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A Facile Synthetic Method for the Synthesis of new 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-trione and its Biological Evaluation

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Abstract

Sixteen new spirocyclic quaternary dérivatives were synthesized with using simple Acetic acid as a catalyst in ethanol solvent by optimized multi component reactions method. Moreover, these new compound were tested in *vitro* for their antimicrobial activity against various *bacterial* and *fungal* strains. Some of these new derivatives exhibits good antibacterial and antifungal activities. All these new products structures are confirmed by spectral analysis. By this new MCR one-pot and three-component synthetic method, we achieved trione derivatives with more operational simplicity, short reaction time and good yields (up to 93%).

Keywords: Spirooxindole, Multi-component reactions, Acetic acid, Quinoline, Antibacterial, Antifungal.

INTRODUCTION

Multi-component reactions are advanced one-pot organic synthetic methods, in which three or more reactants come collectively in a single reaction vessel to form a product consisting of substantial elements [1-8] of all the reactants. Especially, for the synthesis of highly complicated organic molecules and drug molecules, MCRs have been proven to be very efficient and striking synthetic method [9-13]. A notable feature of these MCRs is that, new bonds and new functionalities [14-18] are generated during the outpouring. Moreover, in MCRs the products and the intermediates reacts further in subsequent steps under identical conditions to form new bonds and new functionalities until execution leads to the stable final products.

Indole ring play vital role in heterocyclic compounds, especially middle of the skeleton of the heterocyclic systems are known as bioactive natural products and pharmaceuticals [19-26]. On the other hand, the oxindole framework consists of a spirocyclic quaternary stereo center at the -C_3 position is a privileged heterocyclic scaffold reported in the literature. These types of heterocyclic scaffolds appear in a plethora of natural alkaloids (*E.g:* Spirotryprostatine B and elacomine) and also acts as compelling non-peptide inhibitor [27-28] of the p53–MDM2 interaction. Moreover, among nitrogen-containing heterocyclic compounds, spiro-oxindoles are attracted for huge attention to medicinal and industrial chemists due to their diverse biological activities such as anti-glutamate, anti-parkinson, anti-microbial, anthelmintic, anti-inflammatory, anti-hyperlipidemic, anti-hypertension [29] and antioxidant properties as well as inhibition of enzymes such as acetylcholine esterase, aldose reductase, lipoxygenase, ATPase and HCV helicase [30]. In general, heterocyclic systems surrounding middle ring scaffold have been found application as pharmaceuticals, agrochemicals, veterinary products and molecular devices [31-33].

In view of above interesting biological activity information of spirocyclic quaternary derivatives containing stereo center at the C_3 position is a significant privileged heterocyclic scaffold. In this context, we chose to develop a new and efficient MCR one pot synthetic method for synthesis of new spirocyclic quaternary derivatives. Recently, synthesis of spirooxindole dione derivatives using 4-hydroxy-2*H*-chromene-2-one, isatine and 1H-indazole-3-amine reactants using MCR method was reported with high yield [34]. We were employed different reactants like indoline-2,3-dione and its substituted derivatives, pyrimidine-2,4,6(*1H,3H,5H*)-trione and 1*H*-indazol-6-amine for the synthesis of new spirocyclic quaternary trione derivatives. However to the best of our knowledge there are no synthetic methods for the synthesis of spirocyclic quaternary trione derivatives. In fact, the main objective of our work is to develop new synthetic methodology for the synthesis new heterocyclic compounds exhibiting biological active applications.

Here, we report the new MCR one pot synthetic method for the synthesis of a series of sixteen 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-triones derivatives with more operational simplicity, short reaction time and good yields. And also these compounds were tested in *vitro* for their antimicrobial activity against various *bacterial* and *fungal* strains. Some of these new Spirocyclic quaternary derivatives exhibits good antibacterial and antifungal activities. Moreover, all these new sixteen spirocyclic quaternary derivatives were characterized by ^{1}H & ^{13}C NMR, HRMS and CHN analysis.

EXPERIMENTAL SECTION

General

All the reagents were purchased commercially (SD fine, India) and used without further purification. ^{1}H NMR (300 MHz, DMSO) and ^{13}C NMR (75 MHz, DMSO) were

recorded on Gemini Varian-VXR-unity (300 MHz) instrument spectrometer TMS as internal standard (chemical shifts and ppm). Mass spectra were recorded on a VG micromass70-70H instrument. Melting points were recorded for all the compounds on Stuart SMP3 melting-point apparatus and the values reported are uncorrected. CHN analysis was carried out using Vario Micro Cube Elemental instrument, Germany. The purity of the compounds was checked by TLC on silica gel plates using a mixture of *n*-hexane and ethyl acetate.

Synthesis

General procedure for the Synthesis of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-trione (4a). Equimolar ratio of the three reactants, indoline-2,3-dione (1mmol), pyrimidine-2,4,6(*1H,3H,5H*)-trione (1mmol) and 1*H*-indazol-6-amine (1mmol) were taken in round bottom flask dissolved in the presence of ethanol (6 mL) and acetic acid (10 mol %), then the reaction mixture refluxed for 2hrs. The reaction was monitored by TLC. After completion of the reaction the solvent was removed from crude mixture and extracted with ethyl acetate and water. The final compounds were purified by the column chromatography using silica gel by eluted with ethyl acetate and hexane (30:70) to yield 93 %. mp: 364-366 ^{0}C, Anal. Calcd for $C_{19}H_{12}N_6O_3$ **(4a)**: C, 61.29, H, 3.25, N, 22.57. Found: C, 61.26, H, 3.28, N, 22.54. ^{1}H NMR (300 MHz, DMSO) δ 11.93 (s, 1H), 9.43 (s, 1H), 7.91 (s, 1H), 7.49 (d, *J* = 6.7 Hz, 1H), 7.33 (d, *J* = 2.2 Hz, 1H), 7.10 (t, *J* = 4.2 Hz, 1H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.63 (t, *J* = 6.2 Hz, 1H), 6.10 (d, *J* = 4.6 Hz, 1H), 5.91(s, 1H), 3.93(s, 1H), 3.79(s, 1H), ^{13}C NMR (75 MHz, DMSO) δ 195.51, 161.64, 161.42, 155.04, 149.89, 146.47, 139.56, 135.15, 128.03, 126.13, 122.76, 120.54, 120.15, 120.04, 116.34, 116.21, 105.46, 81.73, 69.56, ESI-HRMS: m/z [M + H]$^+$ = 373.105 (Calcd M$^+$ = 372.097)

Similar experimental procedure of **4a** was employed for all the remaining derivatives, **4b-4p** with yields between 72-93 %.

5-Chloro-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]trione (4b). Yield=84%, mp: 387-389 ^{0}C, Anal. Calcd for $C_{19}H_{11}ClN_6O_3$: C, 56.10, H, 2.73, N, 20.66. Found: C, 56.07, H, 2.7, N, 20.63. ^{1}H NMR (300 MHz, DMSO) δ 11.55 (s, 1H), 9.18 (s, 1H), 7.06 (s, 1H), 6.57 (d, *J* = 4.7 Hz, 1H), 6.27 (s, 1H), 6.10 (d, *J* = 8.4 Hz, 1H), 5.95 (s, 1H), 5.87 (d, *J* = 2.1 Hz, 1H), 5.60 (d, *J* = 8.6 Hz, 1H), 4.10 (s, 1H), 4.04 (s, 1H), ^{13}C NMR (75 MHz, DMSO) δ 192.38, 161.54, 156.56, 150.78, 146.93, 146.11, 145.79, 129.56, 126.78, 125.56, 122.92, 121.39, 120.15, 112.98, 107.65, 104.79, 100.31, 83.77, 69.40, ESI-HRMS: m/z [M + H]$^+$ = 407.065 (Calcd M$^+$ = 406.058).

5-Fluoro-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline] trione (4c). Yield=76%. mp 369-371 °C. Anal. Calcd for $C_{19}H_{11}FN_6O_3$: C, 58.46, H, 2.84, N, 21.53. Found: C, 58.48, H, 2.85, N, 21.51. ^{1}H NMR (300 MHz, DMSO) δ 11.78 (s, 1H), 10.05 (s, 1H), 8.33 (s, 1H), 7.65 (s, 1H), 6.83 (d, *J* = 4.7 Hz, 1H), 6.45 (d, *J* = 8.4 Hz, 1H), 6.41 (d, *J* = 2.1 Hz, 1H), 6.33 (d, *J* = 8.6 Hz, 1H), 6.11 (s, 1H), 4.11 (s, 1H), 4.07 (s, 1H), ^{13}C NMR (75 MHz, DMSO) δ 196.78, 161.33, 158.83, 155.66, 150.05, 139.23, 134.04, 121.45, 120.33, 120.11, 117.80, 117.70, 115.46, 115.18, 114.88, 111.70, 111.39, 105.23, 80.56, ESI-HRMS: m/z [M + H]$^+$ = 391.095 (Calcd M$^+$ = 390.088).

5-bromo-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4d). Yield=79%. mp 360-362 °C.Anal. Calcd for $C_{19}H_{11}BrN_6O_3$: C, 50.57, H, 2.46, N, 18.62. Found: C, 50.57, H, 2.49, N, 18.63. ^{1}H NMR (300 MHz, DMSO) δ 11.31 (s, 1H), 9.83 (s, 1H), 7.92 (s, 1H), 7.75 (s, 1H), 7.66 (d, *J* = 4.7 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 2.1 Hz, 1H), 6.68 (d, *J* = 8.6 Hz, 1H), 5.93 (s, 1H), 4.02 (s, 2H), ^{13}C NMR (75 MHz, DMSO) δ 193.84, 164.71, 161.25, 149.74, 146.15, 139.20, 134.67, 134.04, 130.61, 127.80, 122.23, 120.34, 120.23, 118.30, 115.59, 113.37, 105.24, 81.34, 69.54, ESI-HRMS: m/z [M + H]$^+$ = 451.015 (Calcd M$^+$ = 450.008).

5-Iodo-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4e). Yield=80%, mp 351-353 °C.Anal. Calcd for $C_{19}H_{11}IN_6O_3$: C, 45.80, H, 2.23, N, 16.87. Found: C, 45.78, H, 2.25, N, 16.90. ^{1}H NMR (300 MHz, DMSO) δ 11.62 (s, 1H), 9.85 (s, 1H), 7.99 (s, 1H), 7.52 (s, 1H), 6.85 (d, *J* = 4.7 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 6.63 (d, *J* = 2.1 Hz, 1H), 6.55 (d, *J* = 8.6 Hz, 1H), 6.02 (s, 1H), 4.02 (s, 2H), 3.98 (s, 3H), ^{13}C NMR (75 MHz, DMSO) δ 193.92, 161.20, 149.55, 145.92, 139.19, 136.30, 134.98, 134.08, 133.67, 122.51, 120.35, 120.23, 118.53, 115.63, 105.23, 100.35, 85.09, 81.53, 69.45, ESI-HRMS: m/z [M + H]$^+$ = 499.003 (Calcd M$^+$ = 497.994).

5-Methyl-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4f). Yield=79%, mp 354-356 °C.Anal. Calcd for $C_{20}H_{14}N_6O_3$: C, 62.17, H, 3.65, N, 21.75. Found: C, 62.18, H, 3.66, N, 21.79. ^{1}H NMR (300 MHz, DMSO) δ 11.63 (s, 1H), 9.85 (s, 1H), 7.98 (s, 1H), 7.14 (d, *J* = 4.7 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.67 (s, 1H), 6.47 (d, *J* = 8.6 Hz, 1H), 6.02 (s, 1H), 4.02 (s, 2H), 2.72 (s, 3H), ^{13}C NMR (75 MHz, DMSO) δ 195.35, 165.56, 161.35, 161.23, 155.56, 149.83, 146.32, 139.28, 132.63, 131.13, 128.47, 125.69, 120.25, 119.70, 115.90, 105.22, 101.35, 101.28, 81.17, 20.03, ESI-HRMS: m/z [M + H]$^+$ = 387.121 (Calcd M$^+$ = 386.113).

5-methoxy-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4g). Yield=80%, mp 358-360 °C. Anal. Calcd for $C_{20}H_{14}N_6O_4$: C, 59.70, H, 3.51, N, 20.89, O, 15.91. Found: C, 59.71, H, 3.49, N, 20.86. ^{1}H NMR (300 MHz, DMSO) δ 11.69 (s, 1H), 9.84 (s, 1H), 8.24 (s, 1H), 7.47 (s, 1H), 6.93 (d, *J* = 4.7 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.65 (d, *J* = 2.1 Hz, 1H), 6.54 (d, *J* = 8.6 Hz, 1H), 6.04 (s, 1H), 4.04 (s, 1H), 3.96 (s, 1H), 3.69 (s, 3H), ^{13}C NMR (75 MHz, DMSO) δ 193.07, 165.56, 164.64, 161.28, 154.70, 150.55, 149.29, 145.06, 136.51, 132.13, 127.85, 127.13, 123.88, 121.13, 115.07, 110.29, 107.06, 106.70, 74.70, 54.75, ESI-HRMS: m/z [M + H]$^+$ = 403.115 (Calcd M$^+$ = 402.108).

5-Nitro-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4h). Yield=77%, mp 371-373 °C. Anal. Calcd for

$C_{19}H_{11}N_7O_5$: C, 54.68, H, 2.66, N, 23.49. Found: C, 54.67, H, 2.65, N, 23.47. ^{1}H NMR (300 MHz, DMSO) δ 11.62 (s, 1H), 9.84 (s, 1H), 7.97 (s, 1H), 7.47 (s, 1H), 7.14 (d, *J* = 4.7 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.46 (d, *J* = 8.6 Hz, 1H), 6.02 (s, 1H), 4.01 (s, 2H), ^{13}C NMR (75 MHz, DMSO) δ 191.62, 165.56, 164.64, 161.22, 155.71, 149.54, 145.76, 141.57, 141.29, 139.29, 134.14, 123.91, 121.65, 120.71, 120.46, 116.88, 105.32, 100.08, 72.42, ESI-HRMS: m/z $[M + H]^+$ = 418.090 (Calcd M^+ = 417.082).

6-Methyl-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4i). Yield=72%, mp 356-358 °C. Anal. Calcd for $C_{20}H_{14}N_6O_3$: C, 62.17, H, 3.65, N, 21.75. Found: C, 62.15, H, 3.69, N, 21.76. ^{1}H NMR (300 MHz, DMSO) δ 11.63 (s, 1H), 9.85 (s, 1H), 7.98 (s, 1H), 7.47 (s, 1H), 7.14 (d, *J* = 4.7 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.67 (s, 1H), 6.47 (d, *J* = 8.6 Hz, 1H), 4.01 (s, 2H), 2.72 (s, 3H), ^{13}C NMR (75 MHz, DMSO) δ 193.05, 161.06, 160.93, 155.26, 149.53, 146.03, 138.98, 132.33, 130.83, 128.17, 125.40, 123.54, 120.29, 119.95, 119.40, 115.61, 104.92, 100.48, 80.87, 19.74, ESI-HRMS: m/z $[M + H]^+$ = 387.121 (Calcd M^+ = 386.113).

6-Methoxy-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4j). Yield=85%, mp 359-361 °C. Anal. Calcd for $C_{20}H_{14}N_6O_4$: C, 59.70, H, 3.51, N, 20.89. Found: C, 59.69, H, 3.52, N, 20.88. ^{1}H NMR (300 MHz, DMSO) δ 11.31 (s, 1H), 9.83 (s, 1H), 7.92 (s, 1H), 7.75 (s, 1H), 7.66 (d, *J* = 4.7 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 2.1 Hz, 1H), 6.68 (d, *J* = 8.6 Hz, 1H), 5.93 (s, 1H), 4.02 (s, 2H), 3.55 (s, 3H), ^{13}C NMR (75 MHz, DMSO) δ 194.94, 166.21, 164.71, 161.06, 160.98, 158.86, 156.24, 148.99, 144.76, 138.83, 136.21, 130.84, 127.55, 126.83, 120.84, 116.21, 114.76, 110.00, 102.84, 54.45,ESI-HRMS: m/z $[M + H]^+$ = 403.115 (Calcd M^+ = 402.108).

6-Nitro-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4k). Yield=82%, mp 377-379 °C.Anal. Calcd for $C_{19}H_{11}N_7O_5$: C, 54.68, H, 2.66, N, 23.49. Found: C, 54.65, H, 2.69, N, 23.48. ^{1}H NMR (300 MHz, DMSO) δ 11.65 (s, 1H), 10.08 (s, 1H), 8.33 (s, 1H), 7.67 (s, 1H), 6.85 (d, *J* = 4.7 Hz, 1H), 6.47 (d, *J* = 8.4 Hz, 1H), 6.43 (d, *J* = 2.1 Hz, 1H), 6.35 (d, *J* = 8.6 Hz, 1H), 6.12 (s, 1H), 4.12 (s, 1H), 4.08 (s, 1H), ^{13}C NMR (75 MHz, DMSO) δ 193.32, 164.39, 160.93, 149.24, 145.46, 141.28, 140.99, 138.99, 133.85, 125.26, 123.61, 121.36, 120.41, 120.16, 116.58, 105.02, 103.65, 101.36, 82.12, ESI-HRMS: m/z $[M + H]^+$ = 418.090 (Calcd M^+ = 417.082).

6-Iodo-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4l). Yield=74%, mp 357-359 °C. Anal. Calcd for $C_{19}H_{11}IN_6O_3$: C, 45.80, H, 2.23, N, 16.87. Found: C, 45.77, H, 2.26, N, 16.85. ^{1}H NMR (300 MHz, DMSO) δ 11.63 (s, 1H), 9.85 (s, 1H), 7.99 (s, 1H), 7.55 (s, 1H), 6.85 (d, *J* = 4.7 Hz, 1H), 6.68 (d, *J* = 8.4 Hz, 1H), 6.63 (d, *J* = 2.1 Hz, 1H), 6.55 (d, *J* = 8.6 Hz, 1H), 6.02 (s, 1H), 4.02 (s, 1H), 3.98 (s, 1H), ^{13}C NMR (75 MHz, DMSO) δ 191.62, 165.26, 160.91, 149.25, 145.62, 138.89, 136.00, 134.69, 133.78, 133.37, 122.22, 120.05, 119.93, 118.23, 115.33, 104.93, 102.98, 101.43, 74.16, ESI-HRMS: m/z $[M + H]^+$ = 499.003 (Calcd M^+ = 497.994).

6-Chloro-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4m). Yield=83%, mp 379-381 °C. Anal. Calcd for $C_{19}H_{11}ClN_6O_3$: C, 56.10, H, 2.73, N, 20.66. Found: C, 56.08, H, 2.75, N, 20.63. ^{1}H NMR (300 MHz, DMSO) δ 11.63 (s, 1H), 9.85 (s, 1H), 7.98 (s, 1H), 7.50 (s, 1H), 7.14 (d, *J* = 4.7 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 4.2 Hz, 1H), 6.67 (s, 1H), 6.47 (d, *J* = 6.6 Hz, 1H), 6.02 (s, 1H), 4.02 (s, 2H), ^{13}C NMR (75 MHz, DMSO) δ 197.14, 161.29, 149.15, 148.22, 146.69, 145.86, 145.54, 143.22, 131.15, 129.31, 126.54, 125.31, 122.68, 121.14, 119.90, 112.73, 107.40, 100.07, 73.52, ESI-HRMS: m/z $[M + H]^+$ = 407.065 (Calcd M^+ = 406.058).

6-Fluoro-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4n). Yield=72%, mp 375-377 °C.Anal. Calcd for $C_{19}H_{11}FN_6O_3$: C, 58.46, H, 2.84, N, 21.53. Found: C, 58.46, H, 2.82, N, 21.51. ^{1}H NMR (300 MHz, DMSO) δ 11.68 (s, 1H), 9.83 (s, 1H), 8.24 (s, 1H), 7.47 (s, 1H), 6.93 (d, *J* = 4.7 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.65 (d, *J* = 4.2 Hz, 1H), 6.55 (d, *J* = 6.6 Hz, 1H), 6.03 (s, 1H), 4.07 (s, 1H), 3.96 (s, 1H), ^{13}C NMR (75 MHz, DMSO) δ 194.49, 161.04, 158.54, 155.38, 149.33, 145.94, 138.94, 133.75, 131.43, 120.04, 119.82, 117.51, 117.41, 115.17, 114.89, 114.59, 111.42, 104.95, 74.77,ESI-HRMS: m/z $[M + H]^+$ = 391.095 (Calcd M^+ = 390.088).

6-Bromo-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4o). Yield=79%, mp 365-367 °C. Anal. Calcd for $C_{19}H_{11}BrN_6O_3$: C, 50.57, H, 2.46, N, 18.62. Found: C, 50.56, H, 2.49, N, 18.63. ^{1}H NMR (300 MHz, DMSO) δ 11.43 (s, 1H), 9.33 (s, 1H), 7.99 (s, 1H), 7.33 (s, 1H), 7.10 (d, *J* = 4.7 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.66 (d, *J* = 2.1 Hz, 1H), 6.40 (d, *J* = 8.6 Hz, 1H),6.33 (s, 1H), 4.10 (s, 1H), 3.93 (s, 1H), ^{13}C NMR (75 MHz, DMSO) δ 194.58, 160.99, 155.29, 150.35, 149.48, 138.94, 134.41, 133.79, 130.35, 127.54, 121.97, 120.09, 119.97, 118.05, 115.33, 113.11, 104.98, 101.08, 72.45, ESI-HRMS: m/z $[M + H]^+$ = 451.015 (Calcd M^+ = 450.008).

7-Chloro-7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4p). Yield=86%, mp 374-376 °C.Anal. Calcd for $C_{19}H_{11}ClN_6O_3$: C, 56.10, H, 2.73, N, 20.66. Found: C, 56.11, H, 2.75, N, 20.63. ^{1}H NMR (300 MHz, DMSO) δ 11.31 (s, 1H), 9.83 (s, 1H), 7.92 (s, 1H), 7.75 (s, 1H), 7.56 (d, *J* = 3.7 Hz, 1H), 7.32-7.21 (m, 3H), 7.15 (d, *J* = 16.0 Hz, 1H), 4.02 (s, 2H), ^{13}C NMR (75 MHz, DMSO) δ 196.89, 165.61, 161.04, 155.06, 150.89, 146.44, 145.61, 145.29, 143.90, 129.06, 126.29, 125.06, 122.43, 120.89, 119.65, 112.48, 107.15, 99.82, 73.27, ESI-HRMS: m/z $[M + H]^+$ = 407.065 (Calcd M^+ = 406.058).

In vitro antimicrobial study

As we designed for biological activity of all the titled compounds, antimicrobial activity was performed utilizing agar well dissemination strategy against test creatures [35-37]. The supplement juices (NB) plates were utilizing of 100 ml capacity and 24 hrs old broth culture for testing the

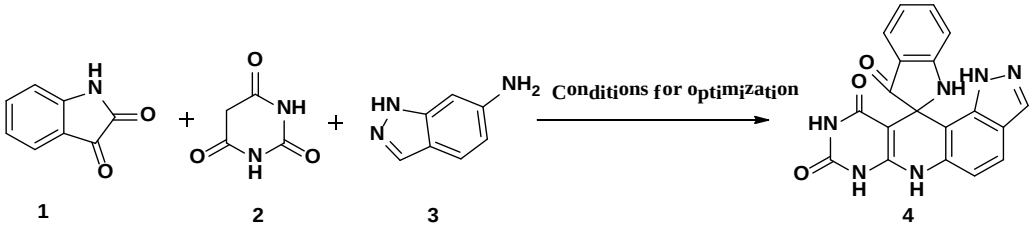
bacteria. Each petri-dish utilizing the sterile stopper borer with made of 6mm size wells. The test samples of various concentrations disintegrated in DMSO solvent. Similar antimicrobial conditions were applied for the reference antibiotic drugs like, amoxicillin (antibacterial) and fluconazole (antifungal). The plates were incubated at 37 ºC for 24 hrs and 28 ºC for 48 hrs, respectively for bacteria and fungi. The zone of inhibition (diameter) for each well was estimated after appropriate incubation period. Three duplicates were maintained for each sample and the normal qualities were figured for more precise antibacterial evaluation value. The minimum inhibitory concentrations (MICs) were performed using freshly prepared broth dilution test for the above mentioned samples. The test microorganisms like bacteria's and fungi (24 hours old) were diluted 100 times in supplement juices (NB) broth. The test samples of rising concentrations were added in to the microorganism cultures contained test tubes. Using NB as control, the tubes were inspected for noticeable turbidity. Among the test samples of various concentrations, the most reduced focus that repressed noticeable development of the tried creatures was accounted for as the MIC value of the compounds, **4a-p**.

RESULTS AND DISCUSSION

Synthesis

Initially to optimize the reaction conditions, we carried out the reactions at the room temperature and as well as at refluxed conditions under different polar solvents (Table 1, Entries 1-12) such as water, methanol and ethanol etc. In our various attempts as mentioned in table 1, we found that under refluxed conditions in ethanol solvent with acetic acid (10 mol %) is as a catalyst, 1 mmol of the reactants provided the best yield. In order to establish the effectiveness of the acetic acid catalyst for the synthesis of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-trione (**4a**), a test reaction was performed without acetic acid catalyst in ethanol solvent using similar reactants under refluxed conditions yielded only 50 % even after 4hrs (Table 1, Entry 5).

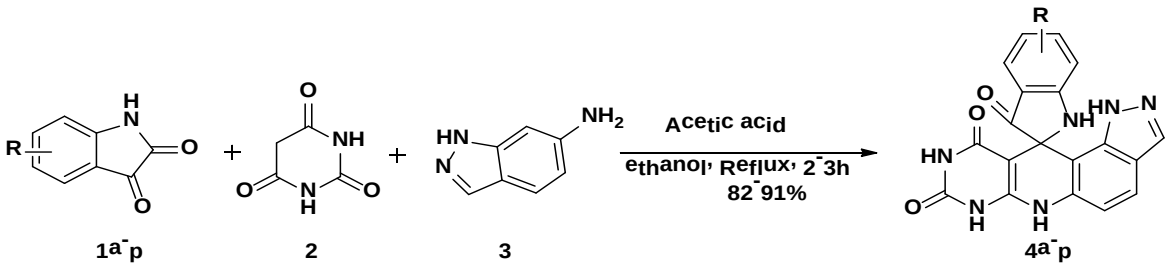
We also carried out few model reactions to check viability of the reaction using different well known other acid catalysts like HCl, *p*-TSA, and $ZnCl_2$ etc. Among all our attempts using different catalysts, acetic acid provided the best yield. Moreover, we also studied the effect of load of the acetic acid catalyst by varying 5, 10 and 15 mol % with similar reaction conditions yielded 80 %, 93 % and 92 %, respectively (Table 1, Entries 11-13). The best optimized conditions were 10 mol % of acetic acid in ethanol solvent under refluxed conditions. In this context, these optimized conditions were employed for the synthesis of derivatives of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-triones (**4a-p**).



Scheme 1. Synthesis of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-trione using various reaction conditions.

Table 1. Reaction conditions for 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-trione.

Entry	Solvent	Temperature (°C)	Catalyst (mol %)	Time (h)	Yield (%)
1	Methanol	rt	--	6	10
2	Ethanol	rt	-	6	12
3	Water	rt	--	6	5
4	Methanol	Reflux	--	6	45
5	Ethanol	Reflux	--	4	50
6	Water	Reflux	--	4	20
7	Ethanol	Reflux	HCl (10%)	4	56
8	Ethanol	Reflux	p-TSA (10%)	4	72
9	Ethanol	Reflux	$ZnCl_2$ (10%)	4	60
10	Ethanol	Reflux	p-TSA (15%)	4	73
11	Ethanol	Reflux	Acetic acid (5 %)	2	80
12	**Ethanol**	**Reflux**	**Acetic acid (10 %)**	**2**	**93**
13	Ethanol	Reflux	Acetic acid (15 %)	2	92
14	Methanol	Reflux	Acetic acid (10 %)	3	80
15	Water	Reflux	Acetic acid (10 %)	3	74



Scheme 2. Synthetic conditions for derivatives of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-triones.

Table 2. Reaction conditions for derivatives of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-triones.

Entry	Substrate	Product	Yield (%)
1	Indoline-2,3-dione	**4a**	**93**
2	5-Chloro indoline-2,3-dione	**4b**	**84**
3	5-Fluoro indoline-2,3-dione	**4c**	**76**
4	5-Bromo indoline-2,3-dione	**4d**	**79**
5	5-Iodo indoline-2,3-dione	**4e**	**80**
6	5-Methyl indoline-2,3-dione	**4f**	**79**
7	5-Methoxy indoline-2,3-dione	**4g**	**80**
8	5-Nitro indoline-2,3-dione	**4h**	**77**
9	6-Methyl indoline-2,3-dione	**4i**	**72**
10	6-Methoxy indoline-2,3-dione	**4j**	**85**
11	6-Nitro indoline-2,3-dione	**4k**	**82**
12	6-Iodo indoline-2,3-dione	**4l**	**74**
13	6-Chloro indoline-2,3-dione	**4m**	**83**
14	6-Fluoro indoline-2,3-dione	**4n**	**72**
15	6-Bromo indoline-2,3-dione	**4o**	**79**
16	7-Chloroindoline-2,3-dione	**4p**	**86**

The optimized reaction conditions described above were employed for a series of substituted indoline-2,3-diones including electron-withdrawing group such as halogen atoms and nitro group at 5th position (Table 2, entries 1-5 and 8), 6th position (Table 2, entries 11-15) and also 7-chloroindoline-2,3-dione (Scheme 2). Similarly, we also carried out the same conditions for electron-donating groups also such as methyl and methoxy for indoline-2,3-diones at both 6th and 7th positions(Table 2, entries 6-7 and 9-10). A variety of substituents in indoline-2,3-diones reacted well in this protocol and delivered good yields (72-93 %) (Table 2). From the above results we can concluded that, this methodology can tolerate both electron-withdrawing and electron-donating groups effectively for the synthesis of corresponding derivatives of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-triones in good yields. Hence, this methodology can apply generally for variety of substituents on indoline-2,3-dione to yield derivatives of spirocyclic quaternary trione derivatives with good yield for cheap acetic acid catalyst, less solvent and less reaction time etc. This study provides a road map for the synthesis of new drug molecules by simple direct one pot MCR method (see supporting information for the spectral data).

Biological Evaluation (In Vitro antimicrobial activity)

All these newly synthesized compounds **4a-p** were screened for their *in vitro* antimicrobial activity [35] against four bacteria like *Staphylococcus aureus* (ATCC 29213) and *Bacillus subtilis* (ATCC 6633), as examples of Gram-positive bacteria and *Proteus vulgaris* (ATCC 29213) and *Escherichia coli* (ATCC 11229), as examples of Gram-negative bacteria, respectively. The results obtained as minimum inhibitory concentration (MIC) in µg/mL and the measurements for all the products **4a-p** are presented in table 3. For the reference purpose, Amoxicillin was employed as a standard antibacterial drug. The compound **4b** (5-chloro), displayed MIC 3.12 µg/mL against all the bacteria except *B. subtilis* (6.25 µg/mL). Similar, **4m** (6-chloro) displayed 3.12 µg/mL against *B. subtilis* and *S. aureus*, 12.5 µg/mL against *E. coli* and *6.* µg/mL against *P. vulgaris*. The compound **4p,** (7-chloro) exhibited MIC 3.12 µg/mL against all the bacteria except *S. aureus* (6.25 µg/mL). It was envisaged from the analysis of antibacterial and antifungal activity results that, electro-negative moiety especially chloro group on aromatic phenyl ring were found to be more potent as compared to standard drug amoxicillin (3.12 µg/ mL). All the other products also exhibited moderate activity against antibacterial.

Later, all the titled compounds **4a-p,** of the present study, were also screened for their *in vitro* antifungal activity against *Candida albicans* (ATCC 10231) and *Aspergillus*

niger (ATCC 9029) fungal strains, the results were listed table 3. For the reference purpose, Fluconazole was used as a standard antifungal drug. Compound **4m** displayed better antifungal activity with MIC 3.12 µg/mL against all the fungal strains. Similarly, **4b** exhibited promising antifungal activity with MIC 3.12 µg/mL against *A. niger* and MIC 6.25 µg/mL against *C. albicans and* **4p** exhibited antifungal activity with MIC 3.12 µg/mL against *C. albicans* and MIC 6.25 µg/mL against *A. niger* fungal strains when compared with the reference drug fluconazole (MIC 3.12 µg/mL). Thus, we can conclude that compounds with electronegative groups especially chloro group on phenyl ring might be the reason for showing high antifungal inhibitory potency similar to antibacterial studies.

Table 3. Antibacterial and antifungal activity studies of synthesized compounds, **4a-p (MIC, µg/mL)**.

Compound	Gram positive bacteria (**µg/mL**)		Gram negative bacteria (**µg/mL**)		Fungal strains (**µg/mL**)	
	B. subtilis	S. aureus	E. coli	P. vulgaris	A. niger	C. albicans
4a	12.5	12.5	25	12.5	50	12.5
4b	6.25	3.12	3.12	3.12	3.12	6.25
4c	12.5	12.5	25	25	12.5	25
4d	100	50	25	100	12.5	12.5
4e	12.5	100	25	12.5	25	50
4f	50	12.5	25	12.5	12.5	12.5
4g	12.5	12.5	6.25	12.5	6.25	12.5
4h	6.25	12.5	25	25	50	25
4i	25	25	12.5	50	50	25
4j	12.5	12.5	25	25	12.5	25
4k	100	50	25	100	12.5	12.5
4l	12.5	3.12	25	12.5	25	50
4m	3.12	3.12	12.5	6.25	3.12	3.12
4n	25	50	25	100	12.5	12.5
4o	12.5	6.25	25	12.5	6.25	50
4p	3.12	6.25	3.12	3.12	6.25	3.12
Amoxicillin	3.12	3.12	3.12	3.12		
Fluconazole	–	–	–	–	3.12	3.12

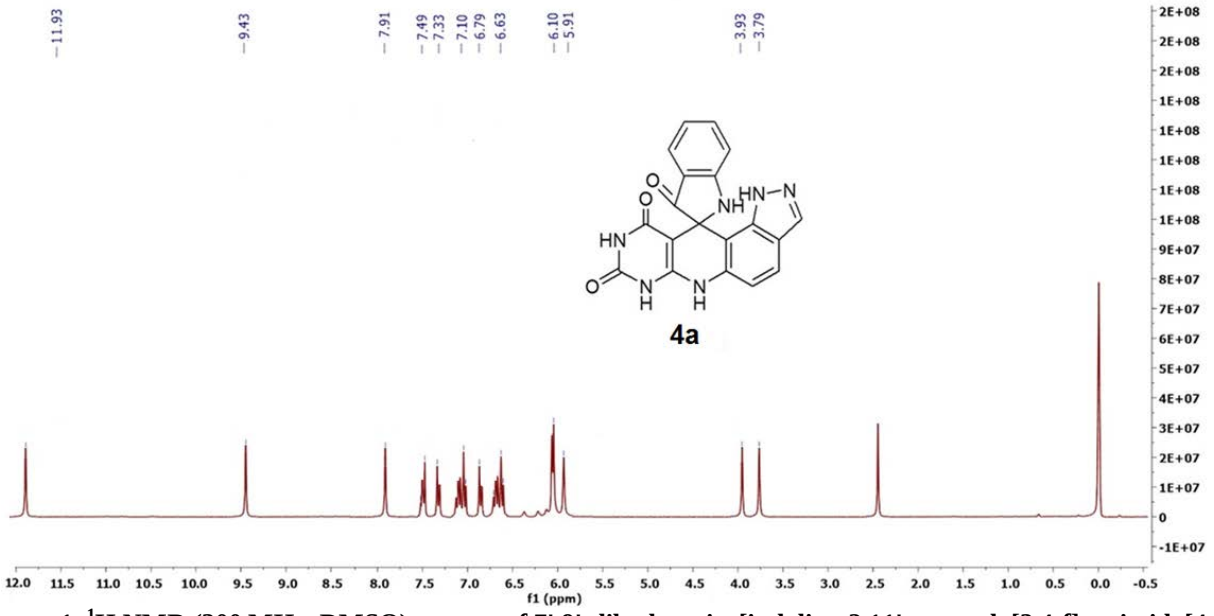


Figure 1. ^{1}H NMR (300 MHz, DMSO) spectra of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4a).

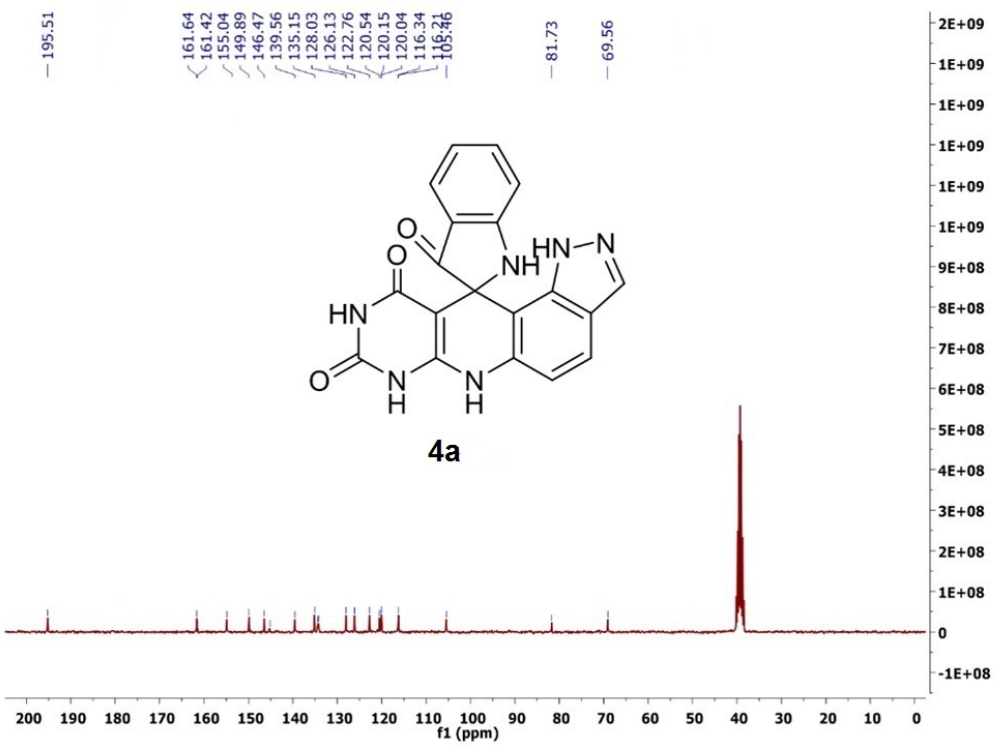


Figure 2. ^{13}**C NMR (75 MHz, DMSO) spectra of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4a)**

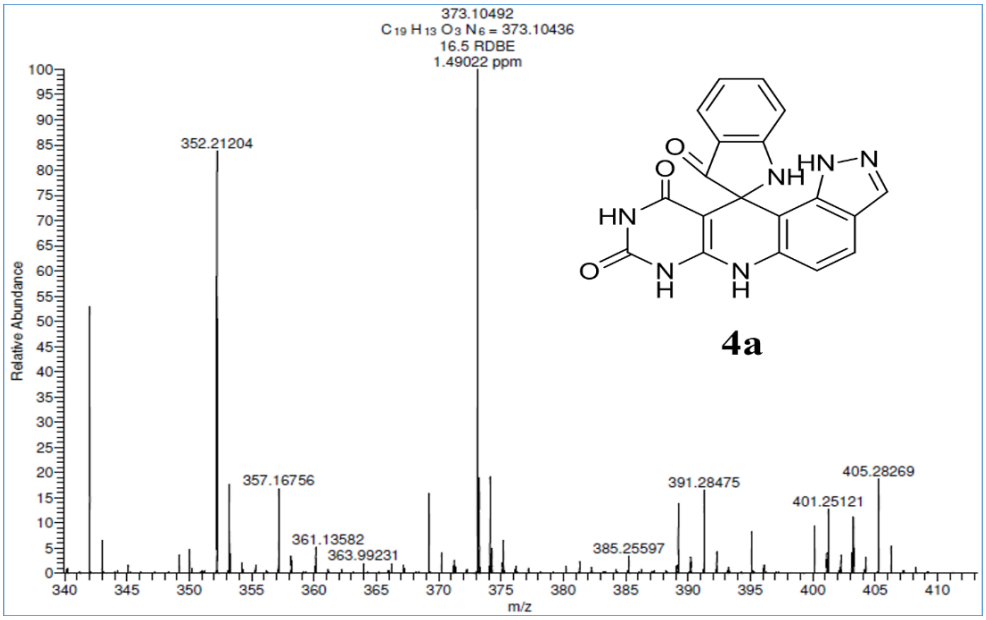


Figure 3. HRMS spectra of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-f]pyrimido[4,5-b]quinoline]-3,8',10'(1'H,6'H)-trione (4a)

CONCLUSIONS

In summary, we have been developed a proto type facile and efficient, mild and straightforward method for the synthesis of 7',9'-dihydrospiro[indoline-2,11'-pyrazolo[3,4-*f*]pyrimido[4,5-*b*]quinoline]-3,8',10'(1'*H*,6'*H*)-triones, **4(a-p),** by using acetic acid as a catalyst in ethanol solvent By this new MCR one-pot and three-component synthetic method, we achieved sixteen new trione derivatives with more operational simplicity, short reaction time and good yields (up to 93 %). Moreover, this methodology also can tolerate both electron-withdrawing and electron-donating groups effectively for the synthesis of corresponding derivatives of **4(a-p)**, in good yields. All the synthesized **4(a-p)** compounds were screened for antimicrobial activity and these are exhibited better microbial inhibition against selected microorganisms compared with the standard drugs. Among the many substitutes on the benzene ring, electronegative group especially chlorine, it shows excellent biological activity.

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