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Original article

Synthesis of novel spirooxindole derivatives by one pot multicomponent reaction and their antimicrobial activity

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ABSTRACT

A series of novel spirooxindoles have been synthesized through 1,3-dipolar cycloaddition of an azomethine ylide generated from isatin and sarcosine or L-proline with the dipolarophile 1,4-naphthoquinone followed by spontaneous dehydrogenation. Synthesised compounds were evaluated for their antimicrobial activities against eight bacteria and three fungi. All the spirooxindole derivatives exhibited significant antibacterial activity against *Staphylococcus aureus*, *S. aureus* (MRSA), *Enterobacter aerogens*, *Micrococcus luteus*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Salmonella paratyphi-B* and anti-fungal activity against *Malassesia pachydermatis*, *Candida albicans* and *Botyritis cinerea* organisms. Among 23 compounds screened, 1′-acetyl-2,5′-dimethyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione was found to be more active against tested bacteria and fungi.

1. Introduction

Heterocyclic compounds particularly spirooxindoles with nitrogen-containing five membered ring have played an important role in the field of medicinal chemistry (Fig. 1). Multicomponent reactions (MCR's) have emerged as a powerful tool for delivering the molecular diversity needed in the combinatorial approaches for the preparation of bioactive compounds [1]. 1,3-Dipolar cycloaddition reaction is an efficient method for the construction of heterocyclic units in a highly regio- and stereo- selective manner [2]. In particular, the chemistry of azomethine ylides have gained significance in recent years as it serves as an expedient precursor for constructing spirooxindoles with nitrogen-containing five membered heterocycles, which constitute the central skeleton of numerous natural products [3].

Synthetic or natural heterocyclic compound containing spirooxindole framework is endowed with a wide range of pharmacological activities [4]. Naturally occurring spirooxindole alkaloids (Fig. 1), such as horsfiline **1** [5–10] isolated from *Horsfieldia superba* and elacomine **2** [11] isolated from *Eleagnus commutata* find use as indigenous medicine. Alstonisine **3** is a useful bioactive natural product. Mitraphylline **4** isolated from *Uncaria tomentosa* possesses anti-tumor activity against human brain cancer cell lines, neuroblastoma SKNBE (2) and malignant glioma GAMG [12]. Spirotryprostatins A **5** and B **6** [13,14] found in the secondary metabolites of *Aspergillus fugimatus* inhibit mammalian cell cycle at G2/M phase. Rhynchophylline **7** isolated from *Uncaria rhynchophylla* has been used as antipyretic, anti-hypertensive and anticonvulsant medications for the treatment of headache, vertigo and epilepsy [15] and as noncompetitive antagonists of the NMDA receptor [16]. Generally, oxindole derivatives possess anti-tumor [17], antimicrobial [18], antiproliferative [19] and anti-fungal activities [20] and protein kinase B/Akt inhibitory activity useful for the treatment of cancer [21].

2. Chemistry

The above mentioned biological importance of spirooxindole alkaloids led us to synthesize novel spiro-heterocycles comprising oxindole related ring systems [22]. In continuation of our studies in the area of 1,3-dipolar cycloaddition reactions and with a view to synthesis of novel spirooxindoles [23,24], we herein report the novel one pot tandem/domino reaction of 1,3-dipolar cycloaddition reaction followed by spontaneous dehydrogenation to form the spirooxindole derivatives. To the best of our knowledge, there have been no reports for the synthesis of spirooxindole derivatives containing 1,4-naphthaquinone moiety.

In the present investigation, the 1,3-dipolar cycloaddition of azomethine ylides, generated *insitu via* decarboxylative condensation of substituted isatins **8a–n** and sarcosine **9** to 1,4-

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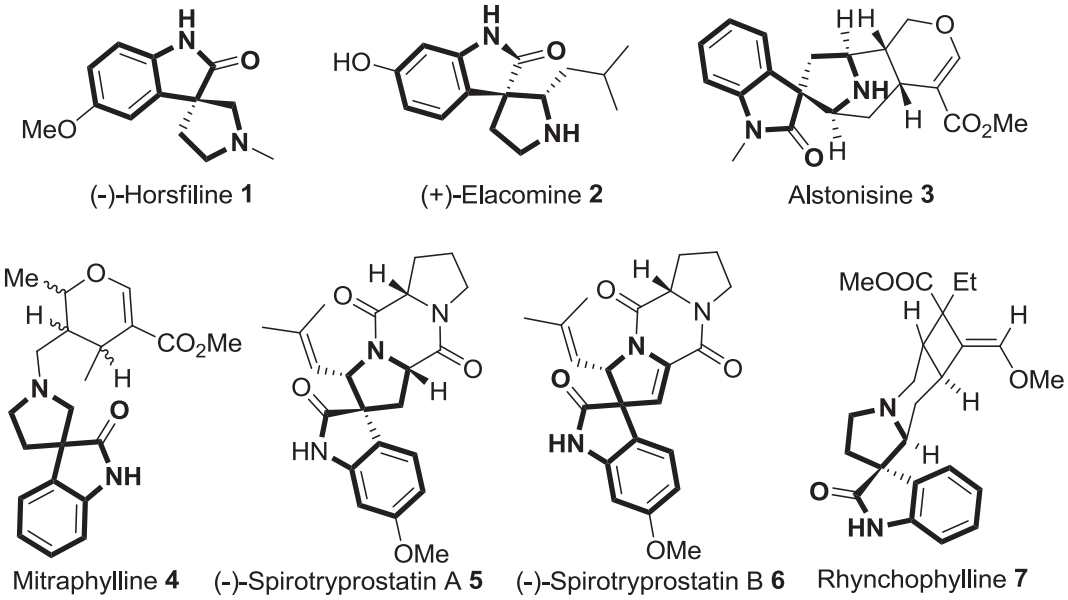


Fig. 1. Naturally occurring spirooxindole compounds.

naphthoquinone **10** followed by spontaneous dehydrogenation in ethanol afforded novel spirooxindoles **12a**–**n** in high yields (Scheme 1).

This reaction was performed by heating an equimolar mixture of substituted isatins **8a**–**n,** sarcosine **9** and 1,4-naphthoquinone **10** in ethanol under reflux for 1.5 h. After completion of the reaction (TLC), the reaction mixture was poured into ice-water, the resulting solid was filtered off and purified by column chromatography to obtain pure spirooxindole derivatives **12a**–**n** in 89–96% yields (Table 1).

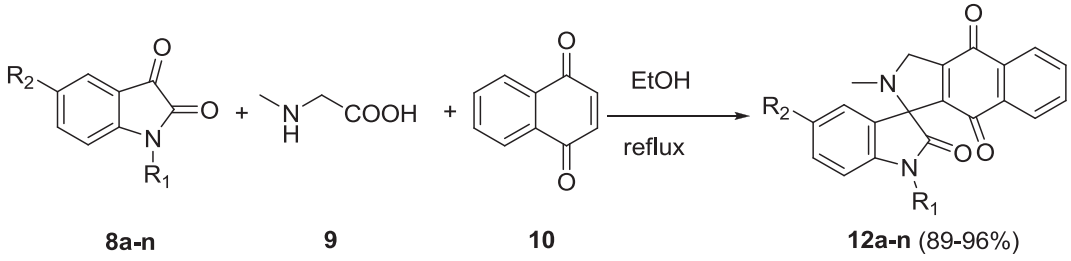
In fact we planned to synthesize 1,3-dipolar cycloaddition product **11**, but we obtained product **12** instead. In ^{1}H NMR, we found that there are no peaks for two C–H aliphatic protons for desired product **11**. ^{13}C NMR exhibited the number of peaks equal to the desired product **11** but two peaks were in the C=C region instead of the aliphatic region. According to DEPT 135 and DEPT 90, presence of two quaternary aromatic carbon signals instead of two aliphatic –CH carbons also prove the formation of product **12**. The mass spectrum also confirmed the dehydrogenated product **12**. Single crystal XRD of compound **14b** (Fig. 2) established the elucidated dehydrogenated product **12** (Scheme 2).

We tried to isolate the 1,3-dipolar cycloaddition product **11l** at nitrogen atmosphere. We found this product **11l** at nitrogen atmosphere and isolated as a pure product and confirmed by spectral data (see supporting information). But we obtained dehydrogenated product in atmospheric reflux condition. So this reaction can be a domino type reaction of 1,3-dipolar cycloaddition followed by spontaneous air oxidation resulting in the novel spirooxindole products **12l** (Scheme 3).

To further explore the potential of this protocol for novel spirooxindole synthesis, this reaction was further explored by heating an equimolar mixture of substituted isatin **8a**–**h**, 1,4-naphthoquinone **10** and L-proline **13** in ethanol under reflux for 1.5 h and obtained pure spirooxindole derivatives **14a**–**h** in 87–93% yields (Scheme 4**).** The results are summarized in Table 2**.**

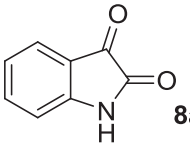
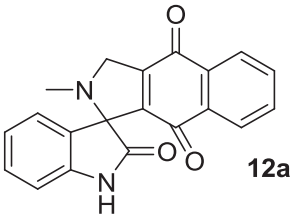
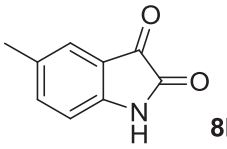
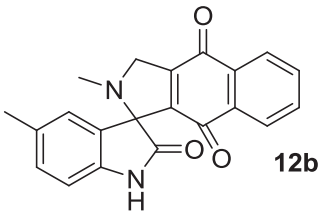
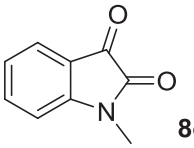
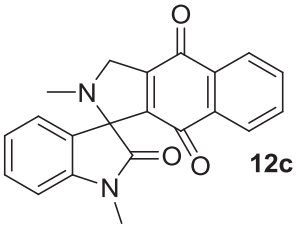
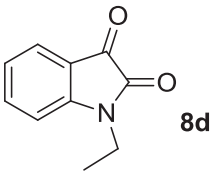
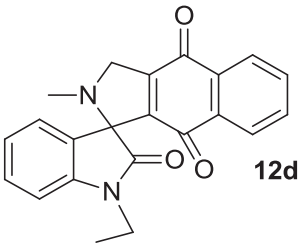
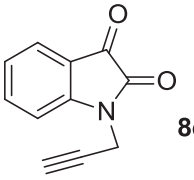
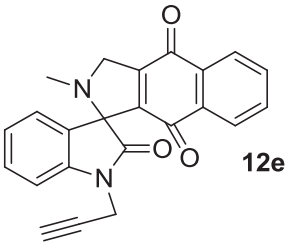
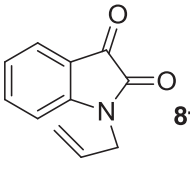
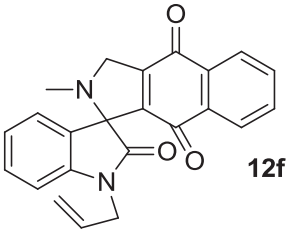
The unexpected promising results prompted us to extend this protocol to synthesize spirooxindole derivatives **17** and **18** involving acenaphthenequinone **15** and ninhydrin **16** under optimized condition gave good yield of respective spirooxindole derivatives in 91 and 93% of yield (Scheme 5).

The structure of unusual dehydrogenative spirooxindole derivatives by 1,3-dipolar cycloaddition of azomethine ylide was elucidated with the help of IR, ^{1}H NMR, ^{13}C NMR and Mass data as illustrated for **12a**. In the IR spectrum, the sharp peak appeared at 3425 cm^{-1} corresponds to the spirooxindole N–H group and the sharp peaks at 1721 and 1628 cm^{-1} correspond to C=O functional groups of the product **12a**. In the ^{1}H NMR spectrum, peaks in the range of δ: 6.85–8.00 ppm shows aromatic protons. A broad singlet in the region δ: 10.60 ppm signal confirmed the presence of –NH proton and the signal at δ: 2.12 ppm for three protons confirmed the presence of $-NCH_3$ group. The ABq peak at δ: 4.21 ppm with the *J* value 16.8 Hz for two protons shows the presence of pyrrolidine ring $-CH_2$ group. The ^{13}C NMR, the peak at δ: 77.6 ppm corresponds to the spiro carbon and the peak at δ: 175.6 ppm shows amide carbonyl carbon. The peaks at δ: 180.8 and 182.1 ppm confirmed the presence of two carbonyl groups. In dept 135 and 90, the peaks at δ: 57.7 & 34.7 ppm confirms the presence of $-CH_2$ and $-NCH_3$ group respectively. A distinguishing peak was observed at *m/z*: 331 in the



Scheme 1. Synthesis of spirooxindole derivatives from substituted isatin, sarcosine and 1,4-napthoquinone.

Table 1
Synthesis of spirooxindole derivatives from substituted isatin, sarcosine and 1,4-naphthoquinone.

Entry	Isatin	Product[a]	Yield (%)[b]
1	 **8a**	 **12a**	91
2	 **8b**	 **12b**	89
3	 **8c**	 **12c**	94
4	 **8d**	 **12d**	92
5	 **8e**	 **12e**	92
6	 **8f**	 **12f**	94

(*continued on next page*)

Table 1 (*continued*)

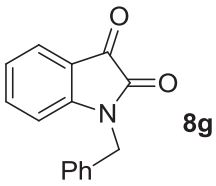
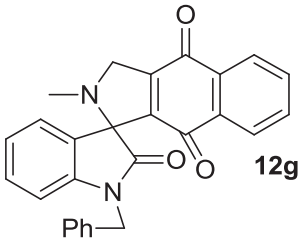
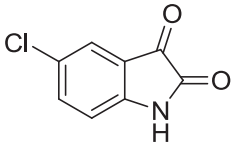
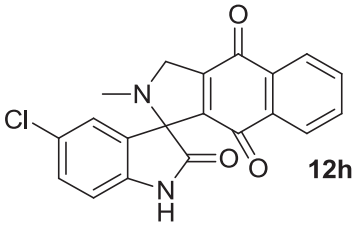
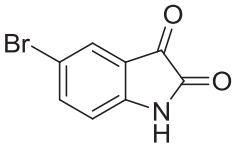
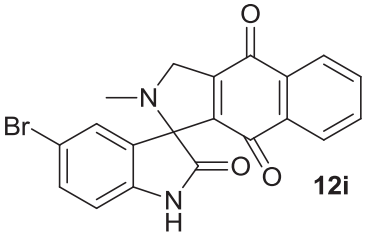
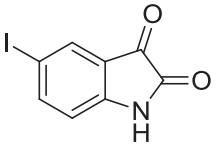
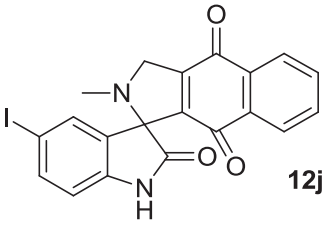
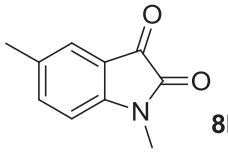
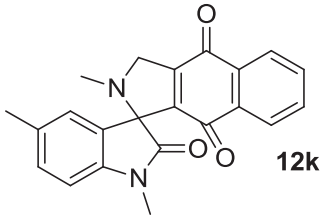
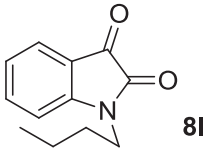
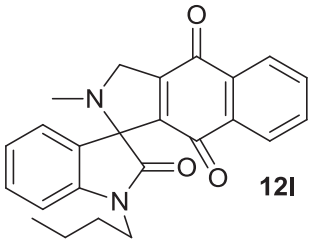
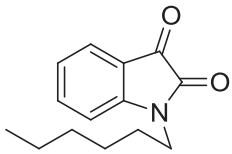
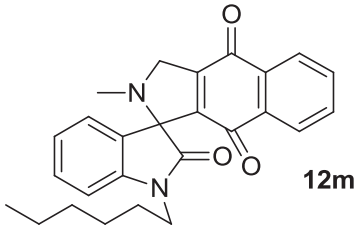
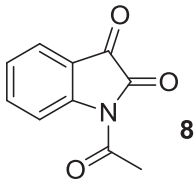
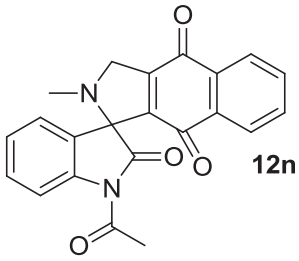
Entry	Isatin	Product[a]	Yield (%)[b]
7	 **8g**	 **12g**	90
8	 **8h**	 **12h**	95
9	 **8i**	 **12i**	94
10	 **8j**	 **12j**	92
11	 **8k**	 **12k**	93
12	 **8l**	 **12l**	91
13	 **8m**	 **12m**	89

Table 1 (*continued*)

Entry	Isatin	Product[a]	Yield (%)[b]
14	 **8n**	 **12n**	96

[a] All the products were characterised by Mass, IR and NMR spectrum.
[b] Isolated yield after purification by column chromatography.

mass spectrum for $[M + H]^+$ ion further conforms the product **12a**. The structure determined from an X-ray crystallographic study of the single crystal of the derivative **14b** confirms the structure that deduced from spectroscopic data (Fig. 2). Table 3 gives crystal data, data collection and refinement parameters of **14b**.

3. Biology

In the present study, the antimicrobial activities of synthesized compounds were screened against eight bacteria and three fungi using *in vitro* disc diffusion method. The results revealed that most of the synthesised compounds exhibited antimicrobial activities against *Staphylococcus aureus*, *S. aureus* (MRSA), *Enterobacter aerogens*, *Micrococcus luteus*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Salmonella paratyphi-B*, *Malassesia pachydermatis*, *Candida albicans* and *Botyritis cinerea* organisms. The results are summarized in Table 4 and Fig. 3. Compounds **12d**, **12i**, **12k**, **12l** and **12n** showed good activity more than standard drug against *S. aureus*. Compound **12n** showed good activity against both Gram-positive and Gram-negative bacteria among all synthesized compounds compare with standards. Compound **12k** showed good activity among N-alkyl substituted compounds **12d, 12l** and **12m**. Halo substituted compounds **12i** and **12j** shows good activity against tested bacteria. Compound **12n** showed significant anti-fungal activity against *C. albicans*, *M. pachydermatis* and *B. cinerea*.

The MIC values of active compounds (**12a**–**e** & **12i**–**n**) against bacteria and fungi are given in Table 5 and Fig. 4. Significant MIC values were observed against Gram positive and Gram negative bacteria. Compounds **12b**, **12d**, **12i** and **12k** showed good activity against *S. aureus*. In comparison to compound **12a**, the presence of methyl group on the phenyl ring (**12b**) increased the potency against *S. aureus* by one fold. Interestingly the presence of methyl groups in both phenyl ring and nitrogen (**12k**) increased the potency against *S. aureus* by two fold. Also compound **12d** (N-ethyl) increased the potency against *S. aureus* by two fold. In comparison to compound **12a**, the presence of acetyl group on the isatin nitrogen (**12n**) increased the potency against bacteria *S. aureus* by one fold, *S. aureus* (MRSA) by two fold, *M. luteus*, *K. pneumonia* and *S. typhimurium* by three fold and *E. aerogens*, *P. vulgaris* and *S. paratyphi*-B by four fold and also against fungi *C. albicans* by two fold and *M. pachydermatis* by four fold. Compound **12n** (N-acetyl) showed good activity than standard drugs for most of the tested bacteria and fungi.

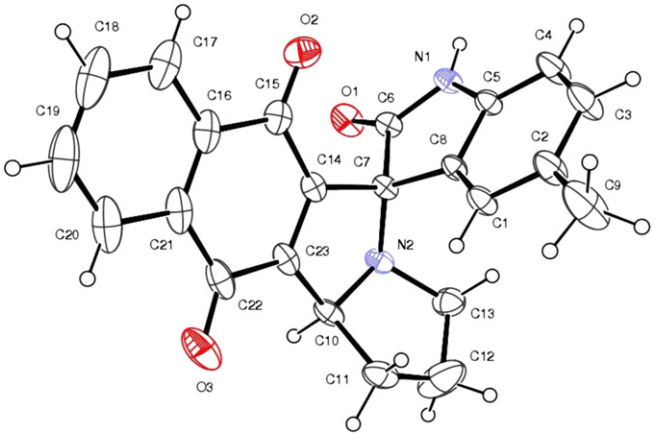


Fig. 2. ORTEP diagram of synthesized compound **14b**.

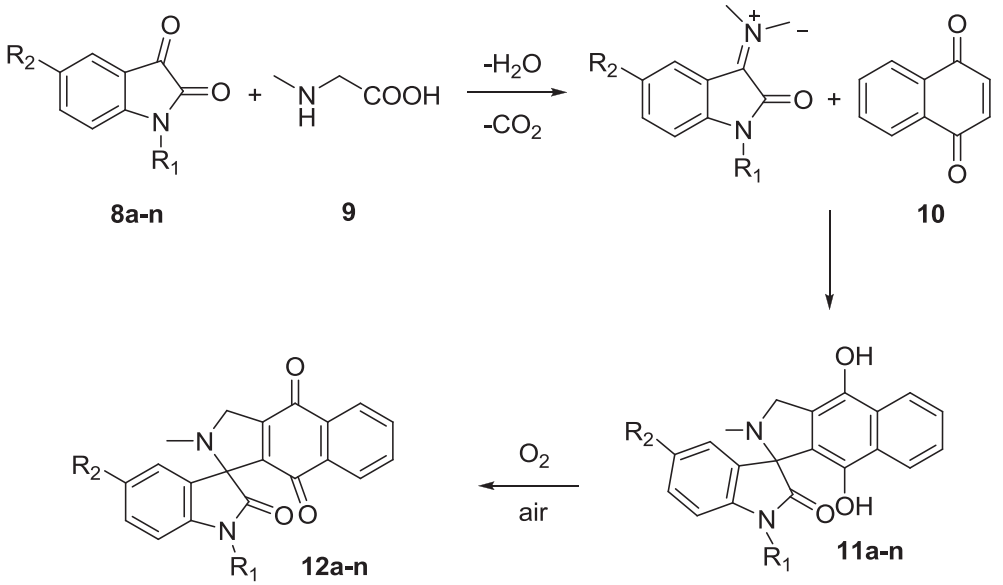
4. Conclusion

In summary, we have reported the synthesis of novel spirooxindole derivatives through 1,3-dipolar cycloaddition of an azomethine ylide generated from isatin and sarcosine or L-proline with the dipolarophile 1,4-naphthoquinone followed by dehydrogenation and characterisation of synthesised spirooxindole derivatives. These novel compounds were evaluated for their activities against eight bacteria and three fungi. Compound **12n** 1′-acetyl-2,5′-dimethyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione was found to be more than 1.6 times active against *S. aureus* (MRSA) bacteria than streptomycin and ciprofloxacin. Also more than 6.4 times active against *M. luteus* and *S. typhimurium* bacteria than ciprofloxacin. Also more than 3.2 times active against *C. albicans* fungi than fluconazole. These results showed that synthesized spirooxindole compound **12n** might be a potential antibacterial and anti-fungal agent.

5. Experimental

5.1. Chemistry

Melting points were determined in capillary tubes and are uncorrected. IR spectra were taken as KBr pellets for solids on a Perkin Elmer Spectrum RXI FT-IR. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in DMSO-d_6 solutions with TMS as an internal standard on a JEOL instrument. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer. Elemental analysis data were recorded using Thermo Finnigan FLASH EA 1112 CHN analyzer.



Scheme 2. Plausible mechanism for the formation of spirooxindoles.

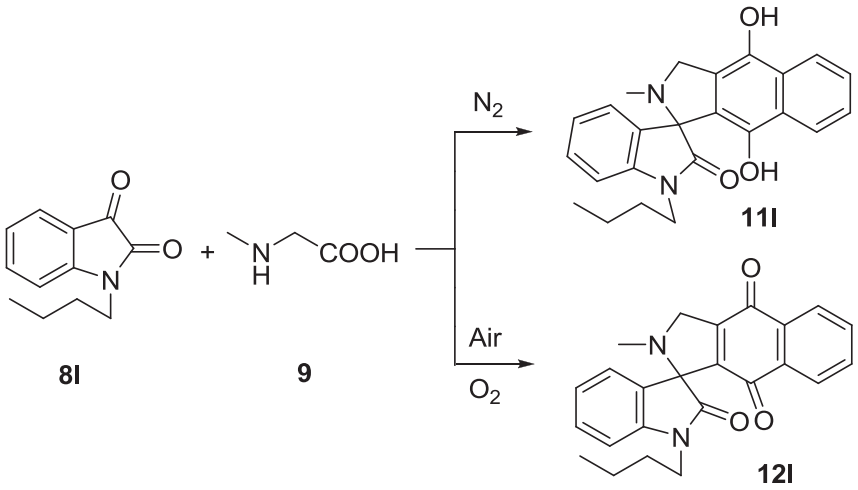
*5.1.1. Experimental procedure for the synthesis of spirooxindole derivatives (**12a**–**n**)*

A mixture of substituted isatin **8a**–**n** (1 mmol), sarcosine **9** (1 mmol) and 1,4-naphthoquinone **10** (1 mmol) was refluxed in ethanol (5 ml). Completion of the reaction was evidenced by TLC analysis. After completion of the reaction, the reaction mixture was poured into ice-water, the resulting solid was filtered off and purified by column chromatography using ethyl acetate: petroleum ether (3:7) as an eluent to afford pure spirooxindoles (**12a**–**n**).

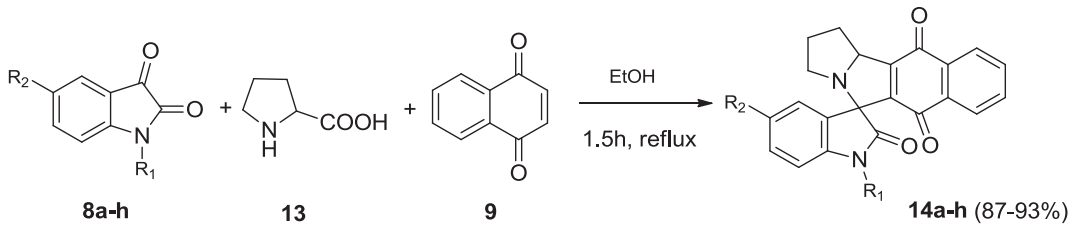
*5.1.1.1. 2-Methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12a**).* Brown solid; mp 218–219 °C; IR (cm^{-1}): 3425, 2926, 1969, 1721, 1628, 1594, 1383, 1221, 1048, 581; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.12 (s, 3H), 4.21 (ABq, 2H, J = 16.8 Hz), 6.85–6.93 (m, 2H), 7.18 (d, 1H, J = 6.9 Hz), 7.22 (t, 1H, J = 7.6 Hz), 7.76–7.85 (m, 3H), 8.00 (d, 1H, J = 6.9 Hz), 10.6 (brs, 1H, -N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6): δ 34.7, 57.7, 77.6, 110.5, 122.4, 125.7, 126.4, 126.6, 128.1, 130.2, 132.4, 132.7, 134.9, 135.0, 142.8, 147.5, 150.9, 175.6, 180.8, 182.1; MS m/z = 331 $[MH]^+$; Anal. Calcd for $C_{20}H_{14}N_2O_3$: C, 72.72; H, 4.27; N, 8.48. Found: C, 72.59; H, 4.39; N, 8.37.

*5.1.1.2. 2,5′-Dimethyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12b**).* Reddish brown solid; mp 210–211 °C; IR (cm^{-1}): 3774, 3419, 2369, 1945, 1713, 1588, 1182, 607; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.14 (s, 3H), 2.15 (s, 3H), 4.21 (ABq, 2H, J = 16.1 Hz), 6.75 (d, 1H, J = 8.4 Hz), 6.94–7.09 (m, 2H), 7.74–7.79 (m, 3H), 7.99 (d, 1H, J = 6.8Hz), 10.4 (brs, 1H, -N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6): δ 21.0, 34.7, 57.7, 77.7, 110.2, 126.3, 126.4, 126.5, 128.3, 130.4, 131.2, 132.5, 132.7, 134.9, 140.4, 147.6, 150.8, 162.9, 175.4, 180.8, 182.1; MS m/z = 345 $[MH]^+$; Anal. Calcd for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13. Found: C, 73.34; H, 4.77; N, 8.01.

*5.1.1.3. 1′,2-Dimethyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12c**).* Brown solid; mp 240–241 °C; IR (cm^{-1}): 3415, 3054, 2931, 2832, 1719, 1662, 1601, 1462, 1336, 1250, 1216, 1085, 1018, 847, 790, 744, 713, 533, 473, 124; ^{1}H NMR (500 MHz,

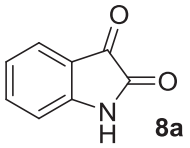
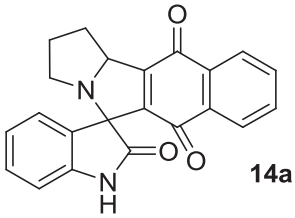
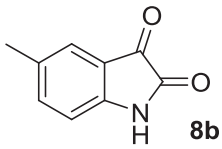
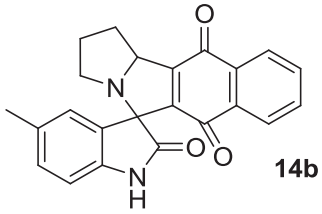
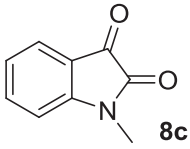
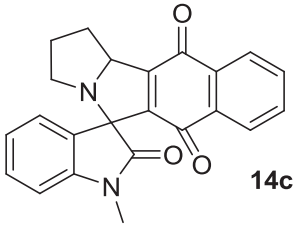
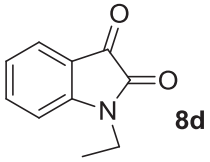
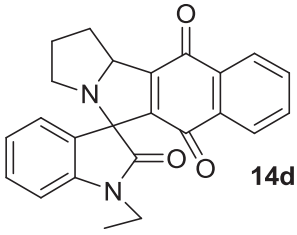
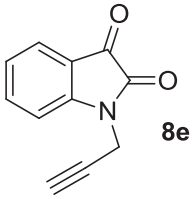
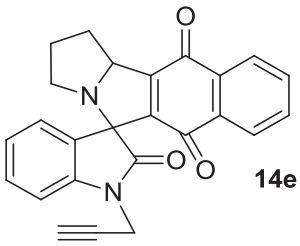
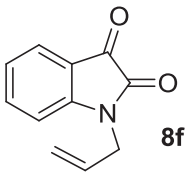
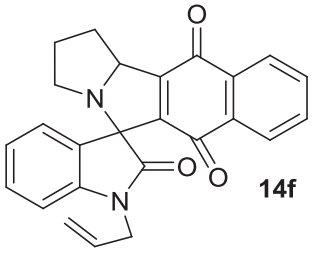


Scheme 3. Synthesis of spirooxindole derivative **11l** at nitrogen atmosphere.



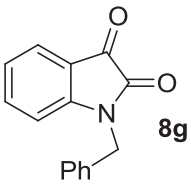
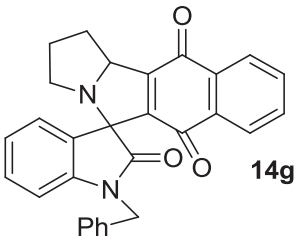
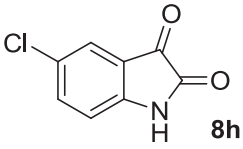
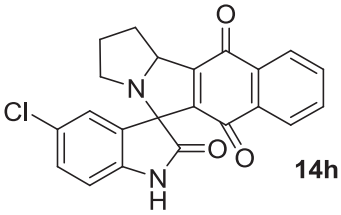
Scheme 4. Synthesis of spirooxindole derivatives from substituted isatin, L-proline and 1,4-napthoquinone.

Table 2
Synthesis of spirooxindole derivatives from substituted isatin, L-proline and 1,4-naphthoquinone.

Entry	Isatin	Product[a]	Yield (%)[b]
1	 8a	 14a	87
2	 8b	 14b	89
3	 8c	 14c	92
4	 8d	 14d	88
5	 8e	 14e	93
6	 8f	 14f	91

(*continued on next page*)

Table 2 (*continued*)

Entry	Isatin	Product[a]	Yield (%)[b]
7	 **8g**	 **14g**	90
8	 **8h**	 **14h**	92

[a] All the products were characterised by Mass, IR and NMR spectrum.
[b] Isolated yield after purification by column chromatography.

DMSO-d_6): δ 2.09 (s, 3H), 3.16 (s, 3H), 4.23 (ABq, 2H, J = 17.6 Hz), 6.98 (t, 1H, J = 6.8 Hz), 7.07 (d, 1H, J = 8.4 Hz), 7.25 (d, 1H, J = 7.6 Hz), 7.34 (t, 1H, J = 7.6 Hz), 7.38–7.76 (m, 3H), 8.01 (d, 1H, J = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.7, 34.8, 57.8, 77.2, 109.4, 122.9, 125.4, 126.4, 126.6, 127.3, 130.3, 132.4, 132.8, 134.8, 134.9, 144.3, 147.3, 151.0, 173.9, 180.7, 182.0; MS m/z = 345[MH]$^+$; Anal. Calcd for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13. Found: C, 73.14; H, 4.61; N, 8.27.

*5.1.1.4. 1-Ethyl-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12d**).* Yellow solid; mp 247–248 °C; IR (cm^{-1}): 3412, 3051, 2931, 1719, 1662, 1462, 1332, 1216, 1084, 1018, 847, 713; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.33 (t, 3H, J = 6.8 Hz), 2.13 (s, 3H), 3.38 (q, 2H, J = 6.8 Hz), 4.25 (ABq, 2H, J = 16.8 Hz), 6.97 (t, 1H, J = 6.8 Hz), 7.07 (d, 1H, J = 8.4 Hz), 7.24 (d, 1H, J = 7.6 Hz), 7.35 (t, 1H, J = 7.6 Hz), 7. 36–7.74 (m, 3H), 8.01 (d, 1H, J = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 10.9, 34.5, 39.5, 57.8, 77.2, 109.3, 122.9, 125.4, 126.3, 126.5, 127.3, 130.1, 132.4, 132.8, 134.8, 134.9, 144.4, 147.2, 151.1, 173.9, 180.7, 182.0; MS m/z = 359 [MH]$^+$; Anal. Calcd for $C_{22}H_{18}N_2O_3$: C, 73.73; H, 5.06; N, 7.82. Found: C, 73.84; H, 5.14; N, 7.72.

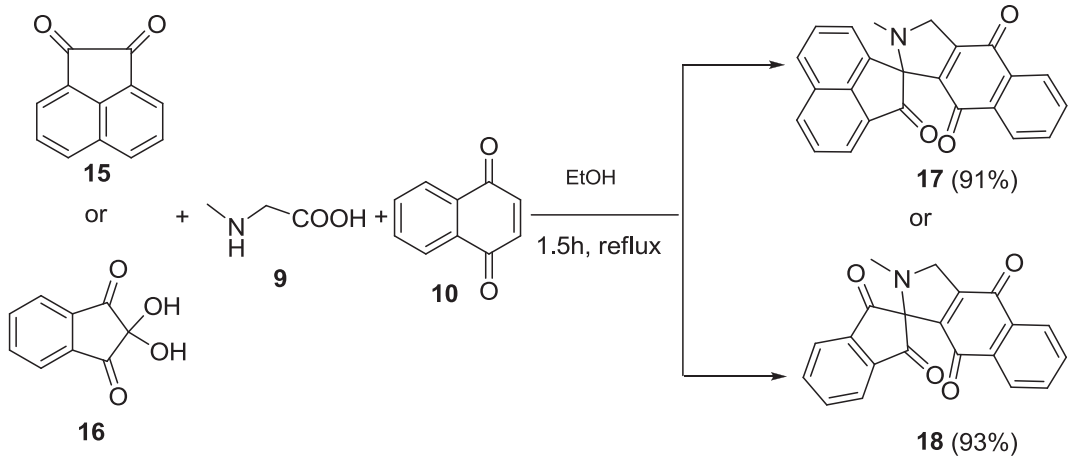
*5.1.1.5. 2-Methyl-1′-(prop-2-ynyl)-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12e**).* Yellow solid; mp 223–224 °C; IR (cm^{-1}): 3779, 3656, 3238, 2934, 2365, 2208, 1724, 1678, 1602, 1460, 1338, 1185, 962, 720, 483; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.08 (s, 3H), 3.26 (t, 1H, J = 2.3 Hz), 4.23 (ABq, 2H, J = 16.8 Hz), 4.57 (ABq, 2H, J = 17.6 Hz), 7.02 (t, 1H, J = 7.6 Hz), 7.16 (d, 1H, J = 8.4 Hz), 7.28 (d, 1H, J = 7.6 Hz), 7.36 (t, 1H, J = 7.6 Hz), 7.77–7.85 (m, 3H), 7.99 (d, 1H, J = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 29.5, 34.6, 57.8, 74.9, 77.0, 78.3, 110.2, 123.3, 125.7, 126.4, 126.6, 127.2, 130.3, 132.4, 132.8, 134.8, 134.9, 142.4, 146.9, 151.2, 173.3, 180.6, 182.0; MS m/z = 369 [MH]$^+$; Anal. Calcd for $C_{23}H_{16}N_2O_3$: C, 74.99; H, 4.38; N, 7.60. Found: C, 75.09; H, 4.45; N, 7.49.

*5.1.1.6. 1′-allyl-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12f**).* Brown solid; mp 212–213 °C; IR (cm^{-1}): 3016, 2886, 1715, 1678, 1609, 1461, 1341, 1169, 962, 728, 483; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.12 (s, 3H), 4.26 (ABq, 2H, J = 16.8 Hz), 4.29–4.39 (m, 2H), 5.17 (d, 1H, J = 10.7 Hz), 5.31 (d, 1H, J = 16.8 Hz), 5.81–5.95 (m, 1H), 6.96–7.01 (m, 2H), 7.26–7.33 (m, 2H), 7.78–7.86 (m, 3H), 8.01 (d, 1H, J = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 34.7, 42.2, 57.8, 77.2, 110.1, 117.1, 123.0, 125.6, 126.4, 126.6, 127.3, 130.2, 132.1, 132.4, 132.8, 134.8, 134.9, 143.4, 147.3, 151.2, 173.7, 180.8, 182.0; MS m/z = 371 [MH]$^+$; Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.67; H, 4.82; N, 7.69.

*5.1.1.7. 1′-Benzyl-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12g**).* Brown solid; mp 219–220 °C; IR (cm^{-1}): 3776, 3430, 3030, 2918, 2843, 1721, 1659, 1598, 1464, 1337, 1173, 969, 793, 698, 618, 550, 469; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.12 (s, 3H), 4.26 (ABq, 2H, J = 16.8 Hz), 4.93 (s, 2H), 6.89 (d, 1H, J = 7.6 Hz), 6.96 (t, 1H, J = 6.9 Hz), 7.21–7.29 (m, 3H), 7.33 (t, 2H, J = 7.6 Hz), 7.40 (d, 2H, J = 7.6 Hz), 7.78–7.86 (m, 3H), 8.01 (d, 1H, J = 6.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 34.8, 43.5, 57.8, 77.2, 110.1, 123.1, 125.6, 126.5, 126.6, 127.3, 127.6, 127.9, 129.1, 130.3, 132.4, 132.8, 134.9, 135.0, 136.6, 143.3, 147.2, 151.3, 174.2, 180.8, 182.0; MS m/z = 421 [MH]$^+$; Anal. Calcd for $C_{27}H_{20}N_2O_3$: C, 77.13; H, 4.79; N, 6.66. Found: C, 77.01; H, 4.86; N, 6.72.

*5.1.1.8. 5′-Chloro-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12h**).* Yellow solid; mp 237–238 °C; IR (cm^{-1}): 3776, 3182, 1869, 1667, 1598, 1450, 1277, 1179, 959, 882, 712, 553; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.16 (s, 3H), 4.24 (ABq, 2H, J = 16.8 Hz), 6.88 (d, 1H, J = 8.4 Hz), 7.27 (d, 1H, J = 8.4 Hz), 7.31–7.33 (m, 1H), 7.78–7.84 (m, 3H), 8.01 (d, 1H, J = 7.6 Hz), 10.7 (brs, 1H, –N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6): δ 34.7, 57.8, 77.6, 111.9, 126.0, 126.4, 126.5, 130.1, 130.3, 132.5, 132.8, 132.9, 134.8, 134.9, 141.8, 146.7, 151.2, 175.3, 180.8, 182.0; MS m/z = 364 [MH]$^+$; Anal. Calcd for $C_{20}H_{13}ClN_2O_3$: C, 65.85; H, 3.59; N, 7.68. Found: C, 65.74; H, 3.67; N, 7.74.

*5.1.1.9. 5′-Bromo-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12i**).* Brown solid; mp 172–173 °C; IR (cm^{-1}): 3249, 2868, 1720, 1596, 1459, 1282, 1203, 806, 708; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.14 (s, 3H), 4.24 (ABq, 2H, J = 16.8 Hz), 6.86 (d, 1H, J = 8.4 Hz), 7.33 (d, 1H, J = 8.4 Hz), 7.31–7.34 (m, 1H), 7.77–7.85 (m, 3H), 8.01 (d, 1H, J = 7.6 Hz), 10.7 (brs, 1H, -N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6): δ 34.7, 57.8, 77.6, 126.1, 126.2, 126.6, 128.2, 130.2, 130.3, 132.7, 132.7, 132.9, 134.4, 134.9, 141.8, 146.6, 151.2, 175.2, 180.8, 182.1; MS m/z = 409 [MH]$^+$;



Scheme 5. Synthesis of spirooxindole derivatives from acenaphthenequinone or ninhydrin with sarcosine and 1,4-naphthoquinone.

Anal. Calcd for $C_{20}H_{13}BrN_2O_3$: C, 58.70; H, 3.20; N, 6.85. Found: C, 58.52; H, 3.13; N, 6.62.

*5.1.1.10. 5′-Iodo-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12j**).* Pale yellow solid; mp 174–175 °C; IR (cm^{-1}): 3259, 2862, 1711, 1596, 1459, 1281, 1204, 806, 708; ^1H NMR (500 MHz, DMSO-d_6): δ 2.14 (s, 3H), 4.24 (ABq, 2H, J = 16.8 Hz), 6.84 (d, 1H, J = 8.4 Hz), 7.31 (d, 1H, J = 8.4 Hz), 7.32–7.35 (m, 1H), 7.79–7.86 (m, 3H), 8.01 (d, 1H, J = 7.6 Hz), 10.7 (brs, 1H, -N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6): δ 34.7, 57.8, 77.6, 98.2, 126.1, 126.2, 126.6, 130.2, 130.3, 132.7, 132.7, 132.9, 134.4, 134.9, 141.8, 146.6, 151.2, 175.2, 180.8, 182.1; MS m/z = 457 $[MH]^+$; Anal. Calcd for $C_{20}H_{13}IN_2O_3$: C, 52.65; H, 2.87; N, 6.14. Found: C, 52.53; H, 2.74; N, 6.27.

*5.1.1.11. 1′,2,5′-Trimethyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12k**).* Brown solid; mp 130–131 °C. IR (cm^{-1}): 3757, 3403, 2934, 2799, 2363, 1710, 1607, 1495, 1344, 1188, 1100, 806, 705; ^1H NMR (500 MHz, DMSO-d_6): ^1H NMR (500 MHz, DMSO-d_6): δ 2.12 (s, 3H), 2.15 (s, 3H), 3.16 (s, 3H), 4.22 (ABq, 2H, J = 16.1 Hz), 6.75 (d, 1H, J = 8.4 Hz), 6.92–7.12 (m, 2H), 7.71–7.74 (m, 3H), 7.78 (d, 1H, J = 6.8Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 21.0, 26.8, 34.7, 57.7, 77.8, 110.2, 126.2, 126.4, 126.5, 128.4, 130.4, 131.1, 132.5, 132.6, 134.9, 140.4, 147.6, 150.9, 162.9, 175.4, 180.8, 182.1; MS m/z = 359 $[MH]^+$; Anal. Calcd for $C_{22}H_{18}N_2O_3$: C, 73.73; H, 5.06; N, 7.82. Found: C, 73.59; H, 5.18; N, 7.77.

Table 3
Crystal data and structure refinement parameters of compounds **14b**.

Empirical formula	$C_{23}H_{18}N_2O_3$
Formula weight	370.39
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, P2(1)/*n*
Unit cell dimensions	a = 9.3713(4) Å; b = 9.7977(4) Å; c = 20.3233(7) Å; $\alpha = \gamma = 90°$; β = 99.495(2)°
Volume	1840.46 (13) $Å^3$
Z, Density (calculated)	4, 1.337 mg m^{-3}
Absorption coefficient	0.090 mm^{-1}
F (000)	776
Crystal size	0.45 × 0.40 × 0.25 mm
θ range for data collection	2.03–32.43°
Limiting indices	$-13 \le h \le 12$, $-14 \le k \le 14$, $-23 \le l \le 26$
Reflections collected/unique	15,052/5097 [R(int) = 0.0212]
Absorption correction	Multi-scan
Max. and min. transmission	0.9779 and 0.9608
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	5097/0/258
Goodness-of-fit on F^2	1.032
Largest diff. peak and hole	0.530 and −0.421 $eÅ^{-3}$
CCDC	852,448

*5.1.1.12. 1′-Butyl-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12l**).* Brown solid; mp 148–149 °C; IR (cm^{-1}): 3054, 2933, 2872, 2802, 1707, 1667, 1602, 1459, 1342, 1218, 1177, 962, 752; ^1H NMR (500 MHz, DMSO-d_6): δ 0.92 (t, 3H, J = 7.5), 1.36 (sex, 2H, J = 7.5 Hz), 1.63 (qt, 2H, J = 6.9 Hz), 2.14 (s, 3H), 3.73 (t, 2H, J = 6.9 Hz), 4.27 (ABq, 2H, J = 16.8 Hz), 7.01 (t, 1H, J = 7.2 Hz), 7.14 (d, 1H, J = 7.8 Hz), 7.29 (d, 1H, J = 7.8 Hz), 7.36 (td, 1H, J = 7.8 Hz, J = 1.2 Hz), 7.81–7.86 (m, 3H), 8.05 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.6, 19.4, 28.9, 34.1, 57.2, 76.6, 109.0, 122.2, 125.0, 125.9, 126.0, 127.0, 129.8, 131.9, 132.2, 134.3, 134.4, 143.2, 146.9, 150.5, 173.2, 180.2, 181.5; MS m/z = 387 $[MH]^+$; Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.43; H, 5.77; N, 7.11.

*5.1.1.13. 1′-Hexyl-2-methyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12m**).* Brown solid; mp 132–133 °C; IR (cm^{-1}): 3055, 2927, 2859, 1715, 1663, 1600, 1463, 1346, 1178, 1022, 965, 744; ^1H NMR (500 MHz, DMSO-d_6): δ 0.93 (t, 3H, J = 7.5), 1.22–1.50 (m, 6H), 1.52–1.81 (m, 2H), 2.14 (s, 3H), 3.72 (t, 2H, J = 6.6 Hz), 4.28 (ABq, 2H, J = 16.8 Hz), 7.01 (q, 1H, J = 7.2 Hz), 7.09 (d, 1H, J = 7.2 Hz), 7.29 (d, 1H, J = 7.2 Hz), 7.36 (td, 1H, J = 7.8 Hz, J = 1.2 Hz), 7.81–7.87 (m, 3H), 8.05 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.5, 14.7, 19.4, 21.3, 28.8, 34.1, 57.1, 76.6, 109.0, 122.3, 125.0, 125.8, 126.0, 127.1, 129.8, 131.8, 132.2, 134.4, 134.4, 143.1, 146.9, 150.5, 173.3, 180.2, 181.7; MS m/z = 415 $[MH]^+$; Anal. Calcd for $C_{26}H_{26}N_2O_3$: C, 75.34; H, 6.32; N, 6.76. Found: C, 75.43; H, 6.67; N, 6.89.

*5.1.1.14. 1′-Acetyl-2,5′-dimethyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-trione (**12n**).* Brown solid; mp 167–168 °C; IR (cm^{-1}): 3340, 3082, 1711, 1664, 1600, 1468, 1371, 1267, 1072, 761; ^1H NMR (500 MHz, DMSO-d_6): δ 2.11 (s, 3H), 2.25 (s, 3H), 4.21 (ABq, 2H, J = 16.8 Hz), 6.84–6.97 (m, 2H), 7.19 (d, 1H, J = 6.9 Hz), 7.32 (t, 1H, J = 7.6 Hz), 7.77–7.84 (m, 3H), 8.00 (d, 1H, J = 6.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.4, 34.8, 57.7, 77.6, 110.5, 122.3, 125.5, 126.4, 126.5, 128.1, 130.7, 132.4, 132.7, 134.9, 135.0, 142.8, 147.5, 150.9, 173.1, 175.6, 180.8, 182.1; MS m/z = 373 $[MH]^+$; Anal. Calcd for $C_{22}H_{16}N_2O_4$: C, 70.96; H, 4.33; N, 7.52. Found: C, 70.85; H, 4.39; N, 7.34.

Table 4
In vitro antimicrobial activity of synthesized compounds.

Compounds	Zone of inhibition in mm										
	Gram positive bacteria				Gram negative bacteria				Fungi		
	S. aureus	*S. aureus* (MRSA)	*M. luteus*	*E. aerogens*	*P. vulgaris*	*K. pneumonia*	*S. typhimurium*	*S. Paratyphi*-B	*M. pachydermatis*	*C. albicans*	*B. cinerea*
12a	15	10	13	10	11	13	12	10	11	10	10
12b	15	12	13	13	9	13	12	11	12	8	9
12c	13	10	12	10	10	18	14	11	11	9	8
12d	22	13	18	12	15	12	19	10	11	10	8
12e	13	10	12	11	9	11	15	10	10	10	10
12f	11	9	10	9	8	9	11	Ni	9	9	8
12g	9	8	9	8	8	10	9	8	9	8	10
12h	14	11	12	10	10	13	11	13	10	9	8
12i	20	12	17	16	12	10	19	8	10	10	11
12j	16	8	15	12	10	11	14	7	14	11	12
12k	22	14	15	17	16	13	22	8	10	12	11
12l	17	8	13	10	10	13	15	9	11	10	9
12m	12	10	10	13	10	10	16	8	12	14	10
12n	18	16	22	21	26	24	24	28	19	18	15
14a	12	8	9	9	8	9	10	Ni	8	Ni	Ni
14b	10	8	11	9	9	10	11	8	9	Ni	9
14c	10	8	9	9	8	9	11	Ni	10	10	8
14e	Ni	Ni	9	Ni	8	Ni	9	8	Ni	8	9
14f	8	Ni	9	Ni	Ni	Ni	9	Ni	Ni	Ni	Ni
14g	10	8	9	8	10	8	9	Ni	9	8	Ni
14h	12	9	10	9	9	11	10	Ni	9	Ni	Ni
17	9	Ni	8	Ni	8	9	10	Ni	8	8	9
18	9	Ni	8	8	8	9	10	9	Ni	Ni	9
Streptomycin	16	20	23	22	22	20	23	17	Na	Na	Na
Ketoconazole	Na	Na	Na	Na	Na	Na	Na	Na	28	22	Ni

NA – not applicable; NI – no inhibition.

*5.1.2. Experimental procedure for the synthesis of spirooxindole derivatives (**14a**–**h**)*

A mixture of substituted isatin **8a**–**h** (1 mmol), L-proline **13** (1 mmol) and 1,4-naphthoquinone **10** (1 mmol) was refluxed in ethanol (5 ml). Completion of the reaction was evidenced by TLC analysis. After completion of the reaction, the reaction mixture was poured into ice-water, the resulting solid was filtered off and purified by column chromatography using ethyl acetate: petroleum ether (3:7) as an eluent to afford pure spirooxindoles (**14a**–**h**).

*5.1.2.1. 1,2,3,11b-Tetrahydrospiro[benzo[f]pyrrolo[2,1-a]isoindole-5,3′-indoline]-2′,6,11-trione (**14a**).* White solid; mp 227–228 °C; IR (cm^{-1}): 3378, 2878, 1735, 1606, 1466, 1304, 1156, 794, 727, 565; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.72–1.88 (m, 3H), 2.14–2.22 (m, 1H), 2.45–2.51 (m, 1H), 2.56–2.63 (m, 1H), 4.74 (t, 1H, J = 7.6 Hz), 6.85–6.91 (m, 2H), 7.09 (d, 1H, J = 7.6 Hz), 7.24 (t, 1H, J = 7.6 Hz), 7.76–7.81 (m, 3H), 8.00 (d, 1H, J = 7.6 Hz), 10.49 (brs, 1H, -N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.8, 30.9, 47.8, 70.7, 77.0, 110.7, 121.7, 126.1, 126.3, 126.5, 127.1, 130.4, 132.7, 133.1,

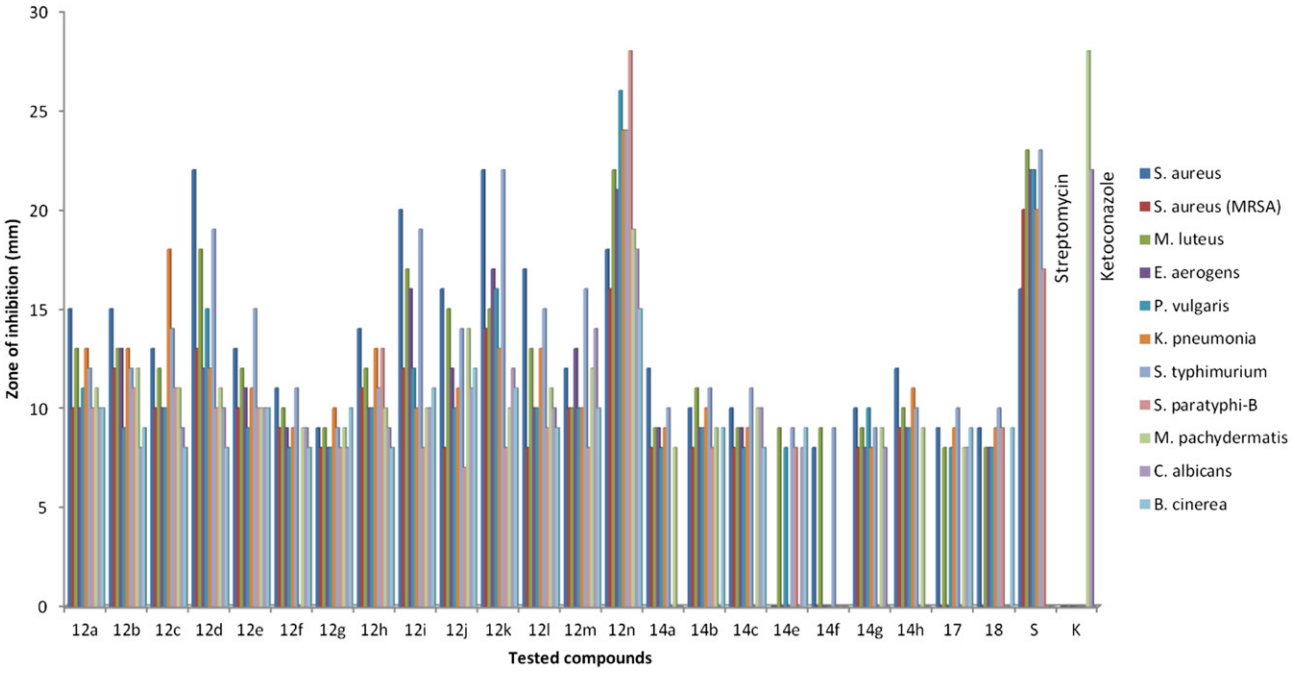


Fig. 3. Comparison of antimicrobial activity of synthesised compounds and standard drugs.

Table 5
MIC (μg/ml) of compounds against tested bacteria and fungi.

Compounds	Minimum inhibitory concentration (μg/ml)										
	Gram positive bacteria				Gram negative bacteria				Fungi		
	S. aureus	*S. aureus* (MRSA)	*M. luteus*	*E. aerogens*	*P. vulgaris*	*K. pneumonia*	*S. typhimurium*	*S. Paratyphi*-B	*M. pachydermatis*	*C. albicans*	*B. cinerea*
12a	62.5	250	125	250	250	125	125	250	250	125	125
12b	31.25	125	62.5	125	250	62.5	250	125	125	250	250
12c	62.5	250	125	125	125	31.25	125	250	125	250	250
12d	15.62	250	31.25	250	62.5	250	31.25	500	250	500	500
12e	125	125	125	250	500	250	62.5	250	250	250	250
12i	15.62	250	62.5	62.5	125	500	31.25	500	500	500	250
12j	62.5	500	15.62	250	500	250	125	500	125	250	250
12k	15.62	125	125	62.5	125	250	15.62	500	500	250	250
12l	62.5	500	250	500	500	250	125	500	250	500	500
12m	250	500	250	250	500	500	62.5	500	250	125	500
12n	31.25	62.5	15.62	15.62	<15.62	15.62	15.62	<15.62	15.62	31.25	125
Streptomycin	6.25	>100	6.25	25	6.25	6.25	30	NI	NA	NA	NA
Ciprofloxacin	<0.78	>100	>100	<0.78	<0.78	<0.78	>100	6.25	NA	NA	NA
Fluconazole	NA	NA	NA	NA	NA	NA	NA	NA	12.5	>100	NI
Ketoconazole	NA	NA	NA	NA	NA	NA	NA	NA	15	25	25

134.7, 134.8, 143.3, 146.1, 152.5, 177.7, 181.5, 182.6; MS m/z = 357 $[MH]^+$; Anal. Calcd for $C_{22}H_{16}N_2O_3$: C, 74.15; H, 4.53; N, 7.86. Found: C, 74.25; H, 4.47; N, 7.92.

*5.1.2.2. 5′-Methyl-1,2,3,11b-tetrahydrospiro[benzo[f]pyrrolo[2,1-a] isoindole-5,3′-indoline]-2′,6,11-trione (**14b**).* White solid; mp 239–240 °C; IR (cm^{-1}): 3172, 2949, 1724, 1638, 1474, 1291, 1178, 716, 448; ^1H NMR (500 MHz, DMSO-d_6): δ 1.71–1.88 (m, 3H), 2.15 (s, 3H), 2.44–2.51 (m, 2H), 2.55–2.65 (m, 1H), 4.74 (t, 1H, J = 6.8 Hz), 6.77 (d, 1H, J = 7.6 Hz), 6.92 (s, 1H), 7.04 (d, 1H, J = 7.6 Hz), 7.76–7.86 (m, 3H), 8.01 (d, 1H, J = 6.9 Hz), 10.37 (brs, 1H, -N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6): δ 21.1, 27.8, 31.0, 47.8, 70.7, 77.1, 110.4, 126.2, 126.3, 126.4, 127.6, 130.6, 130.7, 132.7, 133.1, 134.7, 134.8, 140.8, 146.2, 152.4, 177.7, 181.5, 182.7; MS m/z = 371 $[MH]^+$; Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.49; H, 4.82; N, 7.68.

*5.1.2.3. 1′-Methyl-1,2,3,11b-tetrahydrospiro[benzo[f]pyrrolo[2,1-a] isoindole-5,3′-indoline]-2′,6,11-trione (**14c**).* White solid; mp 268–269 °C; IR (cm^{-1}): 3413, 2934, 2356, 1708, 1609, 1474, 1335, 1123, 1000, 744, 480; ^1H NMR (500 MHz, DMSO-d_6): δ 1.71–1.88 (m, 3H), 2.13–2.23 (m, 1H), 2.44–2.50 (m, 1H), 2.57–2.62 (m, 1H), 3.12 (s, 3H), 4.75 (t, 1H, J = 7.6 Hz), 6.84–6.92 (m, 2H), 7.10 (d, 1H, J = 7.6 Hz), 7.23 (t, 1H, J = 7.6 Hz), 7.75–7.82 (m, 3H), 8.01 (d, 1H, J = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.8, 27.8, 31.0, 47.8, 70.8, 76.6, 109.6, 122.4, 125.3, 126.3, 126.5, 126.8, 130.6, 132.6, 133.1, 134.8, 134.9, 144.6, 146.0, 152.6, 176.1, 181.4, 182.6; MS m/z = 371 $[MH]^+$; Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.48; H, 4.78; N, 7.70.

*5.1.2.4. 1′-Ethyl-1,2,3,11b-tetrahydrospiro[benzo[f]pyrrolo[2,1-a]iso-indole-5,3′-indoline]-2′,6,11-trione (**14d**).* White solid; mp 251–252 °C; IR (cm^{-1}): 3416, 2932, 2359, 1702, 1602, 1472, 1342, 1119, 753; ^1H NMR (500 MHz, DMSO-d_6): δ 1.32 (t, 3H, J = 6.9 Hz), 1.72–1.84 (m, 3H), 2.15–2.24 (m, 1H), 2.47–2.54 (m, 1H), 2.58–2.64 (m, 1H), 3.39 (q, 3H, J = 6.9 Hz), 4.74 (t, 1H, J = 7.6 Hz), 6.82–6.91 (m, 2H), 7.11 (d, 1H, J = 7.6 Hz), 7.25 (t, 1H, J = 7.6 Hz), 7.74–7.81 (m, 3H), 8.02 (d, 1H, J = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.1, 26.8, 27.8, 31.0, 39.3, 47.7, 70.6, 76.7, 109.5, 122.4, 125.4, 126.2, 126.6, 126.7, 130.6, 132.6, 133.1, 134.7, 134.9, 144.5, 146.1, 152.5, 176.1, 181.3,

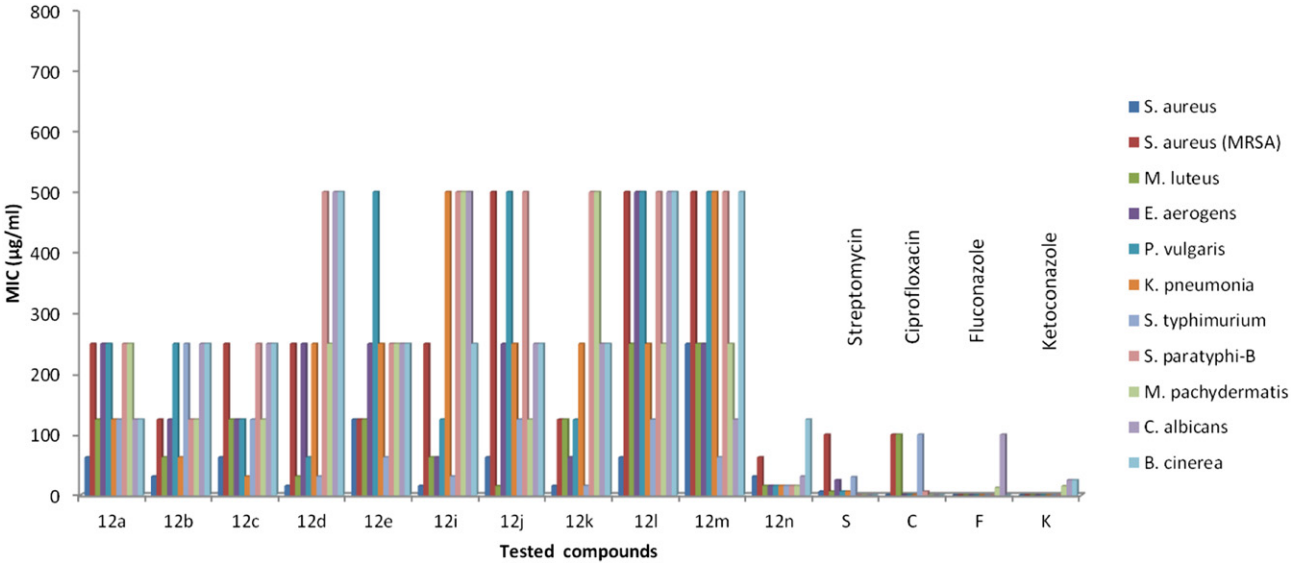


Fig. 4. Comparison of MIC (μg/ml) values of synthesised compounds and standard drugs.

182.6; MS $m/z = 385$ $[MH]^+$; Anal. Calcd for $C_{24}H_{20}N_2O_3$: C, 74.98; H, 5.24; N, 7.29. Found: C, 74.79; H, 5.34; N, 7.37.

*5.1.2.5. 1′-(Prop-2-ynyl)-1,2,3,11b-tetrahydrospiro[benzo[f]pyrrolo [2,1-a]isoindole-5,3′-indoline]-2′,6,11-trione (**14e**).* White solid; mp 231–232 °C; IR (cm^{-1}): 3778, 3433, 3246, 2892, 2365, 1724, 1664, 1605, 1468, 1341, 1180, 731, 486; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.72–1.79 (m, 1H), 1.82–1.89 (m, 2H), 2.15–2.23 (m, 1H), 2.42–2.45 (m, 1H), 2.53–2.55 (m, 1H), 3.27 (m, 1H), 4.53 (ABq, 2H, $J = 17.5$), 4.76 (t, 1H, $J = 7.7$ Hz), 7.01 (t, 1H, $J = 7.6$ Hz), 7.16 (d, 1H, $J = 7.6$ Hz), 7.20 (d, 1H, $J = 7.6$ Hz), 7.38 (t, 1H, $J = 7.6$ Hz), 7.74–7.81 (m, 2H) 7.84 (t, 1H, $J = 7.6$ Hz), 8.01 (d, 1H, $J = 7.6$ Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.8, 29.6, 30.8, 47.8, 70.8, 75.1, 76.6, 78.3, 110.3, 122.8, 125.2, 126.3, 126.5, 127.0, 130.6, 132.6, 133.2, 134.8, 134.9, 142.8, 145.6, 152.8, 175.3, 181.3, 182.6; MS $m/z = 395$ $[MH]^+$; Anal. Calcd for $C_{25}H_{18}N_2O_3$: C, 76.13; H, 4.60; N, 7.10. Found: C, 76.24; H, 4.51; N, 7.02.

*5.1.2.6. 1′-Allyl-1,2,3,11b-tetrahydrospiro[benzo[f]pyrrolo[2,1-a]iso-indole-5,3′-indoline]-2′,6,11-trione (**14f**).* White solid; mp 204–205 °C; IR (cm^{-1}): 3421, 2885, 1711, 1608, 1466, 1341, 1164, 962, 729, 481; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.73–1.79 (m, 1H), 1.82–1.90 (m, 2H), 2.16–2.24 (1H, m), 2.48–2.52 (m, 1H), 2.55–2.62 (m, 1H), 4.31 (2H, m), 4.77 (t, 1H, $J = 7.6$ Hz), 5.16 (d, 1H, $J = 10.7$ Hz), 5.27 (d, 1H, $J = 16.0$ Hz), 5.80 (m, 1H), 6.94–7.00 (m, 2H), 7.18 (d, 1H, $J = 7.6$ Hz), 7.31 (t, 1H, $J = 7.9$ Hz), 7.76–7.80 (m, 2H), 7.81–7.87 (m, 1H), 8.02 (d, 1H, $J = 7.6$ Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.8, 30.9, 42.1, 47.7, 70.8, 76.6, 110.2, 116.8, 122.4, 125.3, 126.4, 126.5, 126.9, 130.5, 132.0, 132.6, 133.2, 134.8, 134.9, 143.7, 145.8, 152.8, 175.9, 181.5, 182.6; MS $m/z = 397$ $[MH]^+$; Anal. Calcd for $C_{25}H_{20}N_2O_3$: C, 75.74; H, 5.08; N, 7.07. Found: C, 75.62; H, 5.18; N, 7.15.

*5.1.2.7. 1′-Benzyl-1,2,3,11b-tetrahydrospiro[benzo[f]pyrrolo[2,1-a] isoindole-5,3′-indoline]-2′,6,11-trione (**14g**).* White solid; mp 241–242 °C. IR (cm^{-1}): 3413, 2934, 2356, 1708, 1609, 1474, 1335, 1123, 1000, 744, 480; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.71–1.92 (3H, m), 2.14–2.26 (1H, m), 2.48–2.53 (m, 1H), 2.54–2.63 (m, 1H), 4.77–4.84 (m, 1H), 4.92 (s, 2H), 6.88 (d, 1H, $J = 6.9$ Hz), 6.95 (t, 1H, $J = 7.6$), 7.20 (d, 1H, $J = 6.9$ Hz), 7.29–7.41 (m, 6H), 7.74–7.89 (m, 3H), 8.02 (d, 1H, $J = 6.9$ Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.8, 30.9, 43.3, 47.7, 70.9, 76.6, 110.3, 122.6, 125.4, 126.4, 126.5, 127.0, 127.4, 127.8, 129.1, 130.5, 132.7, 133.2, 134.8, 134.9, 136.6, 143.6, 145.7, 152.9, 176.4, 181.5, 182.6; MS $m/z = 447$ $[MH]^+$; Anal. Calcd for $C_{29}H_{22}N_2O_3$: C, 78.01; H, 4.97; N, 6.27. Found: C, 77.89; H, 4.87; N, 6.37.

*5.1.2.8. 5′-Chloro-1,2,3,11b-tetrahydrospiro[benzo[f]pyrrolo[2,1-a] isoindole-5,3′-indoline]-2′,6,11-trione (**14h**).* White solid; mp 253–254 °C; IR (cm^{-1}): 3367, 3199, 2954, 2869, 2360, 1737, 1647, 1462, 1282, 1184, 809, 721, 556; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.73–1.80 (m, 1H), 1.82–1.90 (m, 2H), 2.13–2.20 (m, 1H), 2.49–2.53 (m, 1H), 2.55–2.62 (m, 1H), 4.74 (t, 1H, $J = 6.9$ Hz), 6.89 (d, 1H, $J = 7.6$ Hz), 7.26–7.33 (m, 2H), 7.76–7.86 (m, 3H), 8.01 (d, 1H, $J = 6.8$ Hz), 10.60 (brs, 1H, -N*H*, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.8, 30.6, 47.9, 70.9, 76.9, 112.0, 125.9, 126.4, 127.3, 128.0, 128.8, 130.3, 132.7, 133.2, 134.7, 134.8, 142.2, 145.3, 152.8, 177.5, 181.6, 182.6; MS $m/z = 391$ $[MH]^+$; Anal. Calcd for $C_{22}H_{15}ClN_2O_3$: C, 67.61; H, 3.87; N, 7.17. Found: C, 67.71; H, 3.94; N, 7.09.

*5.1.3. Experimental procedure for the synthesis of spirooxindole derivative (**17**)*

A mixture of isatin **8a** (1 mmol), sarcosine **9** (1 mmol) and acenaphthenequinone **15** (1 mmol) was refluxed in ethanol (5 ml). Completion of the reaction was evidenced by TLC analysis. After completion of the reaction, the reaction mixture was poured into ice-water, the resulting solid was filtered off and purified by column chromatography using ethyl acetate: petroleum ether (3:7) as an eluent to afford pure spirooxindole (**17**).

*5.1.3.1. 2′-Methyl-2′,3′-dihydro-2H-spiro[acenaphthylene-1,1′-benzo [f]isoindole]-2,4′,9′-trione (**17**).* Brown solid; mp 274–275 °C; IR (cm^{-1}): 3757, 3023, 2957, 2743, 1708, 1684, 1593, 1456, 1267, 1156, 972, 778, 698, 581; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.27 (s, 3H), 4.29 (ABq, 2H, $J = 16.8$ Hz), 7.25–7.79 (m, 5H), 7.82 (t, 1H, $J = 6.8$ Hz), 7.99 (d, 1H, $J = 6.8$ Hz), 8.01–8.12 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.2, 58.8, 78.7, 123.6, 123.9, 126.1, 126.3, 128.4, 130.7, 131.9, 132.1, 133.4, 134.5, 135.3, 136.4, 138.3, 138.8, 140.1, 141.6, 146.7, 152.9, 181.0, 182.3, 194.4; MS $m/z = 366$ $[MH]^+$; Anal. Calcd for $C_{24}H_{15}NO_3$: C, 78.89; H, 4.14; N, 3.83. Found: C, 78.79; H, 4.27; N, 3.92.

*5.1.4. Experimental procedure for the synthesis of spirooxindole derivative (**18**)*

A mixture of isatin **8a** (1 mmol), sarcosine **9** (1 mmol) and ninhydrin **16** (1 mmol) was refluxed in ethanol (5 ml). Completion of the reaction was evidenced by TLC analysis. After completion of the reaction, the reaction mixture was poured into ice-water, the resulting solid was filtered off and purified by column chromatography using ethyl acetate: petroleum ether (3:7) as an eluent to afford pure spirooxindole (**18**).

*5.1.4.1. 2-Methyl-2,3-dihydrospiro[benzo[f]isoindole-1,2′-indene]-1′,3′,4,9-tetraone (**18**).* Brown solid; mp 260–261 °C; IR (cm^{-1}): 3766, 3023, 2943, 2743, 1711, 1656, 1573, 1464, 1237, 1156, 969, 778, 698, 618, 560, 469; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.29 (s, 3H), 3.89 (s, 1H), 4.28 (s, 2H), 7.22–7.78 (m, 2H), 7.83 (t, 1H, $J = 6.9$ Hz), 7.93 (d, 1H, $J = 6.7$ Hz), 8.01–8.12 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.7, 59.3, 79.8, 123.3, 124.1, 126.5, 126.8, 131.0, 131.8, 132.8, 134.9, 135.3, 137.3, 138.0, 141.3, 146.2, 152.3, 178.5, 181.0, 182.3, 198.4; MS $m/z = 344$ $[MH]^+$; Anal. Calcd for $C_{21}H_{13}NO_4$: C, 73.46; H, 3.82; N, 4.08. Found: C, 73.55; H, 3.71; N, 4.20.

5.2. Biological assays

5.2.1. Materials and methods for the antimicrobial activity

Streptomycin and ciprofloxacin (Sigma) were used as positive controls against bacteria. Fluconazole and ketoconazole (Himedia, Mumbai) were used as positive controls against fungi.

5.2.2. Tested microbes

The following gram positive bacteria were used for the experiments; *S. aureus* MTCC 96, *S. aureus* (MRSA), *E. aerogens* MTCC111, *M. luteus*. The Gram negative bacteria included: *P. vulgaris* MTCC 1771, *K. pneumonia* MTCC 109, *S. typhimurium* MTCC1251, *S. paratyphi-B*. Fungi *M. pachydermatis, C. albicans* MTCC 227 and *B. cinerea*. All cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

5.2.3. Preparation of inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37 °C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 10^4 CFU/ml. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28 °C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on sabouraud dextrose broth (SDB) at 28 °C for 48 h.

5.2.4. Disc diffusion assay

Antibacterial activity was carried out using a disc diffusion method [25]. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Himedia, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at 1000 μg/disc. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 μg/disc) was used as positive control. The plates were incubated for 24 h at 37 °C for bacteria and 48 h at 27 °C for fungi. Zone of inhibition were recorded in millimeters and the experiment was repeated twice.

5.2.5. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration studies of isolated compounds were performed according to the standard reference method for bacteria [26], for filamentous fungi [27] and yeasts [28]. The required concentrations (1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml and 15.62 μg/ml) of the compound was dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 μl from each well was inoculated. The anti-fungal agents ketoconazole, fluconazole for fungi and streptomycin, ciprofloxacin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48–72 h at 28 °C and for bacteria the plates were incubated for 24 h at 37 °C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 μl of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.02.024.

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