Engaging thieno[2,3-b]indole-2,3-dione for the efficient synthesis of spiro[indoline-3,4'-thiopyrano[2,3-b]indole] by reaction with *N*-substituted isatilidenes

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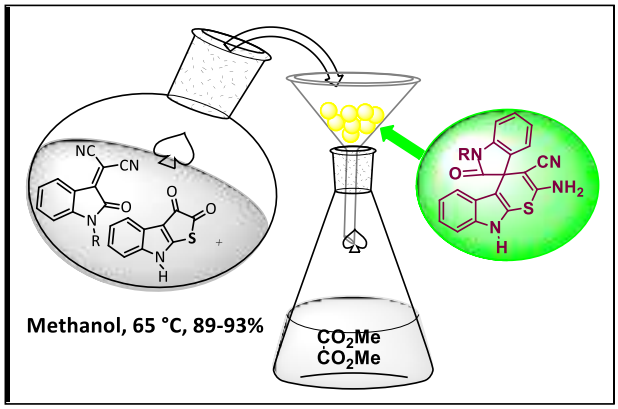
Abstract

A simple and efficient method, proceeding through a new mechanistic pathway, for the synthesis of spiro[indoline-3,4-thiopyrano[2.3-b]indole derivatives have been developed by exploiting the reaction of thieno[2,3-b]indole-2,3-dione with N-substituted isatilidenes. The compounds synthesized have been screened for antibacterial activity. The generality of the reaction and mechanistic rationale are presented.



Keywords: Thieno[2,3-b]indole-2,3-dione, isatilidene, thiopyran, Michael addition, elimination

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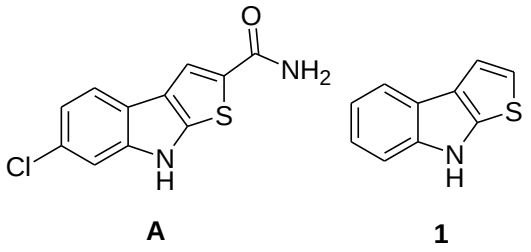
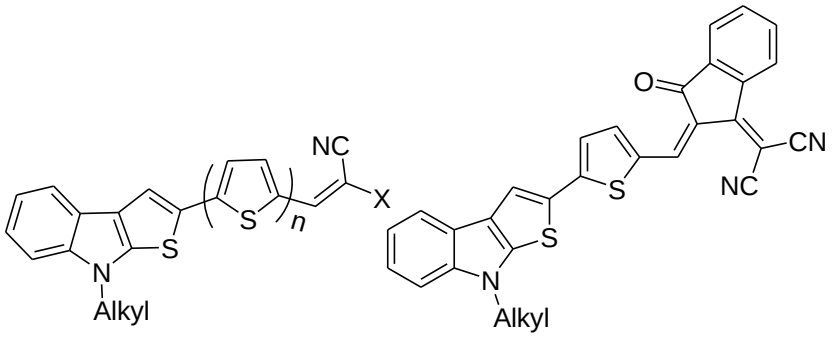
Introduction

The presence of thieno[2,3-b]indole moiety **1** in thienodolin **A**, (Figure 1)[1] an alkaloid with plant growth regulating properties, and the biological activities associated with **1** has triggered scientific interest in this molecule over the years.[2,3] Thienoindole derivatives are known to exhibit various biological properties such as anti-tubercular, anti-inflammatory, acetylcholine esterase inhibitory and CNS disorder inhibition.[4-6] In addition, very recent studies have shown that thienoindoles can be used to prepare antibody-drug conjugates (ADC) which can act as anticancer agents by specifically recognizing ALK protein which appears on the surface of nueroblastoma cells.[7] Principally, indole derivatives are the most widely distributed heterocycles in nature having numerous applications in areas of pharmaceuticals and agrochemicals.[8-10] Apart from the biological interests associated with indoles and thienoindoles, studies have also proved that the latter are suitable for the construction of organic photovoltaic devices as well as they represent an electron-rich heteroaromatic moiety which can be used for the construction of D-π-A type systems (Figure 2).[11]



A **1**

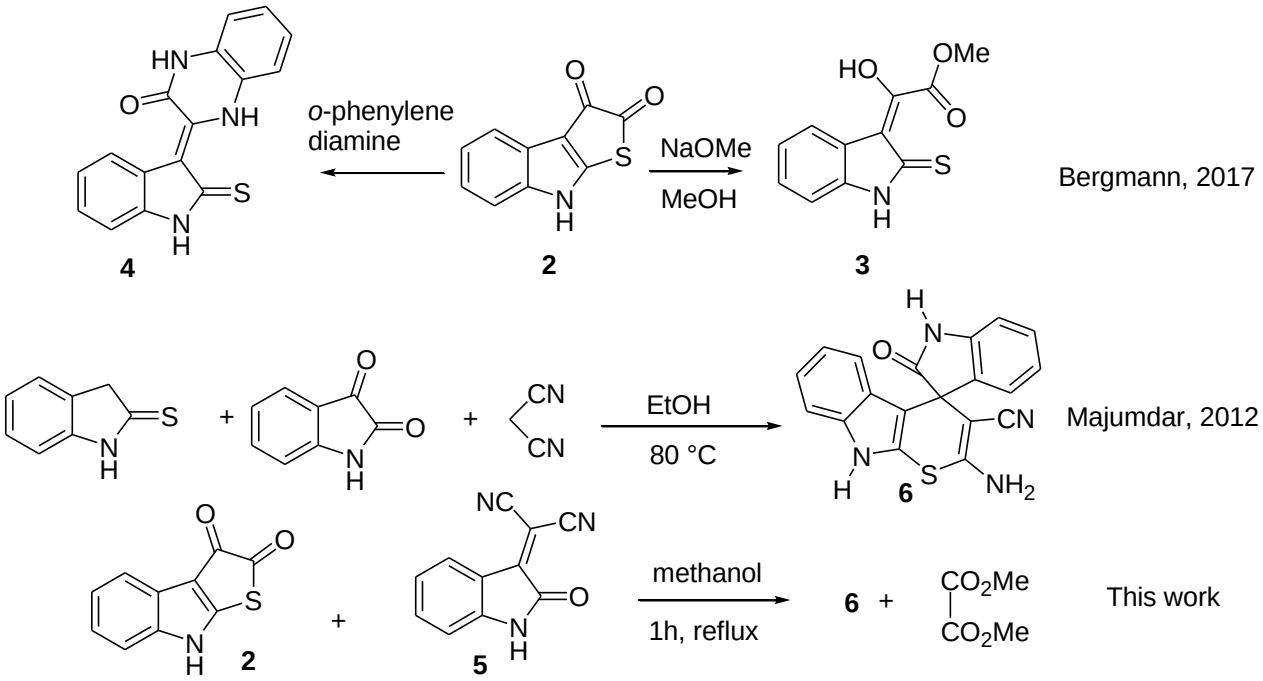
Figure 1. Thienodolin and thieno[2,3-b]indole



Alkyl = Ethyl, 2-ethyl hexyl
X = CN, CO_2R, CO_2H
n = 0, 1, 2, 3

Figure 2. Representative examples for D-π-A systems using thieno[2,3-b]indole

Bergman and Berg have together reported the synthesis of thieno[2,3-b]indole-2,3-dione **2** and found that it undergoes ring expansion on treatment with diazomethane.[12,13] The susceptibility of **2** towards ring opening was also evident when it yielded esterthione **3** on reaction with sodium methoxide in methanol and compound **4** on reaction with *o*-phenylene diamine. In the light of this report and due to our continued interest in the synthesis of spirofused compounds,[14] we decided to explore the reactivity of thieno[2,3-b]indole-2,3-dione towards electrophilic systems like acetylenic esters and isatilidenes. The results of our investigations with isatilidenes is the subject matter of this paper. Coincidentally, the product that we obtained from our reaction was same as that obtained by Majumdar *et.al*. through the one-pot reaction of indole-2-thione, isatin and malononitrile (Scheme 1).[15,16] Although the product was reported, what enthralled us was the unexpected pathway of the reaction resulting in the thiopyran ring formation by loss of two carbon atoms, probably by involvement of the solvent.



Scheme 1. Comparison of reported work and present work

Results and Discussion

As an initial experiment, **2** was treated with N-methyl isatilidene **5b** in methanol at room temperature. The progress of the reaction was monitored using TLC and the reaction was found

to be complete in 12 hours. Addition of a few drops of water to the reaction mixture resulted in the precipitation of a compound **6b**. The reaction conditions were then optimized and it was observed that in solvents like THF, acetonitrile, dichloromethane and toluene, the reaction did not yield any product at all while very good yields of the product was obtained in methanol/ethanol under reflux conditions in 1 hour. It was observed that one pot reaction of thieno[2,3-b]indole-2,3-dione, malononitrile and N-methyl isatin at 80 °C resulted in lower yield for the product, again indicating that the reaction proceeded *via* another pathway in our case, yielding the same product. The generality of the reaction was then tested using various N-alkyl isatins and cyanoacrylates (Table 1). N-methyl-thieno[2,3-b]indole-2,3-dione was also found to react similarly yielding **6j-l**.

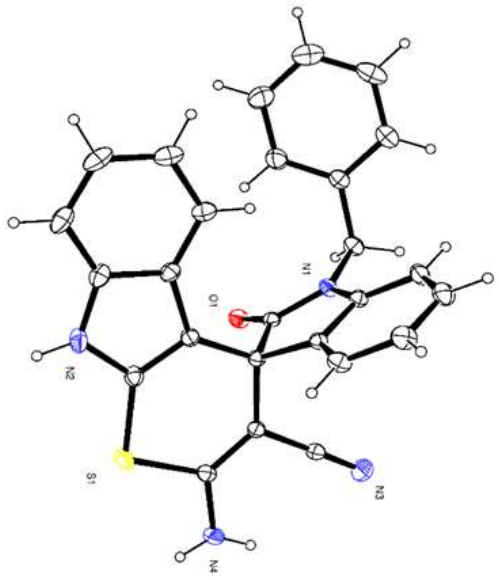
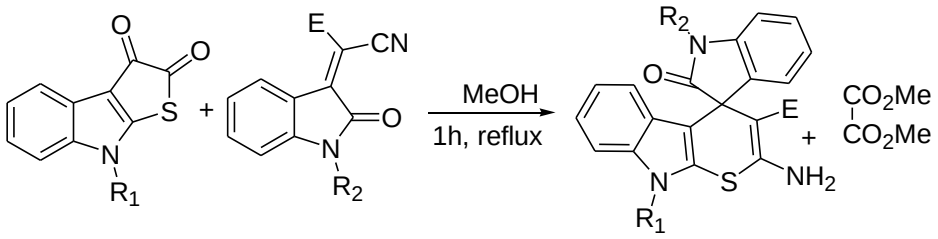


Figure 3. ORTEP diagram of **6g**

In the IR spectrum of **6b**, the ketone carbonyl stretching was seen at 1637 cm^{-1} while nitrile stretch was observed at 2186 cm^{-1} and peak at 3170 cm^{-1} corresponded to the NH stretch. In the ^{1}H NMR spectrum, the singlets at δ 11.6 and δ 3.27 ppm were attributed to the NH and NCH_3 protons respectively. In the ^{13}C NMR spectrum, signals at δ 176.7 and δ 71.7 ppm corresponded to the amide carbonyl and carbon atom bonded to the nitrile group respectively. The quarternary carbon atom was seen as a peak at 51.5 ppm. In the high resolution mass spectrum (ESIMS) the [M+1] peak was observed at m/z 359.0977. The structure was finally confirmed based on single crystal X-ray analysis (CCDC 2020515, Figure 3).

Table 1. Generality of the reaction



2 **5a-l** **6a-l**

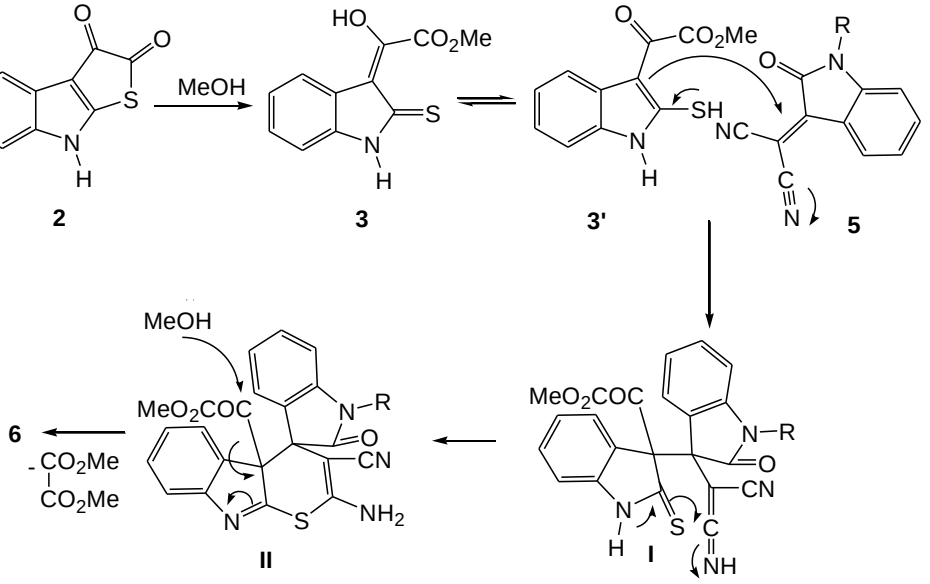
R_1 = H, Me

Entry	Product[a]	R_1	R_2	E	Yield[b] (%)
1	**6a**	H	H	CN	93
2	**6b**	H	Me	CN	91
3	**6c**	H	Et	CN	90
4	**6d**	H	Pr	CN	92
5	**6e**	H	Bu	CN	93
6	**6f**	H	Allyl	CN	89
7	**6g**	H	CH_2Ph	CN	91
8	**6h**	H	H	COOEt	89
9	**6i**	H	CH_2Ph	COOEt	92
10	**6j**	Me	Pr	CN	91
11	**6k**	Me	H	CN	93
12	**6l**	Me	CH_2Ph	CN	92

[a]All reactions were done by refluxing **2** (0.15 mmol) and **5** (0.15 mmol)

in MeOH (4 ml) for 1 h. [b]Isolated yield.

The unusual mechanism of the reaction can be explained along the following lines. Under reflux condition (or at room temperature for longer time) thieno[2,3-b]indole-2,3-dione **2** ring opens to form the enol intermediate **3**.[12] A blank experiment done by stirring **2** in methanol under the same conditions also yielded **3** and its structure was confirmed by FTIR, ^{1}H and ^{13}C NMR analysis (supporting information). The enol **3** can co-exist with its thiol tautomer **3'** which undergoes Michael addition with isatilidene producing thione **I**. The latter probably cyclizes to form the imine **II** which tautomerizes to the enamine **6** *via* elimination of a molecule of dimethyl oxalate in presence of methanol. In a separate experiment, the reaction of enol **3** with isatilidene under the same conditions also furnished the product **6** indicating that the reaction occurs through **3** (Scheme 2). The formation of the by-product dimethyl oxalate (or diethyl oxalate in case the reaction is done with ethanol) was confirmed by titrating the mother liquor, obtained after filtering the product **6** with aqueous $KMnO_4$ which indicated stoichiometric formation of dimethyl oxalate (mother liquor was hydrolysed prior to the titration). Further from the LCMS analysis of the crude reaction mixture, a peak at m/z 118.250 indicated the presence of the anion formed from dimethyl oxalate.[17] The reaction proceeded with slightly lower yields in ethanol probably because the ring opening of **2** in ethanol is not as feasible as it is in methanol.



Scheme 2. Mechanistic rationale

Contrastingly, in the one pot synthesis of spirooxindole annulated thiopyran derivatives reported by Majumdar *et al*., the enol form of indole-2-thione adds in a Michael fashion to the isatilidene formed by the Knoevenagel condensation of N-substituted isatin and ethyl cyanoacetate/malononitrile leading to the intermediate (similar to the proposed thione intermediate in our mechanism) which then undergo intramolecular S-acylation to give the product.[15] Whereas the current reaction is initiated through ring opening by the attack of methanol (a weak nucleophile under reflux conditions) and the product is formed by elimination of dimethyl oxalate.

Table 2 MIC (µg/mL) against ESKAPE pathogen panel

		MIC (µg/mL)				
S.No	Compound	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*K. pneumoniae* BAA 1705	*A. baumannii* BAA 1605	*P. aeruginosa* ATCC 27853
1	6a	>64	>64	>64	>64	>64
2	6b	>64	32	>64	>64	>64
3	6c	>64	8	>64	>64	>64
4	6d	>64	8	>64	>64	>64
5	6e	>64	32	>64	>64	>64
6	6f	>64	8	>64	>64	>64
7	6g	>64	8	>64	>64	>64
8	6h	>64	32	>64	>64	>64
9	6i	>64	16	>64	>64	>64
10	Levo floxacin	0.0156	0.125	64	8	1

The antibacterial activity of the synthesized compounds **6a-i** were screened using microbroth dilution assay at CSIR-Central Drug Research Institute, Lucknow, India and the results are summarized in Table 2. It can be seen that compounds **6c**, **6d**, **6e** and **6f** showed increased activity against *S. aureus* ATCC 29213 with an MIC value of 8 µg/mL. To determine the selectivity Index, **6c, 6d, 6e and 6f** were tested to determine their cytotoxicity against Vero cells. Upon analysis, it was determined that the compounds were cytotoxic at MIC levels, thus precluding further experiments.

Conclusions

In conclusion, we have described a new route for the catalyst-free synthesis of spiro[indoline-3,4'-thiopyrano[2,3-b]indole] in methanol/ethanol and have evaluated their antibacterial property. It was seen that the compounds **6c**, **6d**, **6e** and **6f** showed increased activity against *S. aureus* ATCC 29213 with lower MIC values but were found to be cytotoxic. It has been observed that thieno[2,3-b]indole-2,3-dione is a promising molecule for discovering new reactions and its reactivity towards other electrophilic systems like dimethyl acetylene dicarboxylate and transformations of the products thus obtained are underway in our laboratory.

Experimental Section

NMR spectra were recorded on a 400 MHz Bruker Avance FTNMR spectrometer. Chemical shifts are reported relative to TMS as the internal standard. IR spectra were recorded on an Agilent Cary 630 FTIR spectrometer. The oxindole, isatin, malononitrile and Lawesson's reagent were purchased from Spectrochem Pvt. Ltd and were used as such without further purification. Thieno[2,3-b]indole-2,3-dione was synthesized following the literature.[12] Commercial grade solvents were used. Analytical thin layer chromatography was performed on silica gel coated on aluminium sheets which was visualized using UV light of 254 nm.

General procedure

Procedure for the synthesis of thieno[2,3-b]indole-2,3-dione: Lawesson's reagent (1.74 g, 10 mmol) was taken in a 250 ml RB flask and dissolved in 75 ml dry THF. Oxindole (1.5 g, 11.2 mmol) was added to it and stirred for 12 h at room temperature. The reaction mixture was then dried on rotary evaporator, dissolved in minimum amount of acetonitrile and kept for stirring.

Dropwise addition of oxalyl chloride (1.27 g, 10 mmol) followed by 5 minutes stirring and filtration yields the required thieno[2,3-b]indole-2,3-dione.[12]

Compound 3 (E)-methyl 2-hydroxy-2-(2-thioxoindolin-3-ylidene)acetate:

Synthetic procedure: In a 50 ml dried RB flask, thieno[2,3–b]indole-2,3-dione 50 mg (0.25 mmol) was stirred with 4 ml methanol under room temperature for 4 hours. After the reaction was complete, (monitored by TLC) water was added to the reaction mixture. The yellow precipitate formed was filtered dried and weighed (58 mg, 98%) and was subjected to spectral characterization.

Characterization: Mp 190-191°C: FTIR (cm^{-1}): 3360, 2917, 2186, 1732, 1635, 1624, 1464, 1426. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.90 (s, 3H, -OMe), 7.26-7.33 (m, 2H, ArH), 7.52 (m, 1H, ArH), 7.77 (m, 1H, ArH), 12.80 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ, ppm 53.2, 112.7, 113.1, 119.7, 123.7, 124.6, 126.3, 137.7, 143.2, 166.0, 180.0. Anal. Calculated (%) for $C_{11}H_9NO_3S$: C, 56.16; H, 3.89; N, 5.95; S, 13.63 Found: C, 56.13; H, 3.84; N, 5.97. S, 13.65

Representative procedure for the synthesis of spiro[indoline-3,4'-thiopyrano[2,3-b]indole

Synthesis of 6b: In a 50 ml RB flask, thieno[2,3–b]indole-2,3-dione 30 mg (0.15 mmol) was refluxed with N-methyl isatilidene 32 mg (0.15 mmol) in methanol (10 ml) for 1 h. After completion of the reaction as indicated by TLC, solvent was removed and the residue was collected and washed with minimum amount of methanol-water (1:1) mixture and was filtered. The precipitate thus collected was thoroughly dried and weighed (48 mg) and was subjected to spectral characterization. The mother liquor containing dimethyl oxalate was hydrolysed by adding H_2SO_4 and refluxing for 2h. The concentration of oxalic acid thus obtained was estimated by permanganometric titration with 0.01N $KMNO_4$ solution. Estimated normality of the oxalic acid from the titration was 0.019 N and the weight of dimethyl oxalate in whole of the filtrate was calculated to be 16 mg. Thus, it was found that for every molecule of **6b** formed, I molecule of dimethyl oxalate was generated.

Same procedure was followed for the synthesis of all other derivatives.

Compound 6a 2'-amino-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (47 mg, 93%); mp 266-267 °C (reported melting point of this compound in literature is > 230 °C)[15]; FTIR (cm^{-1}): 3405, 3303, 3167, 2187, 1692, 1620, 1564, 1471, 1438. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 6.37 (d, 1H, J 8.0 Hz, ArH), 6.75 (t, 1H, J 7.6 Hz, ArH), 6,92-701 (m, 4H, ArH), 7.05 (s, 2H, -NH_2), 7.28 (t, 2H, J 8.0 Hz, ArH), 10.71 (s, 1H, -NH), 11.63 (s, 1H, -NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 51.9, 72.1, 103.7, 110.1, 111.4, 117.0, 118.1, 120.0, 121.7, 122.9, 123.0, 124.7, 125.3, 129.4, 134.2, 136.8, 142.0, 153.5, 178.3. Anal. Calculated (%) for $C_{19}H_{12}N_4OS$, C, 66.26; H, 3.51; N, 16.27; S, 9.31; Found: C, 66.22; H, 3.55; N, 16.28, S, 9.34

Compound 6b 2'-amino-1-methyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (48 mg, 91%); mp 229-231 °C; FTIR (cm^{-1}): 3408, 3322, 3170, 2186, 1689, 1637, 1562, 1470, 1432. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.27 (3H, s, N-CH_3), 6.20 (d, 1H, J 8.0 Hz ArH), 6.72 (t, 1H, J 8.0 Hz, ArH), 6.95-7.07 (m, 3H, ArH), 7.10 (s, 2H, -NH_2), 7.23 (d, 1H, J 8.0 Hz, ArH), 7.28 (d, 1H, J 8.0 Hz, ArH), 7.36-7.40 (m, 1H, ArH), 11.6 (s, N-H). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 51.5, 71.7, 103.5, 109.2, 119.2, 120.5, 120.8, 121.8, 122.7, 123.1, 123.6, 124.4, 124.5, 125.0, 129.5, 133.4, 136.6, 136.8, 153.7, 176.7. HRMS: MS Calculated: *m/z* value for [M+1]: 359.0977, observed value for [M+1]: 359.0977. Anal. Calculated (%) for $C_{20}H_{14}N_4OS$: C, 67.02; H, 3.94; N, 15.63; S, 8.95. Found: C, 67.03; H, 3.91; N, 15.59; S, 8.99

Compound 6c 2'-amino-1-ethyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (48 mg, 89%); mp 239-241 °C; FTIR (cm^{-1}): 3389, 3279, 3170, 2186, 1687, 1637, 1624, 1564, 1471, 1436. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.23 (t, 3H, J 7.2 Hz, -$CH_2\underline{CH_3}$), 3.83 (q, 2H, J 7.2 Hz, N-$\underline{CH_2}CH_3$), 6.26 (d, 1H, J 8.0 Hz, Ar-H), 6.72 (t, 1H, J 8.0 Hz, ArH), 6.95-7.06 (m, 3H, ArH), 7.08 (s, 2H, -NH_2), 7.24 (d, 1H, J 8.0 Hz, ArH), 7.29 (d, 1H, J 8.0 Hz, ArH), 7.35-7.39 (m, 1H, ArH), 11.6 (s, 1H, -NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 12.9, 35.0, 51.4, 71.9, 103.4, 109.2, 111.5, 116.9, 117.8, 120.0, 121.8, 123.1, 123.4, 124.6, 125.2, 129.5, 133.6, 136.8, 142.3, 153.5, 176.2. Anal. Calculated for $C_{21}H_{16}N_4OS$: C, 67.72; H, 4.33; N, 15.04; S, 8.61 Found: C, 67.70; H, 4.29; N, 15.07; S, 8.63

Compound 6d 2'-amino-1-propyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (53 mg, 93%); mp 249-251 °C; FTIR (cm^{-1}): 3412, 3328, 3155,

2184, 1688, 1620, 1563, 1475, 1431. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.93 (t, 3H, J = 7.2 Hz, –$CH_2CH_2\underline{CH_3}$), 1.66-1.72 (m, 2H, –$CH_2\underline{CH_2}CH_3$), 3.71 -3.79 (m, 2H, -$\underline{CH_2}CH_2CH_3$), 6.22 (d, 1H, J 8.2 Hz, ArH), 6.72 (t, 1H, J 7.2 Hz, ArH), 6.95–7.09 (m, 3H, ArH), 7.11 (s, 2H, NH_2), 7.23 (d, 1H, J 8.0 Hz, ArH), 7.30 (d, 1H, J 8.4 Hz, ArH), 7.37 (t, 1H, J 7.6 Hz, ArH), 11.68 (s, 1H, -NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 13.7, 20.9, 51.5, 72.0, 103.5, 109.4, 111.5, 116.9, 117.9, 120.0, 121.8, 123.2, 123.4, 124.6, 125.2, 129.5, 133.4, 136.9, 143.0, 143.5, 153.7, 176.7. Anal. Calculated (%) for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50; S, 8.30; Found: C, 68.33; H, 4.67; N, 14.52; S, 8.31

Compound 6e 2'-amino-1-butyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (55 mg, 93%); mp 256-258 °C; FTIR (cm^{-1}): 3402, 3289, 3165, 2186, 1689, 1567, 1473, 1428. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.88 (t, 3H, J 7.2 Hz, –$CH_2CH_2CH_2\underline{CH_3}$), 1.33-1.38 (m, 2H, –$CH_2CH_2\underline{CH_2}CH_3$), 1.61-1.67 (m, 2H, –$CH_2\underline{CH_2}CH_2CH_3$), 3.75-3.80 (m, 2H, -$\underline{CH_2}$ $CH_2CH_2CH_3$), 6.19 (d, 1H, J 8.4 Hz, ArH), 6.70 (t, 1H, J 7.6 Hz, ArH), 6.95-7.05 (m, 2H, ArH), 7.07 (s, 2H, NH_2), 7.15–7.41 (m, 4H, ArH), 11.66 (s, 1H, NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 14.1, 20.0, 29.6, 42.9, 51.4, 71.9, 103.4, 109.3, 111.5, 116.9, 119.9, 120.5, 120.8, 121.8, 122.7, 123.2, 124.6, 125.2, 129.5, 133.4, 136.6, 153.6, 176.6. Anal. Calculated (%) for $C_{23}H_{20}N_4OS$, C, 68.98; H, 5.03; N, 13.99; S, 8.01; Found: C, 68.95; H, 5.07; N, 13.97; S, 8.05

Compound 6f 2'-amino-1-allyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (52 mg, 93%); mp. 257-259 °C; FTIR (cm^{-1}): 3419, 3308, 3164, 2183, 1693, 1627, 1558, 1464, 1424. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 4.34-4.39 (m, 2H, -$\underline{CH_2}$-CH=CH_2), 5.19-5.36 (m, 2H, CH_2-CH=$\underline{CH_2}$), 5.82-5.92 (m, 1H, CH_2-$\underline{CH}$=CH_2), 6.17 (d, 1H, J 8.0 Hz, ArH), 6.69 (t, 1H, J 7.6 Hz, ArH),. 6.94-7.11 (m, 4H, ArH), 7.13 (s, 2H, -NH_2), 7.29-7.38 (m, 2H, ArH), 12.10 (s, 1H, -NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 49.0, 51.5, 71.7, 103.3, 109.8, 111.6, 116.9, 117.5, 118.1, 119.9, 120.8, 121.7, 123.4, 123.6, 124.6, 125.2, 129.4, 131.9, 133.3, 137.0, 154.1, 176.5. Anal. Calculated (%) for $C_{22}H_{16}N_4OS$: C, 68.73; H, 4.19; N, 14.57; S, 8.34. Found: C, 68.70; H, 4.15; N, 14.59; S, 8.38

Compound 6g 2'-amino-1-benzyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (60 mg, 93%); mp 231-233 °C; FTIR (cm^{-1}): 3410, 3311, 3181, 2182, 1691, 1622, 1562, 1466, 1435. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 5.04 (q,

2H, J 9.6 Hz -NCH_2), 6.09 (t, 1H, J 5.6 Hz, ArH), 6.61 (t, 1H, J 5.6 Hz, ArH), 6.95-7.03 (m, 2H, ArH), 7.07–7.11 (m, 2H, ArH), 7.16 (s, 2H, NH_2), 7.28-7.31 (m, 5H, ArH), 7.44 (m, 2H, ArH) 11.70 (s, 1H, NH), ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 43.7, 51.6, 71.7, 79.1, 79.7, 103.5, 109.9, 111.4, 117.1, 118.2, 119.9, 121.8, 123.3, 123.7, 124.5, 125.3, 128.0, 128.7, 128.9, 129.4, 133.3, 136.4, 136.8, 142.5, 154.0, 177.0. Anal. Calculated (%) for $C_{26}H_{18}N_4OS$, C, 71.87; H, 4.18; N, 12.89; S, 7.38; Found: C, 71.83; H, 4.22; N, 12.93; S, 7.35

Compound 6h ethyl 2'-amino-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carboxylate: White powder (52 mg, 90%); mp 237-238 °C; FTIR (cm^{-1}): 3408, 3304, 3259, 2108, 1717, 1685, 1643, 1575, 1453. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.81 (t, 3H, J 9.6 Hz -$OCH_2\underline{CH_3}$), 4.09 (q, 2H, J 5.6 Hz, -$O\underline{CH_2}CH_3$), 6.73-6.79 (m, 2H, ArH) 6.84-6.85 (d, 1H, J 7.2 Hz, ArH) 6.88-6.96 (m, 3H, ArH),), 7.07-7.13 (m, 1H, ArH) 7.22 (d, 1H, J 8.0 Hz ArH), 8.18 (s, 2H, NH_2), 10.49 (s, 1H, NH), 11.48 (s, 1H, NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 13.5, 52.2, 59.4, 90.2, 105.7, 109.1, 117.8, 117.8, 119.7, 121.4, 121.4, 122.0, 123.3, 124.7, 127.8, 136.8, 137.9, 142.4, 154.6, 167.9, 180.6. Anal. Calculated (%) for $C_{21}H_{17}N_3O_3S$, C, 64.43; H, 4.38; N, 10.73; S, 8.19; Found: C, 64.46; H, 4.35; N, 10.77; S, 8.17.

Compound 6i Ethyl 2'-amino-1-benzyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carboxylate: White powder (65 mg, 92%); mp 229-230 °C; FTIR (cm^{-1}): 3417, 3313, 3163, 2188, 1713, 1692, 1623, 1554, 1476, 1432. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.04 (t, 3H, J 8.0 Hz, $OCH_2\underline{CH_3}$), 4.70 (q, 2H, J 8.0 Hz, -$O\underline{CH_2}CH_3$), 5.01(m, 2H, -$N\underline{CH_2}$Ph), 6.34 (d, 2H, J 8.0 Hz, ArH), 6.96 -7.00 (m, 2H, ArH) 7.11-7.16 (m, 2H, ArH), 7.25-7.39 (m, 7H, ArH) 7.30 (s, 2H, -NH_2), 12.43 (s, 1H, -NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 13.1, 43.3, 52.6, 56.0, 89.6, 109.8, 111.8, 112.3, 118.1, 119.6, 120.8, 122.5, 125.3, 126.4, 127.8, 127.9, 128.0, 128.1, 129.0, 132.9, 133.0, 135.9, 136.6, 150.8, 176.9, 183.5 Anal. Calculated for $C_{28}H_{23}N_3O_3S$, C, 69.83; H, 4.81; N, 8.73; S, 6.66; Found C, 69.87; H, 4.83; N, 8.70; S, 6.62.

Compound 6j 2'-amino-9'-methyl-2-oxo-1-propyl-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (55.6 mg, 94%); mp 256-257 °C; FTIR (cm^{-1}): 3315, 3251, 3185, 2179, 1698, 1635, 1561, 1531, 1464. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.841 (t, 3H, J 7.6 Hz, -$NCH_2CH_2\underline{CH_3}$), 1.57-1.64 (m, 2H, -$NCH_2\underline{CH_2}CH_3$), 3.26 (s, 3H,-$NCH_3$), 3.63-3.69 (m, 2H, -$N\underline{CH_2}CH2_2CH_3$), 6.21 (d, 1H, J 8.0 Hz, ArH), 6.65-6.69 (m, 1H,

ArH), 6.92-7.01 (m, 3H, ArH), 7.11 (s, 2H, -NH_2), 7.17 (d, 1H, J 8.0 Hz, ArH), 7.28-7.37 (m, 2H, ArH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 11.6, 20.8, 30.7, 41.7, 51.8, 72.2, 103.1, 109.4, 109.9, 117.0, 117.7, 120.2, 121.7, 123.4, 124.2, 125.3, 125.5, 129.6, 133.3, 137.8, 143.0, 153.9, 176.5. Anal. Calculated (%) for $C_{23}H_{20}N_4OS$, C, 68.98; H, 5.03; N, 13.99; S, 8.01; Found C, 68.94; H, 5.00; N, 13.95; S, 8.03

Compound 6k 2'-amino-9'-methyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (50 mg, 94%); mp 274-275 °C (reported melting point of this compound in literature is > 250 °C)[15] ; FTIR (cm^{-1}): 3431, 3259, 3155, 2186, 1691, 1613, 1568, 1464, 1434. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.70 (s, 3H, -NCH_3) 6.37 (d, 1H, J 8.0 Hz, ArH), 6.79 (q, 1H, J 7.6 Hz, ArH), 6,92-707 (m, 4H, ArH), 7.17 (s, 2H, -NH_2), 7.26-7-44 (m, 2H, ArH), 10.73(s, 1H, -NH). ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 30.7, 52.2, 72.4, 103.3, 109.9, 110.2, 117.1, 117.9, 120.2, 121.8, 122.9, 124.3, 125.2, 125.4, 129.5, 134.1, 137.8, 142.0, 152.7, 178.2. Anal. Calculated (%) for $C_{20}H_{14}N_4OS$, C, 67.02; H, 3.94; N, 15.63; S, 8.95; Found: C, 67.01; H, 3.90; N, 15.67; S, 8.91.

Compound 6l 2'-amino-1-benzyl-9'-methyl-2-oxo-9'H-spiro[indoline-3,4'-thiopyrano[2,3-b]indole]-3'-carbonitrile: White powder (62 mg, 94%); mp 189-191 °C; FTIR (cm^{-1}): 3431, 3271, 2183, 1691, 1605, 1572, 1464. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.72 (s, 3H, -NCH_3), 5.02 (m, 2H, -N$\underline{CH_2}$Ph), 6.08 (d, 1H, J 8.4 Hz, ArH), 6.65 (t, 1H, J 7.2 Hz, ArH), 6.99-7.11 (m, 4H, ArH), 7.26-7.32 (m, 4H, ArH), 7.30 (s, 2H, -NH_2), 7.41-7.44 (t, 3H, J 5.6 Hz, ArH), ^{13}C NMR (DMSO-d6, 100 MHz): δ, ppm 30.75, 52.0, 55.3, 72.0, 103.1, 109.9, 117.3, 118.0, 120.1, 121.8, 123.7, 124.2, 125.4, 125.5, 128.0, 128.9, 129.5, 133.2, 136.4, 137.9, 142.5, 153.2, 176.8. Anal. Calculated (%) for $C_{27}H_{20}N_4OS$, C, 72.30; H, 4.49; N, 12.49; S, 7.15; Found C, 72.26; H, 4.53; N, 12.53; S, 7.11.

Antibacterial studies

Antibiotic susceptibility testing of DSF was conducted according to the CLSI guidelines using the broth microdilution assay. 10 mg/mL stock solutions of test compounds were prepared in DMSO. Bacterial cultures were inoculated in MHBII and optical density (OD) was measured at 600nm, followed by dilution to achieve ~106 CFU/mL. The compounds were tested from 64–0.5 mg/L in two-fold serial diluted fashion with 2.5 μL of each concentration added to well of a 96-

well round bottom microtiter plate. Later, 97.5 µL of bacterial suspension was added to each well containing either test compound or appropriate controls. The plates were incubated at 37°C for 18-24 h following which the MIC was determined. The MIC is defined as the lowest concentration of the compound at which there is absence of visible growth. For each test compound, MIC determinations were carried out independently three times using duplicate samples.

Cell cytotoxicity of DSF

Cell toxicity was performed against Vero cells using the MTT assay. ~103 cells/well were seeded in 96 well plate and incubated at 37°C in an5% CO2 atmosphere. After 24 h, compound was added ranging from 100-12.5 µg/mL concentration and incubated for 72 h. After the incubation was over, MTT was added in each well, incubated at 37°C for further 4 h, residual medium was discarded, 0.1 mL of DMSO was added to solubilise the formazan crystals and OD was taken at 540 nm for the calculation of CC50. CC50 is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Doxorubicin was used as positive control and each experiment was repeated in triplicate.

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Supplementary Material

The ^{1}H NMR spectra, ^{13}C NMR spectra, and CHN data of all new compounds can be found via the ‘Supplementary content’ section of this article’s webpage

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17. The LCMS data of the crude reaction mixture showing dimethyl oxalate peak at m/z 118.250 is included in the supporting information. Our attempts to find literature precedence to dimethyl oxalate elimination type reactions however failed and so we presume this is the first kind of such a report.

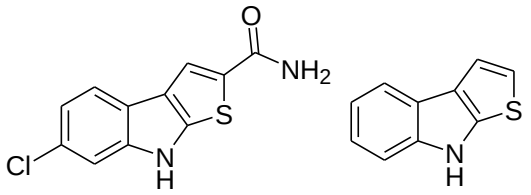


Figure 1. Thienodolin and thieno[2,3-b]indole

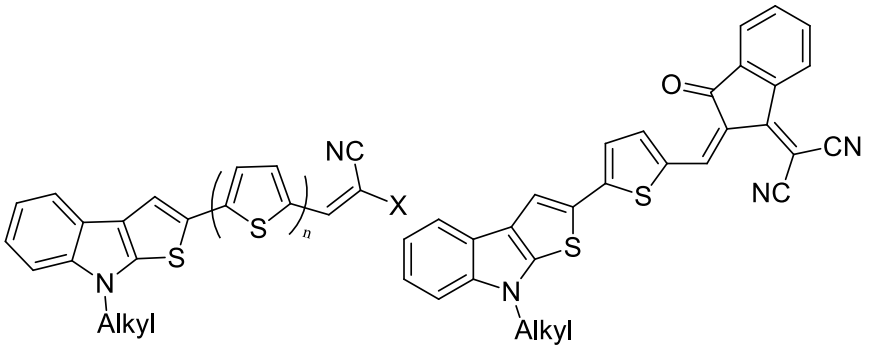
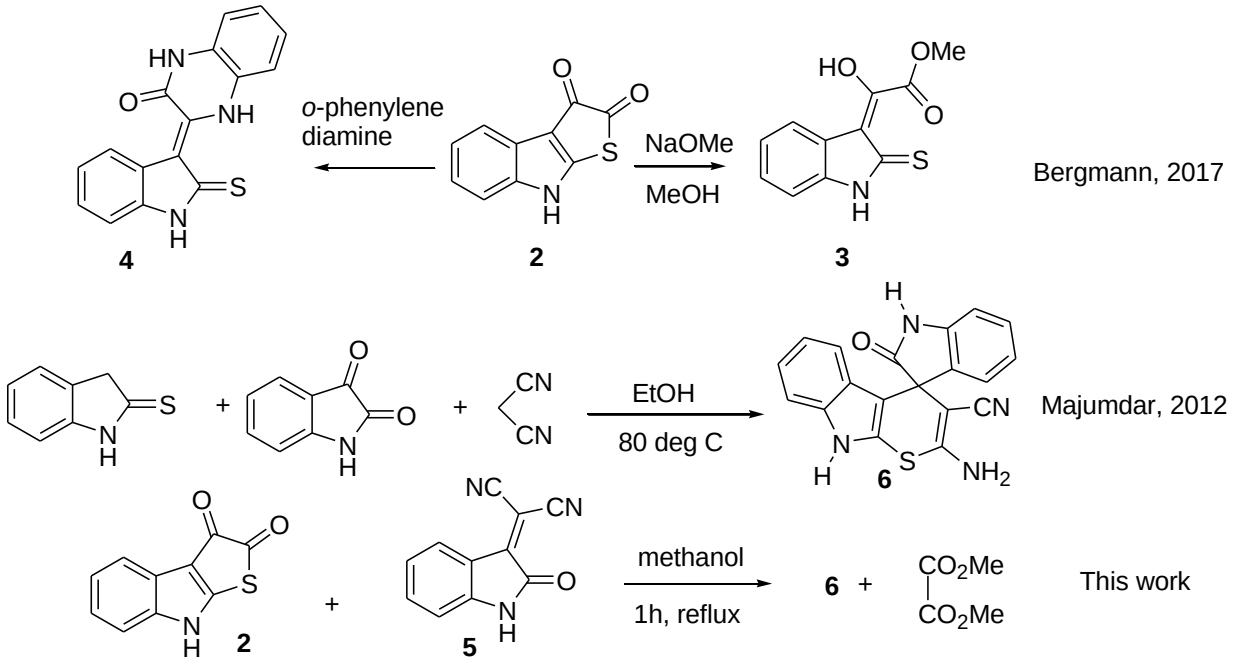


Figure 2. Representative examples for D-π-A systems using thieno[2,3-b]indole



Scheme 1. Ring opening reactions of thieno[2,3-b]indole-2,3-dione

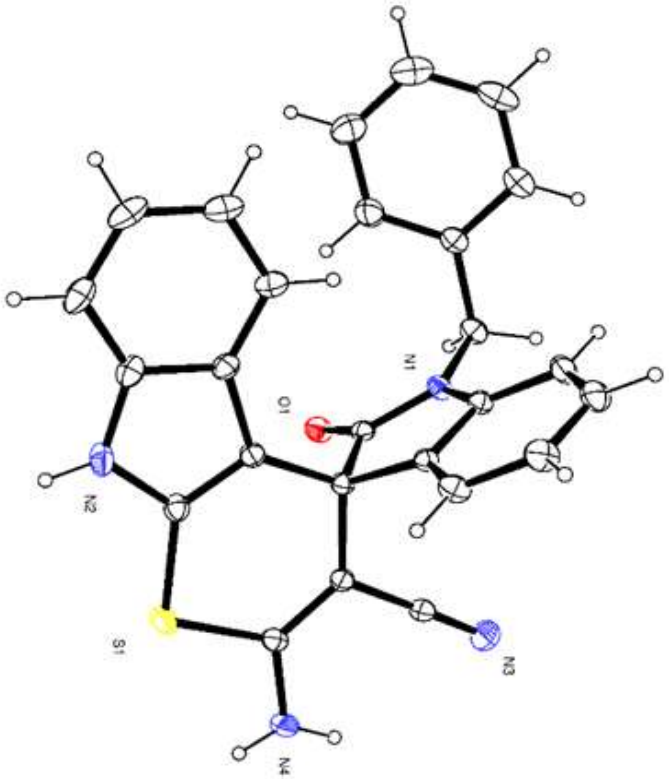
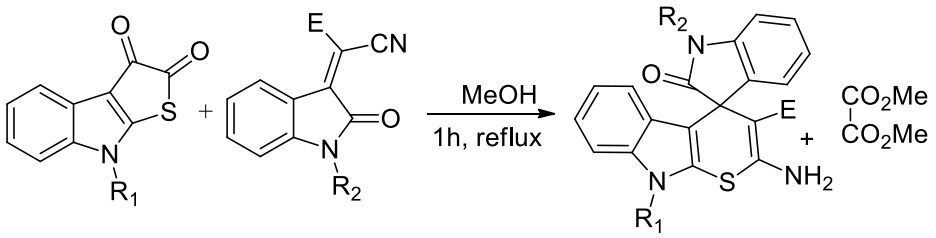


Figure 3. ORTEP diagram of **6g**

Table 1. Generality of the reaction



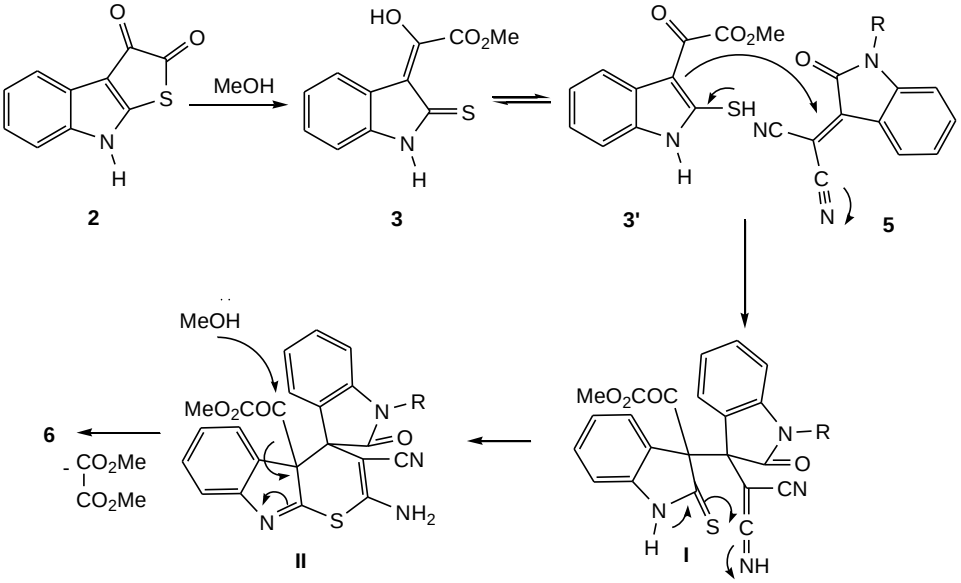
2 **5a-l** **6a-l**

R_1 = H, Me

Entry	Product[a]	R_1	R_2	E	Yield[b] (%)
1	**6a**	H	H	CN	93
2	**6b**	H	Me	CN	91
3	**6c**	H	Et	CN	90
4	**6d**	H	Pr	CN	92
5	**6e**	H	Bu	CN	93
6	**6f**	H	Allyl	CN	89
7	**6g**	H	CH_2Ph	CN	91
8	**6h**	H	H	COOEt	89
9	**6i**	H	CH_2Ph	COOEt	92

10	**6j**	Me	Pr	CN	91
11	**6k**	Me	H	CN	93
12	**6l**	Me	CH_2Ph	CN	92

[a]All reactions were done by refluxing **2** (0.15 mmol) and **5** (0.15 mmol) in MeOH (4 ml) for 1 h. [b]Isolated yield.



Scheme 2. Mechanistic rationale

Table 2 MIC (µg/mL) against ESKAPE pathogen panel

		MIC (µg/mL)				
S.No	Compound	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*K. pneumoniae* BAA 1705	*A. baumannii* BAA 1605	*P. aeruginosa* ATCC 27853
1	6a	>64	>64	>64	>64	>64
2	6b	>64	32	>64	>64	>64
3	6c	>64	8	>64	>64	>64
4	6d	>64	8	>64	>64	>64
5	6e	>64	32	>64	>64	>64
6	6f	>64	8	>64	>64	>64
7	6g	>64	8	>64	>64	>64
8	6h	>64	32	>64	>64	>64
9	6i	>64	16	>64	>64	>64
10	Levo floxacin	0.0156	0.125	64	8	1