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Original article

Synthesis of new class of spirocarbocycle derivatives by multicomponent domino reaction and their evaluation for antimicrobial, anticancer activity and molecular docking studies

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ABSTRACT

A series of 25 new spirocarbocycles were synthesized by a three component reaction that involves few cyclic nucleophiles, vinyl malononitriles and aldehydes with variable substitution patterns. All the synthesized compounds were evaluated for their antimicrobial activity and the compounds showed significant activity. Synthesized compounds **4c, 4i** and **6i** showed good anticancer activity against A549 cancer cell line. Molecular docking studies indicated that compound **4i** had the greatest affinity for DNA gyrase receptor than others and compound **6i** had the greatest affinity for anaplastic lymphoma kinase (ALK) receptor. These compounds can be better therapeutic agents for microbial and cancer cell lines.

1. Introduction

Spiro compounds exhibit a broad spectrum of important bioactivities such as antitumor [1], antidiabetic [2], antibacterial [3], antitubercular [4], antiinflammatory [5] activities etc [6] due to their interesting structures. Spirooxindoles are privileged medicinal scaffolds that are found in natural and unnatural biologically active compounds (Fig. 1). The key paradigm of modern drug discovery and the goal of synthetic organic chemistry are being achieved by the rapid assembly of structurally different compounds. One of the most potential strategies for the synthesis and library production of these spiro compounds is achieved through multicomponent reactions (MCRs) [7]. They have been used widely for carbon–carbon bond formation by synthetic chemists. They also offer an eloquent tool for the one pot synthesis of distinct and complex molecule as well as small and drug like heterocycles.

Electron-deficient dicyanoalkenes have been reported to behave as good hydride acceptors in conjugate reduction reactions [8] and also act as versatile direct vinylogous donors in asymmetric Michael addition reactions with excellent chemo- and stereo selectivity [9]. The strong electron-withdrawing groups activate the γ-position and further it undergoes Michael addition. Vinylogous Michael addition was reported as a key step in the preparation of many spiro compounds [10].

The methodologies that are accessible for the synthesis of spirocyclic compounds are alkylations [11], rearrangement reactions [12], cycloadditions [13], transition metal catalyzed reactions [14] and cleavage of bridged systems [15]. The synthetic and pharmacological importance of spiro compounds are anchored in their spirocyclic motifs. The captivating framework of spirooxindoles increased the thirst of chemists in preparing these compounds by numerous methods. Our group has always been interested on the synthesis of novel spirooxindole compounds and screening for their biocidal activity [16].

2. Result and discussion

2.1. Chemistry

The biological importance of the spiro compounds directed us to synthesize spiro-carbocycles. In continuation of our studies in the

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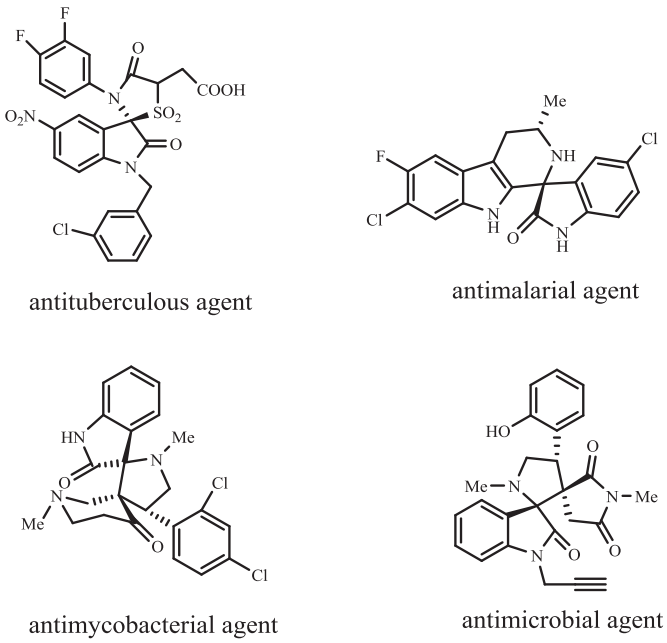


Fig. 1. Some biologically important spirooxindole compounds.

area of multicomponent domino reaction using vinylogous Michael addition methodology [17] we herein report the one pot multicomponent reaction of vinyl malononitriles with different chalcones and their antimicrobial, anticancer activity and molecular docking results of ligands to the DNA gyrase and ALK receptor. To the best of our knowledge, there have been no reports for the synthesis of the spiro moieties derived from oxindole, indanedione and 1,3-cyclohexanedione chalcones. In the present study the vinyl malononitriles undergo vinylogous Michael addition with the *in situ* generated chalcones followed by intramolecular nucleophilic addition and isomerization respectively to afford spirocarbocycles in moderate to high yields (Scheme 1).

We initiated our study by performing the reaction of cyclohexylidene malononitrile **3a** with *in situ* generated indanedione chalcone without any base catalyst and using methanol as solvent which afforded compound **4a** in 40% yield (Table 1, entry 1). Then the investigatory experiment was performed to improve the yield of the product by varying reaction conditions *viz.* solvent, temperature and base catalyst. All the reaction products are purified by recrystallization using ethanol as a solvent. The observations (Table 1) led us to the conclusion that the base has an obvious effect on the reaction. Among the selected bases (Table 1, entries 2–6), commercially available organocatalyst L-proline was proven to be the most suitable (Table 1, entries 6–9) as it gives single diastereomer. The reaction of 1,3-indandione **1** with aldehyde **2a** and alkylidene malononitrile **3a** in the presence of L-proline was completely diastereoselective in affording only the trans diastereomer whereas the other bases yield mixture of diastereomers.

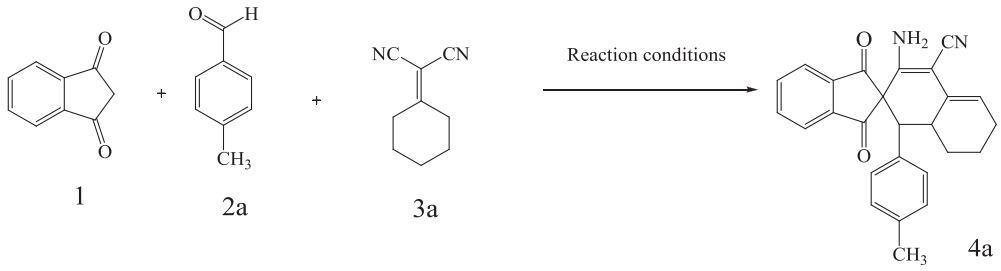
Several solvents were investigated (Table 1, entries 6, 7, 10–12) and methanol was the best one (Table 1, entries 7–9), although ethanol also produced comparable results (Table 1, entry 6). The stoichiometry ratio 15 mol% of the catalyst was chosen to be the best for performing the reaction since further increase had no impact on the yield of the reaction (Table 1 entries 7–9). Thus the best result was obtained when 1,3-indandione **1** and aldehyde **2a** were stirred for ten minutes in the presence of 15 mol% of L-proline as a catalyst for the *in situ* generation of chalcone followed by the addition of cyclohexylidene malononitrile **3a** to provide a precipitate which was filtered off and recrystallized from ethanol to yield the desired product **4a** in 89% yield.

To explain the mechanism of this tandem reaction, a postulated reaction course is depicted in (Scheme 2). In the first step 1,3-indandione **1** undergoes condensation reaction with aldehyde by the removal of water molecule and affords chalcone **1a**. In the second step the proton of vinyl malononitrile **3a** is removed by the mild base to furnish vinylogous carbanion which attacks the activated double bond of chalcone **1a** forming the intermediate **1b**. Among the two paths the first one shows the steric repulsion and fails to afford a product. The nucleophilic addition to the nitrile carbon from the least hindered side through path B results in the formation of intermediate **1c** which on isomerisation gives only one spirocarbocyclic diastereomer.

With these results in hand, we then investigated the substrate scopes and limitations of the synthesis of spirocarbocycles. First we started the reaction with 1,3-indandione by varying aldehydes and alkylidene malononitrile to afford spirocarbocycles (Scheme 3) the results are listed in Table 2. Aromatic aldehydes having electron donating groups like 4-methyl and 4-methoxy benzaldehydes (Table 2, entries 1–5) reacted at faster rates compared with those that substituted with electron withdrawing groups like 2-fluoro and 4-chloro benzaldehydes (Table 2, entries 6–10). Various aromatic aldehydes such as naphthaldehyde were also examined (Table 2, entry 11). Besides, our methodology has been used successfully in order of acid and base sensitive materials such as heteroaromatic aldehydes and corresponding spirocarbocycles were obtained in excellent yields without the formation of any by products (Table 2, entries 12, 13). As it is clear from the obtained results, the presented methodology can be used in oxygen and sulphur containing heteroaromatic aldehydes. Thus the reaction was found to be general and has a broad scope due to its applicability to a variety of substrates and the products **4a**–**4m** were isolated in excellent yields (79–90%) under milder reaction conditions. The results are summarized in Table 2.

To extend the scope of this protocol for this multicomponent reaction, studies were continued with other chalcones like oxindole chalcones using the same protocol which resulted in the formation of corresponding spiroxindoles (Scheme 4). The reaction provided spiroxindoles in good yields (78–86%) and the results are shown in Table 3. The promising results prompted us to further explore the scope of this protocol. We investigated the reaction of cyclohexylidene malononitrile with 1,3-cyclohexanedione chalcone. The spirocarbocycles (Scheme 4) were obtained in moderate yields (72–74%) and the results are mentioned in Table 3.

The structure of all prepared compounds were elucidated with the aid of IR, ^{1}H and ^{13}C NMR, Mass spectroscopy and elemental analysis data were discussed for a representative compound **6a**. The IR spectrum of **6a** exhibited a sharp peak at 3422 cm^{-1} which corresponds to the –NH stretching of –NH$_2$ group and peaks at 2200 and 1702 cm^{-1} corresponded to the nitrile and amide carbonyl respectively. The ^{1}H NMR spectra of 6a showed two singlets at δ 5.38 and 10.35 for the –NH$_2$ and –NH protons (D$_2$O exchangeable) respectively, clearly indicating the incorporation of both moieties in the product. The doublet appearing for the H$_a$ proton of the product **6a** showed a coupling constant value of 12.4 Hz indicative of the trans stereochemistry between the H$_a$ and H$_b$ protons which is also clearly evidenced from the single crystal X-ray analysis of compound **6a** (Fig. 2, Table 4) [18] which further supports the ^{1}H NMR spectroscopy. In ^{13}C NMR spectra, the spiro carbon atom displayed a signal at δ 82.0 and the amide carbonyl carbon atom resonated at δ 176.6. The mass spectra also exhibited a distinguishing peak at *m*/*z* 382 [M+H]$^+$. This shows that the reaction of oxindole **5** with aldehyde **2a** and alkylidene malononitrile



Scheme 1. Synthesis of spirocarbocycle.

3a is completely diastereoselective in affording only the trans diastereomer. The structures of all spirocarbocycles were consistent with the above mentioned data.

2.2. Pharmacology

2.2.1. Antimicrobial activity

In the present work, the antimicrobial activities of 25 synthesized compounds were screened against nine bacteria and two fungi using *in vitro* disc diffusion method. The results revealed that most of the synthesized compounds exhibited antimicrobial activities against *Enterobacter aerogenes*, *Staphylococcus epidermidis*, *Staphylococcus aureus* (MRSA), *Salmonella typhimurium, Klebsiella pneumonia* and *Micrococcus luteus*. The results are summarized in Table 5 and Fig. 3. Compounds **4b, 4c, 4i** and **4k** have shown excellent activities more than the standard drug against both gram-positive and gram-negative bacteria at 1 mg/disc. Moreover the compounds **4a**, **4i, 4l, 4m, 6a, 6b, 6i, 6j** and **8b** showed good antibacterial activity over the others. All tested compounds showed moderate antifungal activity against *Candida albicans and Malassezia pachydermatis*.

The Minimum Inhibitory Concentration (MIC) values of active compounds against bacteria are given in Table 6 and Fig. 4. Significant MIC values were observed against gram positive and gram negative bacteria. The results revealed that the spirocarbocycles **4c, 4i, 4k** and **6i** have shown good antibacterial activity against tested organisms. Among all tested compounds 4-methyl substituted aromatic ring containing compound **4c** has shown significant MIC values against *K. pneumonia*, *Proteus vulgaris*, *Shigella flexneri*, 4-chloro substituted aromatic ring containing compound **4i** is potent against *S. aureus* (MRSA), *P. vulgaris*, *S. flexneri* and *M. luteus*. The naphthyl group containing compound **4k** is active against *S. aureus* (MRSA), *S. flexneri* and *M. luteus*. The 2-thiophenyl containing spirooxindole compound **6i** showed significant MIC values against *S. aureus* (MRSA), *P. vulgaris* and *S. flexneri.*

2.2.2. Anticancer activity

Anticancer activity studies have been performed for the synthesized compounds **4i, 4c** and **6i**. They showed potent anticancer activity *in vitro* against A549 lung adenocarcinoma cancer cell line. Compound **4c** showed 59.6% activity at the dose of 50 μg/mL with IC_{50} value of 50 μg/mL. Compound **4i** showed 78.8% activity at the dose of 50 μg/mL with IC_{50} value of 30 μg/mL. Compound **6i** showed 83.9% activity at the dose of 50 μg/mL with IC_{50} value of 20 μg/mL. All concentrations used in the experiment decreased the cell viability significantly ($P < 0.05$) in a concentration-dependent manner (Table 7).

2.2.3. Molecular docking studies

Docking studies were performed to gain insight into the protein inhibitor interactions inside the enzyme binding sites. Over the past decade DNA gyrase receptor remains one of the most investigated and validated targets for the development of anti bacterials [19]. Most of the synthesized spirocarbocyclic compounds have shown significant activity against *S. aureus* (MRSA) hence it is thought worthwhile to do docking studies to support the *in vitro* activity. All the synthesized new spirocarbocycles were subjected to docking using MOE 2011 software version 7.1. All the prepared compounds were chosen for the docking study of ligands with the DNA gyrase receptor. To find the potential of these molecules against the human lung cancer cells the compounds were also docked to the Anaplastic Lymphoma Kinase (ALK) receptor [20].

To verify the reproducibility of docking calculations the bound ligand was extracted from the complexes and submitted for one ligand run calculation. The final docked conformations fall within 0.5–1 Å root-mean-square deviation [21]. Hence it was concluded that this method could be used for the docking of other compounds (Fig. 5a and 6). We have also performed the docking of the standard ligand Streptomycin with the DNA gyrase receptor for method validation (Fig. 5b).

The docked ligand conformations were analyzed in terms of free energy of binding (FEB), hydrogen bonding and hydrophobic interactions. One hundred (100) docking runs were performed and the best docked representation of the ligand was selected based on the conformation with lowest value of FEB.

Among all compounds docked to the DNA gyrase receptor, compound **4i** was the most active. It had a high binding energy of −11.64 kcal/mol. This compound exactly fits as that of ligand and shows strong interaction with ARG 1122 aminoacid (Fig. 7).

The intermediate active compound **4m** binds with the DNA gyrase receptor and the corresponding binding energy is −9.51 kcal/mol. It shows interaction with the aminoacid GLN 1056 (Fig. 8). The least active compound **6h** has binding energy value −8.14 kcal/mol, and it shows interaction with ALA 1068 aminoacid (Fig. 9).

Molecular docking studies of synthesized molecules to the ALK receptor show that, the most active compound **6i**, has a very high binding energy value, −18.43 kcal/mol and it interacts with the three aminoacids namely, ASP 1249, ASN 1254 and GLY 1272 (Fig. 10). Compound **4h**, a moderately active compound, has a binding energy value of −12.58 kcal/mol and it interacts with GLU 1210 as shown in Fig. 11. The least active compound **6h** has a binding energy of −11.23 kcal/mol. It interacts with ARG 1209 aminoacid (Fig. 12).

The preparation of various other spirooxindoles and the detailed study of biological activity are underway.

3. Conclusion

We have synthesized a new series of 25 spirocarbocycle derivatives through vinylogous Michael addition. A new, simple, efficient and environmentally benign method involving the usage of L-proline for the synthesis of spirocarbocycle was developed. By

Table 1
Optimization of reaction conditions for the preparation of spirocarbocycles.

Entry	Solvent	Catalyst (10 mol%)	Conditions	Time (h)	Yield[a] (%)
1.	MeOH	–	r.t.	48	40
2.	EtOH	K_2CO_3	r.t.	6	62
3.	MeOH	Et_3N	r.t.	4	80
4.	EtOH	DABCO	Reflux	6	54
5.	EtOH	NaOEt	r.t.	5	67
6.	EtOH	L-proline	r.t.	3	85
7.	MeOH	L-proline	r.t.	3	86
8.	**MeOH**	**L-proline**	**r.t.**	**3**	**89**[b]
9.	MeOH	L-proline	r.t.	3	87[c]
10.	CH_3CN	L-proline	r.t.	5	69
11.	H_2O	L-proline	r.t.	6	72
12.	DMF	L-proline	r.t.	8	58

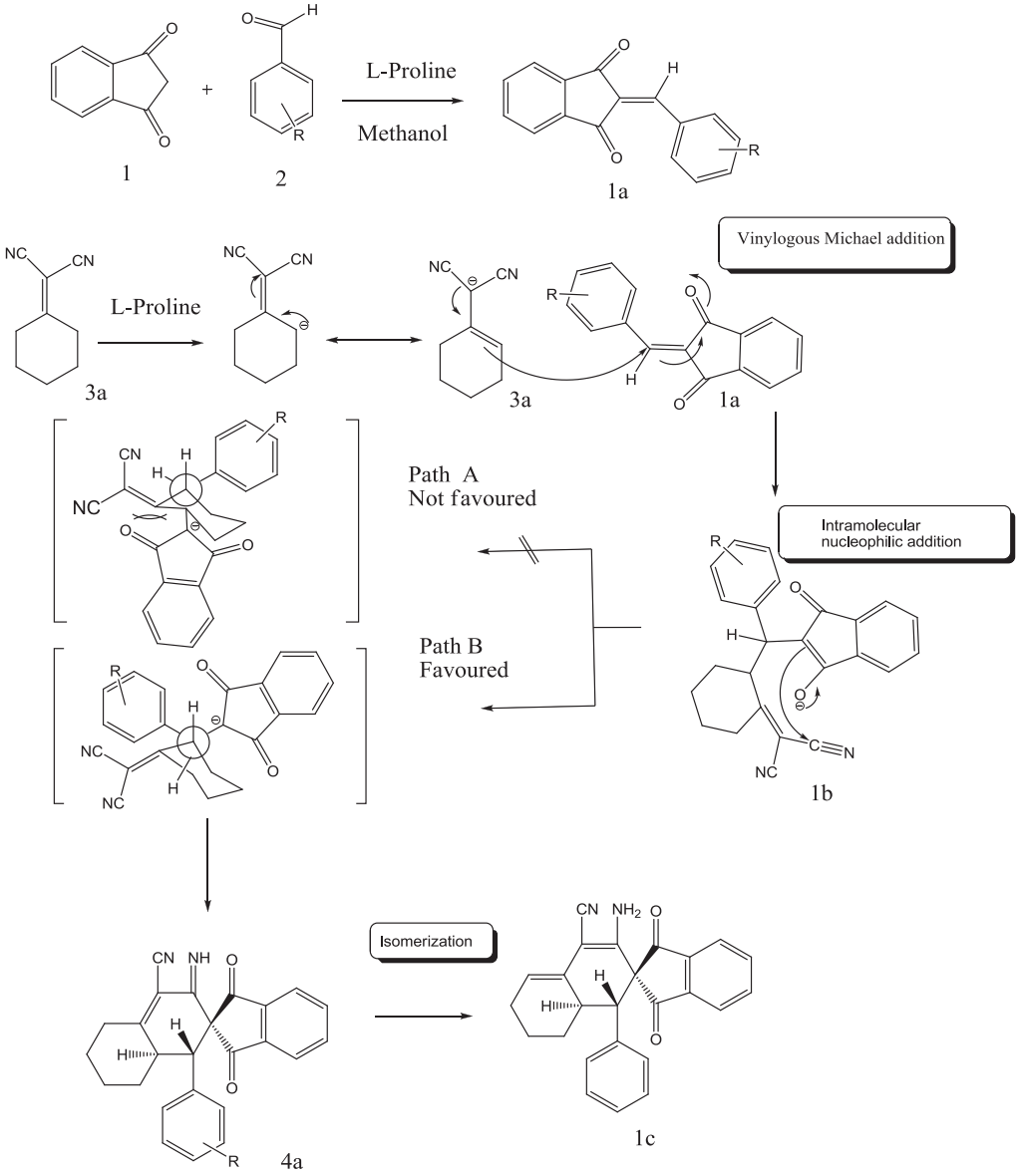
[a] Isolated yield of 4a after recrystallization.
[b] The reaction was performed using 15 mol% of the catalyst.
[c] The reaction was performed using 20 mol% of the catalyst.

this method, a diverse spirocarbocycle library has been rapidly constructed with excellent yields without involving tedious extraction and isolation procedures. All the compounds were evaluated for their activities against 4 gram positive bacteria, 5 gram negative bacteria and 2 fungi. Most of the compounds were found to exhibit significant antimicrobial activity. Among them **4c, 4i** and **6i** have shown excellent activities and hence are promising candidates as antibacterial agents. Compounds **4c, 4i** and **6i** also showed good anticancer activity against A549 lung cancer cell line. The docking scores show that the spirocarbocyclic molecules have good potential against the human lung cancer cells.

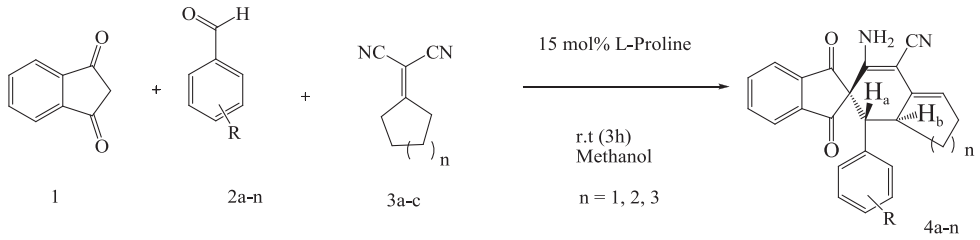
4. Experimental

4.1. Chemistry

Analytical TLC was performed on precoated aluminium sheets of silica gel 60F254 of 0.2 mm thickness (Merck, Germany). Melting points were determined on Gallenkamp melting point apparatus



Scheme 2. Plausible mechanism for the formation of spirocarbocycles.



Scheme 3. Synthesis of spirocarbocycle derivatives from 1,3-indandione, substituted aldehydes and alkylidene malononitrile.

and are uncorrected. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer as KBr pellets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in DMSO-d_6 solutions with TMS as an internal standard on a Bruker Avance DPX-400 MHz instrument. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in parts per million. The number of protons (n) for a given resonance was indicated as nH. Coupling constants (J) are given in hertz. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded under HRMS (ESI) using Thermo Scientific Exactive Orbitrap mass spectrometer and Thermo Finnigan LCQ Advantage MAX 6000 ESI mass spectrometer and Perkin–Elmer GC–MS. Elemental analysis data were recorded using Thermo Finnigan FLASH EA 1112 CHN analyzer.

*4.1.1. Experimental procedure for the synthesis of **(4a–m)***

Indanedione **1** (1 mmol), aldehydes **2** (1 mmol) **2a–h** were stirred in MeOH in the presence of L-proline (20 mol%) at room temperature (r.t.) for ten minutes followed by the addition of alkylidene malononitrile **3a–c** (1 mmol) at r.t. for 3 h. The solid precipitated out, and then was filtered off and purified by recrystallization from ethanol to afford product **4a–m** as yellow crystalline solid.

*4.1.1.1. 3′-Amino-1,3-dioxo-1′-p-tolyl-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4a**).* Yellow solid; mp: 224–226 °C (Decomposes); IR (cm^{-1}): 755, 1246, 1589, 1661, 1704, 1742, 2205, 2921, 3246, 3345, 3408. ^{1}H NMR (400 MHz, DMSO-d_6): δ .0.71 (q, 1H, $J = 12.4$ Hz), 1.32–1.42 (m, 2H), 1.64–1.66 (m, 1H), 1.99 (s, 3H), 2.08–2.20 (m, 2H), 2.98 (d, 1H, $J = 12.8$ Hz), 3.04–3.07 (m, 1H), 5.59–5.61 (m, 1H), 6.12 (brs, 2H, $-NH_2$, D_2O exchangeable), 6.61 (d, 1H, $J = 7.6$ Hz), 6.72–6.79 (m, 3H), 7.67 (d, 1H, $J = 7.6$ Hz), 7.75–7.77 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.0, 22.1, 25.4, 27.9, 33.5, 52.2, 63.6, 82.9, 117.4, 118.1, 123.1, 123.7, 126.6, 129.0, 129.2, 129.2, 131.4, 131.8, 133.1, 136.4, 136.6, 142.7, 143.3, 151.9, 199.7, 200.2. HRMS (ESI): Mass calculated for $C_{26}H_{22}N_2O_2Na$ $[M+Na]^+$ 417.1573, found, $[M+Na]^+$, 417.1573. Anal. Calcd. For: ($C_{26}H_{22}N_2O_2$) C, 79.16; H, 5.62; N, 7.10 Found: C, 79.05; H, 5.73; N, 7.01.

*4.1.1.2. 6-Amino-1′,3′-dioxo-4-p-tolyl-1′,2,3,3a,3′,4-hexahydro-2,5′-spirobi[indene]-7-carbonitrile (**4b**).* Yellow solid; mp 215–216 °C (Decomposes); IR (cm^{-1}): 790, 1244, 1573, 1664, 1703, 1741, 2206, 2925, 3246, 3347, 3406. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.98 (q, 1H, $J = 10.4$), 1.67–1.74 (m, 1H), 1.98 (s, 3H), 2.22–2.34 (m, 2H), 3.03 (d, 1H, $J = 12.4$ Hz), 3.50–3.55 (m, 1H), 5.33–5.35 (m, 1H), 6.48 (brs, 2H, NH_2, D_2O exchangeable), 6.72–6.77 (m, 4H), 7.69–7.75 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.8, 30.4, 31.3, 42.4, 52.8, 65.2, 78.4, 116.0, 117.9, 123.2, 123.7, 129.1, 133.1, 136.4, 136.6, 136.9, 138.5, 142.3, 143.5, 154.6, 199.4, 199.9. MS $m/z = 381$ $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_2O_2$: C, 78.93; H, 5.30; N, 7.36 Found: C, 79.94; H, 5.21; N, 7.24.

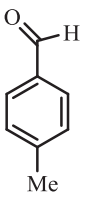
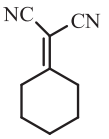
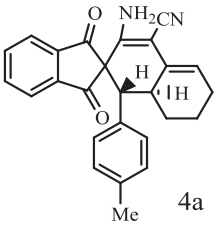
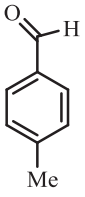
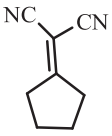
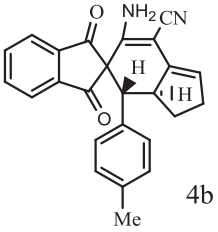
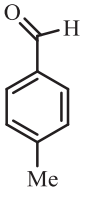
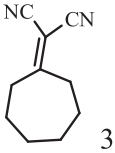
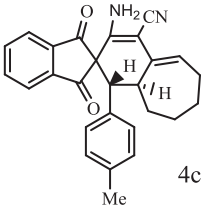
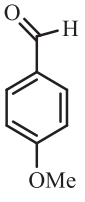
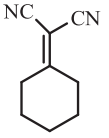
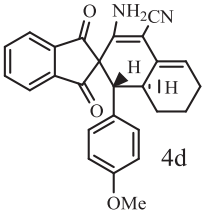
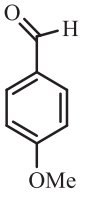
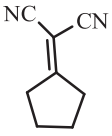
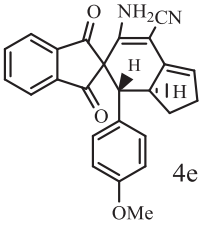
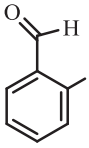
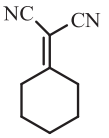
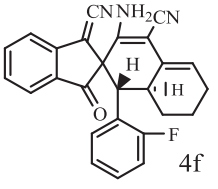
*4.1.1.3. 3-Amino-1′,3′-dioxo-1-p-tolyl-1,1′,3′,6,7,8,9,9a-octahydrospiro[benzo[7]annulene-2,2′-indene]-4-carbonitrile (**4c**).* Yellow solid; mp 210–212 °C (Decomposes); IR (cm^{-1}): 754, 1252, 1590, 1637, 1704, 1741, 2200, 2925, 3253, 3366, 3535. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14–1.24 (m, 3H), 1.34–1.36 (m, 1H). 1.63–1.64 (m, 2H), 1.99 (s, 3H), 2.18–2.31 (m, 2H), 3.13 (d, 1H, $J = 11.6$ Hz), 3.49–3.50 (m, 1H proton merged with solvent peak), 5.76 (t, 1H, $J = 6.2$ Hz), 6.08 (brs, 2H, NH_2, D_2O exchangeable)), 6.70 (d, 2H, $J = 8.4$ Hz), 6.79 (d, 2H, $J = 8.0$ Hz), 7.63–7.66 (m, 1H), 7.74–7.81 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.7, 25.9, 27.9, 29.8, 31.8, 38.0, 52.2,63.0, 83.3, 118.6, 121.2, 123.1, 124.0, 129.2, 134.6, 136.5, 136.7, 136.9, 137.3, 142.2, 143.1, 152.3, 199.0, 199.4. HRMS (ESI): Mass calculated for $C_{27}H_{25}N_2O_2$ $[M+H]^+$ 409.1911 found, $[M+H]^+$ 409.1916; Anal. Calcd for $C_{27}H_{24}N_2O_2$: C, 79.39; H, 5.92; N, 6.86. Found: C, 79.28; H, 6.02; N, 7.94.

*4.1.1.4. 3′-Amino-1′-(4-methoxyphenyl)-1,3-dioxo-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4d**).* Yellow solid; mp 194–197 °C (Decomposes); IR (cm^{-1}): 758, 1246, 1588, 1656, 1702, 1739, 2202, 2927, 3250, 3343, 3429. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.69 (q, 1H, $J = 12.4$ Hz), 1.13–1.17 (m, 2H),1.61–1.67 (m, 1H), 2.14–2.22 (m, 2H),2.95 (d, 1H, $J = 12.4$ Hz), 3.37–3.43 (m, 1H), 3.48 (s, 3H), 5.60–5.62 (m, 1H), 5.94 (brs, 2H, $-NH_2$, D_2O exchangeable), 6.49–6.52 (m, 2H), 6.64 (d, 1H, $J = 8$ Hz), 6.74 (d, 1H, $J = 8.8$ Hz), 7.67 (d, 1H, $J = 7.2$ Hz), 7.73–7.78 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 25.3, 27.7, 30.4, 34.5, 55.3, 63.7, 85.1, 113.6, 114.7, 123.3, 127.5, 131.4, 134.2, 136.8, 140.4, 146.8, 158.3, 172.2, 197.8, 199.0; MS $m/z = 411$ $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_2O_3$: C, 76.08; H, 5.40; N, 6.82 Found: C, 76.19; H, 5.30; N, 6.71.

*4.1.1.5. 6-Amino-4-(4-methoxyphenyl)-1′,3′-dioxo-1′,2,3,3a,3′,4-hexahydro-2,5′-spirobi[indene]-7-carbonitrile (**4e**).* Yellow solid; mp 206–209 °C (Decomposes); IR (cm^{-1}):1254, 1512, 1573, 1661, 1702, 1739, 2204, 2932, 3244, 3345, 3413; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.23–0.28 (m, 1H), 0.98–1.05 (m, 1H), 1.57–1.63 (m, 2H), 2.34 (d, 1H, $J = 7.6$ Hz), 2.77 (s, 3H), 2.82–2.90 (m, 1H), 4.65–4.67 (m, 1H), 5.62 (brs, 2H, $-NH_2$, D_2O exchangeable), 5.80 (d, 2H, $J = 8$ Hz), 6.04–6.08 (m, 2H),7.00–7.06 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.4, 31.3, 42.5, 46.4, 55.3, 65.3, 78.7, 116.8, 117.9, 123.3, 123.6, 127.8, 136.7, 136.9, 138.0, 142.2, 143.3, 154.4, 158.6, 199.6, 200.3; MS $m/z = 397$ $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_2O_3$, C, 75.74; H, 5.08; N, 7.07 Found: C, 75.83; H, 5.19; N, 7.17.

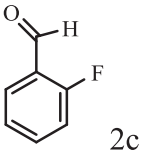
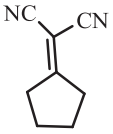
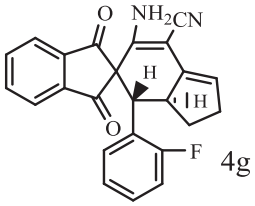
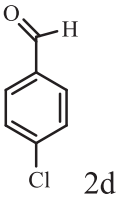
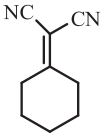
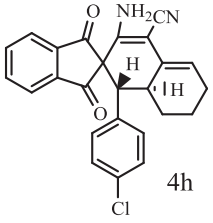
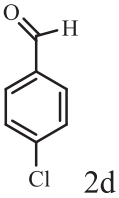
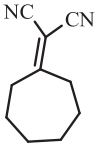
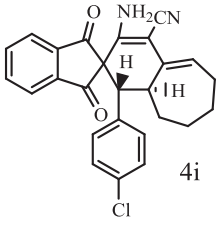
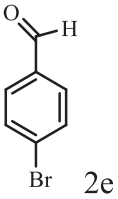
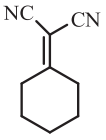
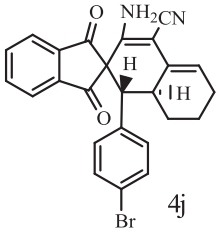
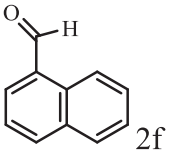
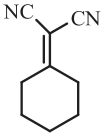
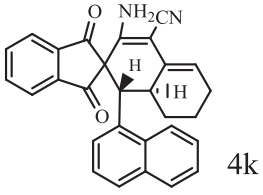
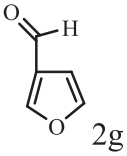
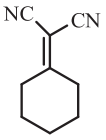
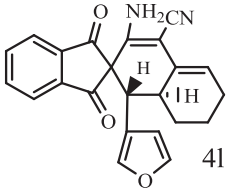
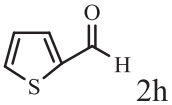
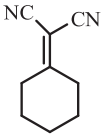
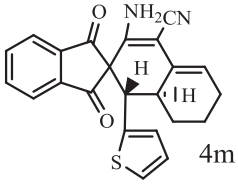
*4.1.1.6. 3′-Amino-1′-(2-fluorophenyl)-1,3-dioxo-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4f**).* Yellow solid; mp 222–224 °C (Decomposes); IR (cm^{-1}): 759, 1247, 1588, 1662, 1704, 1742, 2205, 2919, 3249, 3349, 3410; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.66 (q, 1H, $J = 12.2$ Hz), 1.26–1.34 (m, 2H) 1.62–1.65 (m, 1H), 2.05–2.17 (m, 2H), 3.44 (d, 1H, $J = 12.5$ Hz), 4.48–4.51 (m, 1H), 5.62–5.64 (m, 1H), 6.06 (brs, 2H, $-NH_2$, D_2O exchangeable), 6.79–6.84 (m, 2H), 6.87–6.90 (m, 1H), 6.93–6.96 (m, 1H), 7.70 (d, 1H, $J = 5$ Hz), 7.73–7.76 (m, 3H); ^{13}C NMR

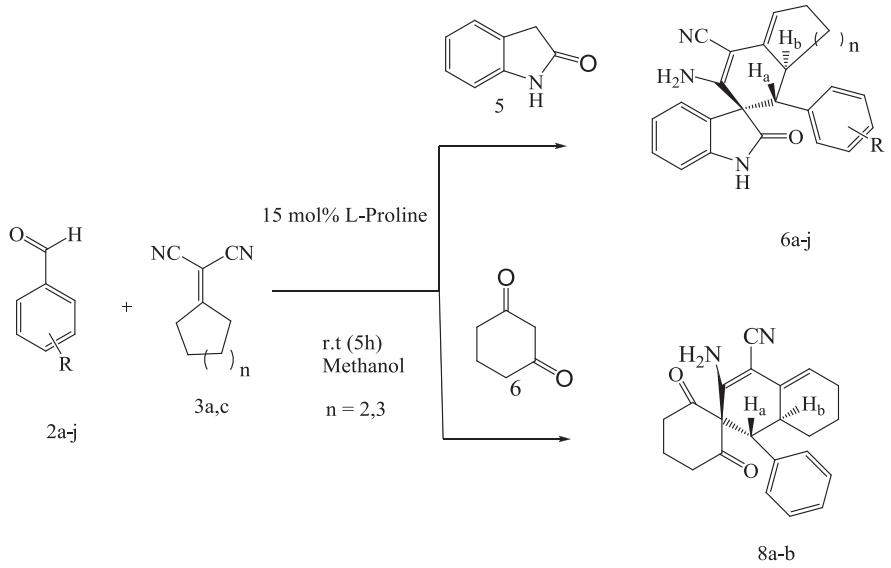
Table 2
Synthesis of spirocarbocycle derivatives from 1, 3-indandione, substituted aldehydes and alkylidene malononitrile.

Entry	Aldehyde	Vinylogous malononitrile	Product	Time	Yield
1.	 2a	 3a	 4a	3	89
2.	 2a	 3b	 4b	2.5	90
3.	 2a	 3c	 4c	4	86
4.	 2b	 3a	 4d	3.5	87
5.	 2b	 3b	 4e	2.5	88
6.	 2c	 3a	 4f	4	81

(*continued on next page*)

Table 2 (*continued*)

Entry	Aldehyde	Vinylogous malononitrile	Product	Time	Yield
7.	 2c	 3b	 4g	3	83
8.	 2d	 3a	 4h	6	82
9.	 2d	 3c	 4i	4.5	84
10.	 2e	 3a	 4j	4	85
11.	 2f	 3a	 4k	5	79
12.	 2g	 3a	 4l	4.5	85
13.	 2h	 3a	 4m	5.5	86



Scheme 4. Synthesis of spirocarbocycle derivatives from oxindole or 1,3-cyclohexanedione, substituted aldehydes and alkylidene malononitrile.

(100 MHz, DMSO-d_6): δ 21.7, 25.3, 27.9, 33.4, 42.9, 63.1, 83.6, 118.1, 118.8, 122.9, 123.6, 124.5, 129.2, 130.2, 131.4, 137.3, 142.8, 151.9, 159.2, 198.8, 200.1; MS *m/z* = 399 $[M+H]^+$; Anal. Calcd for $C_{25}H_{19}FN_2O_2$: C, 75.36; H, 4.81; N, 7.03 Found: C, 75.47; H, 4.72; N, 7.12.

*4.1.1.7. 6-Amino-4-(2-fluorophenyl)-1′,3′-dioxo-1′,2,3,3a,3′,4-hexahydro-2,5′-spirobi[indene]-7-carbonitrile (**4g**).* Yellow solid; mp 222–225 °C (Decomposes); IR (cm^{-1}): 761, 1245, 1573, 1646, 1708, 1742, 2199, 2829, 3258, 3359, 3445; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.25 (q, 1H, *J* = 11.6 Hz), 1.03–1.04 (m, 1H), 1.57–1.66 (m, 2H), 2.42 (d, 1H, *J* = 12.4 Hz), 2.91–2.97 (m, 1H), 4.71–4.72 (m, 1H), 5.78 (brs, 2H, $-NH_2$, D_2O exchangeable), 6.09 (t, 1H, *J* = 9.2 Hz), 6.16–6.20 (m, 1H), 6.27–6.34 (m, 2H), 7.00–7.01 (m, 1H), 7.08 (d, 3H, *J* = 2.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.0, 36.0, 47.2, 48.9, 69.5, 83.4, 120.1, 121.8, 122.6, 128.1, 128.3, 129.6, 133.9, 135.1, 141.6, 141.7, 142.2, 147.0, 147.6, 159.2, 163.2, 203.5, 204.0; MS *m/z* = 385 $[M+H]^+$; Anal. Calcd for $C_{24}H_{17}FN_2O_2$: C, 74.99; H, 4.46; N, 7.29. Found: C, 75.17; H, 4.35; N, 7.38.

*4.1.1.8. 3′-Amino-1′-(4-chlorophenyl)-1,3-dioxo-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4h**).* Yellow solid; mp 246–248 °C (Decomposes); IR (cm^{-1}): 833, 1093, 1244, 1590, 1636, 1697, 1738, 2201, 2923, 3251, 3360, 3452; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.73 (q, 1H, *J* = 12.4 Hz), 1.24–1.32 (m, 1H), 1.40–1.43 (m, 1H), 1.64–1.67 (m, 1H), 1.97–2.07 (m, 1H), 2.15–2.20 (m, 1H), 3.06 (d, 1H, *J* = 12.4 Hz) 3.09–3.10 (m, 1H), 5.60–5.62 (m, 1H), 6.19 (brs, 2H, $-NH_2$, D_2O exchangeable), 6.76 (d, 1H, *J* = 7.2 Hz), 6.86 (d, 1H, *J* = 7.6 Hz), 7.04 (d, 2H, *J* = 8.4 Hz), 7.69 (d, 1H, *J* = 7.6 Hz), 7. 78 (q, 3H, *J* = 7.0 Hz; ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.0, 25.3, 27.7, 33.3, 51.7, 63.5, 82.8, 117.7, 118.0, 123.2, 123.8, 128.5, 128.6, 128.7, 131.4, 132.3, 135.2, 136.7, 136.8, 142.5, 143.2, 151.6, 199.5, 200.0; MS *m/z* = 415 $[M+H]^+$; Anal. Calcd for $C_{25}H_{19}ClN_2O_2$: C, 72.37; H, 4.62; N, 6.75 Found: C, 72.28; H, 4.73; N, 6.65.

*4.1.1.9. 3-Amino-1-(4-chlorophenyl)-1′,3′-dioxo-1,1′,3′,6,7,8,9,9a-octahydrospiro[benzo[7]annulene-2,2′-indene]-4-carbonitrile (**4i**).* Yellow solid; mp 180–181 °C (Decomposes); IR (cm^{-1}): 833, 1091, 1490, 1590, 1635, 1700, 1741, 2199, 2929, 3366, 3455; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.50 (m, 3H), 1.34–1.38 (m, 1H), 1.66–1.69 (m, 2H), 2.02–2.05 (m, 1H), 2.36–2.40 (m, 1H), 2.51 (d, 1H, *J* = 11.7 Hz) 2.98–3.00 (m, 1H), 5.07 (t, 1H, *J* = 5.78 Hz), 5.27 (brs, 2H, $-NH_2$, D_2O exchangeable), 6.09–6.15 (m, 3H), 6.47 (d, 1H, *J* = 8.2 Hz), 6.96 (d, 1H, *J* = 7.4 Hz), 7.07–7.12 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.6, 27.8, 29.6, 30.1, 36.3, 52.4, 62.9, 83.4, 118.5, 122.1, 123.3, 123.9, 126.4, 128.4, 129.3, 132.5, 136.5, 137.1, 141.9, 143.0, 151.9, 199.0, 199.1; MS *m/z* = 429 $[M+H]^+$; Anal. Calcd for $C_{26}H_{21}ClN_2O_2$: C, 72.81; H, 4.94; N, 6.53. Found: C, 72.75; H, 5.15; N, 6.42.

*4.1.1.10. 3′-Amino-1′-(4-bromophenyl)-1,3-dioxo-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4j**).* Yellow solid; mp 254–256 °C (Decomposes); IR (cm^{-1}): 805, 1243, 1589, 1636, 1696, 1738, 2200, 2948, 3250, 3361, 3452; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.72 (q, 1H, *J* = 12 Hz), 1.30 (d, 1H, *J* = 11.2 Hz), 1.39–1.42 (m, 1H), 1.65 (d, 1H, *J* = 9.2 Hz), 2.06–2.07 (m, 1H), 2.15–2.20 (m, 1H), 3.03 (d, 1H, *J* = 11.2 Hz) 3.34–3.41 (m, 1H), 5.60–5.62 (m, 1H), 6.18 (brs, 2H, $-NH_2$, D_2O exchangeable), 6.70 (d, 1H, *J* = 7.6 Hz), 7.17 (d, 1H, *J* = 8.4 Hz), 7.69 (d, 2H, *J* = 8.4 Hz), 7.69 (d, 1H, *J* = 7.6 Hz),7.74–7.81 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.0, 25.3, 27.7, 33.3, 51.8, 63.4, 82.8, 117.7, 118.0, 120.9, 123.3, 123.8, 129.0, 131.4, 131.5, 133.5, 135.6, 136.7, 136.8, 142.5, 143.2, 151.6, 199.5, 200.0; MS *m/z* = 459 $[M+H]^+$; Anal. Calcd for $C_{25}H_{19}BrN_2O_2$: C, 65.37; H, 4.17; N, 6.10. Found: C, 65.26; H, 4.27; N, 6.20.

*4.1.1.11. 3′-Amino-1′-(naphthalen-1-yl)-1,3-dioxo-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4k**).* Yellow solid; mp 213–215 °C (Decomposes); IR (cm^{-1}): 774, 1256, 1488, 1585, 1633, 1721, 2195, 2926, 3420, 3466; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.65 (q, 1H, *J* = 12.1 Hz), 1.23–1.26 (m, 1H), 1.73–1.79 (m, 2H), 2.13–2.22 (m, 1H), 2.37 (d, 1H, *J* = 9.4 Hz), 2.65 (d, 1H, *J* = 10.8 Hz) 3.14–3.20 (m, 1H), 5.65–5.67 (m, 1H), 6.10 (brs, 2H, $-NH_2$, D_2O exchangeable), 7.09–7.11 (m, 2H), 7.23–7.28 (m, 2H), 7.45–7.51 (m, 2H), 7.63–7.70 (m, 4H), 7.79–7.82 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ, 27.3, 35.1,36.9, 44.9, 52.5, 63.8, 83.2, 118.0, 121.8, 123.0, 123.1, 123.7, 125.1, 126.0, 126.7, 128.8, 129.3, 132.1, 133.7, 136.1, 142.7, 152.3, 167.6, 199.5, 200.5; MS *m/z* = 431 $[M+H]^+$; Anal. Calcd for $C_{29}H_{22}N_2O_2$: C, 80.91; H, 5.15; N, 6.51. Found: C, 80.82; H, 5.26; N, 6.60.

Table 3
Synthesis of spirooxindole derivatives from oxindole or 1,3-cyclohexanedione, substituted aldehydes and alkylidene malononitrile.

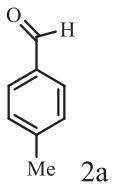
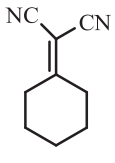
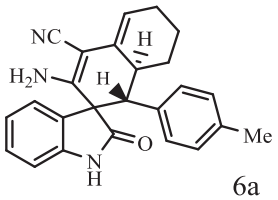
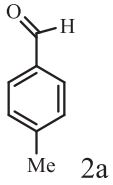
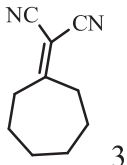
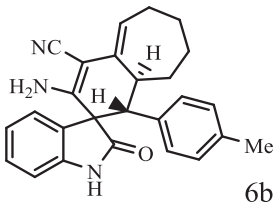
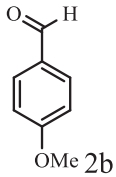
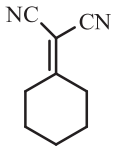
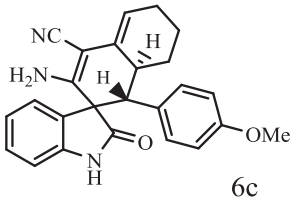
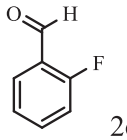
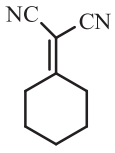
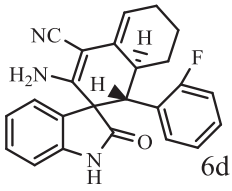
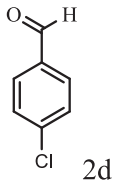
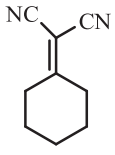
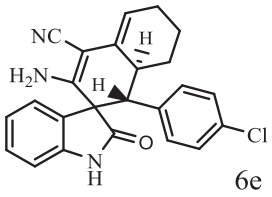
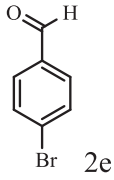
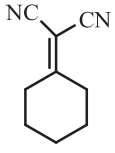
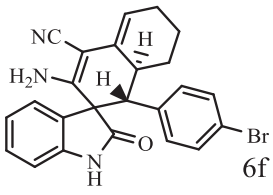
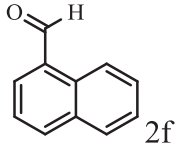
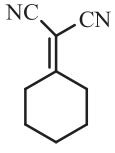
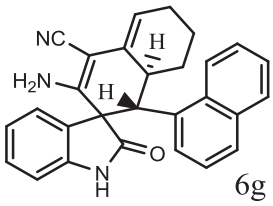
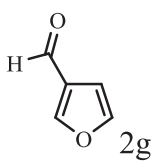
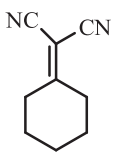
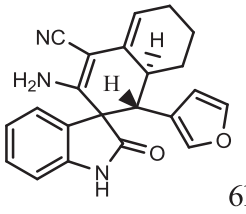
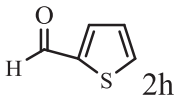
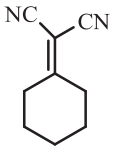
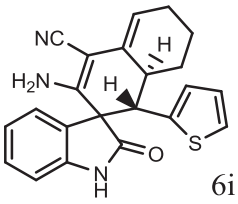
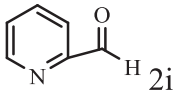
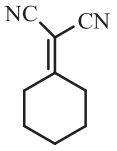
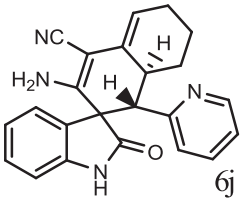
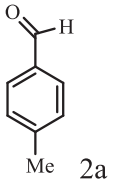
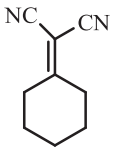
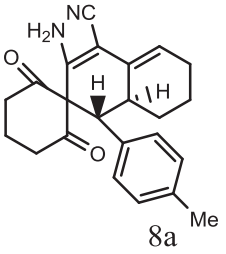
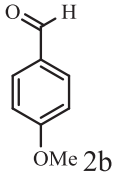
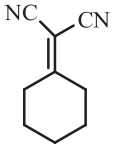
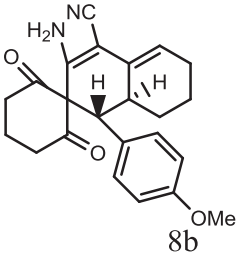
Entry	Aldehyde/ketone	Vinylogous malononitrile	Product	Time	Yield
1.	 2a	 3a	 6a	5	86
2.	 2a	 3c	 6b	6	83
3.	 2b	 3a	 6c	7	84
4.	 2c	 3a	 6d	8	78
5.	 2d	 3a	 6e	10	81
6.	 2e	 3a	 6f	9	80
7.	 2f	 3a	 6g	8	79
8.	 2g	 3a	 6h	7.5	78

Table 3 (*continued*)

Entry	Aldehyde/ketone	Vinylogous malononitrile	Product	Time	Yield
9.	 2h	 3a	 6i	9	81
10.	 2i	 3a	 6j	7	82
11.	 2a	 3a	 8a	6	74
12.	 2b	 3a	 8b	6.5	72

*4.1.1.12. 3′-Amino-1′-(furan-3-yl)-1,3-dioxo-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4l**).* Yellow solid; mp 247–248 °C (Decomposes); IR (cm^{-1}): 1243, 1589, 1660, 1704, 2206, 2923, 3253, 3347, 3422; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.79 (q, 1H, J = 12 Hz), 1.41–1.43 (m, 2H), 1.69–1.72 (m, 1H), 1.94–2.19 (m, 2H), 2.87–2.92 (m, 1H), 2.98 (d, 1H, J = 12.4 Hz), 5.57–5.59 (m, 1H),5.87 (s, 1H) 6.14 (brs, 2H, –NH_2, D_2O exchangeable), 7.13 (s, 1H), 7.20 (s, 1H), 7.79–7.80 (m, 1H), 7.84–7.85 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.7, 30.1, 32.6, 38.2, 47.6, 67.5, 87.6, 122.2, 122.8, 125.1, 128.1, 128.6, 136.2, 141.5, 146.5, 147.4, 148.1, 148.9, 156.3, 204.7, 204.9; MS m/z = 371 $[M+H]^+$; Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.48; H, 4.79; N, 7.65.

*4.1.1.13. 3′-Amino-1,3-dioxo-1′-(thiophen-2-yl)-1,3,6′,7′,8′,8a′-hexahydro-1′H-spiro[indene-2,2′-naphthalene]-4′-carbonitrile (**4m**).* Yellow solid; mp 248–250 °C (Decomposes); IR (cm^{-1}): 1244, 1587, 1662, 1704, 1741, 2205, 2923, 3250, 3347, 3411; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (q, 1H, J = 12.1 Hz), 1.39–1.48 (m, 2H), 1.68–1.69 (m, 1H), 2.08–2.21 (m, 2H), 2.93–2.99 (m, 1H), 3.35–3.40 (m, 1H), 5.59–5.61 (m, 1H),6.15 (brs, 2H, –NH_2, D_2O exchangeable), 6.55–6.61 (m, 2H), 7.08–7.09 (m, 1H), 7.75–7.82 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.0, 25.4, 27.7, 35.5, 47.6, 63.3, 82.8, 117.8, 118.0, 123.4, 124.0, 131.2, 136.6, 136.8, 142.8, 143.3, 151.3, 199.7, 200.0; MS m/z = 387 $[M+H]^+$; Anal. Calcd for $C_{23}H_{18}N_2O_2S$: C, 71.48; H, 4.69; N, 7.25. Found: C, 71.36; H, 4.80; N, 7.16.

*4.1.2. Experimental procedure for the synthesis of (**6a–j**)*

Oxindole **5** (1 mmol), aldehydes **2** (1 mmol) **2a–j** were stirred in MeOH in the presence of L-proline (20 mol%) at room temperature (r.t.) for ten minutes followed by the addition of alkylidene malononitrile **3a–c** (1 mmol) at r.t. for 5 h. The solid precipitated out was filtered off and purified by recrystallization from ethanol to afford product **6a–j** as white crystalline solid.

*4.1.2.1. 3′-Amino-2-oxo-1′-p-tolyl-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6a**).* White solid; mp 248–249 °C (Decomposes); IR (cm^{-1}):752, 1191, 1388, 1580, 1627, 1702, 2200, 2926. 3057, 3299, 3329, 3422; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.72 (q, 1H, J = 12.1 Hz), 1.26–1.29 (m, 1H), 1.37–1.41 (m, 1H), 1.64–1.67 (m, 1H), 2.03–2.07 (m, 1H), 2.12 (s, 3H), 2.16–2.21 (m, 1H), 3.02 (d, 1H, J = 12.4 Hz), 3.37–3.41 (m, 1H), 5.38 (brs, 2H, –NH_2, D_2O exchangeable), 5.58–5.60 (m. 1H), 6.37 (d, 1H, J = 8.8 Hz), 6.49 (d, 1H, J = 8 Hz), 6.66 (d, 1H, J = 7.6 Hz), 6.93–7.00

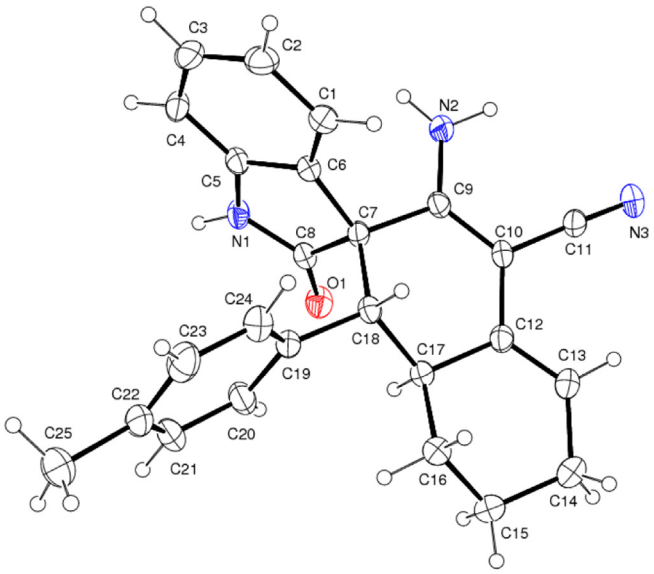


Fig. 2. ORTEP diagram of synthesized compound **6a**.

(m, 2H), 7.06 (t, 1H, J = 7.6 Hz), 7.13 (d, 1H, J = 8 Hz), 7.28 (d, 1H, J = 7.6 Hz), 10.35 (brs, 1H, −N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.9, 22.3, 25.5 28.3, 32.7, 52.6, 57.9, 82.0, 110.0, 116.8, 118.5, 122.2, 124.7, 126.4, 128.1, 128.9, 129.2, 129.7, 132.0, 132.6, 134.5, 135.9, 142.9, 154.5, 176.6; HRMS (ESI): Mass calculated for $C_{26}H_{22}N_2O_2Na$ $[M+Na]^+$: 404.1733, found: 404.1734; Anal. Calcd for $C_{25}H_{23}N_3O$: C, 78.71; H, 6.08; N, 11.02. Found: C, 78.60; H, 6.17; N, 11.12.

*4.1.2.2. 3-Amino-2′-oxo-1-p-tolyl-1,6,7,8,9,9a-hexahydrospiro[benzo[7]annulene-2,3′-indoline]-4-carbonitrile (**6b**).* White solid; mp 238–240 °C (Decomposes); IR (cm^{-1}):755, 1475, 1577, 1622, 1693, 1716, 2200, 2927, 3263, 3350, 3460; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.01 (q, 1H, J = 11.3 Hz), 1.40–1.49 (m, 1H), 1.60–1.67 (m, 3H), 1.71–1.74 (m, 1H), 2.10 (s, 3H), 2.24–2.28 (m, 1H), 2.67–2.69 (m, 1H), 3.20 (d, 1H, J = 12.2 Hz), 3.27–3.28 (m, 1H), 5.26 (brs, 2H, −NH_2, D_2O exchangeable), 5.70–5.74 (m. 1H), 6.92–6.97 (m, 1H), 6.99–7.03 (m, 2H), 7.05–7.10 (m, 2H), 7.14–7.21 (m, 1H), 7.33–7.40 (m, 2H), 10.42 (brs, 1H, −N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.9, 26.0, 28.1, 30.3, 32.3, 37.7, 52.6, 57.6, 82.5, 107.5, 110.0, 119.1, 120.5, 122.2, 125.4, 127.2, 127.6, 128.3, 128.6, 129.0, 129.4, 129.5, 135.9, 136.3, 138.0, 138.3, 142.7, 154.5, 176.0; MS m/z = 396 $[M+H]^+$; Anal. Calcd for $C_{26}H_{25}N_3O$: C, 78.96; H, 6.37; N, 10.62. Found: C, 78.87; H, 6.45; N, 10.53.

Table 4
Crystal data and structure refinement parameters for compound **6a**.

Empirical formula	C_{25} H_{23} N_3 O
Formula weight	381.46
Temperature	293 (2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, P21/n
Unit cell dimensions	a = 9.3858 (3) Å; b = 21.8349 (6) Å; c = 9.6525 (2) Å $\alpha = \gamma = 90°$ β = 93.3610 (10)°
Volume	1974.76 (9) A^3
Z, Calculated density	4, 1.283 Mg m^{-3}
Absorption coefficient	0.080 mm^{-1}
F (000)	808
Crystal size	0.35 × 0.35 × 0.30 mm
θ range for data collection	2.31–25.00°
Limiting indices	$-11 \le h \le 11$, $-25 \le k \le 25$, $-11 \le l \le 11$
Reflections collected/unique	18019/3486 [R (int) = 0.0335]
Completeness to θ = 25.00	100.0%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9865 and 0.9632
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3486/0/275
Goodness-of-fit on F^2	1.030
Final R indices [I > 2sigma (I)]	R1 = 0.0400, wR2 = 0.0965
R indices (all data)	R1 = 0.0587, wR2 = 0.1072
Extinction coefficient	0.0079 (11)
Largest diff. peak and hole	0.240 and −0.158 e A^{-3}
CCDC	972722

*4.1.2.3. 3′-Amino-1′-(4-methoxyphenyl)-2-oxo-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6c**).* White solid; mp 241–243 °C (Decomposes); IR (cm^{-1}): 833, 1185, 1266, 1512, 1573, 1618, 1701, 2195, 2224, 2952, 3276, 3324, 3425; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.72 (q, 1H, J = 12.1 Hz), 1.27–1.30 (m, 1H), 1.37–1.50 (m, 1H), 1.65–1.74 (m, 1H), 2.06–2.08 (m, 1H), 2.16–2.21 (m, 1H), 3.01 (d, 1H, J = 12.8 Hz), 3.31–3.34 (m, 1H),3.60 (s, 3H), 5.43 (brs, 2H, −NH_2, D_2O exchangeable), 5.57–5.59 (m. 1H), 6.39 (s, 2H), 6.50 (d, 1H, J = 7.6 Hz), 6.76 (d, 1H, J = 8.5 Hz), 6.93–6.97 (m, 1H), 7.05–7.09 (m, 1H), 7.14 (d, 1H, J = 8.6 Hz), 7.28 (d, 1H, J = 7.3 Hz), 10.37 (brs, 1H, −N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.3, 25.5, 28.3, 32.8, 52.2, 55.2, 58.0, 81.8, 110.0, 112.6, 113.9, 114.3, 116.6, 118.5, 122.2, 124.7, 127.6, 129.2, 129.3, 129.7, 129.8, 132.6, 133.1, 142.9, 154.5, 158.1, 176.7; MS m/z = 398 $[M+H]^+$; Anal. Calcd for $C_{25}H_{23}N_3O_2$: C, 75.54; H, 5.83; N, 10.57. Found: C, 75.45; H, 5.72; N, 10.66.

*4.1.2.4. 3′-Amino-1′-(2-fluorophenyl)-2-oxo-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6d**).* White solid; mp 265–267 °C (Decomposes); IR (cm^{-1}): 753, 1203, 1227, 1489, 1577, 1625, 1701, 2198, 2831, 2939, 3335, 3427; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.74 (q, 1H, J = 12.0 Hz), 1.20–1.23 (m, 1H), 1.36–1.44 (m, 1H), 1.65–1.67 (m, 1H), 2.04–2.21 (m, 2H),3.39–3.43 (m, 1H), 3.50 (d, 1H, J = 12.5 Hz), 5.52 (brs, 2H, −NH_2, D_2O exchangeable), 5.59–5.61 (m. 1H), 6.50 (d, 1H, J = 7.7 Hz), 6.77 (t, 1H, J = 9.0 Hz), 6.92 (t, 1H, J = 7.6 Hz), 7.04–7.10 (m, 3H), 7.21 (d, 1H, J = 7.4 Hz), 7.31 (t, 1H, J = 7.4 Hz), 10.56 (brs, 1H, −N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 25.4, 28.0, 32.5, 43.5, 57.4, 81.7, 109.8, 115.0, 115.2, 117.0, 118.4, 122.2, 124.6, 124.8, 128.5, 128.7, 129.1, 129.2, 129.4, 132.2, 142.7, 154.4, 176.6; HRMS (ESI): Mass calculated for $C_{24}H_{20}FN_3NaO$ $[M+Na]^+$ 408.1488, found, $[M+Na]^+$ 408.1489. Anal. Calcd for $C_{24}H_{20}FN_3O$: C, 74.79; H, 5.23; N, 10.90. Found: C, 74.68; H, 5.14; N, 10.79.

*4.1.2.5. 3′-Amino-1′-(4-chlorophenyl)-2-oxo-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6e**).* White solid; mp 258–259 °C (Decomposes); IR (cm^{-1}): 747, 1487, 1579, 1629, 1725, 2195, 2923, 3334, 3453; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.76 (q, 1H, J = 12.1 Hz), 1.24–1.27 (m, 1H), 1.38–1.42 (m, 1H), 1.66–1.68 (m, 1H), 2.04–2.08 (m, 1H),2.17–2.21 (m, 1H), 3.14 (d, 1H, J = 12.4 Hz), 3.37–3.41 (m, 1H) 5.52 (brs, 2H, −NH_2, D_2O exchangeable), 5.59–5.60 (m. 1H), 6.50 (d, 2H, J = 7.8 Hz), 6.91–6.97 (m, 2H), 7.06–7.08 (m, 1H), 7.22–7.29 (m, 2H), 7.30–7.32 (m, 1H),10.43 (brs, 1H, −N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 25.5, 28.2, 32.4, 52.2, 57.7, 81.7, 110.0, 116.8, 118.5, 122.3, 124.8, 127.4, 128.3, 128.5, 129.4, 131.7, 132.3, 133.8, 136.6, 142.8, 154.3, 176.4; MS m/z = 402 $[M+H]^+$; Anal. Calcd for $C_{24}H_{20}ClN_3O$: C, 71.73; H, 5.02; N, 10.46. Found: C, 71.63; H, 5.11; N, 10.35.

*4.1.2.6. 3′-Amino-1′-(4-bromophenyl)-2-oxo-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6f**).* White solid; mp 264–266 °C (Decomposes); IR (cm^{-1}): 748, 1485, 1577, 1631, 1725, 2195, 2922, 3331, 3446; ^{1}H NMR (400 MHz, DMSO-d_6):

Table 5
In vitro antimicrobial activity of synthesized compounds.

Compounds	Zone of inhibition in mm										
	Gram positive bacteria				Gram negative bacteria					Fungi	
	S. epidermidis	*S. aureus*	*S. aureus (MRSA)*	*M. luteus*	*E. aerogenes*	*S. typhimurium*	*K. pneumonia*	*P. vulgaris*	*S. flexneri*	*C. albicans*	*M. pachydermatis*
4a	10	10	11	9	13	14	15	10	8	10	11
4b	14	17	12	12	15	10	15	16	13	10	13
4c	16	13	17	10	18	19	20	22	23	10	10
4d	12	11	NI	11	10	NI	10	NI	NI	NI	10
4e	NI	NI	10	8	NI	NI	11	NI	NI	NI	NI
4f	14	14	13	15	12	10	16	12	14	10	NI
4g	17	14	16	12	15	12	15	14	10	NI	NI
4h	8	9	13	NI	13	10	NI	NI	8	NI	NI
4i	17	13	21	21	18	18	17	23	22	13	12
4j	NI	14	13	NI	NI	12	10	NI	NI	10	NI
4k	15	12	21	22	17	18	17	19	21	10	9
4l	12	14	13	18	15	10	12	15	14	13	12
4m	12	10	18	10	15	16	14	22	24	10	11
6a	12	11	9	8	13	9	10	12	9	8	10
6b	14	13	15	13	12	16	12	12	11	9	8
6c	9	10	11	8	10	8	9	NI	8	10	13
6d	13	17	12	10	14	11	10	8	9	10	NI
6e	13	NI	NI	9	10	12	16	17	10	9	11
6f	10	12	12	8	15	11	10	9	8	10	10
6g	15	NI	NI	10	18	14	16	NI	12	NI	9
6h	NI	NI	10	8	NI	NI	NI	NI	NI	NI	NI
6i	19	13	21	15	17	15	16	23	25	10	12
6j	9	NI	NI	NI	8	11	NI	10	NI	12	11
8a	NI	NI	NI	NI	NI	NI	10	9	NI	NI	NI
8b	13	14	15	21	14	11	16	17	19	10	9
Streptomycin	26	14	30	26	22	18	20	30	30	NA	NA
Ketoconazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	28	26

NA – not applicable NI – no inhibition.

δ 0.75 (q, 1H, J = 12.1 Hz), 1.24–1.26 (m, 1H), 1.38–1.41 (m, 1H), 1.65–1.68 (m, 1H), 2.06–2.08 (m, 1H),2.16–2.20 (m, 1H), 3.11 (d, 1H, J = 12.4 Hz), 3.34–3.37 (m, 1H) 5.46 (brs, 2H, –N*H*$_2$, D_2O exchangeable), 5.58–5.60 (m. 1H), 6.45 (d, 1H, J = 8.2 Hz), 6.52 (d, 1H, J = 7.6 Hz) 6.96 (t, 1H, J = 7.5 Hz)), 7.03–7.10 (m, 2H), 7.17 (d, 1H, J = 8.4 Hz), 7.30 (d, 1H, J = 7.4 Hz), 7.39 (d, 1H, J = 8.4 Hz), 10.42 (brs, 1H, –N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 25.5, 28.2, 32.4, 52.3, 57.7, 81.9, 110.1, 117.0, 118.4, 120.3, 122.4, 124.8, 128.9, 129.3, 129.4, 130.4, 131.2, 132.2, 134.1, 137.0, 142.8, 154.2, 176.5; MS m/z = 446 $[M+H]^+$; Anal. Calcd for $C_{24}H_{20}BrN_3O$: C, 64.58; H, 4.52; N, 9.41. Found: C, 64.69; H, 4.43; N, 9.52.

*4.1.2.7. 3′-Amino-1′-(naphthalen-1-yl)-2-oxo-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6g**).* White solid; mp 245–247 °C (Decomposes); IR (cm^{-1}): 783, 1391, 1580, 1631, 1703, 2220, 2948, 3333, 3426; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.68 (q, 1H, J = 12.1 Hz), 1.00–1.06 (m, 1H), 1.32–1.34 (m, 2H), 1.97–2.18 (m, 2H), 3.41–3.46 (m, 1H), 4.24 (d, 1H, J = 12.0 Hz), 5.37 (brs, 2H, –N*H*$_2$, D_2O exchangeable), 5.61–5.64 (m. 1H), 6.34 (d, 1H, J = 7.7 Hz), 6.60–6.64 (m, 1H) 6.78 (t, 1H, J = 7.6 Hz)), 7.27–7.37 (m, 2H), 7.42–7.48 (m, 2H), 7.54–7.56 (m, 1H), 7.59–7.67 (m, 2H), 8.16 (d, 1H, J = 8.0 Hz), 10.56 (brs, 1H, –N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 25.5, 27.6, 34.5, 44.6, 58.2, 82.1, 109.7, 116.9, 118.6, 121.9, 124.2, 124.3, 125.3, 125.4, 125.5, 125.8, 126.0, 126.6, 127.6, 128.6, 128.7, 129.1, 130.9, 132.7, 132.9, 133.3, 133.8, 135.0, 142.4, 155.1, 177.4; MS m/z = 418 $[M+H]^+$; Anal. Calcd for $C_{28}H_{23}N_3O$: C, 80.55; H, 5.55; N, 10.06. Found: C, 80.65; H, 5.44; N, 10.17.

*4.1.2.8. 3′-Amino-1′-(furan-3-yl)-2-oxo-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6h**).* White solid; mp 236–238 °C (Decomposes); IR (cm^{-1}): 754, 1476, 1583, 1633, 1702, 2199, 2930, 3279, 3422; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (q, 1H, J = 12.4 Hz), 1.40–1.48 (m, 2H), 1.70–1.73 (m, 1H), 2.03–2.21 (m, 2H), 2.97 (d, 1H, J = 12.0 Hz), 3.17–3.24 (m, 1H), 5.44 (brs, 2H, –N*H*$_2$, D_2O exchangeable), 5.55–5.57 (m. 1H), 6.07 (s, 1H), 6.62 (d, 1H, J = 7.7 Hz) 6.81 (s, 1H), 6.97 (t, 1H, J = 7.5 Hz), 7.12–7.19 (m, 1H), 7.36 (s, 1H), 10.44 (brs, 1H, –N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 25.5, 28.3, 32.6, 44.1, 57.2, 81.8, 110.1, 116.8, 118.5, 121.2, 122.4, 124.2, 129.3, 130.2, 132.1, 141.2, 143.1, 143.3, 154.0, 177.1; MS m/z = 358 $[M+H]^+$; Anal. Calcd for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76. Found: C, 73.85; H, 5.45; N, 11.67.

*4.1.2.9. 3′-Amino-2-oxo-1′-(thiophen-2-yl)-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6i**).* White solid; mp 267–269 °C (Decomposes); IR (cm^{-1}): 696, 1203, 1476, 1578, 1629, 1701, 2200, 2935, 3278, 3423; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (q, 1H, J = 12.2 Hz), 1.39–1.42 (m, 2H), 1.69–1.71 (m, 1H), 2.07–2.21 (m, 2H), 3.30 (d, 1H, J = 10.8 Hz), 3.46–3.52 (m, 1H), 5.44 (brs, 2H, –N*H*$_2$, D_2O exchangeable), 5.57–5.59 (m. 1H), 6.57 (d, 2H, J = 7.6 Hz), 6.72 (s, 1H) 6.96 (t, 1H, J = 7.4 Hz), 7.10 (t, 1H, J = 7.6 Hz), 7.15–7.16 (m, 1H), 7.27 (d, 1H, J = 7.4 Hz), 10.48 (brs, 1H, –N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 25.5, 28.1, 34.6, 48.9, 57.9, 81.8, 110.1, 117.0, 118.4, 122.5, 124.6, 125.5, 126.7, 129.4, 129.7, 132.0, 140.3, 143.2, 153.9, 176.6; MS m/z = 374 $[M+H]^+$; Anal. Calcd for $C_{22}H_{19}N_3OS$: C, 70.75; H, 5.13; N, 11.25. Found: C, 70.64; H, 5.02; N, 11.34.

*4.1.2.10. 3′-Amino-2-oxo-1′-(pyridin-2-yl)-6′,7′,8′,8a′-tetrahydro-1′H-spiro[indoline-3,2′-naphthalene]-4′-carbonitrile (**6j**).* White solid; mp 270–271 °C (Decomposes); IR (cm^{-1}): 751, 1199, 1475, 1574, 1627, 1699, 2199, 2830, 2937, 3325, 3424; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (q, 1H, J = 12.0 Hz), 1.00–1.03 (m, 1H), 1.35–1.38 (m, 1H), 1.63–1.66 (m, 1H), 2.05–2.20 (m, 2H), 3.28 (d, 1H,

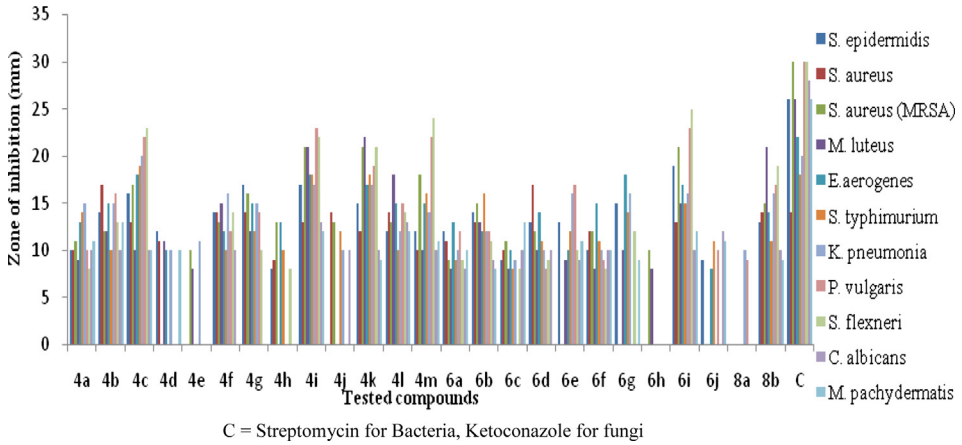


Fig. 3. Comparison of antimicrobial activity of synthesized compounds and standard drugs.

Table 6
MIC (μg/ml) of compounds tested against bacteria.

Compounds	Minimum inhibitory concentration (μg/ml)								
	Gram positive bacteria				Gram negative bacteria				
	S. epidermidis	*S. aureus*	*S. aureus* (MRSA)	*M. luteus*	*E. aerogenes*	*S. typhimurium*	*K. pneumonia*	*P. vulgaris*	*S. flexneri*
4a	500	250	250	1000	250	250	250	500	1000
4b	250	125	250	250	250	500	250	125	250
4c	250	250	125	250	125	125	62.5	62.5	62.5
4f	250	250	250	250	250	500	125	250	250
4g	125	250	125	250	250	250	250	250	250
4i	125	250	62.5	62.5	125	125	125	62.5	62.5
4k	125	250	62.5	62.5	125	125	125	125	62.5
4l	250	250	250	125	250	500	250	250	250
4m	250	250	125	250	250	125	250	62.5	62.5
6a	250	250	1000	1000	250	1000	500	250	1000
6b	250	250	250	250	250	125	250	250	250
6d	250	125	250	250	250	250	500	1000	1000
6e	250	NI	NI	1000	250	250	125	125	250
6f	500	250	250	1000	250	250	500	1000	1000
6g	125	NI	NI	250	125	250	125	NI	500
6i	125	250	62.5	250	125	250	125	62.5	62.5
8b	250	250	250	250	250	250	125	125	125
Streptomycin	6.25	6.25	6.25	6.25	25	30	6.25	6.25	6.25

NI – no inhibition.

J = 12.1 Hz), 3.53–3.61 (m, 1H), 5.39 (brs, 2H, –NH_2, D_2O exchangeable), 5.56–5.58 (m. 1H), 6.46 (d, 1H, J = 7.7 Hz), 6.63–6.74 (m, 1H) 6.86–6.89 (m, 1H), 6.99–7.03 (m, 2H), 7.24 (d, 1H, J = 7.3 Hz), 7.30–7.37 (m, 1H), 8.26–8.33 (m, 1H), 10.26 (brs, 1H, –N*H*, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 25.4, 27.8, 32.5, 54.8, 56.8, 81.7, 109.7, 116.4, 118.5, 121.9, 122.2, 124.9, 128.7, 129.2, 133.0, 135.7, 143.1, 148.8, 155.1, 157.9, 175.9; HRMS (ESI): Mass calculated for $C_{23}H_{21}N_4O$ $[M+H]^+$ 369.1710, found, $[M+H]^+$ 369.1717; Anal. Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 74.88; H, 5.36; N, 15.10.

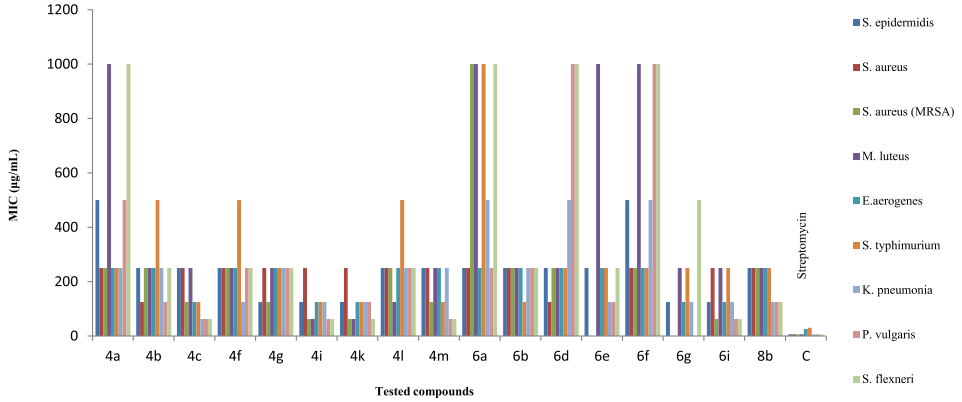


Fig. 4. Comparison of MIC (μg/ml) values of synthesized compounds and standard drugs.

Table 7
Anticancer activity of synthesised compounds against A549 cancer cell line and calculated binding energy with ALK receptor of synthesized spirocarbocycles.

Concentration (μg/mL)	Cell inhibition					
	4c		4i		6i	
	%	Mean ± S.D	%	Mean ± S.D	%	Mean ± S.D
5	3.7	0.341 ± 0.00406	12.1	0.311 ± 0.00435	25.7	0.263 ± 0.00491
10	10.5	0.317 ± 0.00296	28.1	0.255 ± 0.00648	45.8	0.192 ± 0.00322
20	18.6	0.288 ± 0.00586	47.5	0.186 ± 0.00435	62.4	0.133 ± 0.00296
30	30.8	0.245 ± 0.00346	56.2	0.155 ± 0.00529	67.8	0.114 ± 0.00291
40	46.6	0.189 ± 0.00462	62.4	0.133 ± 0.00520	75.9	0.085 ± 0.00364
50	59.6	0.143 ± 0.00230	78.8	0.075 ± 0.00491	83.9	0.057 ± 0.00462
Free energy of binding (kcal/mol)	4c		4i		6i	
	−14.55		−16.13		−18.43	

*4.1.3. Experimental procedure for the synthesis of (**8a & 8b**)*

1,3-Cyclohaxanedione **7** (1 mmol), aldehydes **2** (1 mmol) **2a**–**b** were stirred in MeOH in the presence of L-proline (20 mol%) at room temperature (r.t.) for ten minutes followed by the addition of alkylidene malononitrile **3a** (1 mmol) at r.t. for 3 h. The solid precipitated out, and then filtered off and purified by recrystallization from ethanol to afford product **8a** and **8b** as white crystalline solid.

*4.1.3.1. 3′-Amino-2,6-dioxo-1′-p-tolyl-6′,7′,8′,8a′-tetrahydro-1′H-spiro[cyclohexane-1,2′-naphthalene]-4′-carbonitrile (**8a**).* White solid; mp 245–246 °C (Decomposes); IR (cm^{-1}):822, 1394, 1588, 1657, 1688, 1717, 2202, 2923, 3250, 3345, 3422; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.11 (q, 1H, J = 11.7 Hz), 0.57–0.65 (m, 1H), 1.29–1.32 (m, 2H), 1.42–1.48 (m, 1H), 1.59–1.64 (m, 1H), 1.95–2.13 (m, 4H), 2.24 (s, 3H), 2.47–2.52 (m, 1H), 2.73–2.82 (m, 1H), 2.89 (s, 2H), 5.52–5.54 (m, 1H), 5.91 (brs, 2H, −NH_2, D_2O exchangeable), 6.71 (d, 1H, J = 7.6 Hz), 6.83 (d, 1H, J = 8.0 Hz) 7.08 (d, 1H, J = 7.6 Hz), 7.16 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.6, 21.1, 22.1, 25.4, 27.5, 33.3, 54.9, 71.2, 83.1, 117.1, 118.2, 127.5, 129.9, 130.1, 131.3, 132.0, 133.9, 137.8, 154.3, 209.8, 210.9; HRMS (ESI): Mass calculated for $C_{23}H_{24}N_2NaO_2$ $[M+Na]^+$ 383.1730, found, $[M+Na]^+$ 383.1731 Anal. Calcd for $C_{23}H_{24}N_2O_2$: C, 76.64; H, 6.71; N, 7.77. Found: C, 76.75; H, 6.60; N, 7.68.

*4.1.3.2. 3′-Amino-1′-(4-methoxyphenyl)-2,6-dioxo-6′,7′,8′,8a′-tetrahydro-1′H-spiro[cyclohexane-1,2′-naphthalene]-4′-carbonitrile (**8b**).* White solid; mp 241–243 °C (Decomposes); IR (cm^{-1}): 830, 1262, 1512, 1591, 1659, 1684, 1715, 2204, 2951, 3246, 3346, 3416; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.24 (q, 1H, J = 13.3 Hz), 0.62 (q, 1H, J = 10.8 Hz), 1.32–1.38 (m, 2H), 1.49–1.55 (m, 1H), 1.63–1.66 (m, 1H), 1.99–2.16 (m, 4H), 2.53–2.58 (m, 1H), 2.76–2.84 (m, 1H), 2.88–2.91 (m, 2H), 3.72 (s, 3H), 5.52–5.54 (m, 1H), 5.93 (brs, 2H, −NH_2, D_2O exchangeable), 6.77 (d, 1H, J = 8.2 Hz), 6.87 (t, 2H, J = 7.8 Hz) 6.94 (d, 1H, J = 8.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.7, 22.1, 25.4, 27.5, 33.4, 54.4, 55.5, 83.1, 114.2, 115.3, 117.1, 118.2, 128.6, 128.7, 132.0, 132.4, 154.3, 159.3, 209.9, 211.0; HRMS (ESI): Mass calculated for $C_{23}H_{25}N_2O_3$ $[M+H]^+$ 377.1860, found, $[M+H]^+$ 377.1860; Anal. Calcd for $C_{23}H_{24}N_2O_3$: C, 73.38; H, 6.43; N, 7.44. Found: C, 73.47; H, 6.34; N, 7.54.

4.2. Biological assays

4.2.1. Materials and methods for antimicrobial activity

Streptomycin (Sigma) was used as positive control against bacteria. Ketoconazole (Himedia, Mumbai) was used as positive control against fungi.

4.2.2. Tested microbes

The following bacteria and fungi were used for the experiment. Bacteria; *S. flexneri* MTCC 1457, *M. luteus* MTCC 106, *E. aerogenes* MTCC 111, *S. aureus* MTCC 96, *K. pneumonia* MTCC 109, *S. epidermidis* MTCC 3615, *P. vulgaris* MTCC 1771, *S. typhimurium* MTCC 1251 and *S. aureus* (MRSA – methicillin resistant). The reference cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India-160 036; fungi: *M. pachydermatis* and *C. albicans* MTCC 227. All the cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

4.2.3. Preparation of inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton broth (MHB) (Himedia) for 24 h at 37 °C. 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 48–72 h.

4.2.4. Disc diffusion assay

Antimicrobial activities were carried out using disc diffusion method [22]. Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min and a specific amount of synthesised compound at 1 mg/disc was added to each disc separately. 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 solvents. Streptomycin was used as positive control against bacteria. Ketoconazole was used as positive control for fungi. The plates were incubated for 24 h at 37 °C for bacteria and for 48 h at 28 °C for fungi. Zones of inhibition were recorded in millimetres and the experiment was repeated twice.

4.2.5. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration studies of 17 compounds were performed according to the standard reference methods for antibacterial activity [23]. The required concentrations (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL, 15.62 μg/mL and 7.81 μg/mL) of the compound were 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 antifungal agent Ketoconazole for fungi and Streptomycin for bacteria were included in the assay 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.

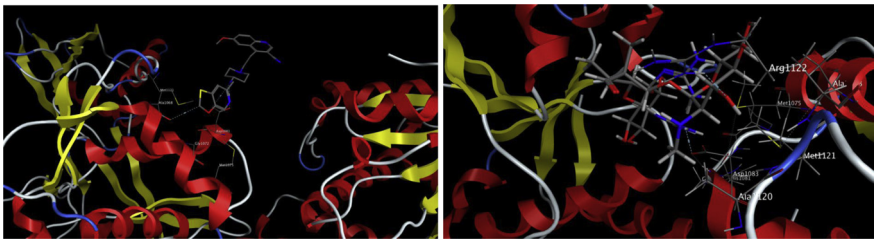


Fig. 5. Docking of co-crystallized ligand (**5a**) and standard drug Streptomycin with the DNA gyrase receptor for method validation (**5b**)

Fig. 6. Docking of co-crystallized ligand (crizotinib) with the ALK receptor for method validation.

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.

4.2.6. Cytotoxic properties

A549 lung adenocarcinoma cancer cell line was obtained from National Institute of Cell Sciences, Pune. A549 cell line was maintained in complete tissue culture medium Dulbecco's Modified Eagle's Medium with 10% Fetal Bovine Serum and 2 mM L-Glutamine, along with antibiotics (about 100 International Unit/mL of penicillin, 100 μg/mL of streptomycin) with the pH adjusted to 7.2. The cytotoxicity was determined according to the method of Balachandran et al. [24] with some changes. Cells (5000 cells/well) were seeded in 96 well plates containing medium with different

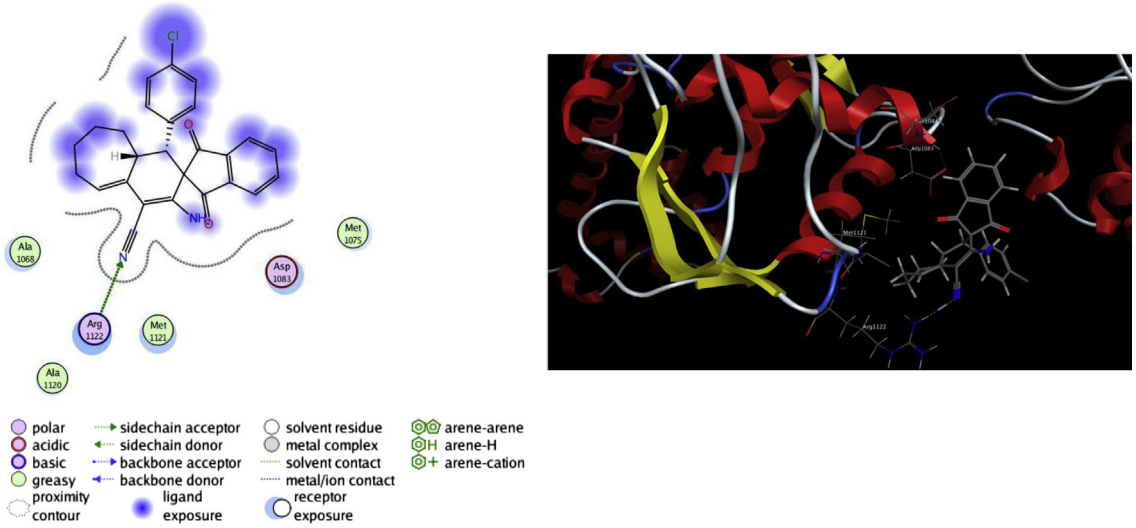


Fig. 7. 2D and 3D binding mode of most active compound **4i** (FEB = −11.64 kcal/mol) with DNA gyrase receptor.

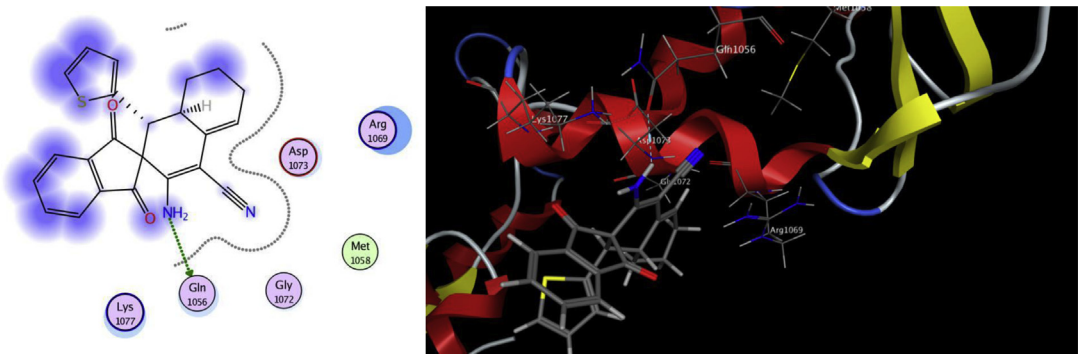


Fig. 8. 2D and 3D binding mode of moderate active compound **4m** (FEB = −9.51 kcal/mol) with DNA gyrase receptor.

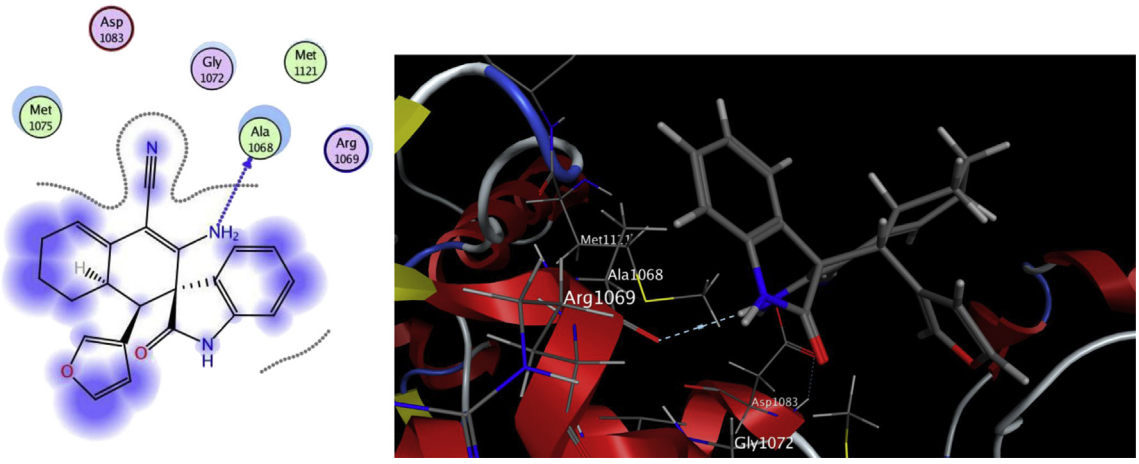


Fig. 9. 2D and 3D binding mode of least active compound **6h** (FEB = −8.14 kcal/mol) with DNA gyrase receptor.

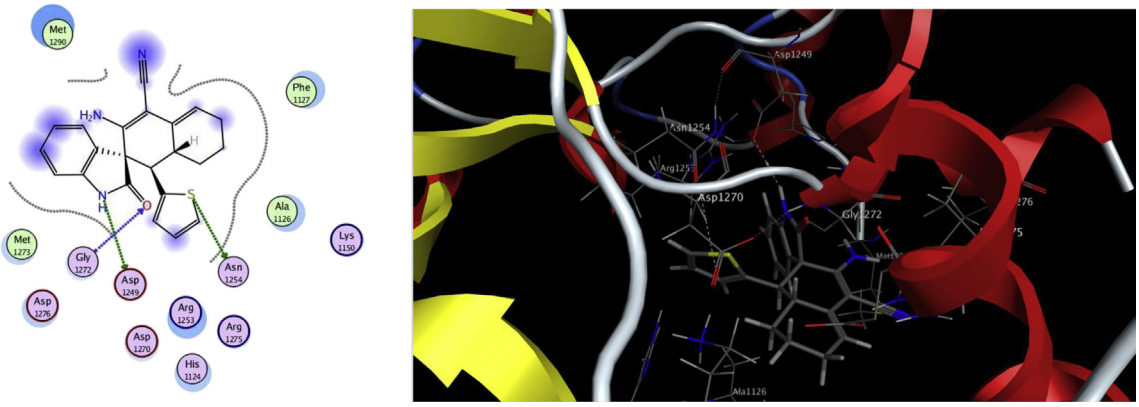


Fig. 10. 2D and 3D binding mode of most active compound **6i** (FEB = −18.43 kcal/mol) with ALK receptor.

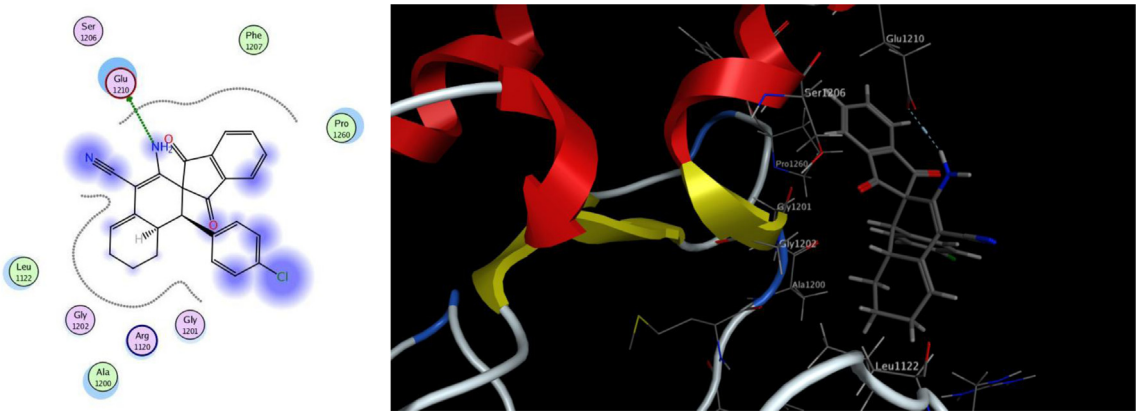


Fig. 11. 2D and 3D binding mode of intermediate active compound **4h** (FEB = −12.58 kcal/mol) with ALK receptor.

concentrations such as 50, 40, 30, 20, 10 and 5 μg/mL. The cells were cultivated at 37 °C with 5% CO_2 and 95% air in 100% relative humidity. After various durations of cultivation, the solution in the medium was removed. An aliquot of 100 μL of medium containing 1 mg/mL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide was loaded in the plate. The cells were cultured for 4 h and then the solution in the medium was removed. An aliquot of 100 μL of DMSO was added to the plate, which was shaken until the crystals were dissolved. The cytotoxicity against cancer cells was determined by measuring the absorbance of the converted dye at 540 nm in an Enzyme linked immune sorbant assay reader. Cytotoxicity of each sample was expressed as the half maximal inhibitory concentration (IC_{50}) value. The IC_{50} value is the concentration of test sample that causes 50% inhibition of cell growth, averaged from three replicate experiments.

4.3. Molecular docking studies

Protein and ligand preparation were carried out using MOE (Molecular Operating Environment) 2011 software tool version 7.1.

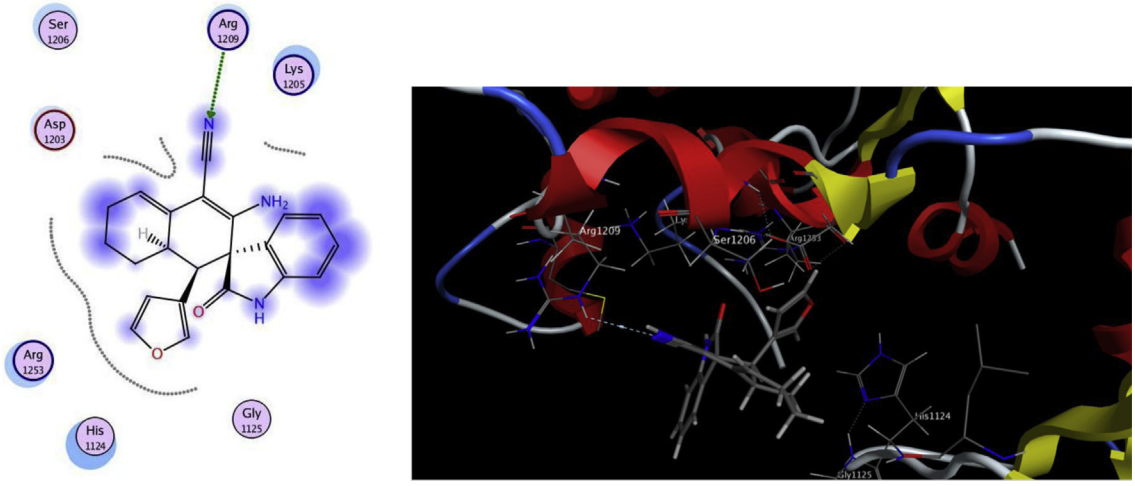


Fig. 12. 2D and 3D binding mode of least active compound **6h** (FEB = −11.23 kcal/mol) with ALK receptor.

A PDB entry 2XCS was selected for antibacterial docking study and was processed with MOE software. The structural issues such as capping, completing residues with missing atoms and selecting appropriate alternate locations were corrected automatically using structure preparation application available with the MOE software. An additional step was carried out to delete extraneous cofactors or unbound water and the co-crystallized ligand. The active site of protein was identified by using MOE's site finder application. Ligand 2D structures were drawn using ChemBioDraw Ultra 11.0 (ChemOffice 2008). Ligand 2D structures were converted to mol2 format using the file convertor Open Babel GUI. Energy minimization was performed to adjust hydrogen and lone pairs and to calculate partial charges. The Protein and ligand structures were energy minimized using of MMFF94x force field implemented in MOE. The energy was minimized to the minimum gradient of 0.05. The final refined poses were ranked by the MM/GBVI binding free energy estimation. The best docked representation of the ligand was chosen based on the conformation with lowest binding free energy out of 100 docking runs. The final docked conformations were within the range of 0.5–1 Å root-mean-square deviation.

For the docking of synthesized molecules to the ALK structure with the PDB id 2XP2, the same procedure followed as for the DNA gyrase receptor.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.065.

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application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 01223 336033 or email: deposit@ccdc.cam.ac.uk

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