecular cyclizations (**Figure 2).**

<Figure 2>

Biological studies

Cytotoxic activity

To estimate the cytotoxicity of spiro-benzodiazepines (**4a-4u**) MTT assay was carried out on the selected human cancer cell lines, such as lung cancer (A549), breast cancer (MCF-7), prostate cancer (DU-145) and cervical cancer (HeLa). The cytotoxicity results in comparison to the positive controls like doxorubicin and etoposide are expressed in IC_{50} values and are tabulated in **Table 4**. The results revealed that the all the synthesized compounds **4a-u** exhibited moderate to good cytotoxic activities with IC_{50} values ranging from 1.349- 50.00 µM. Based on the antiproliferative data the SAR of these spiro benzodiazepines was examined. All the compounds have shown pronounced activity against A549 (lung cancer) and DU-145 (prostate cancer) cancel cell lines as compared to other cell lines. The most active derivatives in this series are **4f, 4h, 4i, 4j** and **4q**, interestingly derivatives **4h** and **4f** were found to be the promising derivatives with IC_{50} values 1.14 and 1.69 µM respectively against DU-145 cell line. The cytotoxicity of the series depends on the nature of substituents present on the diamines and isatins. Unsubstituted (**4q**) and electron rich substitute like 5-CH_3 (**4f**) isatins showed increased cytotoxicity, on the other hand simple and electron deficient substituent such as 5-Cl diamine enhanced cytotoxicity and introduction of benzyl group on isatin-NH (**4h, 4i and 4j**) lead to increased cytotoxic potency on DU-145 and A549 cell lines.

< Table-4>

Conclusion

In conclusion, we have developed a simple, efficient and environmentally benign method for the synthesis of spiro-benzodiazepine derivatives via C-C and C-N bond formation in water and using sulphamic acid as a greener catalyst. The advantage of the present methodology is the use of water and the absence of environmentally harmful

conventional organic solvents. Another important aspect is that the product is isolated by filtration, washing, drying without further purification. Moreover, using this method a series of twenty one spiro-benzodiazepines have been synthesized and evaluated for their cytotoxic potential. Some of these compounds like **4f, 4h**, **4i, 4j** and **4q** showed IC_{50} values ranging between 1.14 to 1.69 µM on selected human cancer cell lines. Hence, these compounds will be considered as a leads for the future drug designing.

Experimental section

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 vapor. ^{1}H and ^{13}C NMR spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) or Innova Varian-VXR-unity (400, 500 MHz) instruments. Chemical shifts were expressed in parts per million (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 procedure for the synthesis of N-benzyl isatins

Isatin (1 mmol), corresponding benzyl bromide (1 mmol) and K_2CO_3 (3 mmol) were heated in acetonitrile for 1-2 h. After completion of the reaction, solvent was removed under vacuum and the product was extracted using ethyl acetate. The organic layer was dried over

anhydrous Na_2SO_4 and concentrated. The product obtained was used for next step without further purification.

1-Benzylindoline-2, 3-Dione (2h)

Light red solid; Yield: 99 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.93 (s, 2H), 6.78 (d, *J* = 8.07 Hz, 1H), 7.09 (t, *J* = 7.47 & 7.62 Hz, 1H), 7.28-7.36 (m, 5H), 7.48 (dd, *J* = 1.37 & 7.93 Hz, 1H), 7.61 (d, *J* = 7.47 Hz, 1H); ESI-MS: *m/z* = 238 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{15}H_{12}O_2N$ calculated m/z: 238.0789, found *m/z*: 238.0782 $(M+H)^+$.

1-Benzyl-5-chloroindoline-2, 3-Dione (2i)

Light red solid; Yield: 98%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.93 (s, 2H), 6.72 (d, *J* = 8.39 Hz, 1H), 7.29-7.37 (m, 5H), 7.43 (dd, *J* = 2.13 & 8.39 Hz, 1H), 7.57 (d, *J* = 8.39 Hz, 1H); ESI-MS: *m/z* = 272 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2NCl$ calculated m/z: 272.0400, found *m/z*: 272.0394 $(M+H)^+$.

1-Benzyl-5-bromoindoline-2, 3-Dione(2j)

Red solid; Yield: 98%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.92 (s, 2H), 6.67 (dd, *J* = 1.67 & 8.39 Hz, 1H), 7.30-7.37 (m, 5H), 7.59 (dd, *J* = 2.13 & 8.39 Hz, 1H), 7.70 (d, *J* = 2.89 Hz, 1H); ESI-MS: *m/z* = 315 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{15}H_{11}O_2NBr$ calculated m/z: 315.9967, found *m/z*: 315.9962 $(M+H)^+$.

***General procedure for the synthesis of 4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione derivatives* (4a-4u)**

In 25ml a round bottom flask with minimum amount of water as solvent at 80℃, to this add *o*-phenylenediamine (1 mmol), tetronic acid (1 mmol), isatin (1 mmol) and sulphamic acid (4 mol %) it was stirred, refluxed at 100℃ for 3h. The progress of reaction was monitored by

TLC. After completion of the reaction, ice-cold water was added and stirred for 5 min. The precipitated solid collected by filtration, washed with water and recrystalised using ethanol.

4,9-Dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4a)**

Light yellow solid; Mp: 285-287 ˚C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.87 (s, 2H), 5.86 (s, 1H), 6.61 (d, *J* = 7.17 Hz, 1H), 6.77-6.94 (m, 5H), 7.04 (d, *J* = 7.55 Hz, 1H), 7.15 (t, *J* = 7.36 & 14.35 Hz, 1H), 10.02 (s, 1H), 10.45 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 63.13, 66.18, 93.91, 109.36, 119.14, 120.97, 121.24, 122.92, 123.35, 128.25, 131.47, 131.80, 135.91, 142.70, 158.52, 170.55, 175.01; ESI-MS: *m/z* = 320 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{18}H_{14}O_3N_3$ calculated m/z: 320.1029, found *m/z*: 320.1038 $(M+H)^+$.

5'-Fluoro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4b)**

White solid; Mp: 269-271 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 4.88 (d, *J* = 12.9 Hz, 2H), 5.89 (s, 1H), 6.48 (d, *J* = 7.3 Hz, 1H), 6.83 (dd, *J* = 16.8, 9.3 Hz, 4H), 7.01 (t, *J* = 8.7 Hz, 2H), 10.01 (s, 1H), 10.95 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.68, 28.88, 63.35, 66.25, 93.35, 109.88, 111.15, 114.29, 119.16, 121.33, 122.93, 123.27, 131.70, 132.78, 135.37, 138.65, 158.59, 170.53, 175.09; ESI-MS: *m/z* = 360 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{18}H_{12}O_3N_3Na$ calculated m/z: 360.0746, found *m/z*: 360.0754 $(M+Na)^+$.

7'-Fluoro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4c)**

Cream solid; Mp: 275-277 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$+ DMSO-d_6): δ 4.84 (s, 2H), 5.85 (s, 1H), 6.41 (d, *J* = 8.0 Hz, 1H), 6.80 – 6.91 (m, 5H), 7.03 (d, *J* = 7.5 Hz, 1H), 10.01 (s, 1H), 10.47 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 59.48, 63.24, 66.27, 93.47, 115.06, 115.29, 119.13, 119.27, 121.28, 121.55, 122.88, 123.27, 129.78, 131.72, 134.16, 135.42, 144.68, 147.88, 158.43, 170.54, 174.78; ESI-MS: *m/z* = 360 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{18}H_{12}O_3N_3FNa$ calculated *m/z*: 360.0745, found *m/z*: 360.0754 $(M+Na)^+$.

***5'-Chloro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione* (4d)**

White solid; Mp: 289-291 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 4.84 (s, 2H), 5.87 (s, 1H), 6.64 (s, 1H), 6.87 (dd, *J* = 17.8, 7.2 Hz, 4H), 7.03 (d, *J* = 7.4 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 10.01 (s, 1H), 10.57 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 63.12, 66.26, 93.14, 110.55, 119.16, 121.30, 122.92, 123.22, 123.39, 125.12, 127.86, 131.64, 132.95, 135.34, 141.45, 158.60, 170.51, 174.90; ESI-MS: *m/z* = 376 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{18}H_{12}O_3N_3ClNa$ calculated *m/z*: 376.0459, found *m/z*: 376.0453 $(M+Na)^+$.

***5'-Bromo-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione* (4e)** [12]

Gray solid; Mp: 310-312˚C; ^{13}C NMR (75 MHz, DMSO-d6); δ 61.55, 64.72, 91.56, 109.58, 111.17, 117.60, 119.75, 121.67, 124.57, 129.16, 130.09, 131.77, 133.78, 140.35, 157.05, 168.97, 173.28; ESI-MS: *m/z* = 398 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{18}H_{13}O_3N_3Br$ calculated *m/z*: 398.01348, found *m/z*: 398.0131 $(M+H)^+$.

***5'-Methyl-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione* (4f)**

Cream solid; Mp: 259-262 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d6): δ 2.20 (d, *J* = 9.7 Hz, 3H), 4.82 (s, 1H), 5.64 (s, 1H), 6.61 – 6.85 (m, 4H), 7.13 (s, 1H), 9.91 (s, 1H), 10.43 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.08, 63.08, 66.07, 109.23, 118.99, 120.80, 121.85, 123.27, 127.91, 129.28, 130.17, 135.50, 142.55, 158.40, 170.54; ESI-MS: *m/z* = 334 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{19}H_{16}O_3N_3$ calculated *m/z*: 334.1186, found *m/z*: 334.1186 $(M+H)^+$.

***5'-Nitro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione* (4g)**

Light yellow solid; Mp: 295-298˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 2.44 (d, *J* = 9.4 Hz, 2H), 3.65 (s, 1H), 4.38 – 4.46 (m, 3H), 4.54 – 4.59 (m, 2H), 4.96 (s, 1H), 5.70 (d, *J*= 8.6 Hz, 1H), 7.72 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 62.73, 66.49, 92.47, 109.47, 118.75, 119.41, 121.66, 123.25, 125.75, 131.75, 131.90, 135.15, 141.66, 149.34, 159.09, 170.65, 175.66; ESI-MS: *m/z* = 365 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{18}H_{13}O_5N_4$ calculated *m/z*: 365.0881, found *m/z*: 365.0880 $(M+H)^+$.

***1'-Benzyl-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione* (4h)**

White solid; Mp: 257-259 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 4.80 (s, 1H), 4.87 (s, 2H), 5.02 (s, 1H), 5.83 (s, 1H), 6.71 (s, 1H), 6.76 – 6.90 (m, 5H), 7.08 (d, *J* = 8.7 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 3H), 7.46 (d, *J* = 7.4 Hz, 2H), 10.05 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 43.16, 62.87, 66.31, 93.57, 108.75, 119.13, 121.39, 121.70, 122.95, 123.24, 123.36, 126.79, 128.11, 130.40, 131.88, 135.46, 135.63, 143.02, 158.70, 170.60, 173.77; ESI-MS: *m/z* = 432 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{25}H_{19}O_3N_3Na$ calculated *m/z*: 432.1318, found *m/z*: 432.1307 $(M+Na)^+$.

***1'-Benzyl-5'-chloro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione* (4i)**

Cream solid; Mp: 265-267 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 4.78 (d, *J*= 6.3 Hz, 1H), 4.90 (s, 2H), 5.02 (d, *J* = 16.3 Hz, 1H), 6.08 (s, 1H), 6.76 (d, *J* = 11.8 Hz, 2H), 6.90 (dd, *J* = 18.1, 8.2 Hz, 3H), 7.06 (d, *J* = 7.5 Hz, 1H), 7.26 (dt, *J* = 9.6, 7.6 Hz, 4H), 7.42 (d, *J* = 7.1 Hz, 2H), 10.14 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.76, 61.35, 64.99, 65.11, 107.83, 117.46, 117.87, 118.25, 120.94, 121.93, 122.57, 123.97, 127.76, 129.38, 130.44, 130.86, 131.05, 133.35, 140.32, 147.72, 157.46, 157.57, 169.29, 174.26; ESI-MS: *m/z* = 466

$(M+Na)^+$; HRMS (ESI) *m/z* for $C_{25}H_{18}O_3N_3ClNa$ calculated *m/z*: 466.0928, found *m/z*: 466.0919 $(M+Na)^+$.

1'-Benzyl-5'-bromo-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4j)** [12]

Cream colour solid; Mp: 271-273 ˚C; ^{1}HNMR (300 MHz, DMSO-d_6): δ 4.78 (d, *J* = 6.24 Hz, 1H), 4.89 (s, 2H), 5.02 (d, *J* = 16.24 Hz, 1H), 6.00 (s, 1H), 6.64 (d, *J* = 8.30 Hz, 1H), 6.84-6.94 (m, 4H), 7.04 (d, *J* = 7.55 Hz, 1H), 7.22-7.33 (m, 4H), 7.40 (d, *J* = 6.98 Hz, 1H), 10.11 (s, 1H); ESI-MS: *m/z* = 488 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{25}H_{19}O_3N_3Br$ calculated *m/z*: 488.0604, found *m/z*: 488.0600 $(M+H)^+$.

7-Methyl-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4k)**

Gray solid; Mp: 232-234˚C; ^{1}HNMR (300 MHz, DMSO-d_6): δ 2.19 (d, *J* = 9.64 Hz, 3H), 4.82 (s, 2H), 5.62 (brs, 1H), 6.53-6.96 (m, 6H), 7.13 (t, *J* = 7.36, 14.16 Hz, 1H), 9.88 (s, 1H), 10.43 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.15, 63.08, 109.23, 118.99, 121.85, 127.91, 129.28, 130.17, 131.71, 133.23, 135.50, 142.55, 158.40, 170.54, 174.94; ESI-MS: *m/z* = 334 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{19}H_{16}O_3N_3$ calculated *m/z*: 334.1186, found *m/z*: 334.1196 $(M+H)^+$.

5'-Chloro-7-methyl-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4l)**

Gray solid; Mp: 247-249˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d6): δ 2.22 (d, *J* = 4.9 Hz, 3H), 4.82 (s, 2H), 5.66 (s, 1H), 6.64 – 6.92 (m, 5H), 7.14 (d, *J* = 7.0 Hz, 1H), 9.89 (s, 1H), 10.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.12, 63.14, 66.30, 92.66, 93.33, 110.46, 119.48, 122.08, 123.43, 125.31, 127.72, 129.08, 130.52, 132.04, 131.54, 132.63, 134.92, 141.25, 158.61, 170.59, 174.95; ESI-MS: *m/z* = 390 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{19}H_{14}O_3N_3ClNa$ calculated *m/z*: 390.0615, found *m/z*: 390.0613 $(M+Na)^+$.

5'-Methoxy-7-methyl-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4m)**

Gray solid; Mp: 239-241˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 2.20 (d, *J* = 7.3 Hz, 3H), 3.58 (s, 3H), 4.82 (s, 2H), 5.65 (s, 1H), 6.21 (d, *J* = 9.9 Hz, 1H), 6.64 – 6.92 (m, 6H), 9.89 (d, *J* = 6.8 Hz, 1H), 10.26 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.18, 28.90, 29.34, 55.01, 63.43, 66.22, 93.42, 109.47, 110.64, 112.42, 119.41, 121.90, 123.49, 129.28, 130.25, 131.72, 132.64, 133.17, 135.84, 154.25, 158.50, 170.56, 174.95; ESI-MS: *m/z* = 386 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{20}H_{17}O_4N_3Na$ calculated *m/z*: 386.1111, found *m/z*: 386.1107 $(M+Na)^+$.

7-methyl-5'-nitro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4n)**

Light yellow solid; Mp: 248-250˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 2.22 (d, *J* = 9.1 Hz, 3H), 4.87 (s, 2H), 5.89 (d, *J* = 13.2 Hz, 1H), 6.69 – 7.04 (m, 4H), 7.52 (d, *J* = 5.8 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 10.04 (s, 1H), 11.14 (s, 1H);^{13}C NMR (75 MHz, DMSO-d_6): δ 20.12, 62.68, 66.47, 109.19, 118.82, 119.61, 122.30, 123.93, 125.34, 130.74, 131.77, 132.42, 134.71, 141.68, 149.08, 158.93, 170.65, 175.67; ESI-MS: *m/z* = 401 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{19}H_{14}O_5N_4Na$ calculated *m/z*: 401.0856, found *m/z*: 401.0849 $(M+Na)^+$.

6,7-Dichloro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4o)**

White solid; Mp: 255-257˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 2.85 (s, 2H), 4.19 (s, 1H), 4.84 (d, *J* = 6.4 Hz, 3H), 5.16 (s, 3H), 8.15 (s, 1H), 8.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.16, 20.45, 28.84, 62.95, 66.02, 94.44, 109.40, 119.71, 121.08, 122.36, 123.43, 123.51, 123.97, 128.38, 130.29, 131.58, 135.88, 142.56, 157.76, 170.08, 174.96; ESI-MS: *m/z* = 410 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{18}H_{11}O_3N_3Cl_2Na$ calculated *m/z*: 410.0069, found *m/z*: 410.0061 $(M+Na)^+$.

6,7-Dichloro-5'-fluoro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4p)**

Graysolid; Mp: 258-260˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 3.78 (s, 2H), 5.40 (s, 1H), 5.72 – 5.97 (m, 5H), 9.50 (s, 1H), 9.75 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.61, 64.90, 94.30, 112.02, 114.07, 115.20, 116.43, 117.20, 118.02, 124.60, 126.79, 131.48, 132.36, 156.47, 159.66, 164.86, 170.86; ESI-MS: *m/z* = 427 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{18}H_{10}O_3N_3Cl_2FNa$ calculated *m/z*: 427.9975, found *m/z*: 427.9969 $(M+Na)^+$.

7-chloro-4,9 dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4q)**

white solid; Mp: 272-276˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 4.84 (s, 2H), 5.87 (s, 1H), 6.64 (s, 1H), 6.83 – 6.92 (m, 4H), 7.03 (d, *J* = 7.4 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 10.01 (s, 1H), 10.57 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 63.12, 66.26, 93.14, 110.55, 119.16, 121.30, 122.92, 123.39, 125.12, 127.86, 131.64, 132.95, 141.45, 158.60, 170.51, 174.90; ESI-MS: *m/z* = 376 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{18}H_{12}O_3N_3ClNa$ calculated *m/z*: 376.0567, found *m/z*: 376.0561 $(M+Na)^+$.

5'-chloro-7-nitro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4r)** [12]

^{13}C NMR (75 MHz, DMSO-d_6): δ 27.40, 57.97, 61.51, 64.57, 93.00, 107.95, 118.27, 119.64, 122.53, 126.94, 130.13, 134.44, 141.11, 156.32, 168.64, 173.51; ESI-MS: *m/z* = 399 $(M+H)^+$; HRMS (ESI) *m/z* for $C_{18}H_{12}O_5N_4Cl$ calculated *m/z*: 399.04904, found *m/z*: 399.04907 $(M+H)^+$.

5-methyl-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione **(4s)**

Cream white solid; Mp: 232-234˚; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 2.16 (s, 3H), 2.56 (s, 1H), 4.82 (s, 2H), 5.61 (s, 1H), 6.52 (s, 1H), 6.93 – 6.70 (m, 4H), 6.99 (dd, *J* = 16.9,

7.3 Hz, 2H), 9.88 (s, 1H), 10.31 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.85, 27.67, 61.52, 64.41, 92.13, 117.30, 119.32, 121.03, 121.54, 126.66, 127.86, 129.57, 129.97, 134.07, 138.46, 156.62, 168.80, 173.52; ESI-MS: *m/z* = 356 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{19}H_{15}O_3N_3Na$ calculated *m/z*: 356.1113, found *m/z*: 356.1109 $(M+Na)^+$.

5',6,7-trichloro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione (*4t**) [12]

White solid; Mp: 272-276˚C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 3.78 (s, 2H), 5.39 (s, 1H), 5.72-5.97 (m, 5H), 9.50 (s, 1H), 9.75 (s, 1H); ESI-MS: *m/z* = 443 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{18}H_{10}O_3N_3Cl_3Na$ calculated *m/z*: 443.9787, found *m/z*: 443.9782 $(M+Na)^+$.

***5'-methoxy-7-nitro-4,9-dihydrospiro[benzo[b]furo[3,4-e][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione* (4u)** [12]

Yellow solid; Mp: 280-283˚C; ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.76, 20.45, 28.84, 59.41, 62.95, 66.02, 94.44, 109.40, 119.71, 121.08, 122.36, 123.43, 123.97, 128.38, 130.29, 131.58, 135.88, 142.56, 157.76, 170.08, 174.96; ESI-MS: *m/z* = 417 $(M+Na)^+$; HRMS (ESI) *m/z* for $C_{19}H_{14}O_6N_4Na$ calculated *m/z*: 417.0913, found *m/z*: 417.0907 $(M+Na)^+$.

Biology

Cytotoxicity evaluation (MTT assay)

The cytotoxicity of these spiro compounds was determined using MTT assay.[25] Cancer cells (DU-145, MCF-7, HeLa and A549) were used in this assay. 1×10^4 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 ^{o}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 and etoposide 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).

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Figures

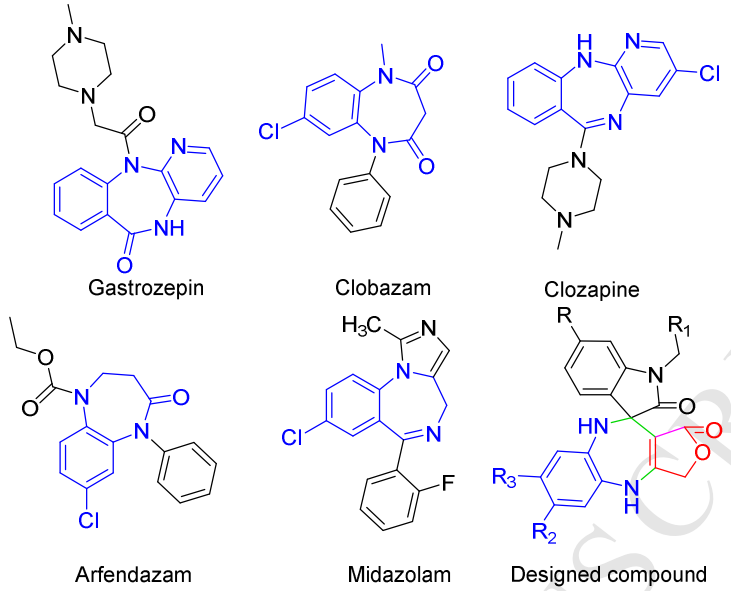


Figure 1: Representative examples of benzodiazepines scaffolds found in some drug candidates.

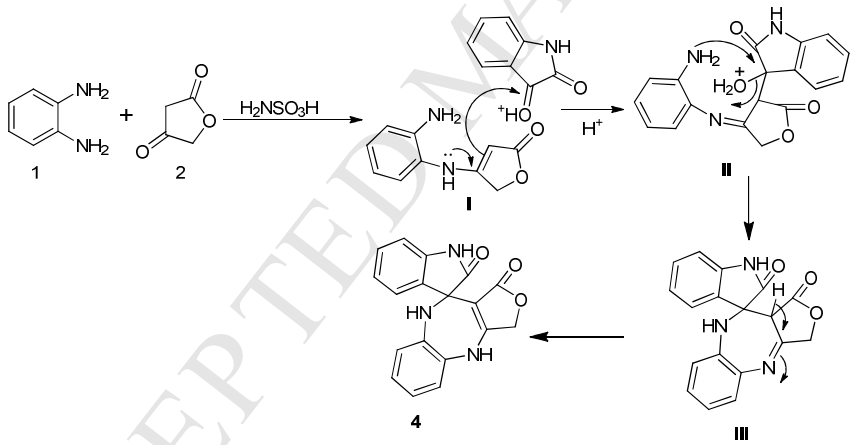
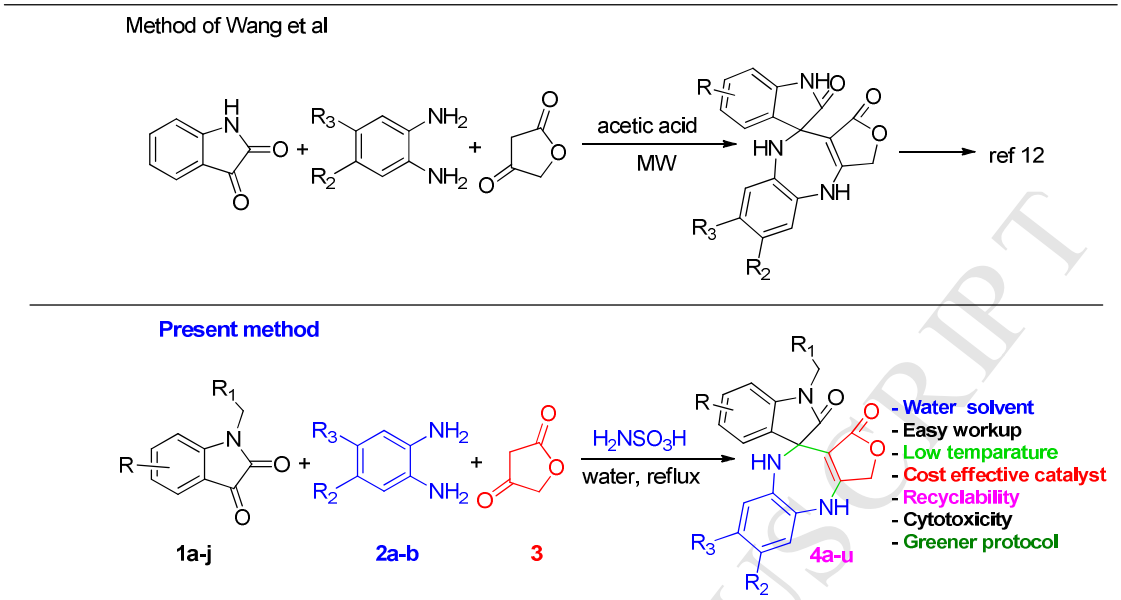
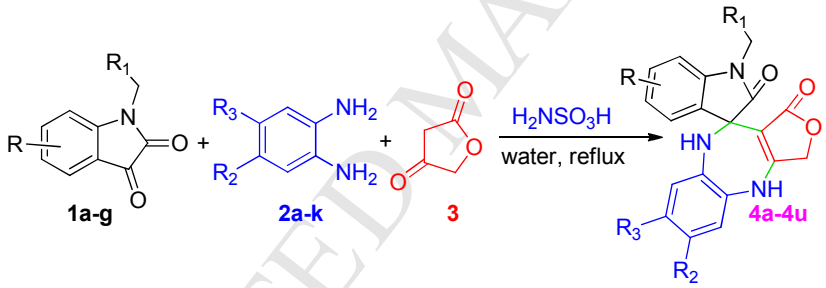


Figure 2: Plausible mechanism

Schemes



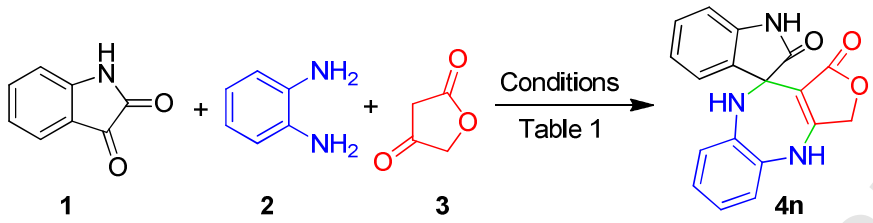
Scheme 1: Synthesis of spiro-benzodiazepines.



Scheme 2: Sulfamic acid catalysed synthesis of 4,9-dihydrospiro[benzo[*b*]furo[3,4-*e*][1,4]diazepine-10,3'-indoline]-1,2'(3H)-dione derivatives

Tables

Table 1: Optimization of reaction conditions



1 2 3 4n

Entry	Solvent	Temperature(°C)	Catalyst	Time (h)	Yield[a] (%)
1	--	rt	--	12	--
2	acetonitrile	rt	--	12	-
3	methanol	rt	--	12	--
4	ethanol	rt	--	12	--
5	water	rt	--	12	--
6	--	100	--	12	--
7	acetonitrile	reflux	--	12	--
8	methanol	reflux	--	12	--
9	ethanol	reflux	--	12	--
10	water	reflux	--	12	--
11	ethanol	reflux	HCl	12	20
12	ethanol	reflux	$ZnCl_2$	12	20
13	ethanol	reflux	CH_3SO_3H	6	28
14	water	reflux	CH_3SO_3H	6	36
15	ethanol	reflux	*p*-TSA	6	35
16	water	reflux	*p*-TSA	6	48
17	--	120	Amberlite IR-120	6	32
18	ethanol	reflux	Amberlite IR-120	6	28
19	water	reflux	Amberlite IR-120	6	42
20	ethanol	reflux	SA	6	68
21	**water**	**reflux**	**SA**	**3**	**87**
22	--	120	SA	12	28
23	chloroform	reflux	SA	6	32
24	acetonitrile	reflux	SA	6	42
25	THF	reflux	SA	6	44
26	methanol	reflux	SA	6	60
27	water	rt	SA	12	20

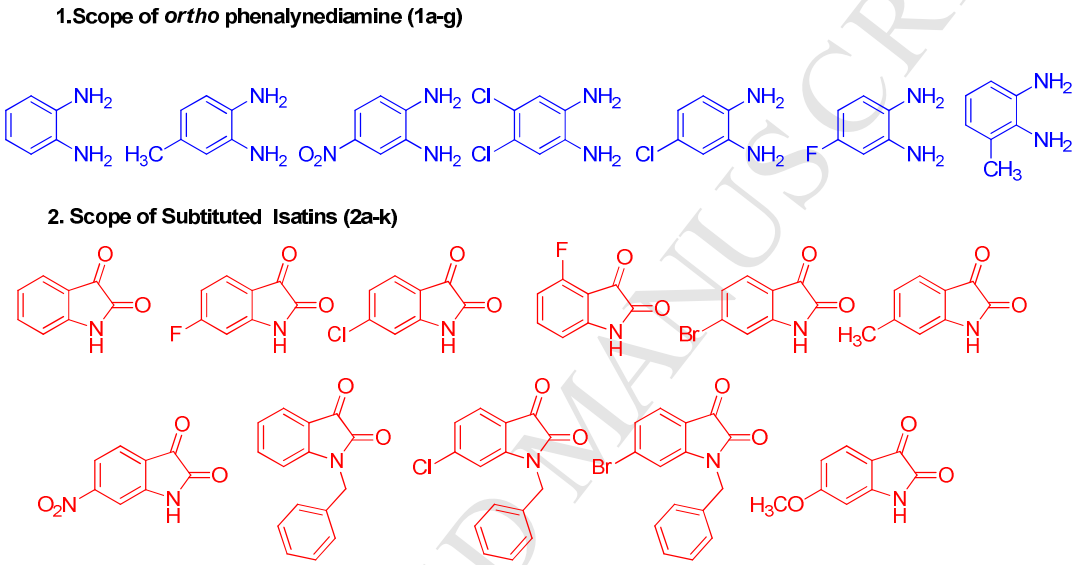
[a] - isolated yields

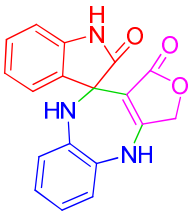
Table 2: Optimization of amount of catalyst required.

Entry	Catalyst (mole %)	Yield[a](%)
1	2	46
2	5	60
3	10	72
4	15	84
5	20	87
6	25	87

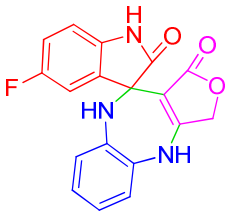
[a]isolated yields

Table 3: The scope and limitations for the synthesis of spiro-benzodiazepines derivatives

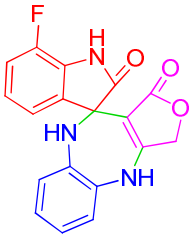




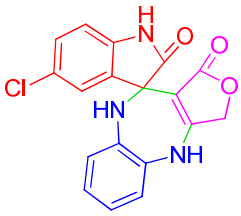
4a, 87%



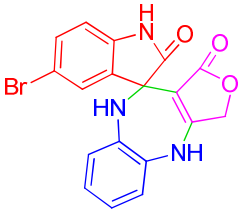
4b, 85%



4c, 83%



4d, 85%



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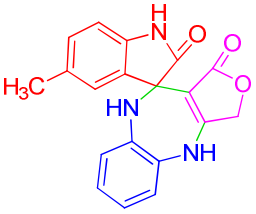

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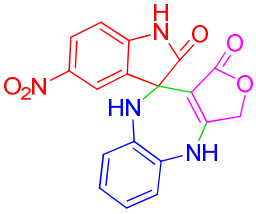

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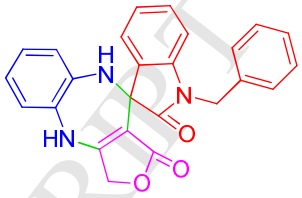

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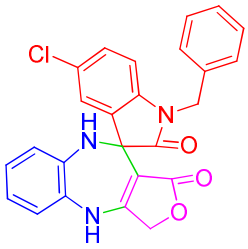

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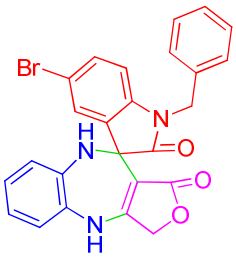

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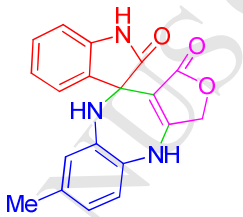

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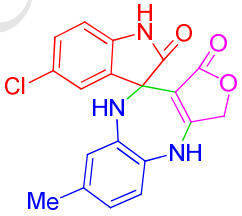

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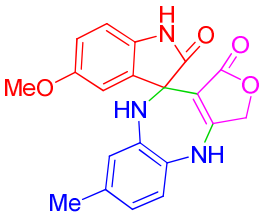

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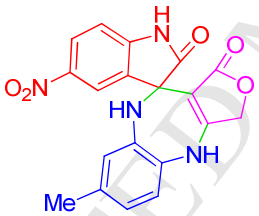

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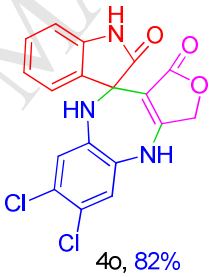

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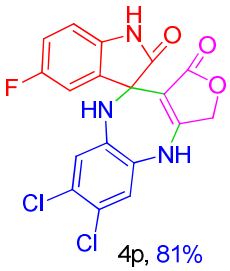

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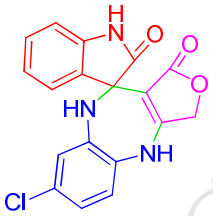

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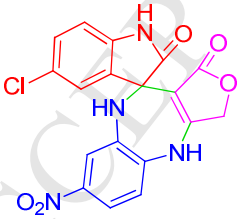

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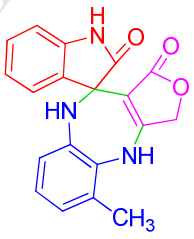

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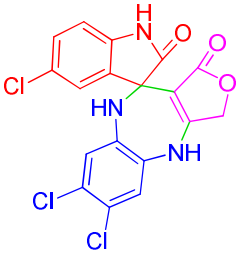

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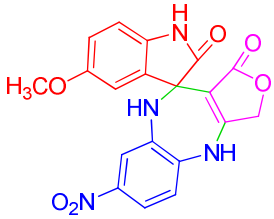

4u, 82%

Table 4: Cytotoxicity evaluation for compounds in selected human cancer cell lines.

Compound	IC_{50} values(in µM)[a]			
	HeLa[b]	A549[c]	MCF-7[d]	DU-145[e]
4a	32.40	16.75	31.63	14.72
4b	44.50	28.22	21.77	22.62
4c	13.93	9.071	13.50	16.43
4d	22.24	17.12	16.60	16.78
4e	23.65	20.50	46.70	18.19
4f	**9.576**	**1.549**	**2.138**	**1.349**
4g	42.83	13.84	40.50	17.69
4h	**2.291**	**1.349**	**2.239**	**1.148**
4i	**15.88**	**3.890**	**9.281**	**2.188**
4j	**11.90**	**3.802**	**2.344**	**3.162**
4k	15.04	9.655	15.36	9.194
4l	25.88	22.05	31.87	21.95
4m	18.17	16.14	10.44	9.960
4n	16.89	19.40	15.31	15.64
4o	53.00	23.46	30.88	16.71
4p	36.85	13.03	23.09	12.88
4q	**5.370**	**2.951**	**9.514**	**1.698**
4r	12.69	18.77	12.45	15.45
4s	26.94	17.21	23.21	13.45
4t	28.35	18.79	21.00	12.33
4u	35.86	16.89	15.84	11.18
Doxorubicin	1.032	1.514	1.445	1.820
Etoposide	1.905	2.238	1.633	2.070

[a] 50% Inhibitory concentration after 48 h of drug treatment.
[b] Human cervical cancer. [c] Human lung cancer.
[d] Human breast cancer. [e] Human prostate cancer.