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One-pot multicomponent synthesis and anti-microbial evaluation of 2′-(indol-3-yl)-2-oxospiro(indoline-3,4′-pyran) derivatives

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ABSTRACT

A simple and efficient method for the one-pot three-component synthesis of new spirooxindoles in room temperature is described. The newly synthesized spirooxindoles were screened for anti-microbial activity and the results are good on comparison with of standard antibacterial compounds.

Functionalized nitrogen and oxygen containing heterocycles play a predominant role in medicinal chemistry and they have been intensively used as scaffolds for drug development. 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] Multicomponent reactions, such as the Biginelli,[2] Passerini,[3] Ugi[4] and Hantzsch, provide a wide variety of important heterocycles.[5] For example, the Hantzsch reaction provides dihydropyridines with activity against calcium channels, multidrug resistance (MDR) proteins, 5-hydroxytryptamine(5-HT) receptors and anti-inflammatory targets.[1b,6,7]

The spirooxindole framework[8] represents important structural organization present in a number of bioactive natural products such as coerulescine, horsfiline, welwitindolinone A, spirotryprostatin A, elacomine and alstonisine. Spirotryprostatin A,[9] a natural alkaloid isolated from the fermentation broth of *Aspergillus fumigatus*, has been identified as a novel inhibitor of microtubule assembly, and pteropodine and isopteropodine have been shown to modulate the function of muscarinic serotonin receptors[10] (Fig. 1).

The 3-substituted indole nucleus substructure is one of the most important heterocycles found in natural products, pharmaceuticals and important medicinal chemistry.[11,12] It is found as a scaffold in a number of biologically active compounds especially with anticancer, anti-tumour,[13] anti-inflammatory, hypoglycemic, analgesic and anti-pyretic activities.[14] Many indole alkaloids are recognized as one of the rapidly growing groups of marine invertebrate metabolites for their broad spectrum of biological properties.[15,16]

The wide-ranging biological activity associated with many spiroxindole and 3-substituted indole derivatives, both naturally occurring and synthetic, ensures that the synthesis of this important ring system remains a topic of current interest. Various

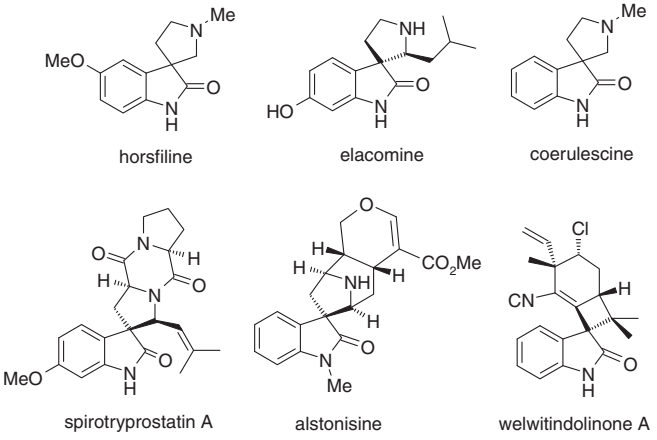

Figure 1. Representative structure of spiroxindole derivatives.

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Table 1
Screening of various bases in methanol

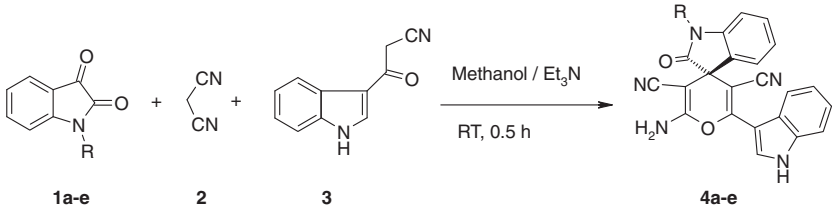
Entry	Bases	Yield[a,d] (%)
1	K_2CO_3	21[b]
2	NaOH	10[b]
3	Piperidine	69[c]
4	Triethylamine	89[c]
5	Diethylamine	74[c]

[a] Isolated yield.
[b] The reactions were carried out with 1.5 equivalents of bases.
[c] The reactions were carried out in 20 mol% of bases.
[d] All the reactions were carried out for 1.5 h at ambient temperature.

Table 2
Screening of various solvents for the formation substituted spiroxindole using Et_3N

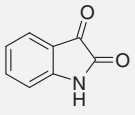
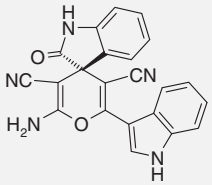
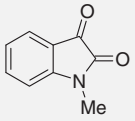
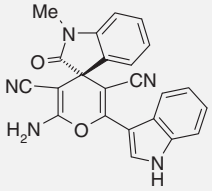
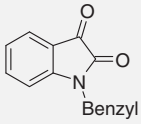
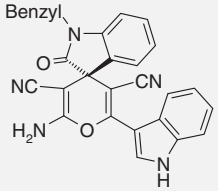
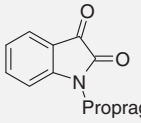
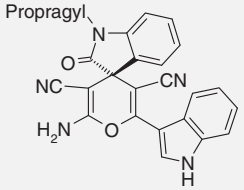
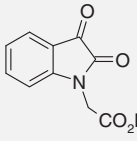
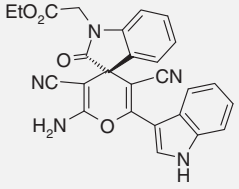
Entry	Solvent	Yield[a, b] (%)
1	DMF	69
2	Dichloroethane	48
3	Acetonitrile	71
4	Water	54
5	Ethanol	83
6	Methanol	89

[a] Isolated yield.
[b] All reactions were carried out with 20 mol% Et_3N for 0.5 h in methanol.

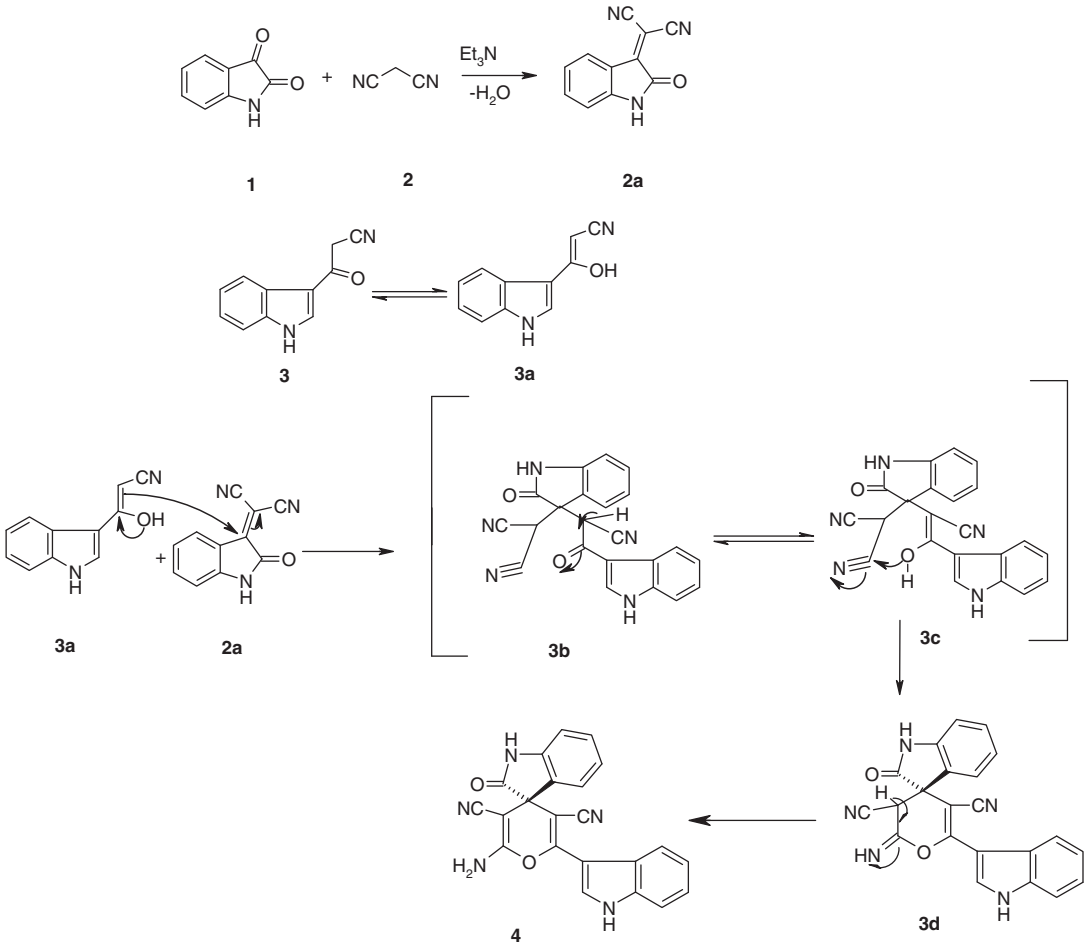


Scheme 1. Synthesis of spiroxindoles from isatin, malanonitrile and 3-cyanoacetyl indole.

Table 3
Synthesis of spiroxindoles from isatin, malanonitrile and 3-cyanoacetyl indole

Entry	Compound	Product[a]	Time (min)	Yield[b] (%)
1	 1a	 4a	30	88
2	 1b	 4b	30	89
3	 1c	 4c	45	85
4	 1d	 4d	40	83
5	 1e	 4e	60	85

[a] All the products characterized by NMR, IR and mass spectroscopy.
[b] Isolated yield.



Scheme 2. Plausible mechanism for the formation spiroxindole derivatives.

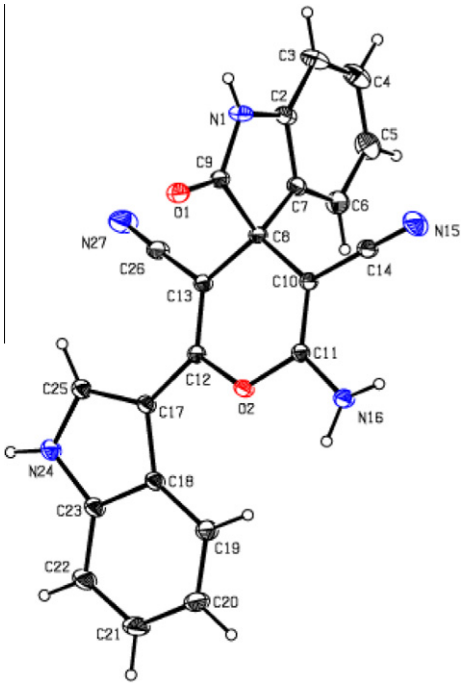
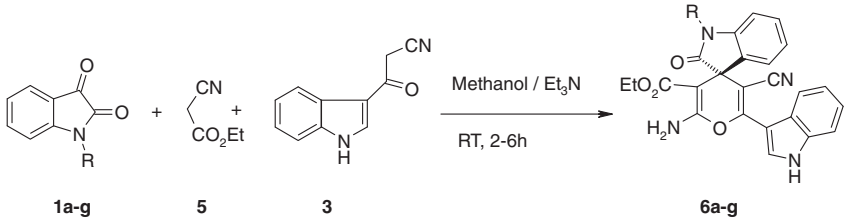


Figure 2. ORTEP diagram of compound **4a**.

methods for the preparation of these compounds have been reported. However these methods suffer from tedious synthetic routes, longer reaction time, drastic reaction conditions, as well as narrow substrate scope.[17–20] To the best of our knowledge, there have been no reports for the synthesis of indol-3-yl derivatives including spiroxindole moieties. As part of our ongoing research on the development of novel synthetic routes for the synthesis of biologically active heterocyclic compounds and use of green chemical techniques in organic synthesis,[21–23] herein, we report a simple and facile one pot procedure for the synthesis of indol-3-yl spirooxindole derivatives in methanol at ambient temperature.

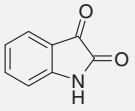
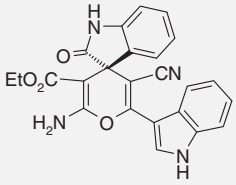
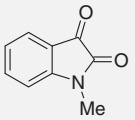
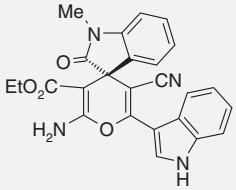
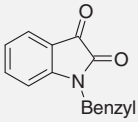
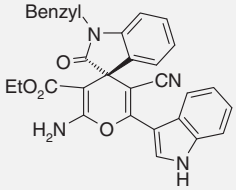
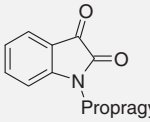
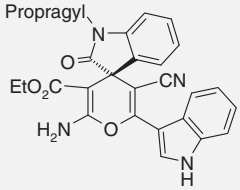
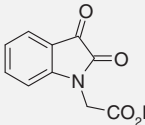
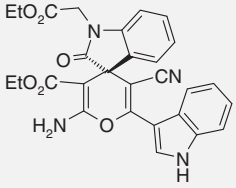
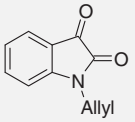
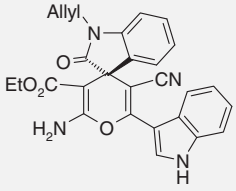
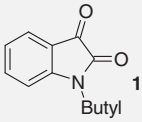
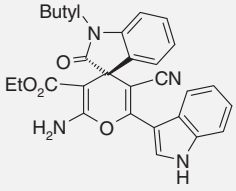
On continuation of earlier work,[24] we carried out a reaction of isatin (**1a**), malononitrile (**2**) and 3-cyanoacetyl indole (**3**) in the presence of in various bases and solvents at ambient temperature (Tables 1 and 2). Excellent results were obtained when triethyl amine was used as a base under ambient temperature condition in methanol with high yield of the product in a shorter reaction time. So we followed the reaction by stirring a mixture of isatin (**1a**), malononitrile (**2**), 3-cyanoacetyl indole (**3**) and triethyl amine (20 mol%) in methanol under ambient temperature condition (Scheme 1). Under these conditions, the reaction preceded smoothly with a wide range of functionalized isatins, including those containing benzyl, propagyl, ester and methyl groups (Table 3).[25]

Based on the above results, a plausible mechanism is proposed (Scheme 2). Initially, isatin **1** reacts with malononitrile **2** to give a corresponding isatin–malononitrile adduct **2a** and 3-cyanoacetyl indole **3** in the presence of base (Et_3N) enolise to give **3a**. **3a** further reacts with isatin–malononitrile adduct **2a** to give intermediates **3b** and **3c**. The intermediate **3c** further rearranges via proton transfer to give **3d**. Finally, the intermediate **3d** affords to yield spiroxindole derivative (**4**) via proton transfer.

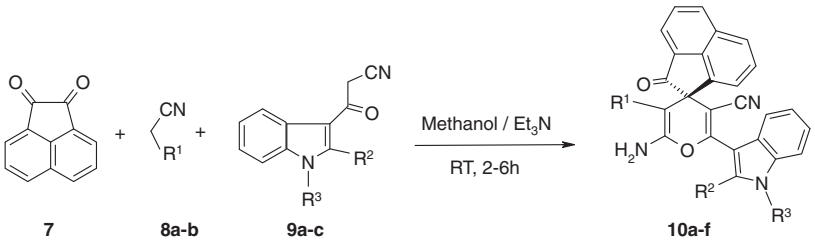


Scheme 3. Synthesis of spiroxindoles from isatin, ethylcyano acetate and 3-cyanoacetyl indole.

Table 4
Synthesis of spiroxindoles from isatin, ethylcyano acetate and 3-cyanoacetyl indole

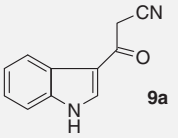
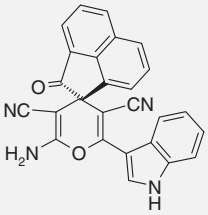
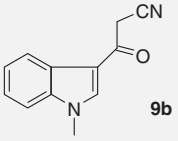
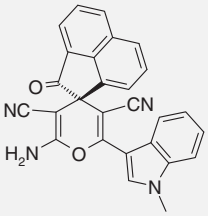
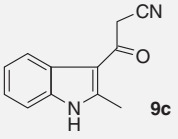
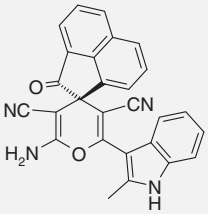
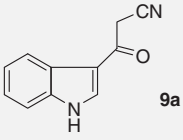
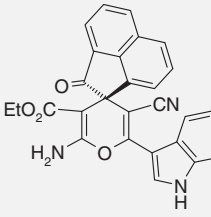
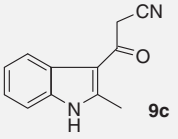
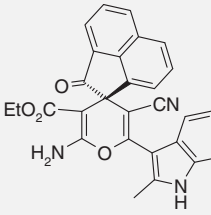
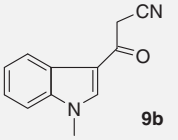
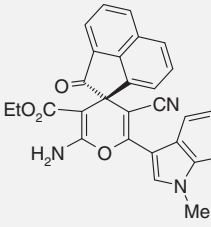
Entry	Compound	Product[a]	Time (min)	Yield[b] (%)
1	 **1a**	 **6a**	4.0	78
2	 **1b**	 **6b**	4.5	79
3	 **1c**	 **6c**	5.0	74
4	 **1d**	 **6d**	5.5	73
5	 **1e**	 **6e**	5.5	75
6	 **1f**	 **6f**	6.0	72
7	 **1g**	 **6g**	6.0	74

[a] All the products characterized by NMR, IR and mass spectroscopy.
[b] Isolated yield.



Scheme 4. Synthesis of spiroxindole derivatives from acenapthenequinone precursor.

Table 5
Synthesis of spiroxindole derivatives from acenapthenequinone precursor

Entry	8	9	Product[a]	Time (h)	Yield[b] (%)
1	 **8a**	 **9a**	 **10a**	1.5	76
2	 **8a**	 **9b**	 **10b**	1.5	75
3	 **8a**	 **9c**	 **10c**	1.5	71
4	 **8b**	 **9a**	 **10d**	4.5	77
5	 **8b**	 **9c**	 **10e**	4.0	78
6	 **8b**	 **9b**	 **10f**	4.5	76

[a] All the products characterized by NMR, IR and mass spectroscopy.
[b] Isolated yield.

The structure of products **4a–e** were confirmed by spectral studies and elemental analysis as exemplified for compound **4a** as follows (Table 3, entry 1): the IR spectrum of **4a** showed absorptions at 3343, 2205 and 1707 cm^{-1} indicating the presence of $–NH_2$, cyano and carbonyl functionalities, respectively. In the ^1H NMR spectrum, aromatic signals were seen at δ 6.91–8.15, a broad singlet at δ 7.60, 10.76 and 12.05 showed the presence of $–NH_2$ and –NH groups (D_2O exchangeable). The carbonyl carbon resonated at δ 177.7 in the ^{13}C NMR spectrum. Mass spectral analysis also supported the structural assignment (*m/z* 380.07 $[M+H]^+$). Finally, the structure of **4a** was confirmed unambiguously by single crystal X-ray analysis[28] (Fig. 2).

Based on the above results, we extended our protocol to the synthesis spiroxindole derivatives using various substituted isatin, 3-cyanoacetyl indole and cyanoethylacetate under optimized conditions (Scheme 3). The reaction proceeded very smoothly and gave high yield of the product in short reaction times without the formation of any side products. The substrate scope of the reaction under the optimized conditions was investigated, and the reaction was found to be amenable to a variety of substituents on isatin (Table 4).[26]

The structures of the compounds **6a–g** were investigated with spectral studies and elemental analysis as demonstrated for compound **6b** as follows (Table 4, entry 2): in the IR spectrum, the C≡N stretching frequency appeared at 2203 and 2369 cm^{-1}, respectively, and the stretching frequency at 3251 and 3370 correspond to –NH and $–NH_2$ functional groups, respectively. In the ^1H NMR spectrum of aromatic protons resonated in the range of δ: 7.01–8.12. A broad singlet in the region δ: 12.00 signal confirmed the presence of –NH proton. A characteristic peak at δ: 94.5 corresponding to cyano group attached carbon in ^{13}C NMR spectrum and the aromatic carbons appeared in the region δ: 114.1–161.1. A methoxy carbon characteristic peak appeared at δ: 54.8. A distinguishing peak was observed at *m/z*: 322.27 in the mass spectrum for $[M+H]^+$ion.

To further explore the potential of this protocol for heterocyclic synthesis, we investigated one-pot reactions involving acenaphthenequinone, 3-cyanoacetyl indole and malononitrile or cyanoethyl acetate under optimized condition gave good yield of spirooxindole derivatives (Scheme 4). The reaction proceeded smoothly with a wide range of functionalized substituted 3-cyanoacetyl indoles and malononitrile or cyanoethyl acetate (Table 5).[27]

The structure of compounds (**10a–f**) were established based on detailed spectroscopic studies and elemental analysis as exemplified for compound **10c** as follows (Table 5, entry 3): in the IR spectrum of compound (**10c**) stretching frequencies at 3446, 3276 and 2364 cm^{-1} confirm the presence $–NH_2$, –NH and C≡N functional groups. The ^1H NMR spectrum showed chemical shift of δ: 12.04 (br s, D_2O exchangeable) which corresponds to NH protons. The aromatic protons resonated the region of δ: 7.04–9.13. The sharp singlet appeared in the region δ: 15.5 correspond to CH_3 carbon. The aromatic carbons appeared in the region of δ: 101.2–162.8. The mass spectrum displayed the molecular ion $[M+H]^+$ peak at *m/z* 429.00.

All the synthesized compounds were screened for their in vitro anti-microbial (antibacterial and antifungal) activities against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger* by disc diffusion method (CUP Plate Method).

The preliminary studies were carried out for total aerobic microbial count by using plate count method for four microorganisms. The microorganism's suspensions at concentration of 10^{-4} CFU/ml were incubated to the corresponding plates with the aerobic microgram 500 counts. The plates were incubated at 36 °C for 24–48 h in the incubation chamber kept sufficiently humid. At the end of the incubation period the zone of inhibition was tested. Out of 17 compounds, four compounds were taken for reference for preliminary screening of microorganisms. The four compounds namely **4a**, **4b**, **4d** and **4e** were screened with 12 microorganisms in various concentrations of the compounds namely 25, 50, 75, 100 and 150 μg/ml, respectively. The samples were prepared in DMSO solution at a concentration of 1000 μg/ml.

From the stock solution, various concentration of test solutions (25, 50, 75, 100 and 150 μg/ml) were prepared by serial dilution. It was found that the four compounds were effective against four microorganisms namely *S. epidermidis*, *S. aureus*, *B. subtilis* and *A. niger* in 100 μg/ml concentration.

Hence we screened anti-microbial activity of all 18 compounds with concentration of 100 μg/ml against the four microorganisms and the results are summarized in Table 6. The diameter of zone of inhibition was measured in millimetre. All the compounds showed good (**4a**, **4b**, **4c**, **4e**, **10a** and **10b**) and moderate inhibition against *S. epidermidis*, *S. aureus*, *B. subtilis* and *A. niger* on comparison with standards.

In conclusion, we have developed a simple and efficient one-pot synthesis for the formation of substituted spiroxindole derivatives. A privileged medicinal scaffold synthesized through three-component reactions of structurally diverse isatin with 3-cyanoacetyl indole and malononitrile (or) cyanoethyl acetate. Spiroxindole derivatives showed a good antifungal and antibacterial activity. Further studies to delineate the scope and limitations of the present methodology are underway.

Table 6
Anti-microbial activity of compounds **4a–10f**[a]

S. No	Product and reference drug	Zone of inhibition in diameter (mm)			
		Staphylococcus aureus	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Aspergillus niger*
1.	**4a**	**29**	**30**	**28**	**28**
2.	**4b**	**27**	**28**	**26**	**27**
3.	**4c**	**29**	**31**	**30**	**24**
4.	**4d**	18	19	20	25
5.	**4e**	24	25	26	25
6.	**6a**	18	18	19	23
7.	**6b**	18	14	13	23
8.	**6c**	16	15	14	24
9.	**6d**	16	16	17	25
10.	**6e**	14	10	12	21
11.	**6f**	18	13	14	21
12.	**6g**	16	14	17	27
13.	**10a**	**38**	**39**	**40**	**29**
14.	**10b**	**39**	**38**	**36**	**28**
15.	**10c**	20	16	19	26
16.	**10d**	20	18	17	27
17.	**10e**	20	16	18	26
18.	**10f**	19	18	18	22
19.	Gentamicin sulphate	26	28	30	28
20.	Chloramphenicol	29	31	32	25
21.	Nysatin	NA	NA	NA	28

These bold font shows that these compounds are more active than the standard drugs. NA, not applicable.

[a] No zone of inhibition was obtained, when blank experiment was performed with the same concentration of DMSO.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.025.

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25. Typical experimental procedure for **4b**: To a stirred solution of *N*-methyl isatin (0.147 g, 1 mmol), malononitrile or ethylcyano acetate (0.066 g, 1 mmol) and 3-cyanoacetyl indole (0.184 g, 1 mmol) in methanol (10 ml), triethyl amine (20 mol%) was added and stirring was continued for 30 min. On completion, the reaction mixture was poured into crushed ice and the precipitate formed was filtered, dried and purified by column chromatography to afford the pure product. The isolated product was further purified by recrystallisation in ethanol and the appropriate yield of the product is 89%. *2′-Amino-6′-(1H-indol-3-yl)-1-methyl-2-oxospiro[indoline-3,4′-pyran]-3′,5′-dicarbonitrile* (Table 3, entry 2) white solid mp 205–208 °C; R_f 0.27 (40% AcOEt/petroleum ether); IR (KBr): 1152, 1250, 1356, 1416, 1471, 1526, 1617, 1666, 2202, 2368, 2929, 3171, 3360 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.19 (s, 3H, N–CH_3), 7.12–7.18 (m, 3H, Ar-*H*), 7.23 (t, *J* = 6.85 Hz, 1H, Ar-*H*), 7.38–7.42 (m, 2H, Ar-*H*), 7.49 (d, *J* = 8.4 Hz, 1H, Ar-*H*), 7.65 (s, 2H, –NH_2), 7.96 (d, *J* = 8.45 Hz, 1H, Ar-*H*), 8.15 (d, *J* = 3.05 Hz, 1H, Ar-*H*), 12.06 (br s, 1H, –N*H*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.1, 50.1, 54.4, 81.4, 105.5, 109.7, 113.0, 117.5, 117.8, 121.8, 122.1, 123.4, 124.2, 124.9, 125.4, 130.6, 131.5, 136.5, 143.5, 158.5, 160.3, 176.0; MS (EI): *m*/*z* 394.00 [M^++H^+]; Anal. Calcd for $C_{23}H_{15}N_5O_2$: C, 70.22; H, 3.84; N, 17.90. Found: C, 70.31; H, 3.85; N, 17.92.
26. Typical experimental procedure for **6b**: A reaction mixture of 1-methyl isatin (1 mmol), ethylcyano acetate (0.066 g, 1 mmol) and 3-cyanoacetyl indole (0.184 g, 1 mmol) in methanol (10 ml), triethyl amine (20 mol%) was added and stirred for 4.5 h. On completion, the reaction mixture was poured into crushed ice and the precipitate formed was filtered, dried and purified by column chromatography to afford the pure product. The isolated product was further purified by recrystallisation in ethanol and the appropriate yield of the product is 79%. *Ethyl 2′-amino-5′-cyano-6′-(1H-indol-3-yl)-1-methyl-2-oxospiro[indoline-3,4′-pyran]-3′-carboxylate* (Table 4, entry 2) white solid; mp 232–235 °C; R_f 0.25 (40% AcOEt/petroleum ether); IR (KBr): 1020, 1153, 1297, 1352, 1439, 1509, 1623, 1693, 2203, 2369, 2929, 3251, 3370 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 0.66 (t, *J* = 6.9 Hz, 3H, –CH_3), 3.14–3.17 (m, 3H, N–CH_3), 3.65–3.69 (m, 2H, –CH_2 –), 7.01–7.03 (m, 2H, Ar-*H*), 7.16–7.31 (m, 4H, Ar-*H*), 7.49 (d, *J* = 8.4 Hz, 1H, Ar-*H*), 7.97–8.12 (m, 4H, –NH_2, Ar-*H*), 12.00 (br s, 1H, –N*H*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 14.0, 26.9, 51.3, 59.5, 73.1, 84.1, 105.5, 108.7, 112.9, 117.6, 121.6, 122.8, 123.3, 123.8, 124.0, 129.3, 130.1, 134.6, 134.9, 136.5, 144.0, 156.7, 160.0, 167.4,177.7; MS (EI): *m*/*z* 441.13 [M^++H^+]; Anal. Calcd for $C_{25}H_{20}N_4O_4$: C, 68.17; H, 4.58; N, 12.72. Found: C, 68.23; H, 4.58; N, 12.74.
27. Typical experimental procedure for **10c**: A reaction mixture of acenapthenequinone (1 mmol), ethylcyano acetate (0.066 g, 1 mmol) and 2-methyl 3-cyanoacetyl indole (0.184 g, 1 mmol) in methanol (10 ml), triethyl amine (20 mol%) was added and stirred for 4.5 h. On completion, the reaction mixture was poured into crushed ice and the precipitate formed was filtered, dried and purified by column chromatography to afford the pure product. The isolated product was further purified by recrystallisation in ethanol and the appropriate yield of the product is 71%. *2′-Amino-6′-(2-methyl-1H-indol-3-yl)-2-oxo-2H spiro[acenaphthylene-1,4′-pyran]-3′,5′-dicarbonitrile* (Table 5, entry 3) blue solid; mp 165–168 °C; R_f 0.48 (40% AcOEt/petroleum ether); IR (KBr): 1229, 1302, 1457, 1540, 1578, 1621, 1708, 2199, 2364, 3011, 3276, 3446 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.64 (s, 3H, –CH_3), 4.47 (br s, 2H, NH_2), 7.04–7.13 (m, 3H, Ar-*H*), 7.34 (s, 1H, Ar-*H*), 7.51 (s, 1H, Ar-*H*), 7.68 (s, 1H, Ar-*H*), 7.91 (s, 1H, Ar-*H*), 8.43 (s, 1H, Ar-*H*), 8.84 (s, 1H, Ar-*H*), 9.13 (s, 1H, Ar-*H*), 12.04 (br s, 1H, –N*H*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 15.5, 57.3, 77.7, 88.5, 101.2, 112.0, 114.6, 116.8, 117.6, 119.4, 120.9, 122.1, 122.4, 122.8, 126.2, 126.9, 128.9, 131.0, 131.8, 132.2, 134.0, 135.2, 146.4, 153.0, 162.8, 176.1, 183.4; MS (EI): *m*/*z* 429.00 [M^++H^+]; Anal. Calcd for $C_{27}H_{16}N_4O_2$: C, 75.69; H, 3.76; N 13.08. Found: C, 75.73; H, 3.75; N, 13.09.
28. Crystallographic data of compound **4a** in this paper have been deposited with the Cambridge Crystallographic Data centre as supplemental Publication No. CCDC-772945. Copies of the data can be obtained, free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 01223 336033 or email: deposit@ccdc.cam.ac.uk).