# Accepted Manuscript


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

N. Sudhapriya , P.T. Perumal , C. Balachandran , S. Ignacimuthu , M. Sangeetha , Mukesh Doble

PII: S0223-5234(14)00489-9

DOI: 10.1016/j.ejmech.2014.05.065

Reference: EJMECH 7023

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 5 March 2014

Revised Date: 9 May 2014

Accepted Date: 26 May 2014

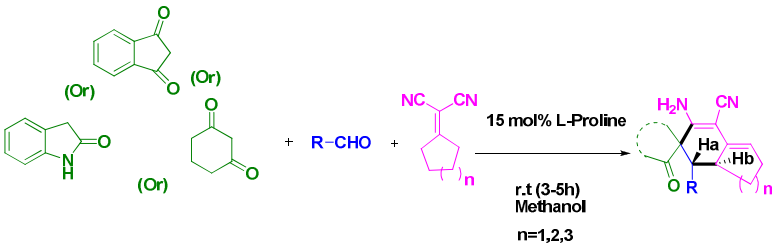
Please cite this article as: N. Sudhapriya, P.T. Perumal, C. Balachandran, S. Ignacimuthu, M. Sangeetha , M. Doble, Synthesis of new class of spirocarbocycle derivatives by multicomponent domino reaction and their evaluation for antimicrobial, anticancer activity and Molecular docking studies, *European Journal of Medicinal Chemistry* (2014), doi: 10.1016/j.ejmech.2014.05.065.

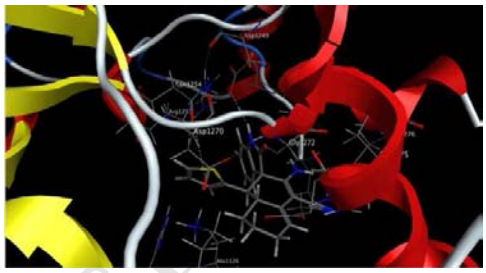
## Highlights

1. One pot synthesis of spirocarbocycles was developed using L-proline.
2. Ordinary reaction conditions, wide substrate scope, excellent yield (72-90%).
3. Most of the compounds exhibited moderate to good activity against bacteria.
4. Docking study of ligands with receptor was further supported by *in vitro* results.
5. Compound 6i showed very good affinity towards the ALK receptor.

# Graphical Abstract

Synthesis of spirocarbocycles was achieved by a three component reaction of cyclic nucleophiles, vinyl malononitriles and aldehydes with variable substitution patterns.



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

N. Sudhapriya[a], P.T. Perumal[a,]*, C. Balachandran[b], S. Ignacimuthu[b], M. Sangeetha [c], Mukesh Doble[c]

[a]*Organic Chemistry Division, CSIR-Central Leather Research Institute, Adyar, Chennai 600 020, India*

[b]*Division of Microbiology, Entomology Research Institute, Loyola College, Chennai 600 034, India*

[c]*Department of Biotechnology, Indian Institute of Technology, Chennai 600 036, India*

*Fax: +91 44 24911589. E-mail: ptperumal@gmail.com*

**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.

*Keywords*: Spirocarbocycle, multicomponent reaction, vinylogous Michael addition, antimicrobial activity, anticancer activity, Molecular docking.

*Corresponding author: Tel./fax: +91-44-24913289; email: ptperumal@gmail.com

## 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 [Figure 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.

<<**Figure 1**>>

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 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**).

<<**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.

<< **Table 1**>>

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.

<<**Scheme 2**>>

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-n** were isolated in excellent yields (79-90%) under milder reaction conditions. The results are summarized in **Table 2.**

<<**Scheme 3**>>

<<**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 (74-86%) 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 5)** were obtained in moderate yields (74-76%) and the results are mentioned in

**Table 4.**

<<**Scheme 4**>>

<<**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 carbonyls 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 ($D_2O$ 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** (**Figure 2**) [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 **3a** is completely diastereoselective in affording only the trans diastereomer. The structures of all spirocarbocycles were consistent with the above mentioned data.

<<**Figure 2**>>

<<**Table 4**>>

## 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 *E. aerogens, S. epidermidis, S. aureus* (MRSA), *S. typhimurium, K. pneumonia* and *M. luteus*. The results are summarized in **Table 5** and **Figure 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 1mg/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 *C. albican and M. pachydermatis*.

<<**Table 5**>>

<<**Figure 3**>>

The Minimum Inhibitory Concentration (MIC) values of active compounds against bacteria are given in **Table 6** and **Figure 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, P. vulgaris, S. 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.*

<<**Table 6**>>

<<**Figure 4**>>

### 2.2.2. Anticancer activity

Anti cancer 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).

<<**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 to 1 Å root-mean-square deviation [21]. Hence it was concluded that this method

could be used for the docking of other compounds **(Figure 5a& b).** We have also performed the docking of the standard ligand Streptomycin with the DNA gyrase receptor for method validation **(Figure 6).**

<<**Figure 5**>>

<<**Figure 6**>>

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. (**Figure 8)**

<<**Figure 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 **(Figure 8).** The least active compound **6h** has binding energy value -8.14 kcal/mol, and it shows interaction with ALA 1068 aminoacid **(Figure 9).**

<<**Figure 8**>>

<<**Figure 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 **(Figure10)**. 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 **Figure 11**. The least active compound **6h** has a binding energy of -11.23 kcal/mol. It interacts with ARG 1209 aminoacid. **(Figure 12)**

<<**Figure 10**>>

<<**Figure 11**>>

<<**Figure 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 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. Componds **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 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 Brucker Avance DPX-400 MHz instrument. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS,

δ = 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$^{o}$C (Decomposes); IR (cm$^{-1}$): 755, 1246, 1589, 1661, 1704, 1742, 2205, 2921 3246, 3345, 3408. $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ .0.71 (q, 1H, $J$ =12.4Hz), 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, $J$ = 12.8Hz), 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 (100MHz, 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.

*4.1.1.2.2 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 $^{o}$C (Decomposes); IR (cm$^{-1}$): 790, 1244, 1573, 1664, 1703, 1741, 2206, 2925, 3246, 3347 3406. $^{1}$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 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$_2$O exchangeable), 6.72 – 6.77 (m, 4H), 7.69 – 7.75 (m, 4H); $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 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 $^{o}$C (Decomposes); IR (cm$^{-1}$): 754, 1252, 1590, 1637, 1704, 1741, 2200, 2925, 3253, 3366, 3535. $^{1}$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 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 prton merged with solvent peak), 5.76 (t, 1H, $J$ = 6.2 Hz), 6.08(brs, 2H, NH$_2$, D$_2$O exchangeable)), 6.70 (d, 2H, $J$ = 8.4 Hz), 6.79 (d, 2H, $J$ =8.0Hz), 7.63-7.66 (m, 1H), 7.74-7.81 (m, 3H); $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 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 $^{o}$C (Decomposes) ; IR (cm$^{-1}$): 758, 1246, 1588, 1656, 1702, 1739, 2202, 2927, 3250, 3343, 3429. $^{1}$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 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, -N*H*$_2$, D$_2$O 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$_6$): $\delta$ 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 $^{o}$C (Decomposes) ; IR (cm$^{-1}$):1254, 1512, 1573, 1661, 1702, 1739, 2204, 2932, 3244, 3345, 3413; $^{1}$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 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, -N*H*$_2$, D$_2$O 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$_6$): $\delta$ 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 $^{o}$C (Decomposes) ; IR (cm$^{-1}$): 759, 1247, 1588, 1662, 1704, 1742, 2205, 2919, 3249, 3349, 3410; $^{1}$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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 (100 MHz, DMSO-$d_6$): $\delta$ 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 $^{o}$C (Decomposes); IR (cm$^{-1}$): 761, 1245, 1573, 1646, 1708, 1742, 2199, 2829, 3258, 3359, 3445; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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-$d_6$): $\delta$ 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 $^{o}$C (Decomposes); IR (cm$^{-1}$): 833, 1093, 1244, 1590, 1636, 1697, 1738, 2201, 2923, 3251, 3360, 3452; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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-$d_6$): $\delta$ 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 $^o$C (Decomposes); IR (cm$^{-1}$): 833, 1091, 1490, 1590, 1635, 1700, 1741, 2199, 2929, 3366, 3455; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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-$d_6$): $\delta$ 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 $^o$C (Decomposes); IR (cm$^{-1}$): 805, 1243, 1589, 1636, 1696, 1738, 2200, 2948, 3250, 3361, 3452; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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 $^{o}$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, -N*H*$_2$, $D_2O$ 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.

*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 $^{o}$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, -N*H*$_2$, $D_2O$ 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 $[MH]^+$; Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.48; H, 4.79; 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 $^oC$ (Decomposes); IR ($cm^{-1}$): 1244, 1587, 1662, 1704, 1741, 2205, 2923, 3250, 3347, 3411; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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-$d_6$): $\delta$ 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 $^oC$ (Decomposes); IR ($cm^{-1}$):752, 1191, 1388, 1580, 1627, 1702, 2200, 2926. 3057, 3299, 3329, 3422; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$,

$D_2O$ exchangeable), 5.58-5.60 (m. 1H), 6.37 (d, 1H, *J* = 8.8 Hz), 6.49 (d, 1H, *J* = 8Hz), 6.66 (d, 1H, *J* = 7.6 Hz), 6.93-7.00 (m, 2H), 7.06 (t, 1H, *J* = 7.6 Hz), 7.13 (d, 1H, J = 8Hz), 7.28 (d, 1H, J = 7.6 Hz), 10.35 (brs, 1H, -N*H*, $D_2O$ exchangeable); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 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 $^oC$ (Decomposes); IR ($cm^{-1}$):755, 1475, 1577, 1622, 1693, 1716, 2200, 2927, 3263, 3350, 3460; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N$H_2$, $D_2O$ 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-$d_6$): $\delta$ 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

*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 $^oC$ (Decomposes); IR ($cm^{-1}$): 833, 1185, 1266, 1512, 1573,1618, 1701, 2195, 2224, 2952, 3276, 3324, 3425; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ exchangeable), 5.57-5.59 (m. 1H), 6.39 (s, 2H), 6.50 (d, 1H, *J* = 7.6Hz), 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-$d_6$): $\delta$ 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 $^{o}$C (Decomposes); IR ($cm^{-1}$):753, 1203, 1227, 1489, 1577, 1625, 1701, 2198, 2831, 2939, 3335, 3427; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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-$d_6$): $\delta$ 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 $^{o}$C (Decomposes); IR ($cm^{-1}$): 747, 1487, 1579, 1629, 1725, 2195,

2923, 3334, 3453; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, -N*H*$_2$, $D_2O$ 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-$d_6$): $\delta$ 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 $^o$C (Decomposes); IR (cm$^{-1}$): 748, 1485, 1577, 1631, 1725, 2195, 2922, 3331, 3446; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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-$d_6$): $\delta$ 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 $^o$C (Decomposes); IR (cm$^{-1}$): 783, 1391, 1580, 1631, 1703, 2220, 2948, 3333, 3426; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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-$d_6$): $\delta$ 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 $^o$C. (Decomposes); IR (cm$^{-1}$): 754, 1476, 1583, 1633, 1702, 2199, 2930, 3279, 3422; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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-$d_6$): $\delta$ 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 $^o$C (Decomposes); IR (cm$^{-1}$): 696, 1203, 1476, 1578, 1629, 1701, 2200, 2935, 3278, 3423; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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-$d_6$): $\delta$ 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 $^{o}$C (Decomposes); IR (cm$^{-1}$): 751, 1199, 1475, 1574, 1627, 1699, 2199, 2830, 2937, 3325, 3424; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 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, *J* = 12.1 Hz), 3.53-3.61 (m, 1H), 5.39 (brs, 2H, -N*H*$_2$, $D_2O$ 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-$d_6$): $\delta$ 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.

*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 $^{o}$C (Decomposes); IR (cm$^{-1}$):822 1394, 1588, 1657, 1688, 1717, 2202, 2923, 3250, 3345, 3422; $^{1}$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 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, -N*H*$_2$, D$_2$O 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$_6$): $\delta$ 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 $^{o}$C (Decomposes); IR (cm$^{-1}$):830 1262, 1512, 1591, 1659, 1684, 1715, 2204, 2951, 3246, 3346, 3416; $^{1}$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 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, -N*H*$_2$, D$_2$O 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$_6$): $\delta$ 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; *Shigella flexneri* MTCC 1457, *Micrococcus luteus* MTCC 106, *Enterobacter aerogenes* MTCC 111, *Staphylococcus aureus* MTCC 96, *Klebsiella pneumoniae* MTCC 109, *Staphylococcus epidermidis* MTCC 3615, *Proteus vulgaris* MTCC 1771, *Salmonella typhimurium* MTCC 1251 and *Staphylococcus aureus* (MRSA- methicillin resistant). The reference cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India-160 036; fungi: *Malassesia pachydermatis* and *Candida 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 1mg/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 7.81µg/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 to 72 hours at 28°C and for bacteria the plates were incubated for 24 h at 37°C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 µl of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

*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 2mM 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 concentrations such as 50, 40, 30, 20, 10 and 5 µg/mL. The cells were cultivated at 37 °C with 5% CO2 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 (IC50) value. The IC50 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. 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 to 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**

N.S.P thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, India for the research fellowship and SAIF, IITM, Chennai for single crystal XRD analysis.

**References**

[1] (a) A.S. Girgis, Eur. J. Med. Chem. 44 (2009) 91-100.

(b) C. Young-Won, S. Angela, S. Bao-Ning, M. Qiuwen, C. Hee-Byung, R. Soedarsono, K. Leonardus, R. Agus, F. Norman, S. Steven, K. Douglas, J. Nat. Prod. 71 (2008) 390-395.

[2] R. Murugan, S. Anbazhagan, S. S. Narayanan, Eur. J. Med. Chem. 44 (2009) 3272-3279.

[3] S.A. Van der Sar, J.W. Blunt, M.H. Munro, Org. Lett. 8 (2006) 2059-2061.

[4] (a) P. Prasanna, K. Balamurugan, S. Perumal, P. Yogeeswari, D. Sriram, Eur. J. Med. Chem. 45 (2010) 5653-5661.

(b) S.V. Karthikeyan, B. D. Bala, V.P.A. Raja, S. Perumal, P. Yogeeswari, D. Sriram,

Bioorg. Med. Chem. Lett. 20 (2010) 350-353.

[5] K.M. Amin, M.M. Kamel, M.M. Anwar, M. Khedr, Y.M. Syam, Eur. J. Med. Chem. 45 (2010) 2117-2131.

[6] (a) M.J. Pawar, A.B. Burungale, B.K. Karale, Arkivoc Part XIII. (2009) 97-107.

(b) B. Thadhaney, D. Sain, G. Pernawat, G.L. Talesara, Indian J. Chem. 49B (2010) 368-373.

[7] (a)M. Li, F.M. Gong, L.R. Wen, Z.R. Li, Eur. J. Org. Chem. (2011) 3482-3490.

(b) S. Bruch, S.S.V. Berkel, B. Westermann, Chem. Soc. Rev. 42 (2013) 4948-4962.

[8] (a) Y.C. Chen, D. Xue, J.G. Deng, X. Cui, J. Zhu, Y.Z. Jiang, Tetrahedron Lett. 45(2004), 1555-1558.

(b) D. Xue, Y.C. Chen, X. Cui, Q.W. Wang, J. Zhu, J.G. Deng, J. Org. Chem. 70 (2005) 3584-3591.

[9] (a) D. Xue, Y.C. Chen, L.F. Cun, Q.W. Wang, J. Zhu, J.G. Deng, Org. Lett. 7 (2005) 5293-5296.

(b) J.W. Xie, L. Yue, D. Xue, X.L. Ma, Y.C. Chen, Y. Wu, J. Zhu, J.G. Deng, Chem. Commun. (2006) 1563-1565.

(c) J.W. Xie, W. Chen, R. Li, M. Zeng, W. Du, L. Yue, Y.C. Chen, Y. Wu, J. Zhu, , J.G. Deng, Angew. Chem. Int. Ed. 46 (2007) 389-392.

(d) T.B. Poulsen, C. Alemparte, K.A. Jørgensen, J. Am. Chem. Soc. 127 (2005) 11614-11615.

(e) T.B. Poulsen, M. Bell, K. A Jørgensen, Org. Biomol. Chem. 4 (2006) 2001-2011.

[10] (a) M.N. Elinson, A.I. Ilovaisky, V.M. Merkulova, F. Barba, B. Batanero,Tetrahedron,

69 (2013) 7125-7130.

(b) Chatani, Naoto, Hanafusa, Terukiyo, Bulletin of the Chemical Society of Japan, 63 (1990) 2134-2135.

(c) A.K. El-Shafei, A.A. Sultan, A.M. Soliman, E. A. Ahmed, Synthetic Commun, 25 (1995) 3211-3217.

[11] (a) J. Ficini, G. Revial, J.P. Genêt, Tetrahedron Lett. 22 (1981) 629-632.

(b) R. Kaiser, P. Naegeli, Tetrahedron Lett. 13 (1972) 2009-2012.

[12] B.M. Trost, D.C. Lee, J. Am. Chem. Soc. 110 (1988) 6556-6558.

[13] B. List, R.A. Lerner, C.F. Barbas, J. Am. Chem. Soc. 122 (2000) 2395-2396.

[14] (a) T.L. Liu, Z.L. He, H.Y. Tao, Y.P Cai, C.J. Wang, Chem. Commun. 47 (2011) 2616-2618.

(b) B.M. Trost, N. Cramer, S.M. Silverman, J. Am. Chem. Soc. 129 (2007) 12396-12397.

[15] (a) S. Chikaoka, A. Toyao, M. Ogasawara, O. Tamura, H. Ishibashi, J. Org. Chem. 68 (2003) 312-318.

(b) M. Inui, A. Nakazaki, S. Kobayashi, Org. Lett. 9(2007) 469-472.

(c) F. Gonzalez-Lopez de Turiso, D.P. Curran Org. Lett . 7 (2005) 151-154.

(d) T. Lanza, R. Leardini, M. Minozzi, D. Nanni, P. Spagnolo, G. Zanardi, Angew. Chem. Int. Ed. 47 (2008) 9439-9442.

[16] (a) A. Nandakumar, P. Thirumurugan, P.T. Perumal, P. Vembu, M.N. Ponnuswamy, P. Ramesh, Bioorg. Med. Chem. Lett. 20 (2010) 4252-4258.

(b) K. Karthikeyan, P.M. Sivakumar, M. Doble, P.T. Perumal, Eur. J. Med. Chem. 45 (2010) 3446-3452.

(c) G. Bhaskar, Y. Arun, C. Balachandran, C. Saikumar, P.T. Perumal, Eur. J. Med. Chem. 51(2012) 79-91.

[17] (a) T.H. Babu, P. Sujeet, D. Muralidharan, P.T. Perumal Synlett. 14 (2010) 2125-2129.

(b) T.H. Babu, A.J. Abragam, D. Muralidharan, P.T. Perumal, Tetrahedron Lett. 51 (2010) 994-996.

(c) T.H. Babu, P.T. Perumal, Synlett 3 (2010) 341-344.

[18] Crystallographic data for compound 6a in this paper have been deposited with the Cambridge Crystallographic Data centre as supplemental publication no. CCDC- 972722. Copies of the data can be obtained, free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 01223 336033 or email: deposit@ccdc.cam.ac.uk).

[19] B. Matjaz, P. Andrej, R. Miha, A. Gregor, T. Dusan J. Med. Chem. 55 (2012) 6413–6426.

[20] J.J. Cui, M. Tran-Dube, H. Shen, M. Nambu, P.P. Kung, M. Parish, L. Jia, J. Meng, L. Funk, I. Botrous, M. McTigue, N. Grodsky, K. Ryan, E. Padrique, G. Alton, S. Timofeevski, S. Yamazaki, Q. Li, H. Zou, J. Christensen, B. Mroczhowski, S. Bender , R. S. Kania, M. P. Edwards, J. Med. Chem. 54 (2011) 6342-6363.

[21] (a) C.R. Corbeil, C.I. Williams, P. Labute, J. Comput. Aided. Mol. Des. 26 (2012) 775-786.

(b) R. Ottana, R. Maccari, J. Mortier, A. Caselli, S. Amuso, G. Camici, A. Rotondo, G. Wolber, P. Paoli Eur. J. Med. Chem 71 (2014) 112-127.

[22] C. Balachandran, V. Duraipandiyan, N.A. Al-Dhabi, K. Balakrishna, N.P. Kalia, V.S. Rajput, I.A. Khan, S. Ignacimuthu, Indian J Microbiol 52 (2012) 676–681.

[23] C. Balachandran, V. Duraipandiyan, K. Balakrishna, R. Lakshmi Sundaram, A. Vijayakumar, S. Ignacimuthu, N.A. Al-Dhabi, Environ Chem Lett 11 (2013) 303-308.

[24] C. Balachandran, V. Duraipandiyan, S. Ignacimuthu. Asian Pac J Trop Biomed (2012) 712-716.

**Table, Figures and Schemes captions**

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

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

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

**Table 4.** Synthesis of spirocarbocycle derivatives from substituted aldehydes and alkylidene malononitrile.

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

**Table 5.** *In vitro* antimicrobial activity of synthesized compounds.

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

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

**Figure 1.** Some biologically important spirooxindole compounds.

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

**Figure 3.** Comparison of antimicrobial activity of synthesized compounds and standard drugs.

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

**Figure 5.** Docking of co-crystallized ligand and standard drug Streptomycin with the DNA gyrase receptor for method validation

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

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

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

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

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

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

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

**Scheme 1.** Synthesis of spirocarbocycle.

**Scheme 2.** Plausible mechanism for the formation of spirocarbocycles.

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

**Scheme 4.** Synthesis of spirooxindole derivatives from oxindole, substituted aldehydes and alkylidene malononitrile

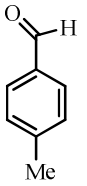
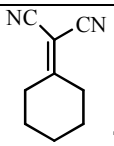
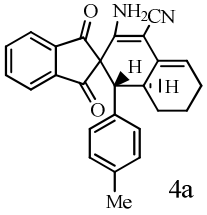
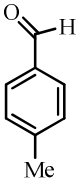
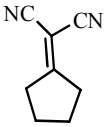
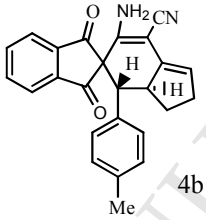
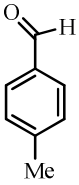
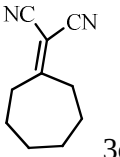
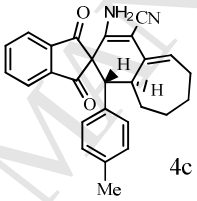
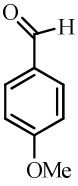
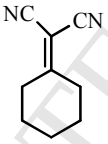
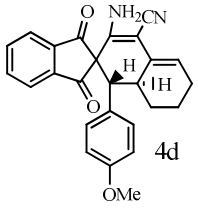
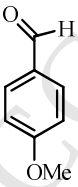
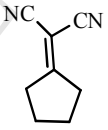
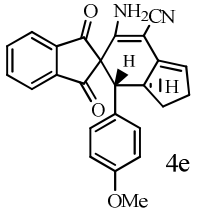
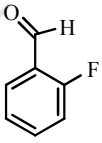
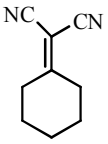
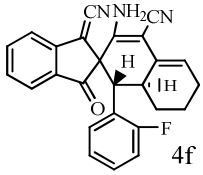
**Scheme 5.** Synthesis of spirocarbocycle derivatives from 1,3-cyclohexanedione, substituted aldehydes and alkylidene malononitrile**.**

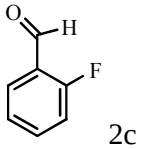
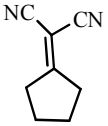
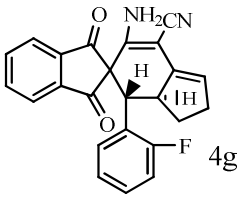
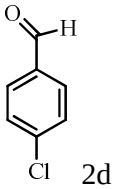
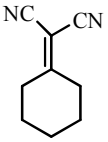
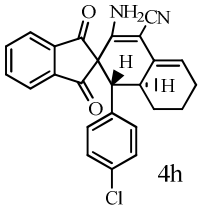
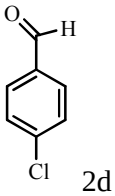
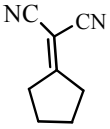
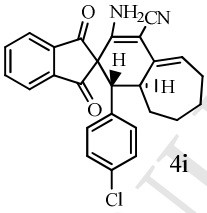
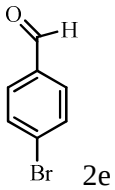
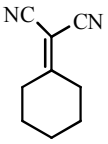
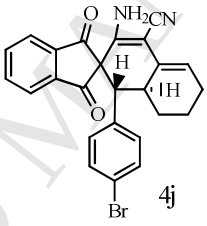
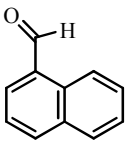
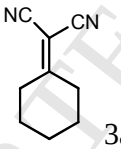
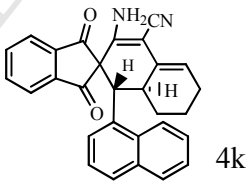
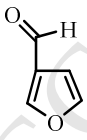
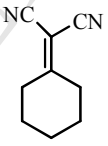
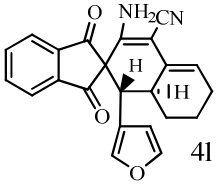
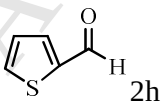
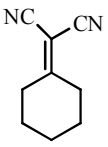
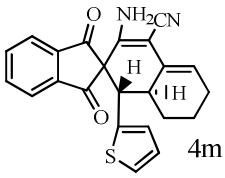
**Table 1**

| 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. | H2O | 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.

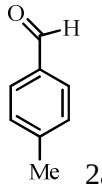
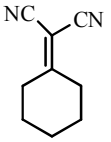
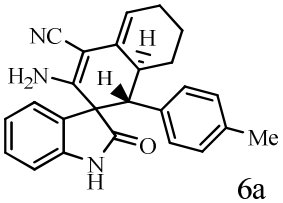
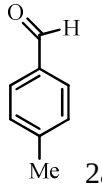
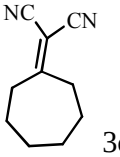
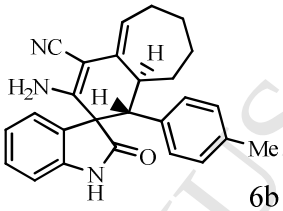
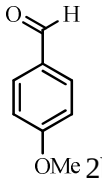
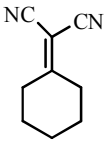
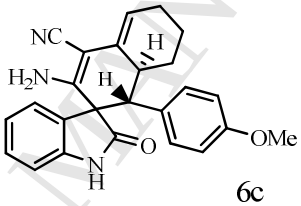
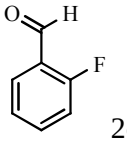
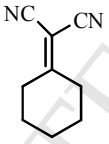
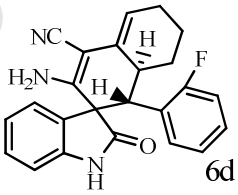
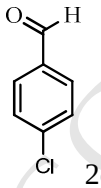
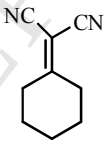
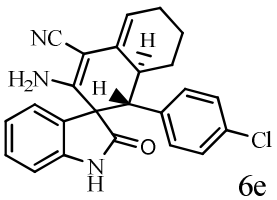
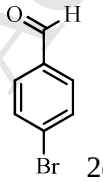
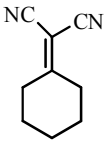
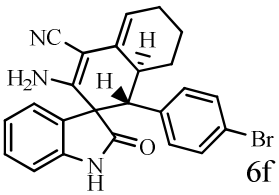
**Table 2**

| 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 |

| | | | | | |
|---|---|---|---|---|---|
| 7. |  2c |  3b |  4g | 3 | 83 |
| 8. |  2d |  3a |  4h | 6 | 82 |
| 9. |  2d |  3b |  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 |

1

**Table 3**

| 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 |

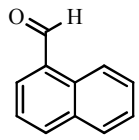
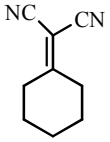
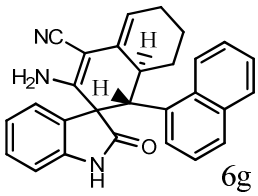
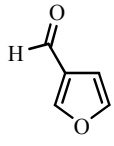
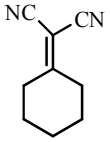
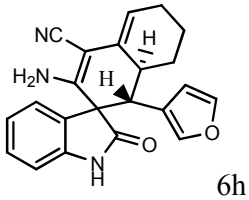
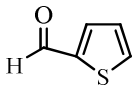
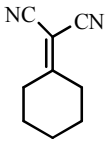
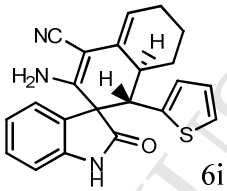
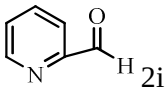
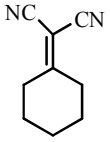
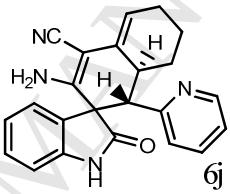
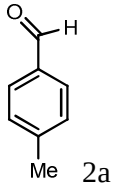
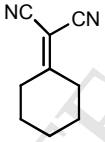
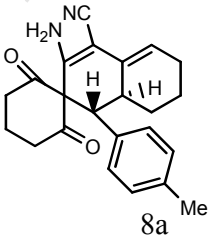
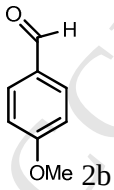
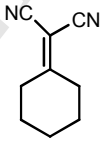
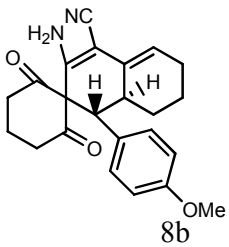
| | | | | | |
|---|---|---|---|---|---|
| 7. |  2f |  3a |  6g | 8 | 79 |
| 8. |  2g |  3a |  6h | 7.5 | 78 |
| 9. |  2h |  3a |  6i | 9 | 81 |
| 10. |  2i |  3a |  6j | 7 | 82 |
| 11. |  2a |  3a |  8a | 6 | 74 |
| 12. |  2b |  3a |  8b | 6.5 | 72 |

**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 |
| | $\alpha = \gamma = 90°$ |
| | $\beta$ = 93.3610(10)° |
| Volume | 1974.76(9) $A^3$ |
| Z, Calculated density | 4, 1.283 $Mgm^{-3}$ |
| Absorption coefficient | 0.080 $mm^{-1}$ |
| F(000) | 808 |
| Crystal size | 0.35 x 0.35 x 0.30 mm |
| $\theta$ range for data collection | 2.31 to 25.00° |
| Limiting indices | -11<=h<=11, -25<=k<=25, -11<=l<=11 |
| Reflections collected / unique | 18019 / 3486 [R(int) = 0.0335] |
| Completeness to $\theta$ = 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 |

**Table 5**

| Compounds | Zone of inhibition in mm | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | | Gram negative bacteria | | | | | Fungi | |
| | *S. epidermidis* | *S. aureus* | *S. aureus (MRSA)* | *M. luteus* | *E. aerogens* | *S. typhimurium* | *K. pneumonia* | *P. vulgaris* | *S. flexneri* | *C. albicans* | *M. pachyderm atis* |
| 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.

**Table 6**

| Compounds | Minimum inhibitory concentration (µg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | | Gram negative bacteria | | | | |
| | *S. epidermidis* | *S. aureus* | *S. aureus* (MRSA) | *M. luteus* | *E. aerogens* | *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 |

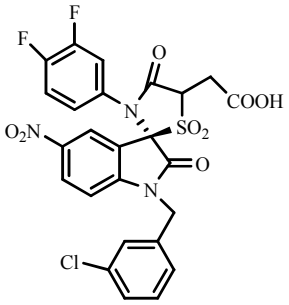
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4l | 250 | 250 | 250 | 125 | 250 | 500 | 250 | 250 | 250 |
| 4m | 250 | 250 | 125 | 250 | 250 | 125 | 250 | 62.5 | 62.5 |
| 6a | 250 | 250 | 1000 | 1000 | 250 | 1000 | 500 | 250 | 1000 |
| 6b | 250 | 250 | 250 | 250 | 250 | 125 | 250 | 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

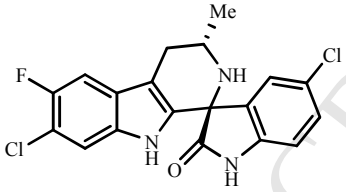
**Table 7**

| 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 | |

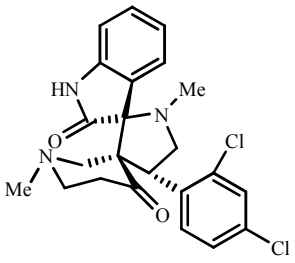
**Figure 1**



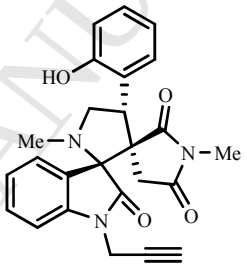

antituberculous agent




antimalarial agent




antimycobacterial agent




antimicrobial agent

**Figure 2**

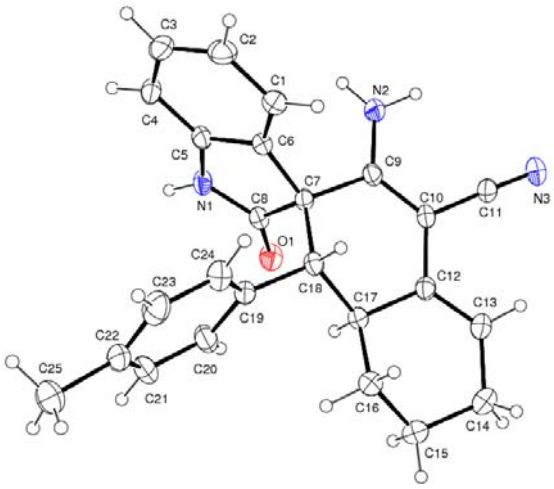
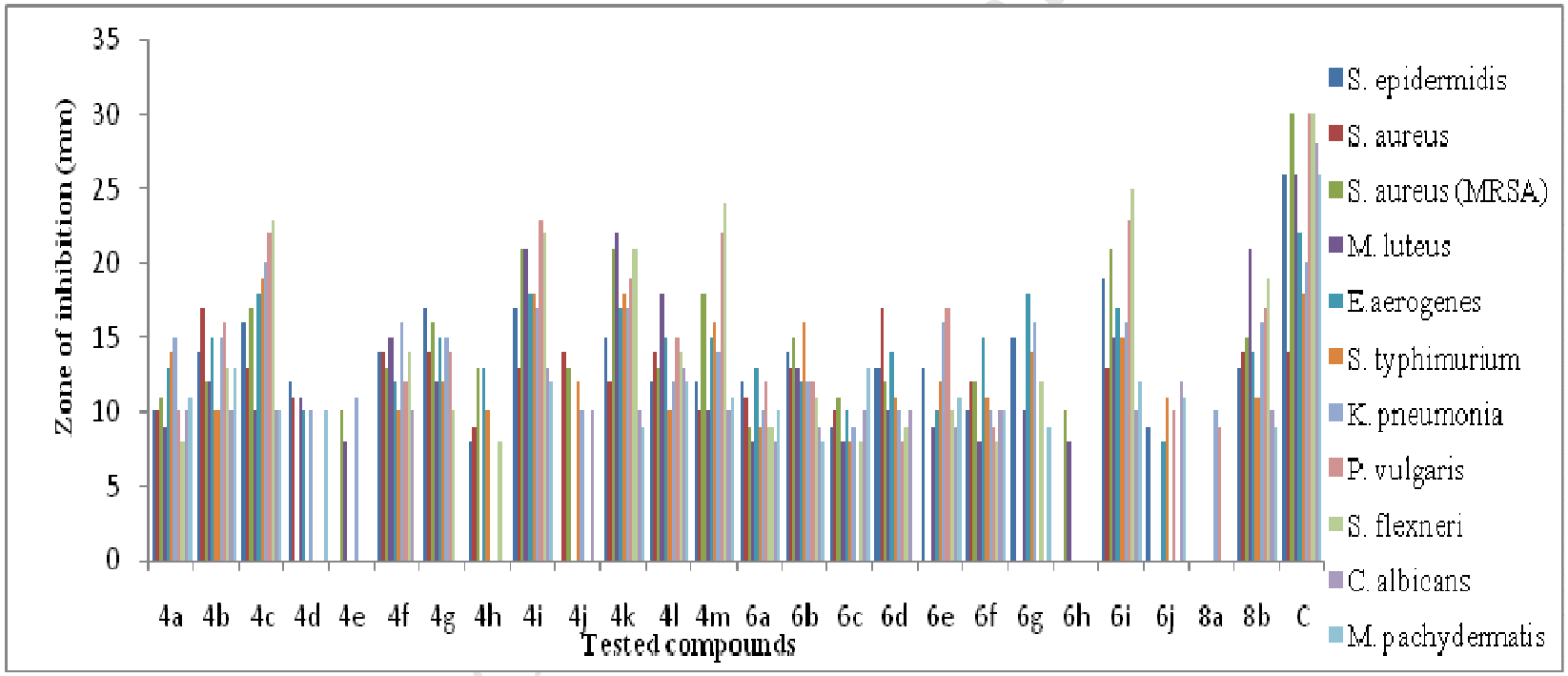
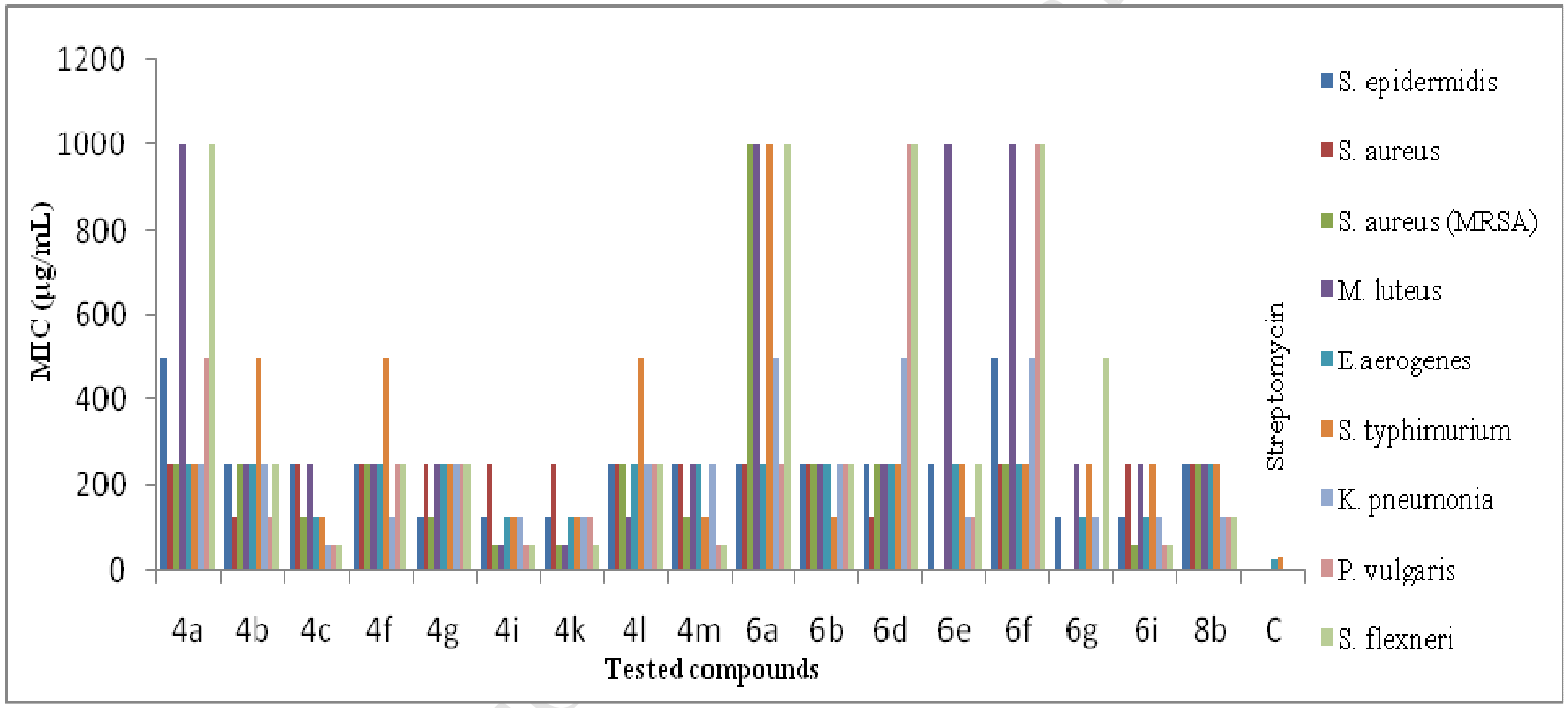


**Figure 3**




C = Streptomycin for Bacteria, Ketoconazole for fungi

**Figure 4**


1200
1000
800
600
400
200
0
MIC (µg/mL)
4a
4b
4c
4f
4g
4i
4k
4l
4m
6a
6b
6d
6e
6f
6g
6i
8b
C
Tested compounds
Streptomycin
S. epidermidis
S. aureus
S. aureus (MRSA)
M. luteus
E.aerogenes
S. typhimurium
K. pneumonia
P. vulgaris
S. flexneri

50

**Figure 5a & 5b**

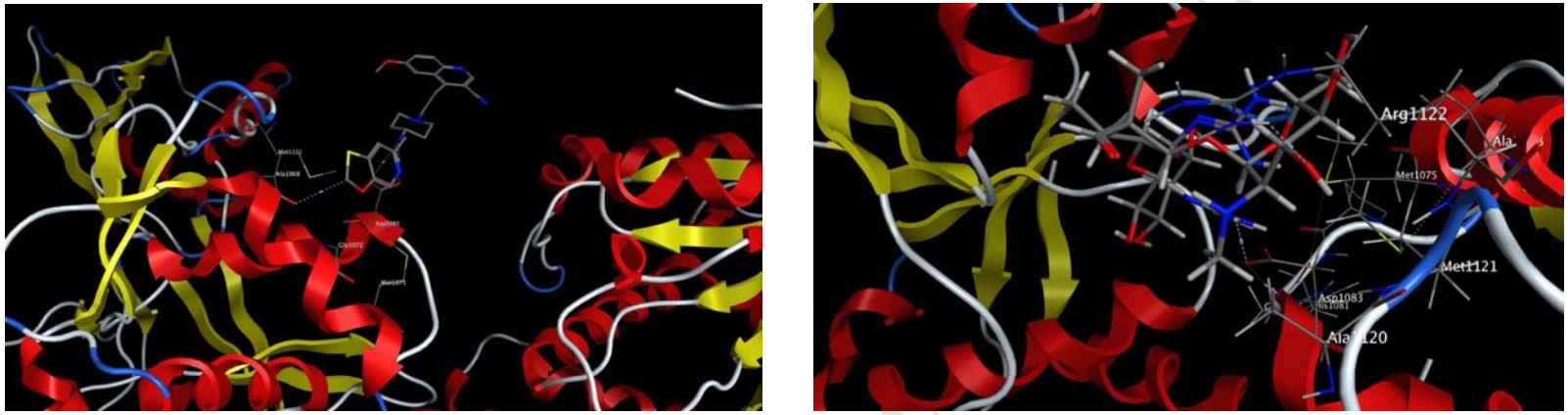



**Figure 6**

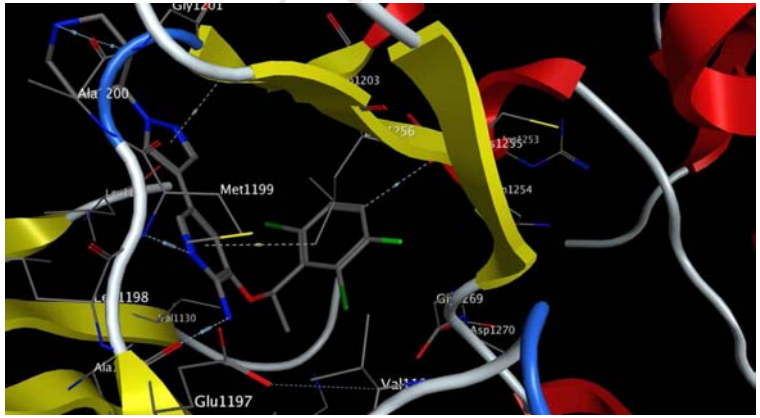
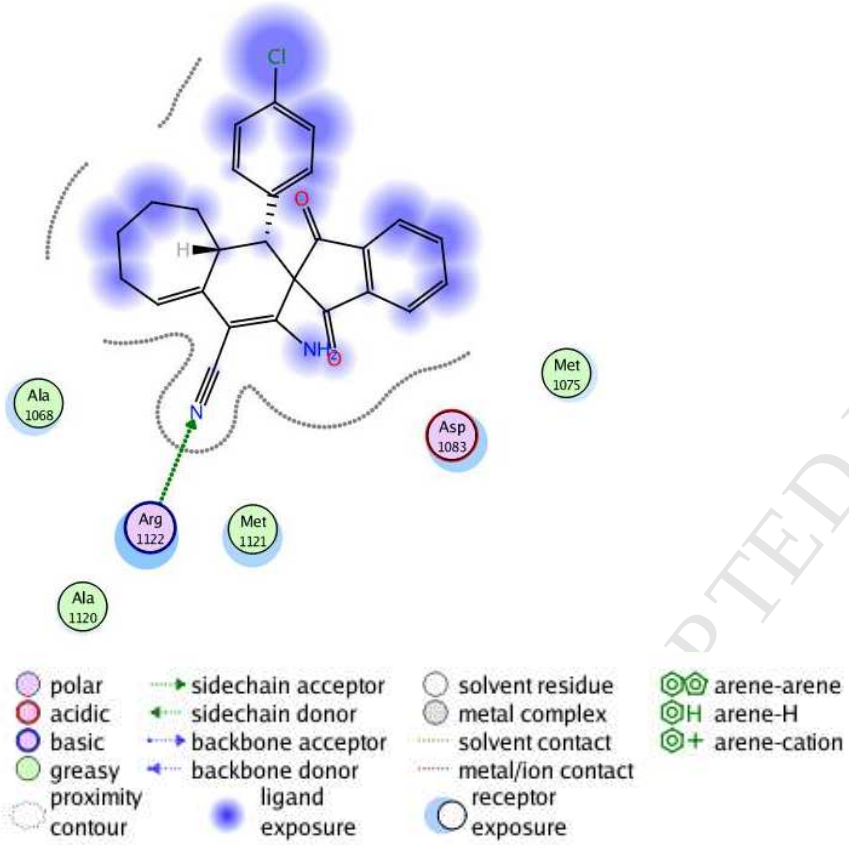


**Figure 7**


Cl
O
H
NH
O
N
Ala
1068
Met
1075
Asp
1083
Arg
1122
Met
1121
Ala
1120
polar
acidic
basic
greasy
proximity
contour
sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand
exposure
solvent residue
metal complex
solvent contact
metal/ion contact
receptor
exposure
arene-arene
arene-H
arene-cation

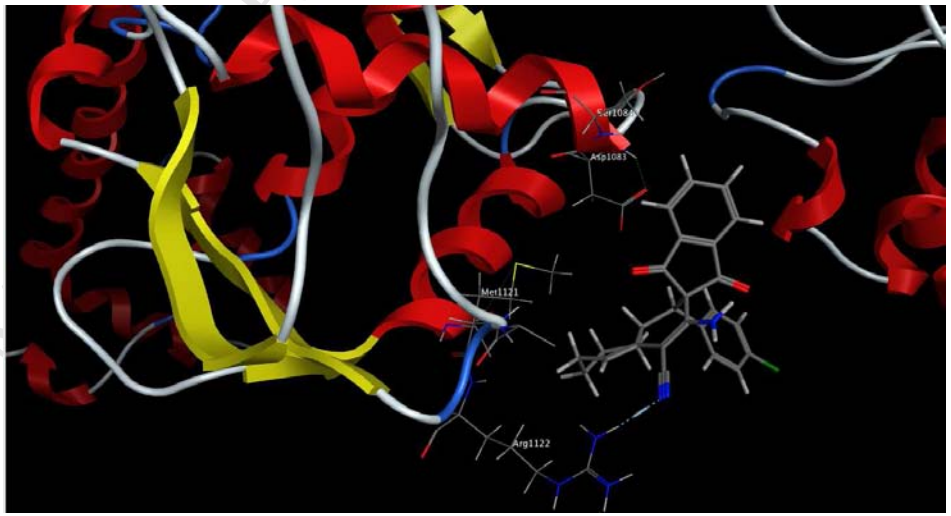

Asp1083
Met1121
Arg1122

**Figure 8**

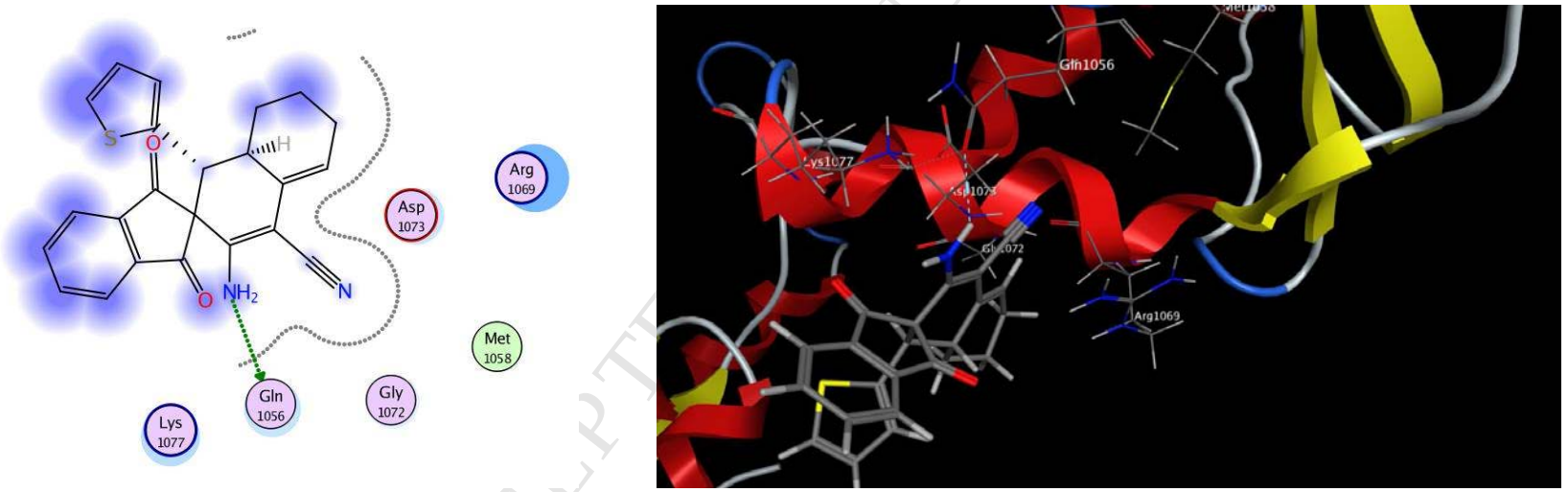

S
O
H
NH2
O
N
Arg
1069
Asp
1073
Met
1058
Gln
1056
Gly
1072
Lys
1077
Gln1056
Arg1069

**Figure 9**

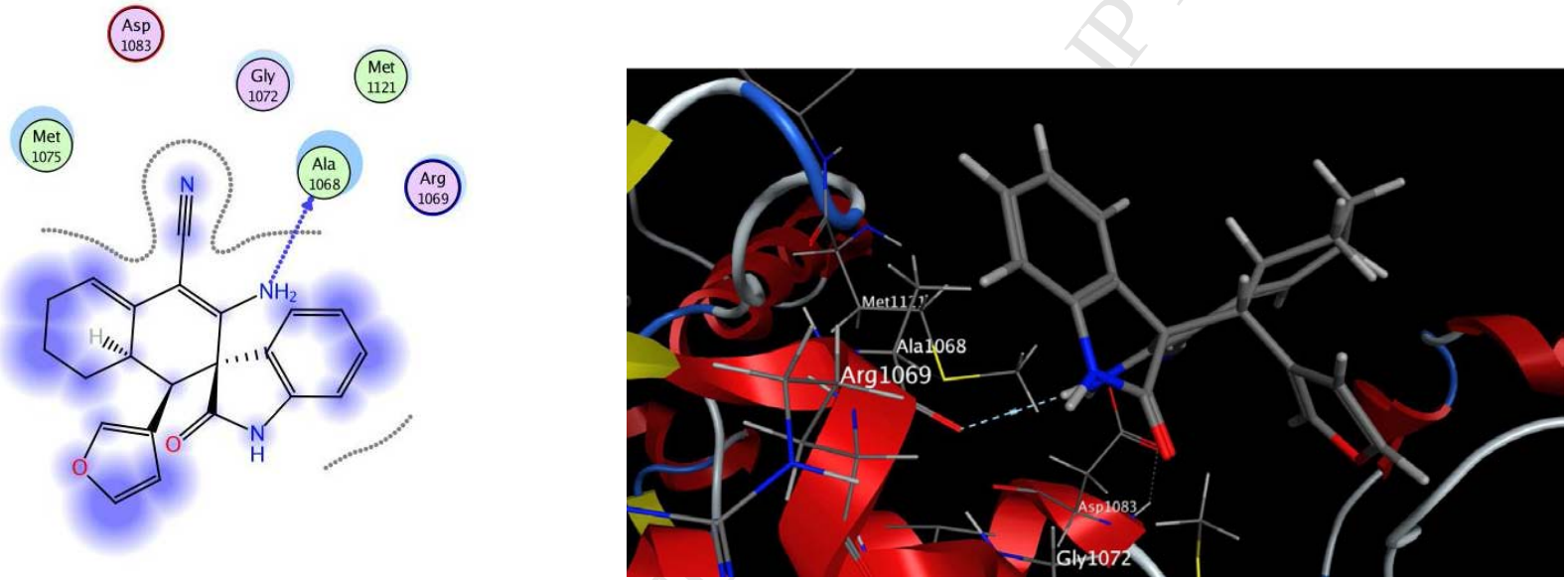
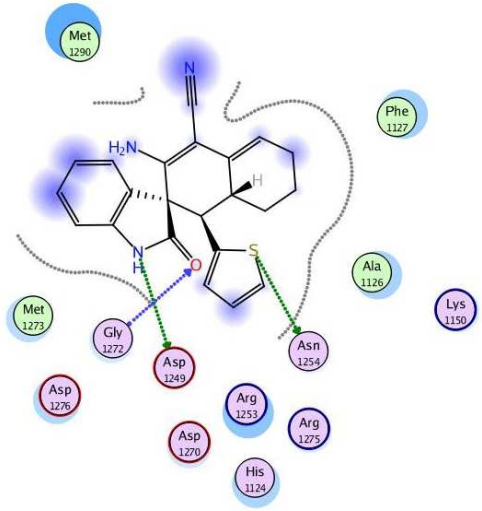


**Figure 10**



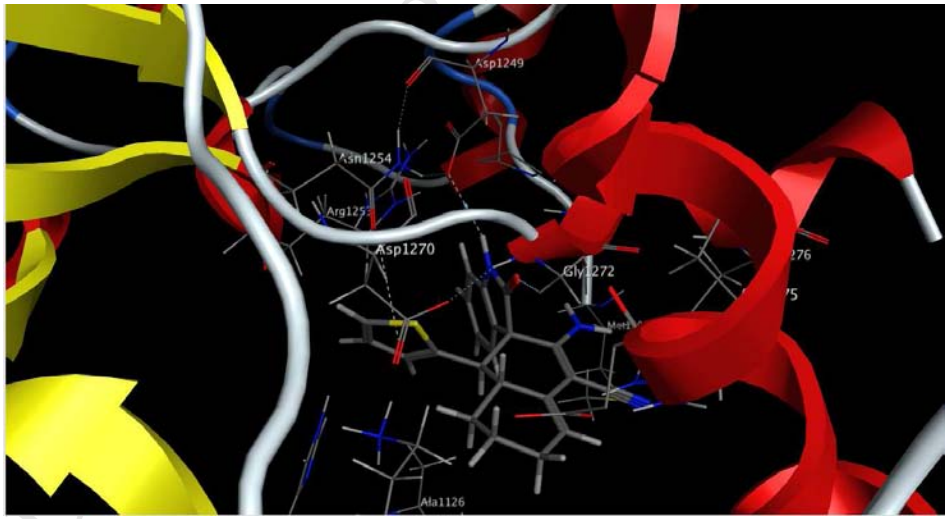



**Figure 11**

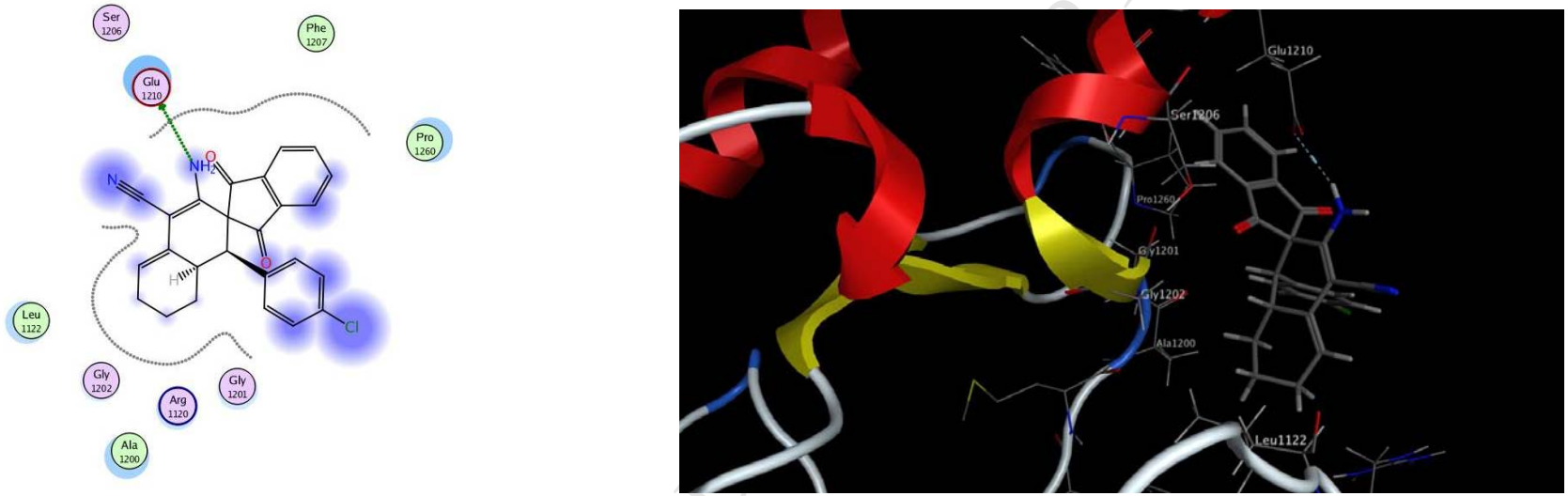
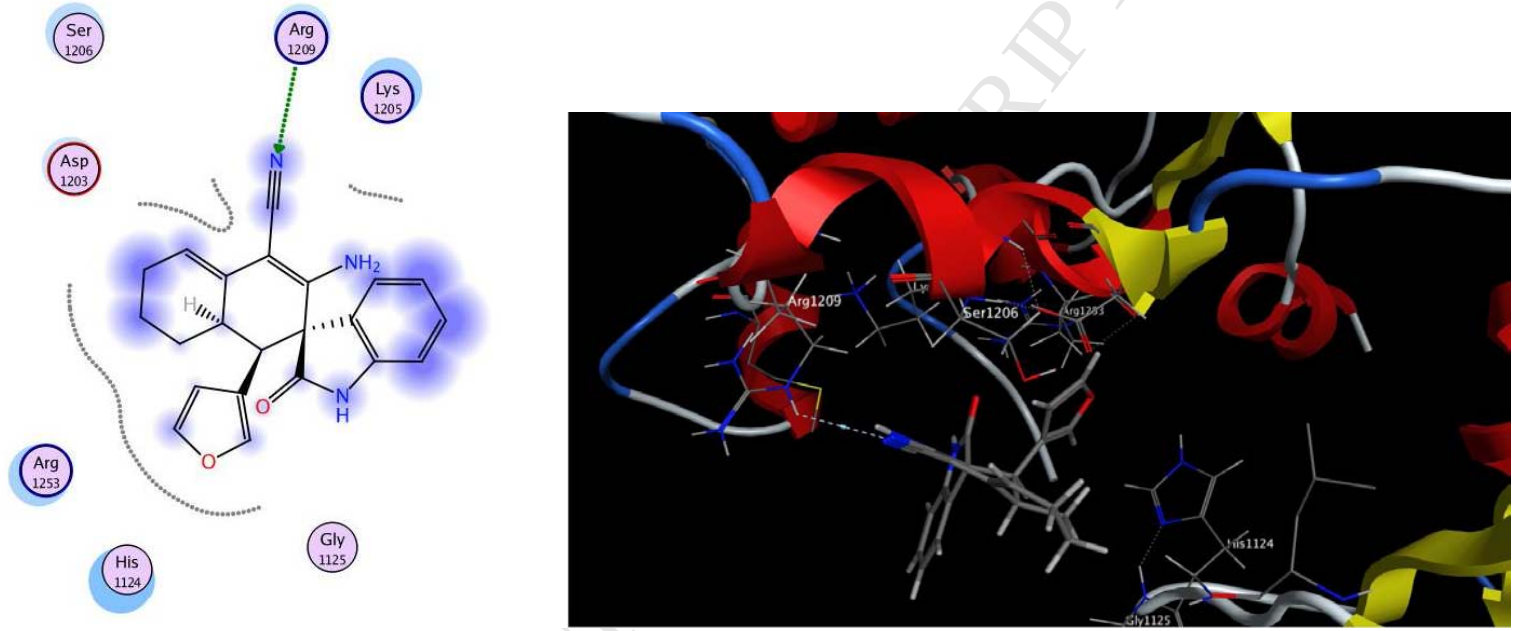
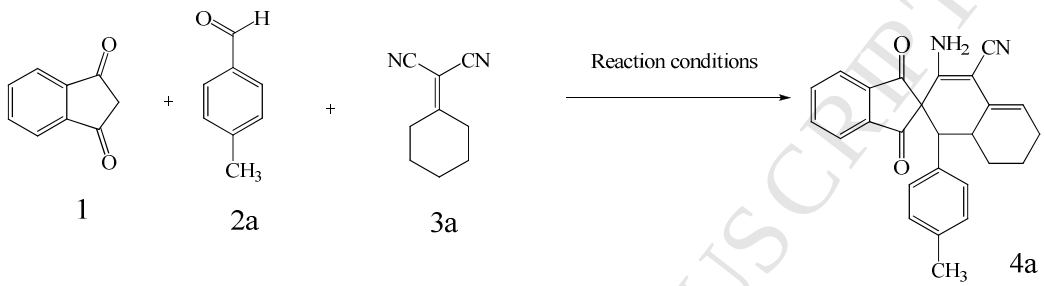


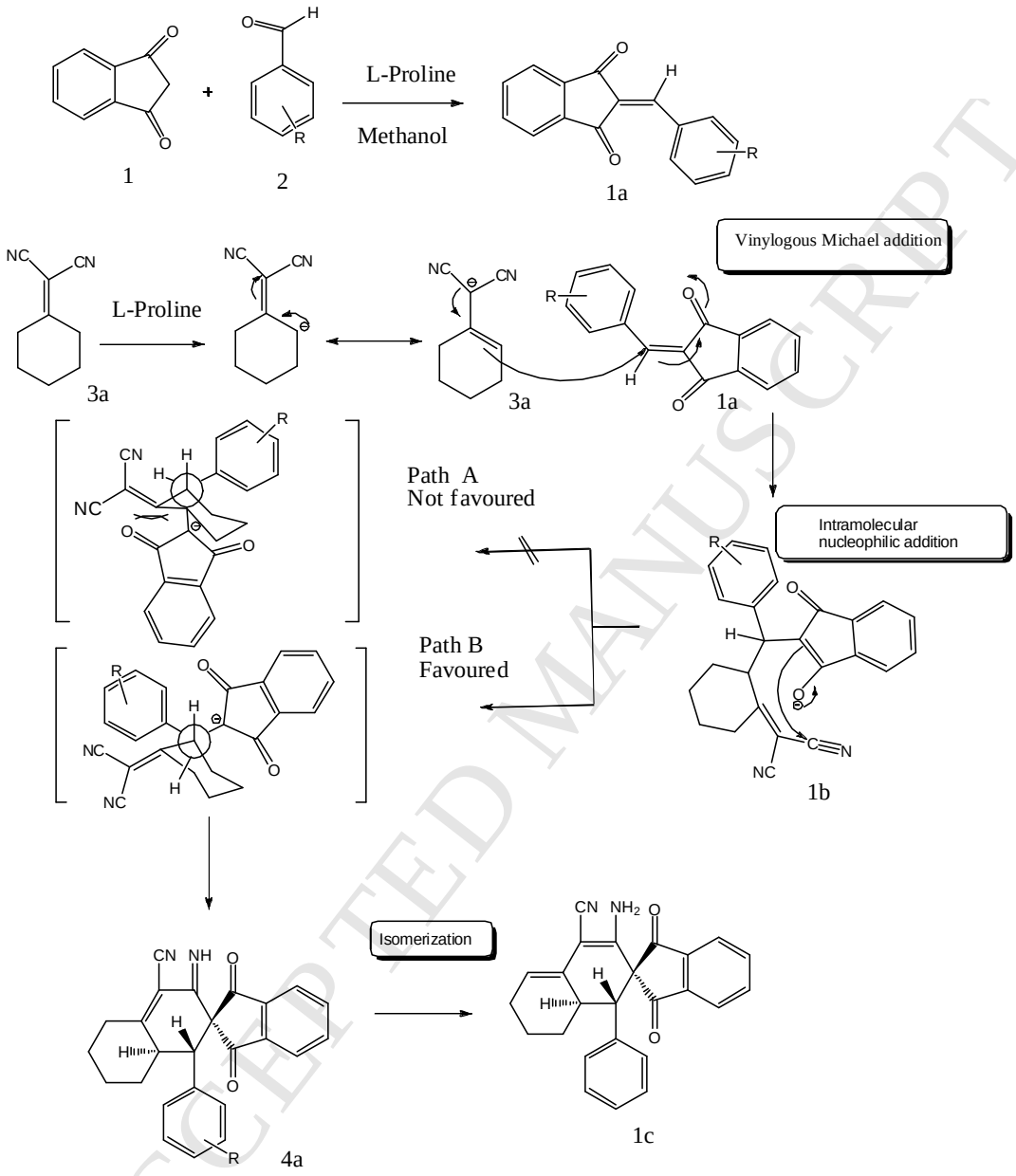
**Figure 12**



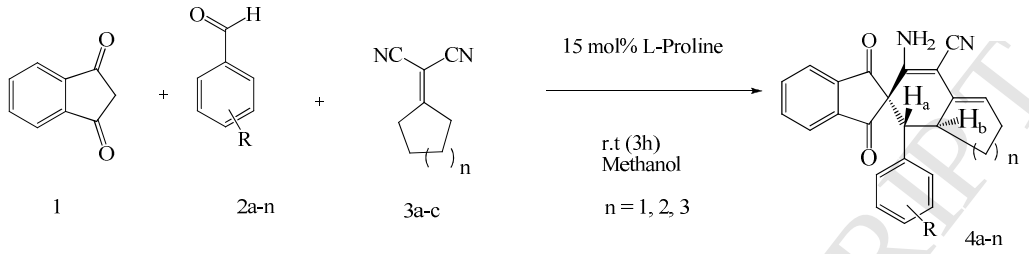
**Scheme 1**



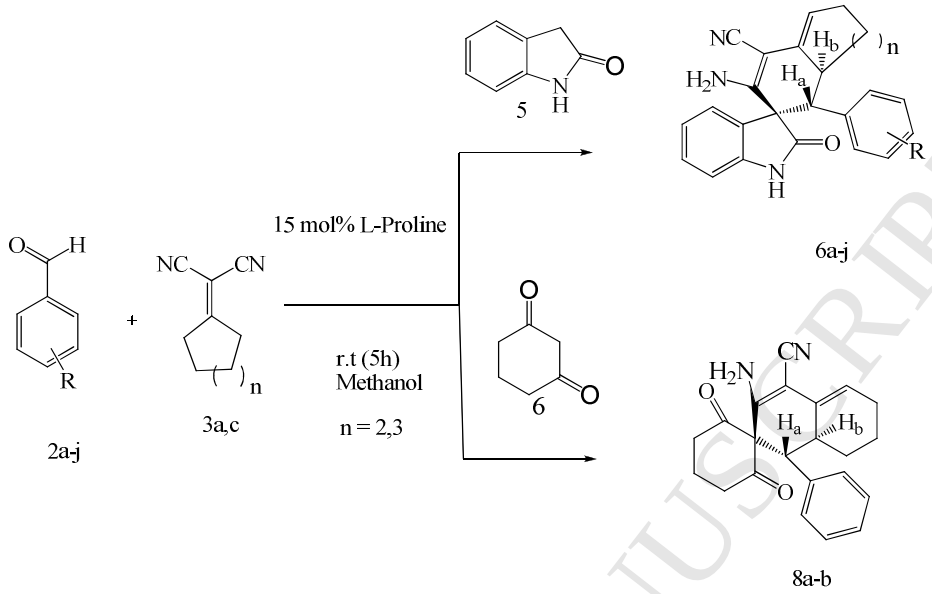
**Scheme 2**



**Scheme 3**



**Scheme 4**



**Figures and Schemes captions**

**Figure 1.** Some biologically important spirooxindole compounds.

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

**Figure 3.** Comparison of antimicrobial activity of synthesized compounds and standard drugs.

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

**Figure 5.** Docking of co-crystallized ligand and standard drug Streptomycin with the DNA gyrase receptor for method validation

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

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

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

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

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

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

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

**Scheme 1.** Synthesis of spirocarbocycle.

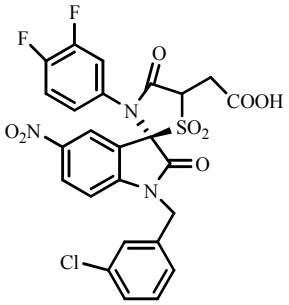
**Scheme 2.** Plausible mechanism for the formation of spirocarbocycles.

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

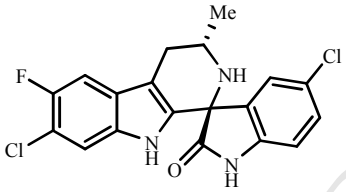
**Scheme 4.** Synthesis of spirooxindole derivatives from oxindole, substituted aldehydes and alkylidene malononitrile

**Scheme 5.** Synthesis of spirocarbocycle derivatives from 1,3-cyclohexanedione, substituted aldehydes and alkylidene malononitrile**.**

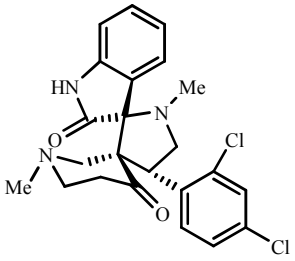
**Figure 1**



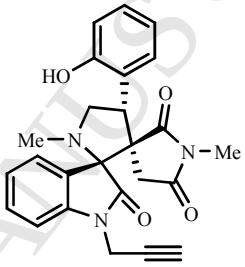

antituberculous agent




antimalarial agent




antimycobacterial agent




antimicrobial agent

**Figure 2**

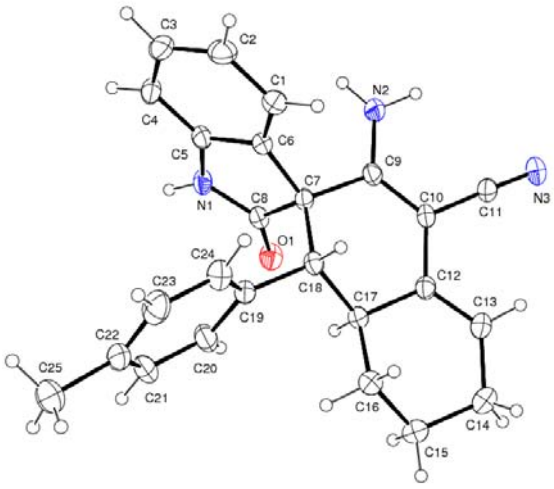
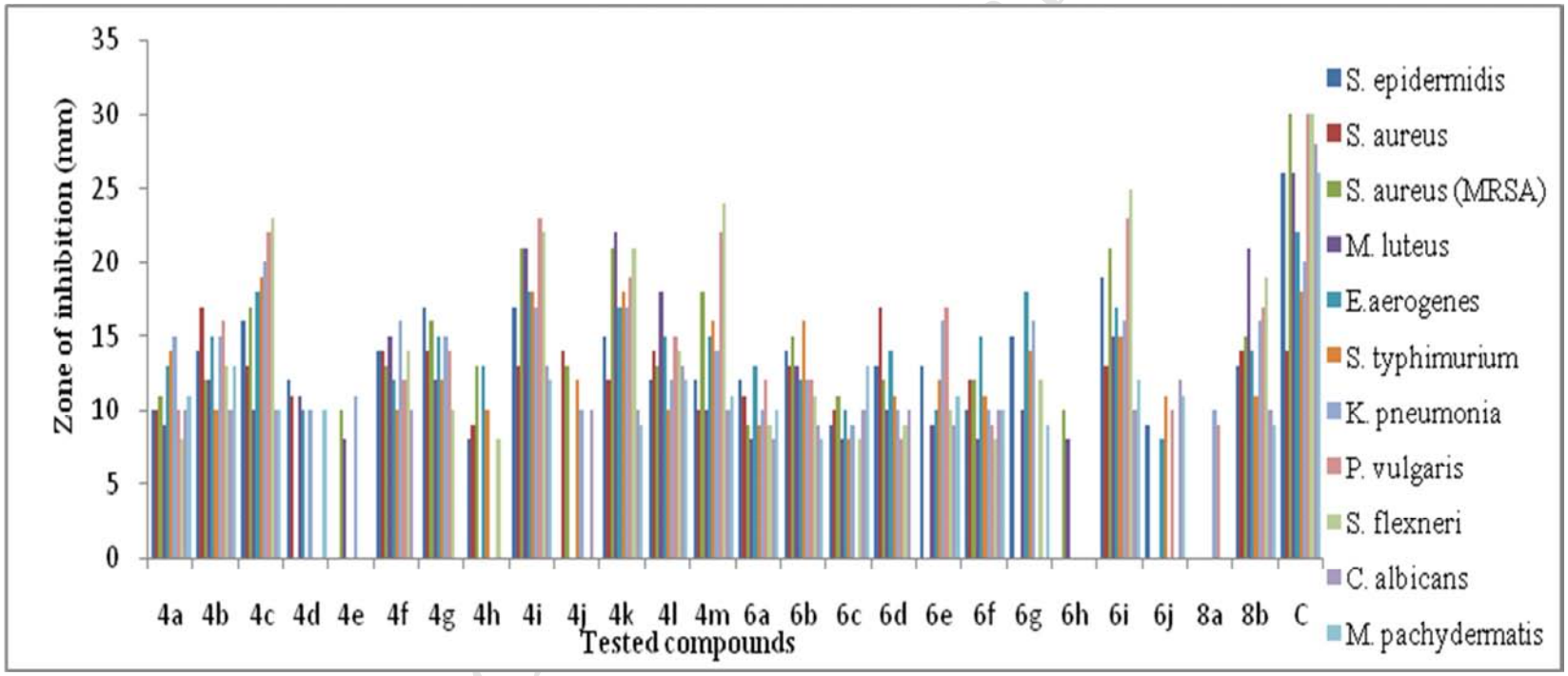


**Figure 3**




C = Streptomycin for Bacteria, Ketoconazole for fungi

**Figure 4**

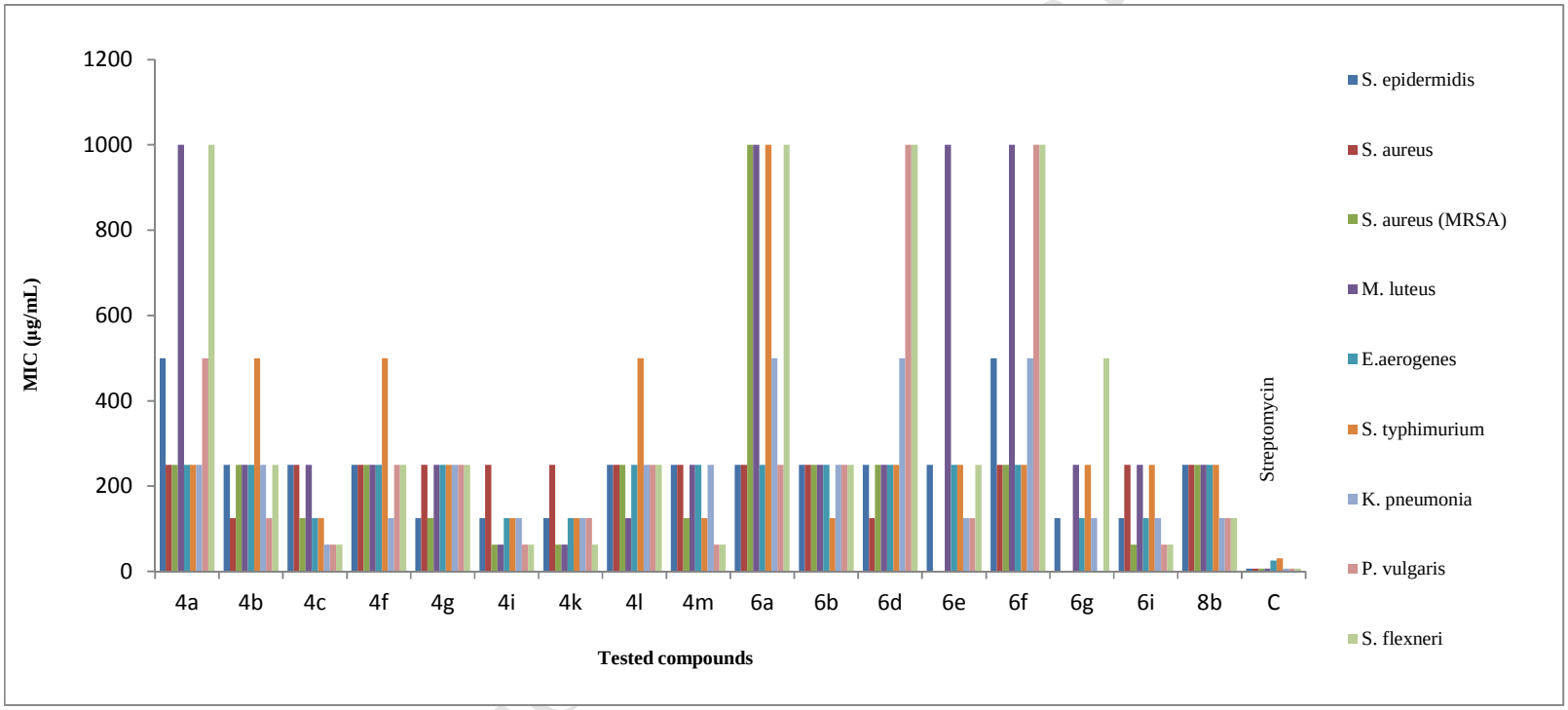

1200
1000
800
600
400
200
0
MIC (µg/mL)
4a
4b
4c
4f
4g
4i
4k
4l
4m
6a
6b
6d
6e
6f
6g
6i
8b
C
Streptomycin
Tested compounds
S. epidermidis
S. aureus
S. aureus (MRSA)
M. luteus
E.aerogenes
S. typhimurium
K. pneumonia
P. vulgaris
S. flexneri

**Figure 5a & 5b**

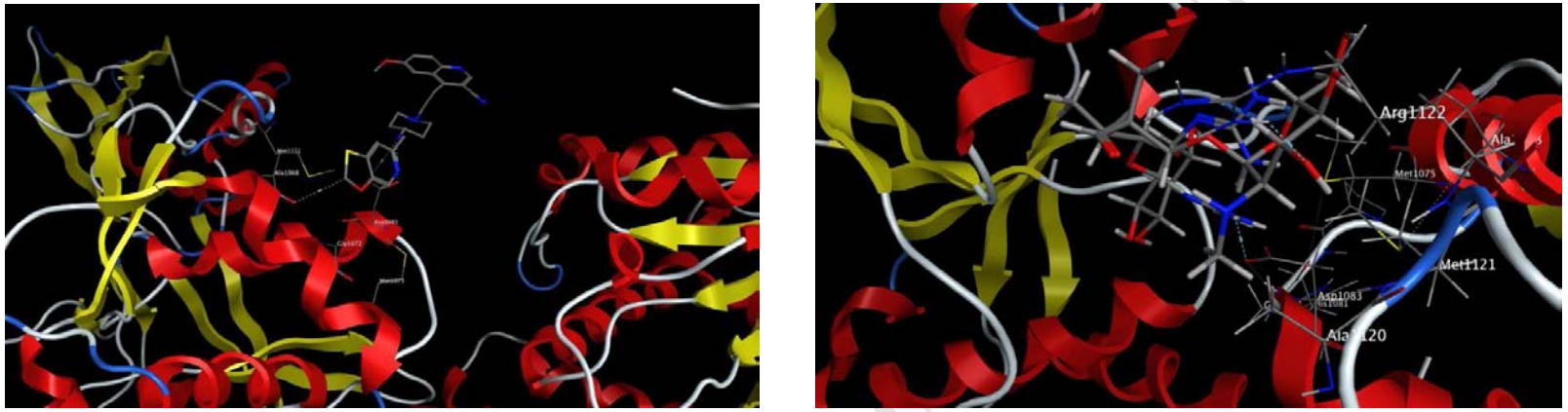
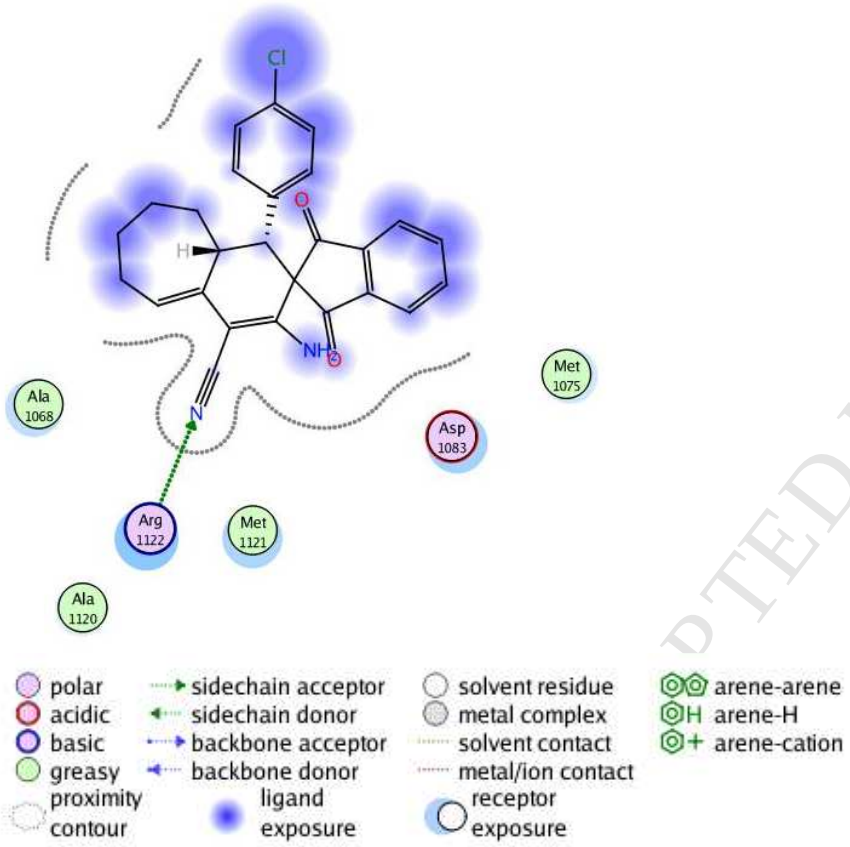



**Figure 6**

**Figure 7**


Cl
O
H
NH
N
Ala
1068
Met
1075
Asp
1083
Arg
1122
Met
1121
Ala
1120
polar
acidic
basic
greasy
proximity
contour
sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand
exposure
solvent residue
metal complex
solvent contact
metal/ion contact
receptor
exposure
arene-arene
arene-H
arene-cation

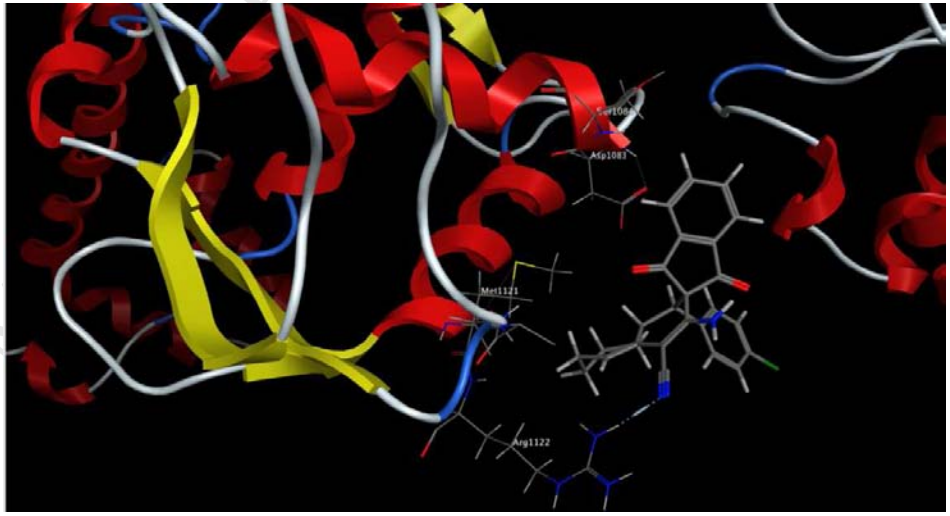
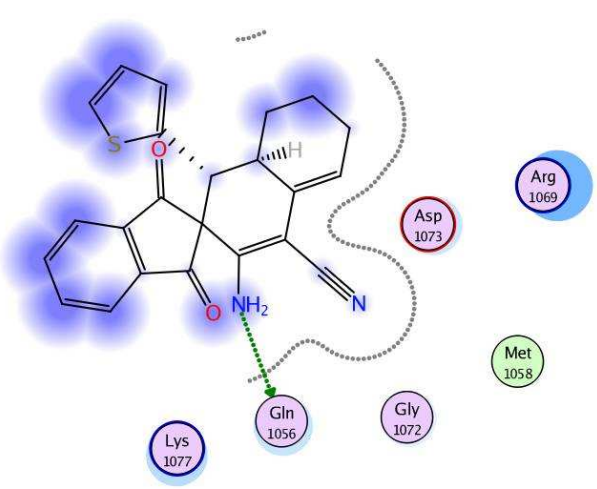


**Figure 8**



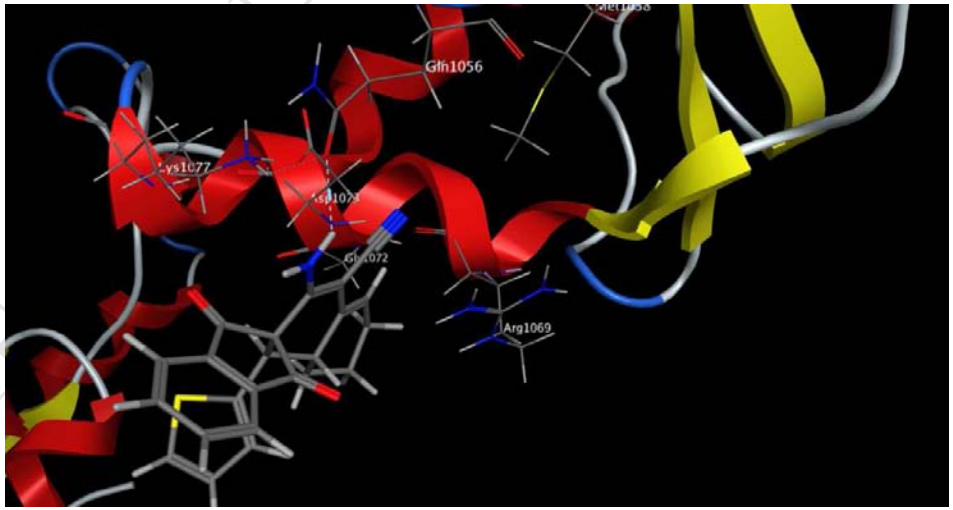
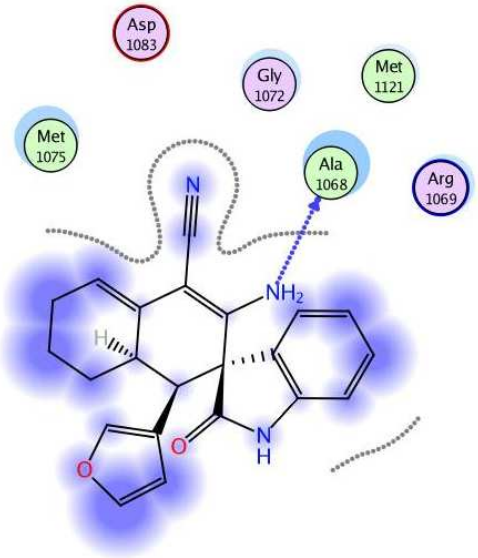



**Figure 9**



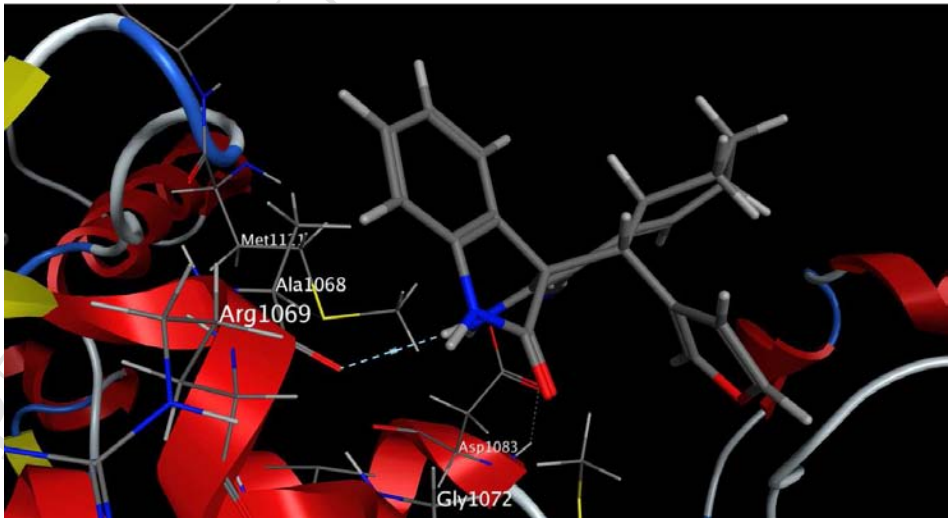
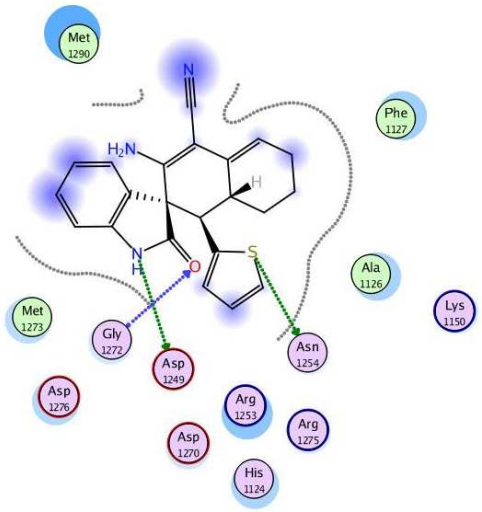



**Figure 10**



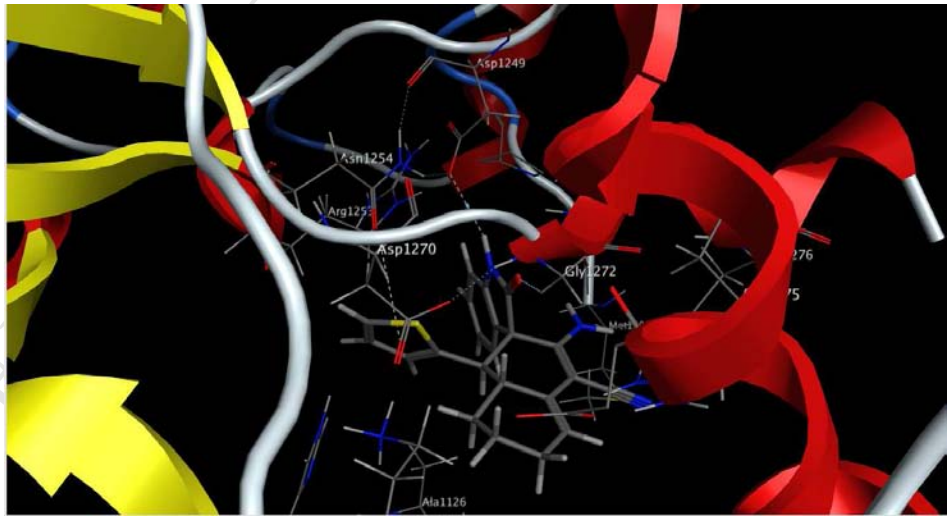
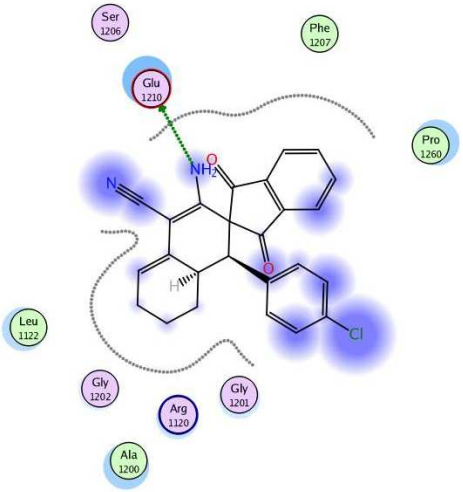



**Figure 11**



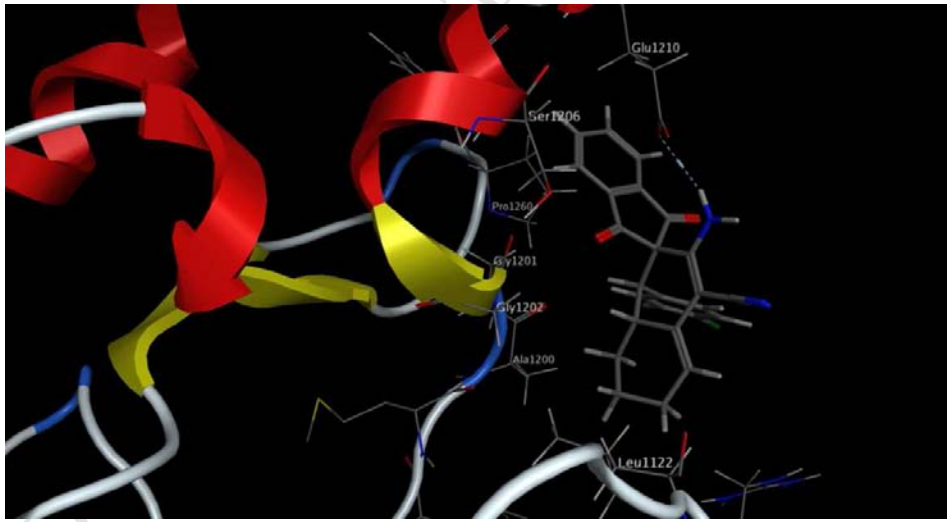
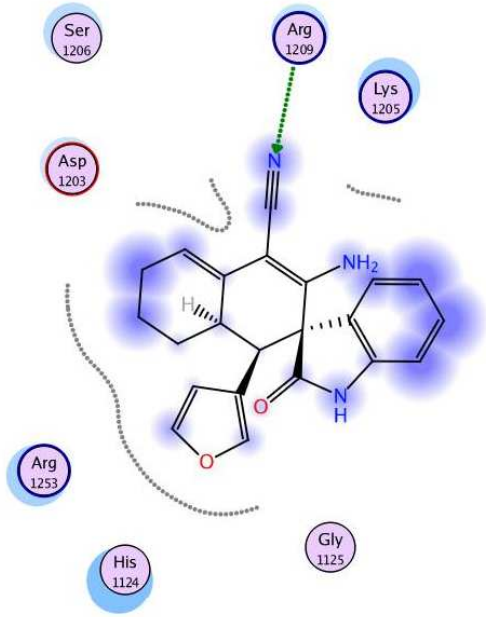
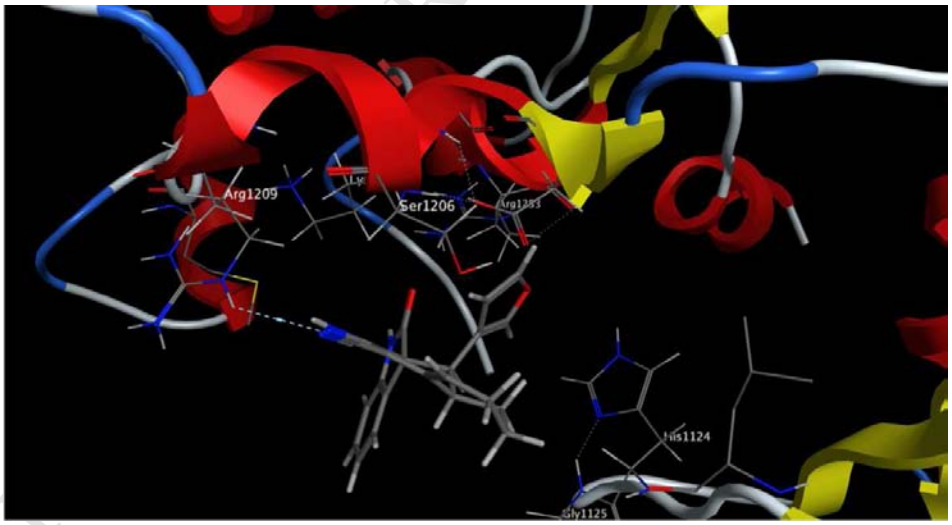
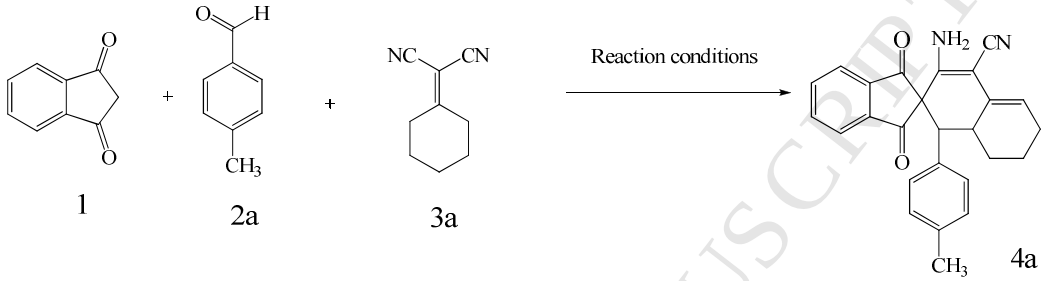



**Figure 12**

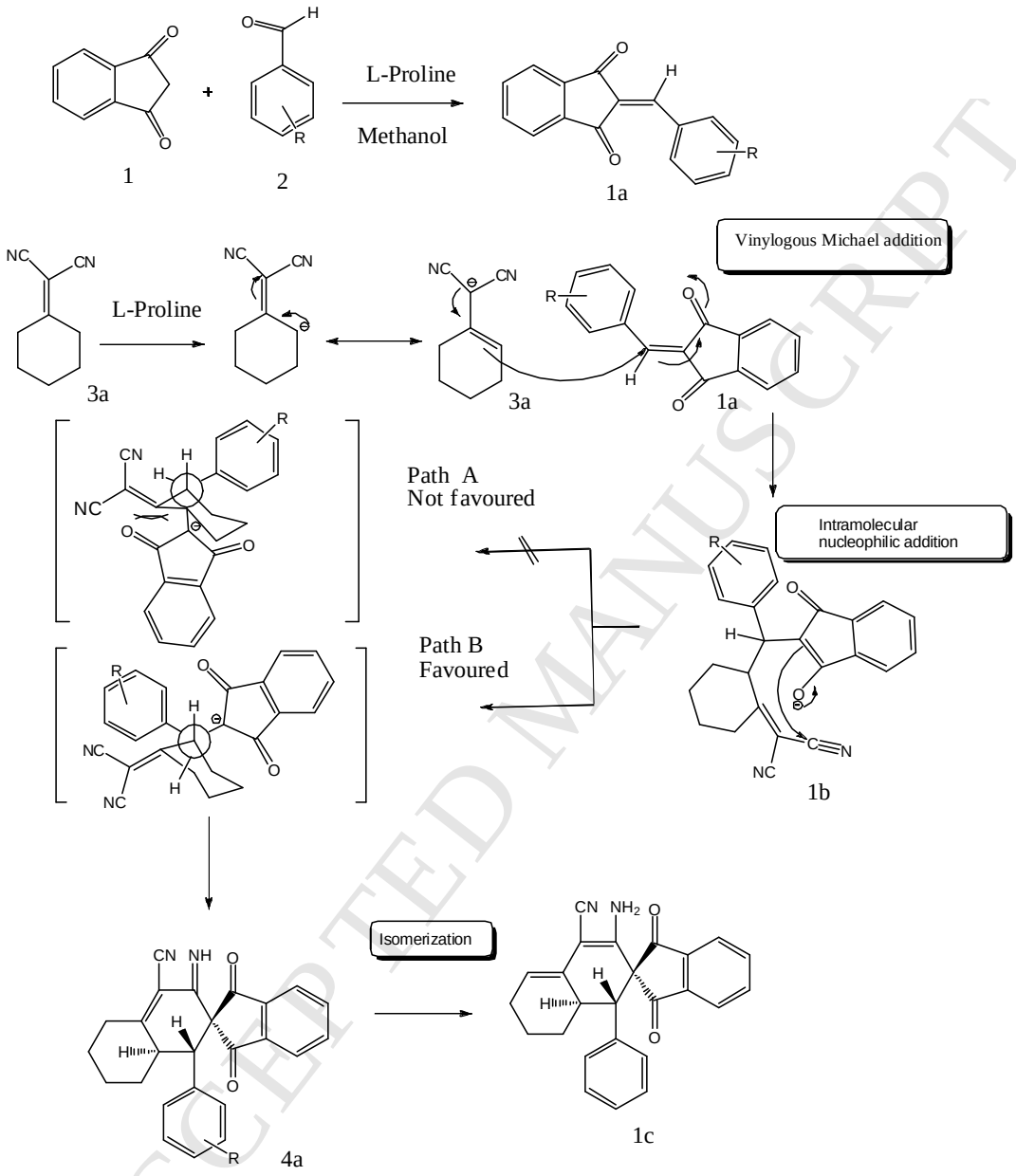


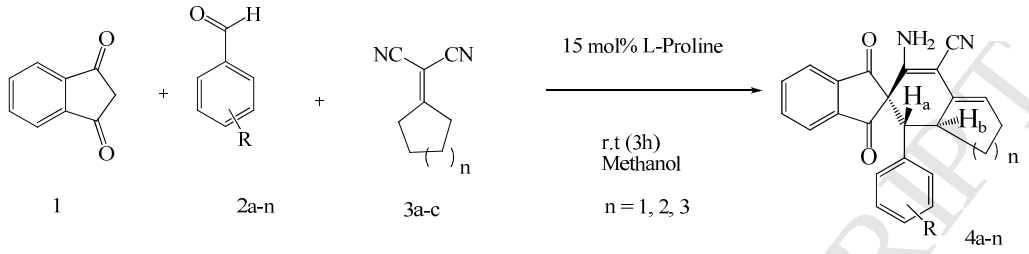
**Scheme 1**



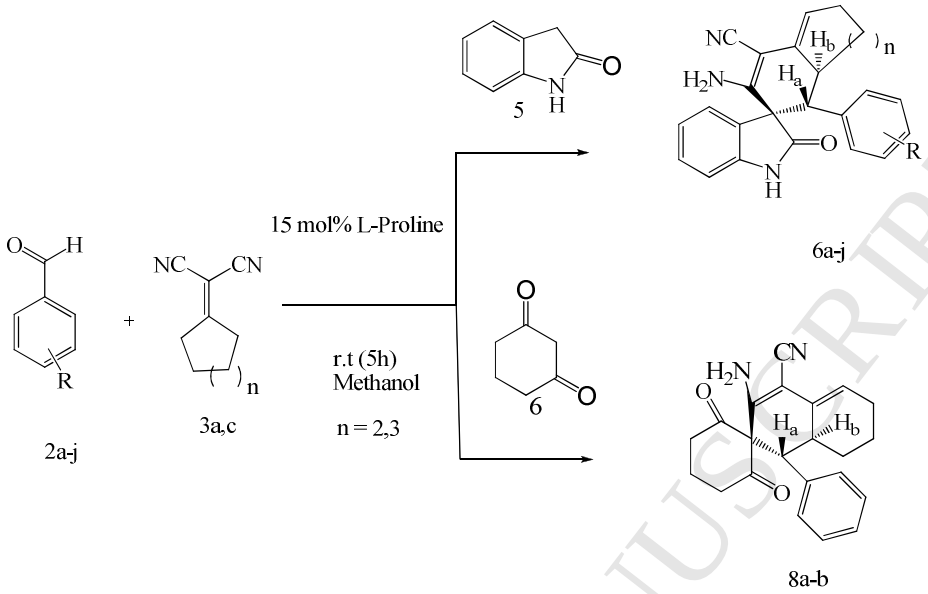
**Scheme 2**



**Scheme 3**



**Scheme 4**



# Tables

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

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

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

**Table 4.** Synthesis of spirocarbocycle derivatives from substituted aldehydes and alkylidene malononitrile.

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

**Table 5.** *In vitro* antimicrobial activity of synthesized compounds.

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

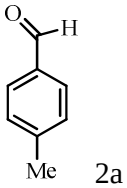
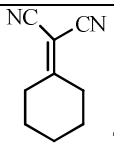
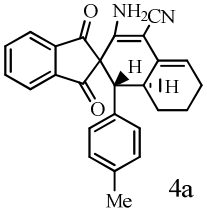
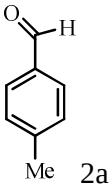
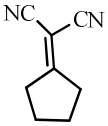
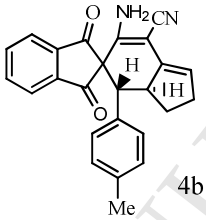
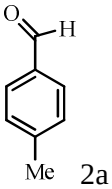
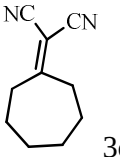
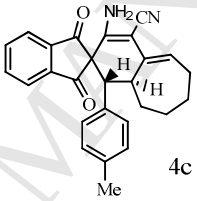
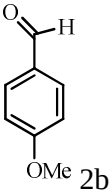
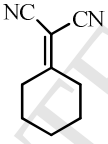
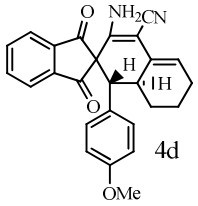
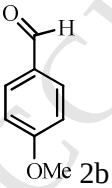
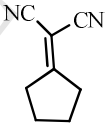
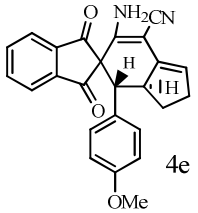
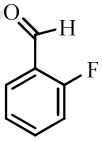
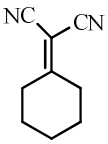
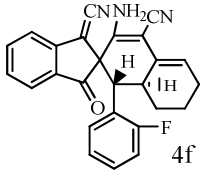
**Table 7.** Anticancer activity of synthesised compounds against A549 cancer cell line and calculated binding energy with ALK receptor of synthesized spirocarbocycles.

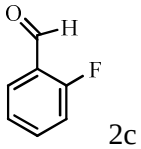
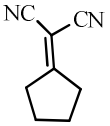
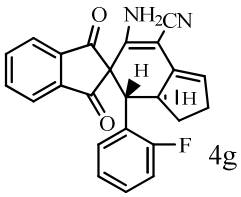
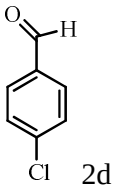
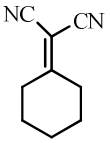
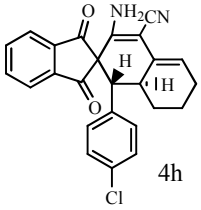
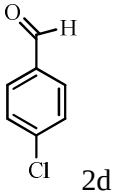
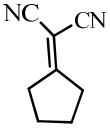
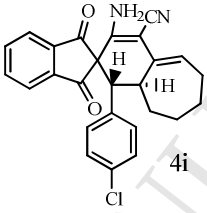
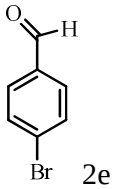
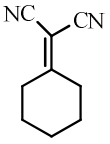
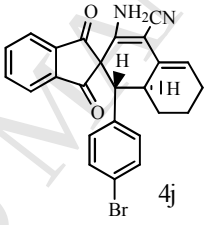
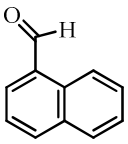
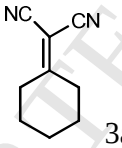
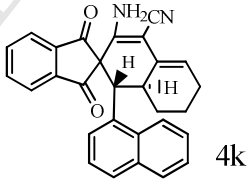
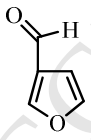
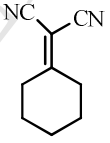
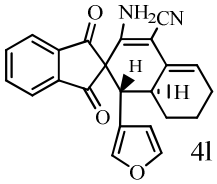
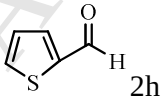
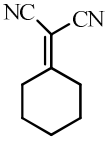
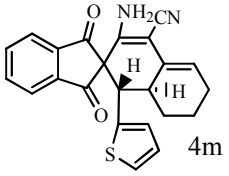
**Table 1**

| 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. | H2O | 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.

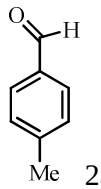
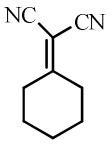
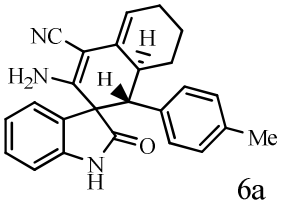
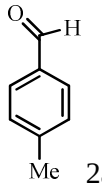
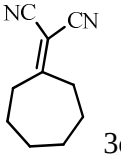
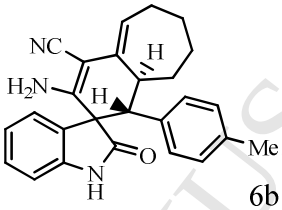
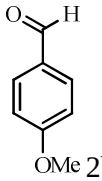
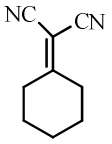
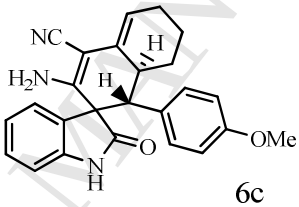
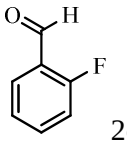
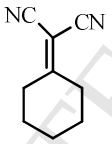
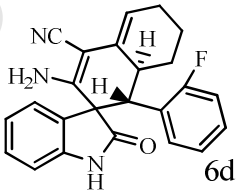
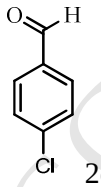
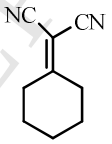
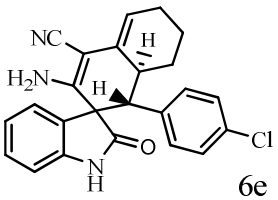
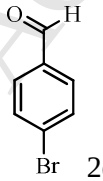
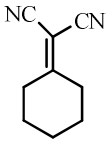
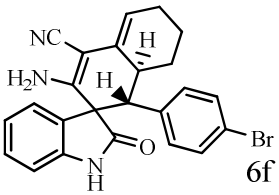
**Table 2**

| 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 |

| | | | | | |
|---|---|---|---|---|---|
| 7. |  2c |  3b |  4g | 3 | 83 |
| 8. |  2d |  3a |  4h | 6 | 82 |
| 9. |  2d |  3b |  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 |

1

**Table 3**

| 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 |

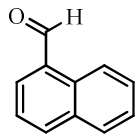
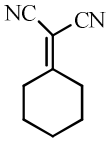
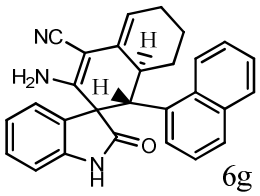
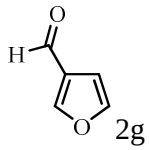
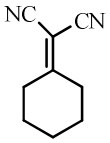
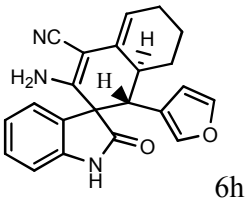
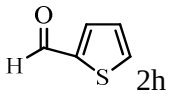
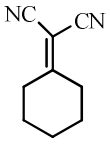
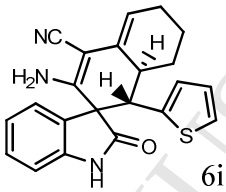
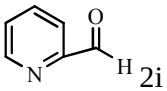
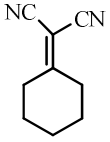
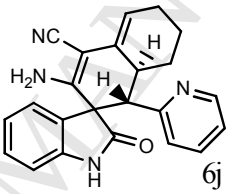
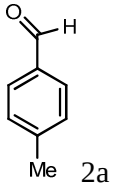
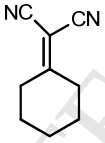
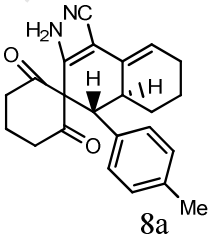
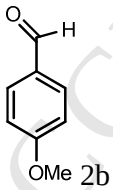
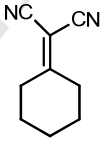
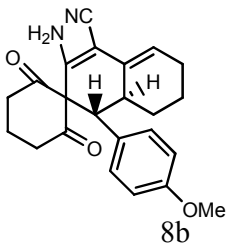
| | | | | | |
|---|---|---|---|---|---|
| 7. |  2f |  3a |  6g | 8 | 79 |
| 8. |  2g |  3a |  6h | 7.5 | 78 |
| 9. |  2h |  3a |  6i | 9 | 81 |
| 10. |  2i |  3a |  6j | 7 | 82 |
| 11. |  2a |  3a |  8a | 6 | 74 |
| 12. |  2b |  3a |  8b | 6.5 | 72 |

**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 |
| | $\alpha = \gamma = 90°$ |
| | $\beta$ = 93.3610(10)° |
| Volume | 1974.76(9) $A^3$ |
| Z, Calculated density | 4, 1.283 $Mgm^{-3}$ |
| Absorption coefficient | 0.080 $mm^{-1}$ |
| F(000) | 808 |
| Crystal size | 0.35 x 0.35 x 0.30 mm |
| $\theta$ range for data collection | 2.31 to 25.00° |
| Limiting indices | -11<=h<=11, -25<=k<=25, -11<=l<=11 |
| Reflections collected / unique | 18019 / 3486 [R(int) = 0.0335] |
| Completeness to $\theta$ = 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 |

**Table 5**

| Compounds | Zone of inhibition in mm | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | | Gram negative bacteria | | | | | Fungi | |
| | *S. epidermidis* | *S. aureus* | *S. aureus (MRSA)* | *M. luteus* | *E. aerogens* | *S. typhimurium* | *K. pneumonia* | *P. vulgaris* | *S. flexneri* | *C. albicans* | *M. pachyderm atis* |
| 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.

**Table 6**

| Compounds | Minimum inhibitory concentration (µg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | | Gram negative bacteria | | | | |
| | *S. epidermidis* | *S. aureus* | *S. aureus* (MRSA) | *M. luteus* | *E. aerogens* | *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

**Table 7**

| 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 | |

# Synthesis of new class of spirocarbocycle derivatives by multicomponent domino reaction and their evaluation for antimicrobial activity and Molecular docking studies

N. Sudhapriya[a], P.T. Perumal[a,]*, C Balachandran[b], S Ignacimuthu[b], M. Sangeetha [c], Mukesh Doble[c]

[a]*Organic Chemistry Division, CSIR-Central Leather Research Institute, Adyar, Chennai 600 020, India*

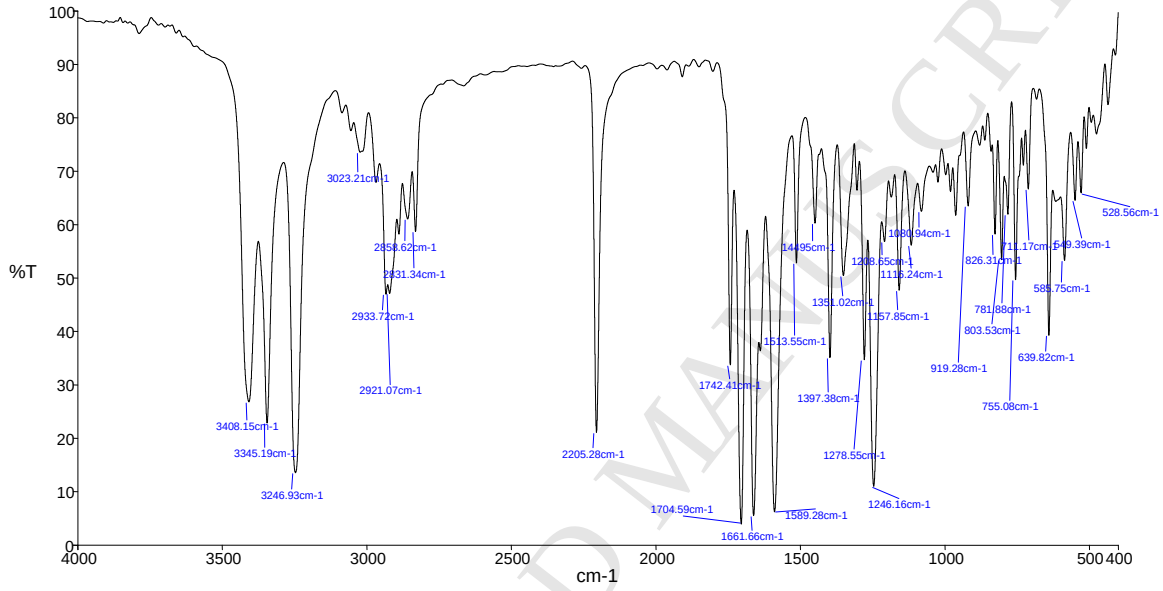
[b]*Division of Microbiology, Entomology Research Institute, Loyola College, Chennai 600 034, India*

[c]*Department of Biotechnology, Indian Institute of Technology, Chennai 600 036, India*

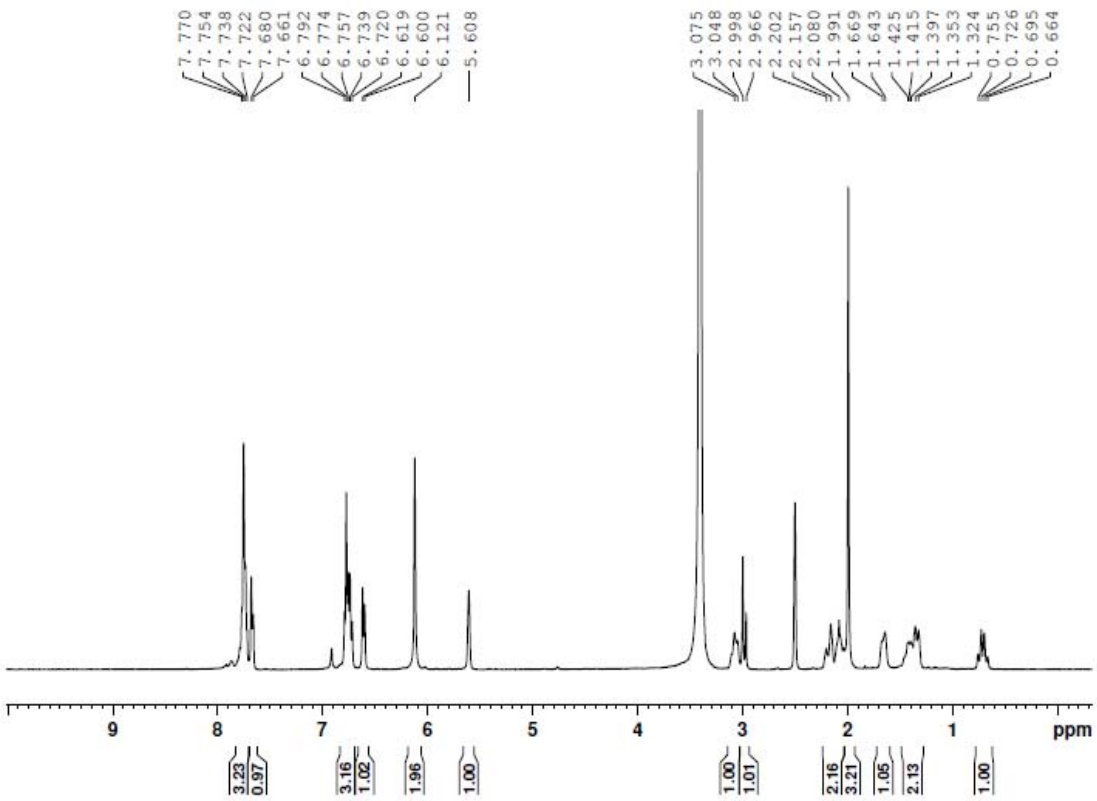
*Fax: +91 44 24911589. E-mail: ptperumal@gmail.com*

## Supplementary material

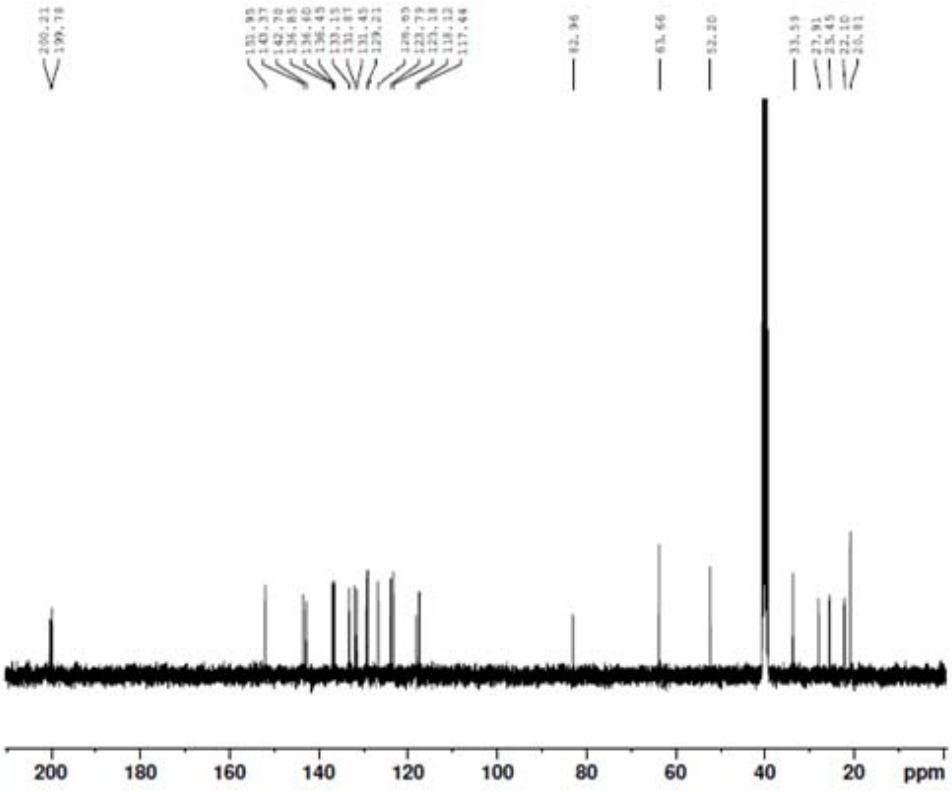
# IR Spectrum of compound 4a



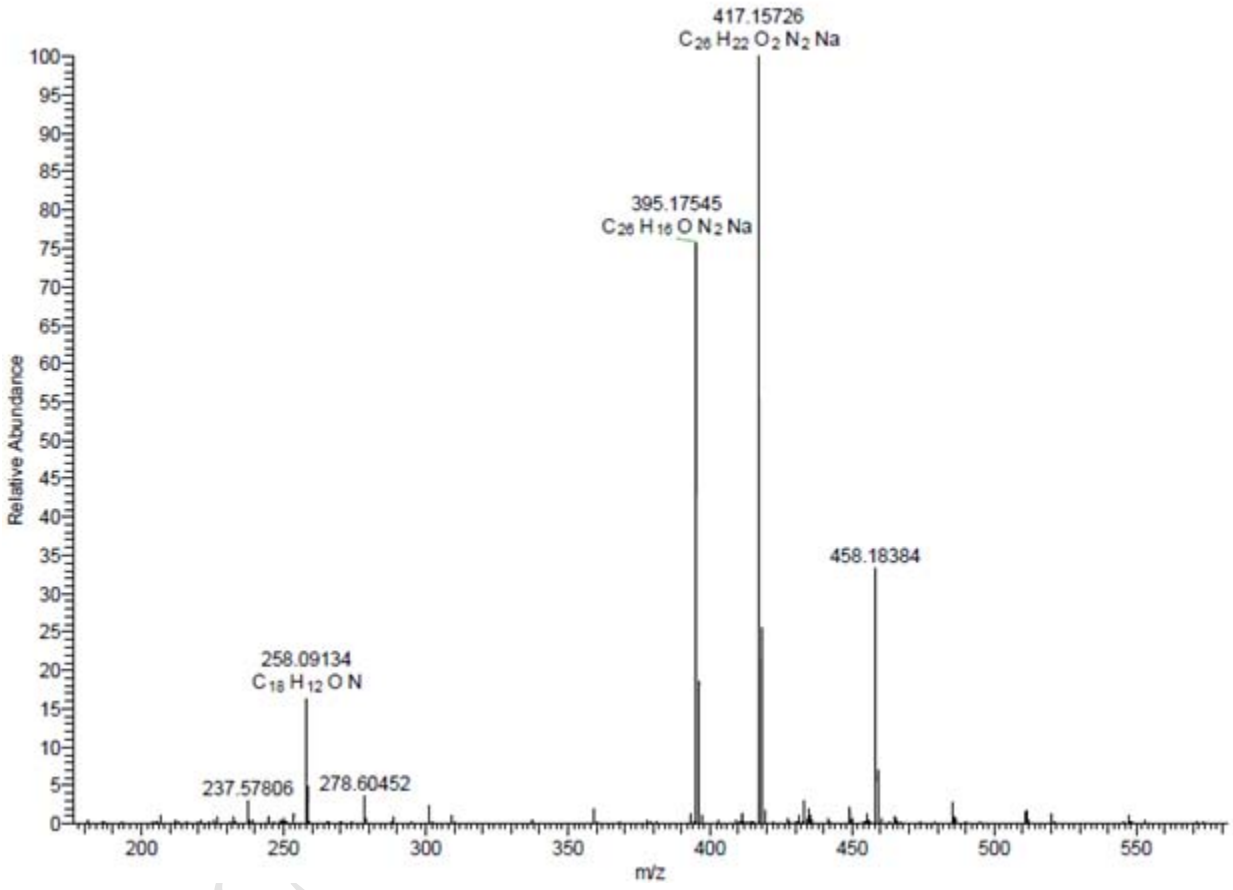
$^{1}$H NMR spectrum of 4a



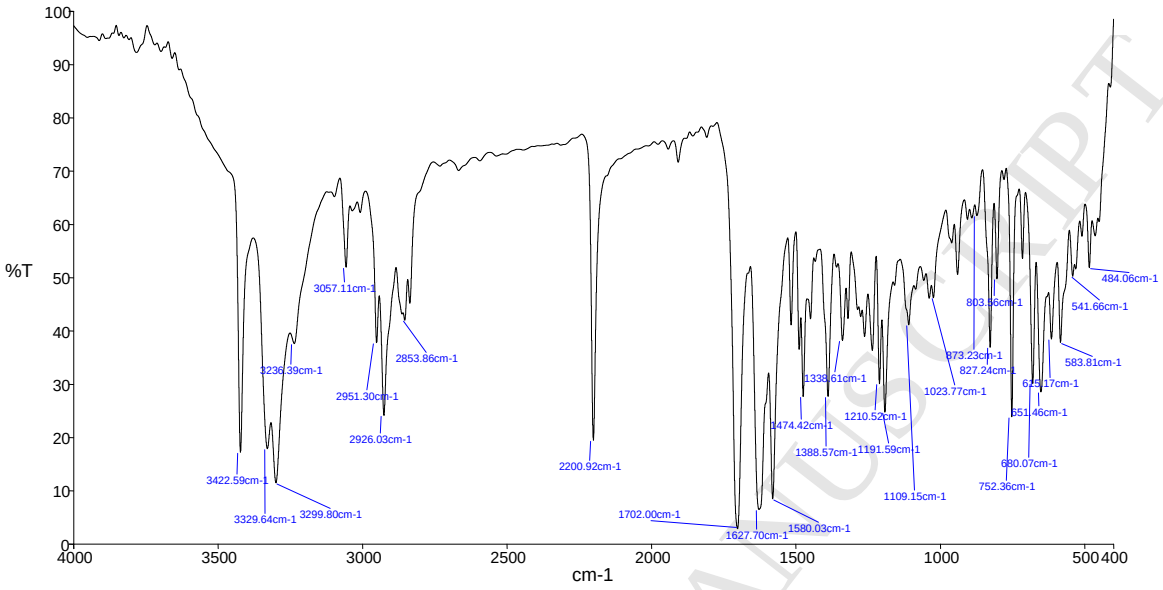
$^{13}$C NMR spectrum of 4a



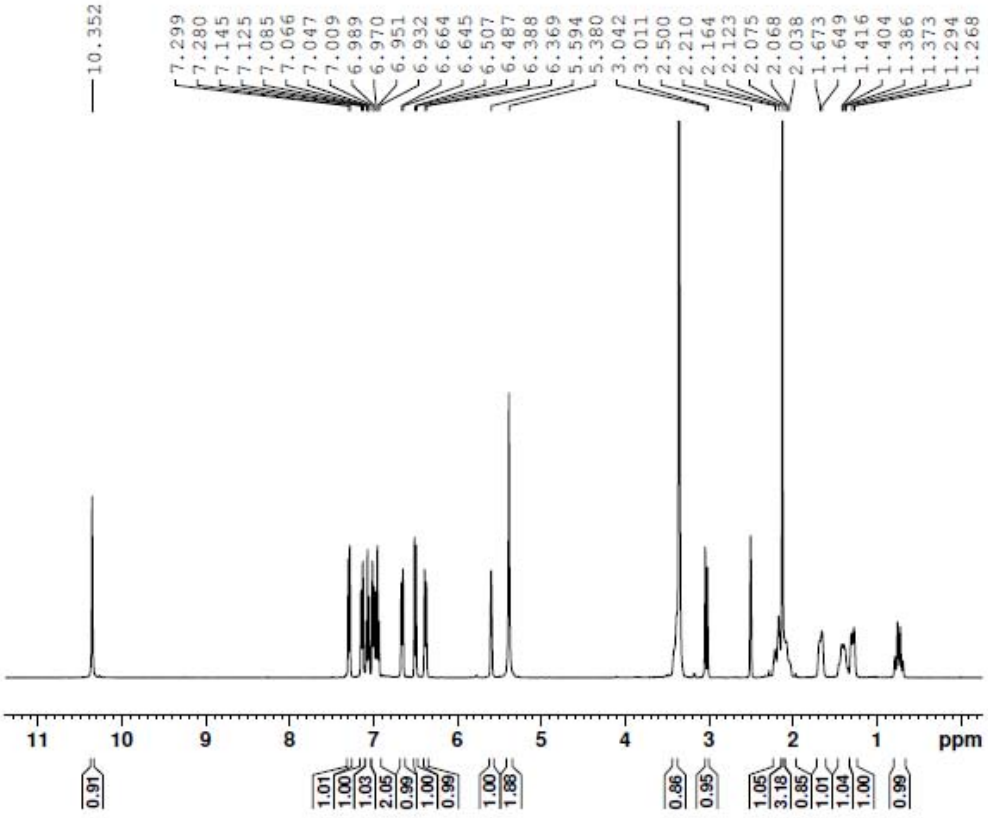
Mass spectrum of 4a



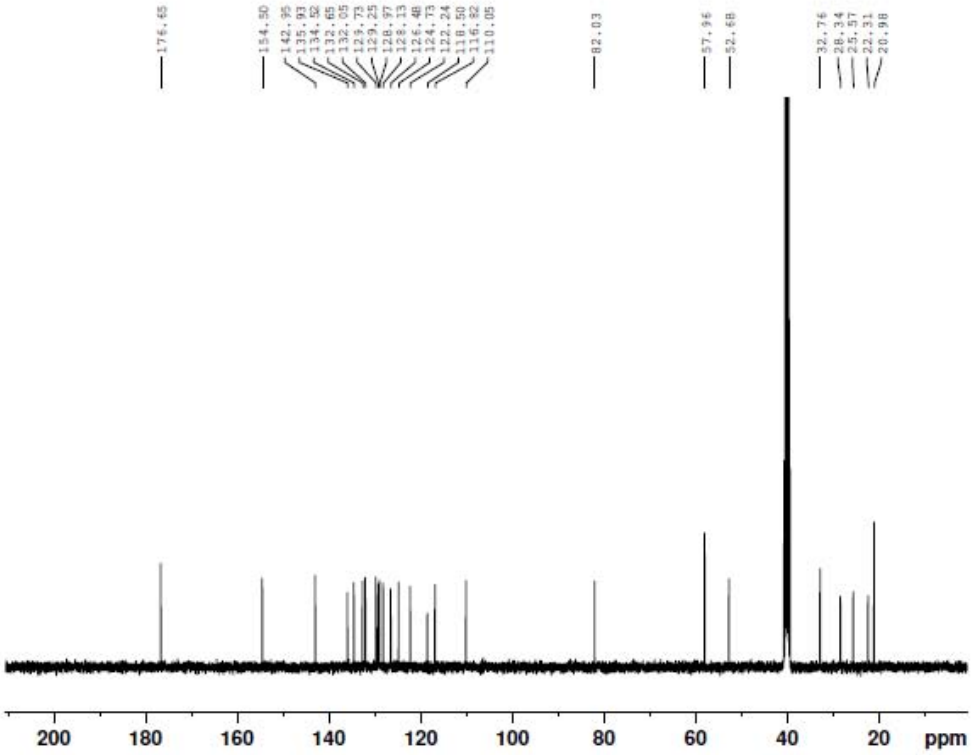
# IR Spectrum of compound 6a



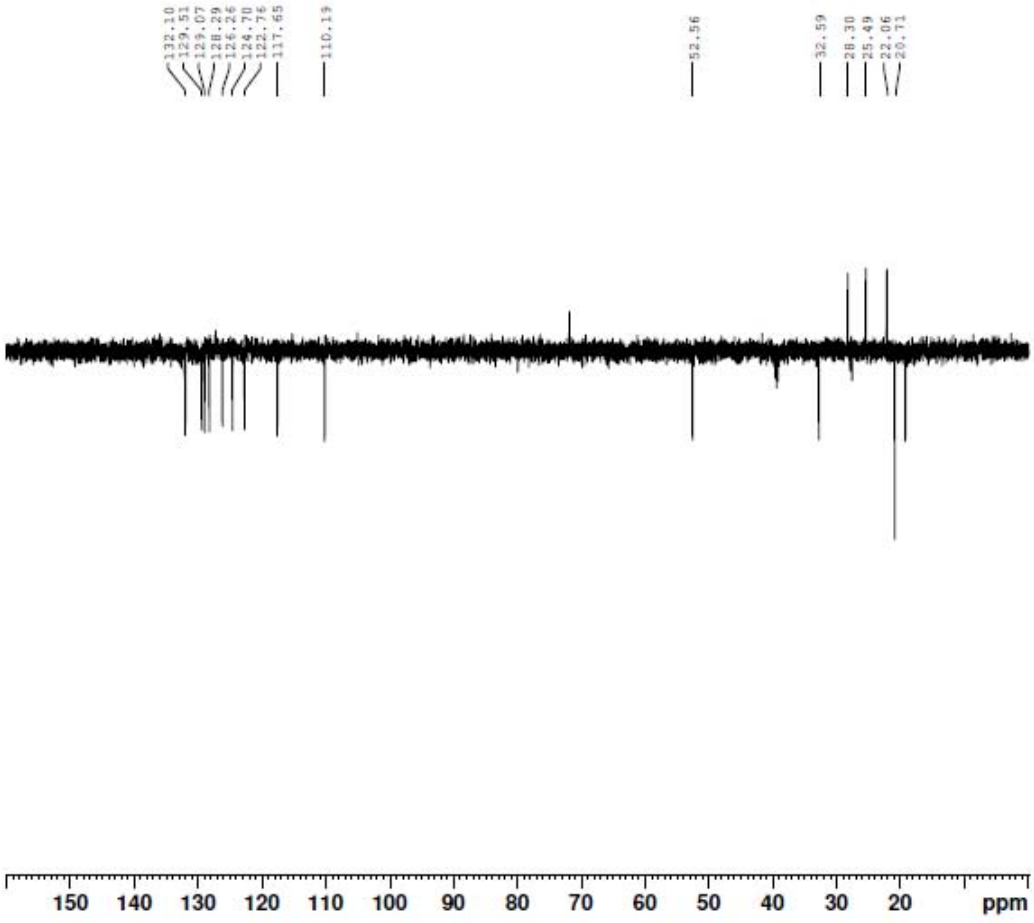
$^{1}$H NMR spectrum of 6a



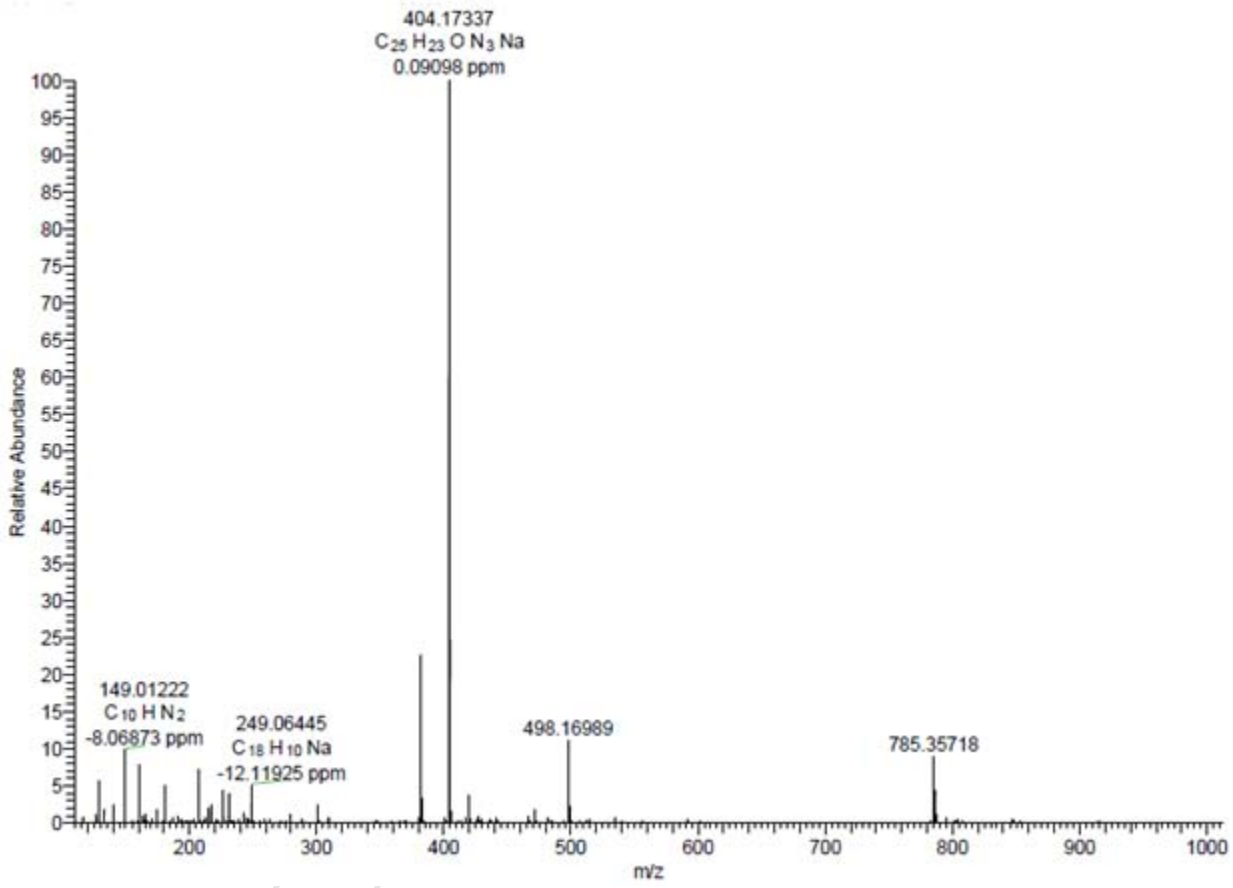
$^{13}$C NMR spectrum of 6a



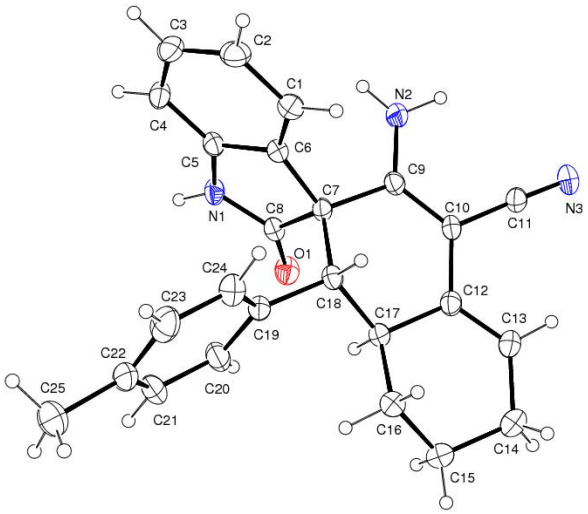
DEPT of compound 6a



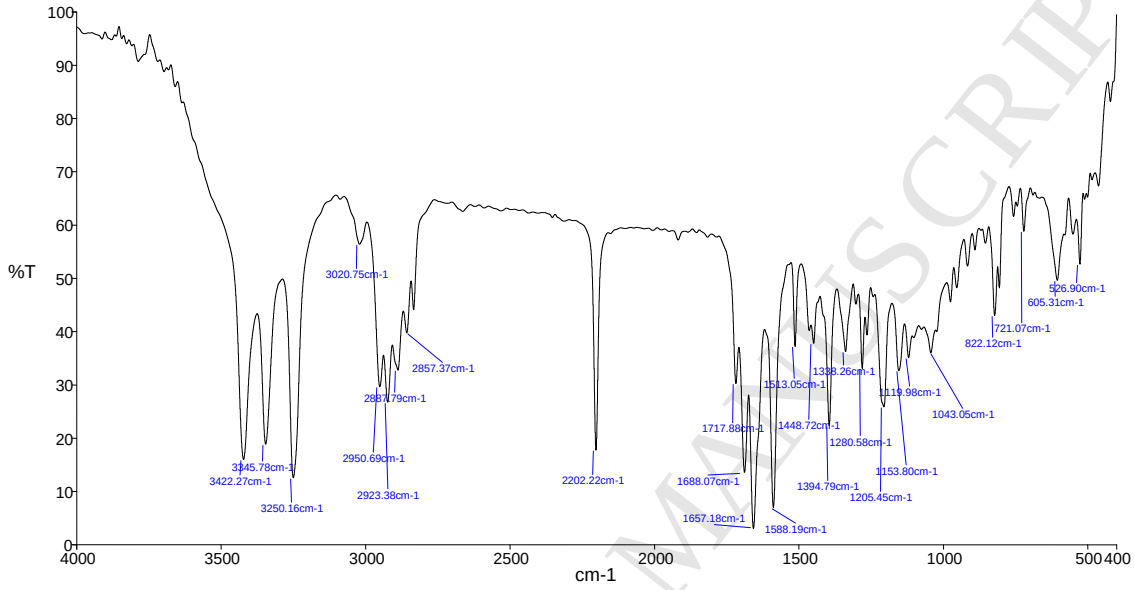
Mass spectrum of 6a



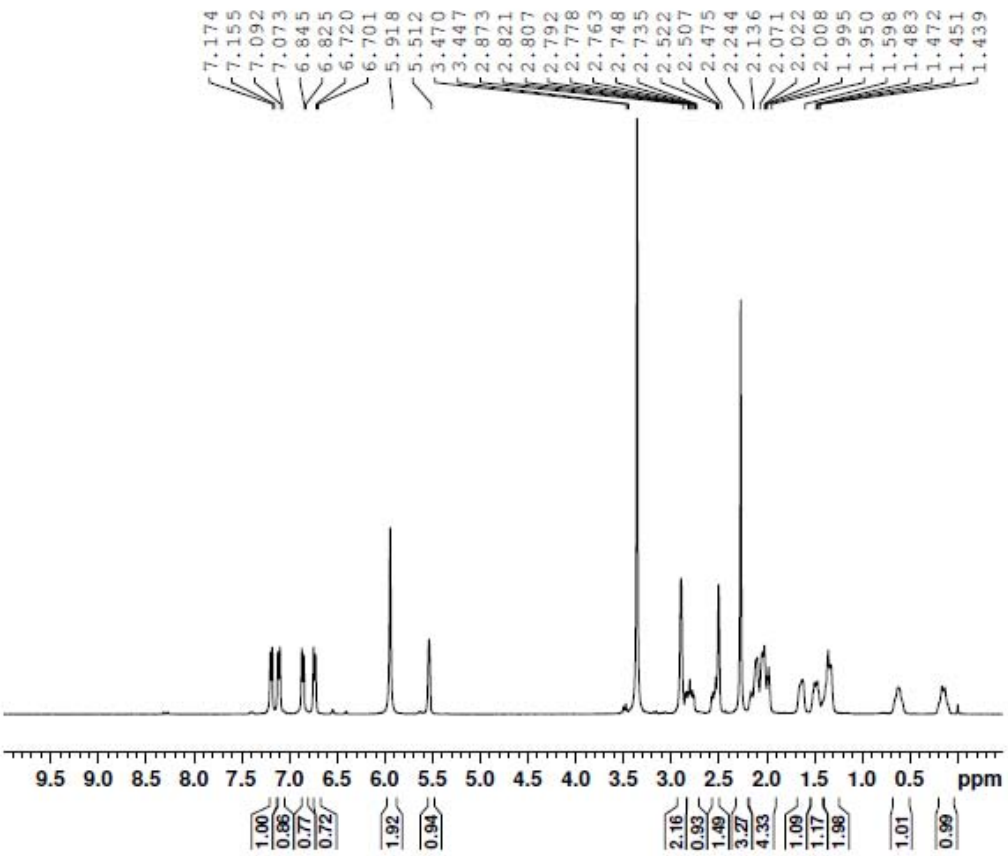
ORTEP diagram of compound **6a**



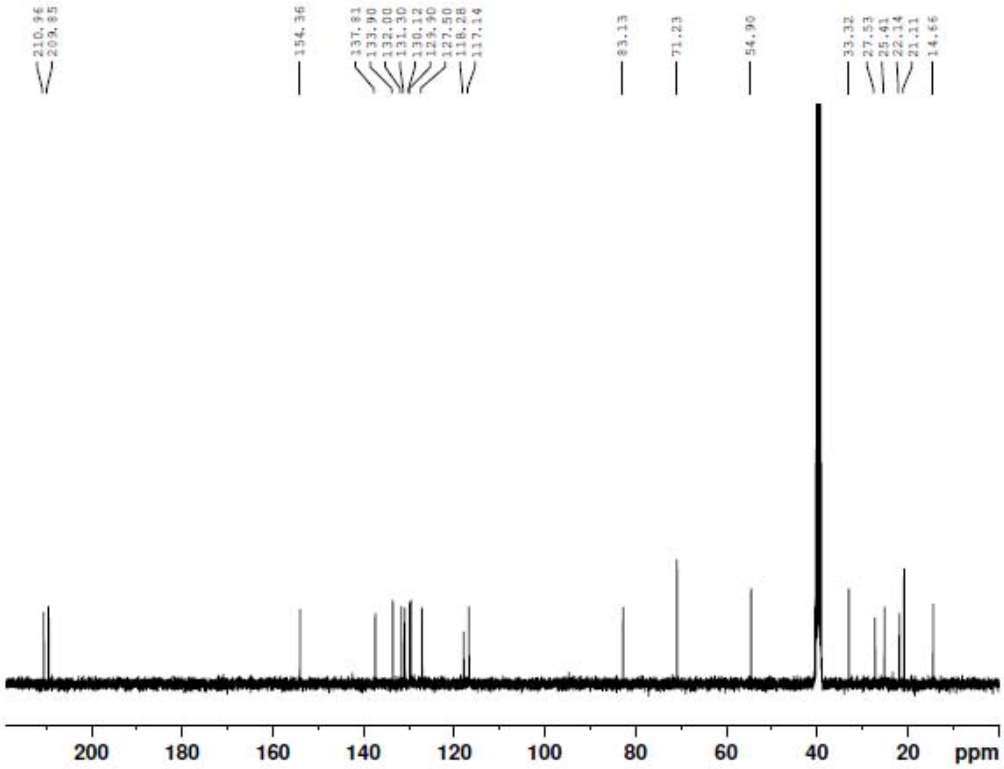
# IR Spectrum of compound 8a



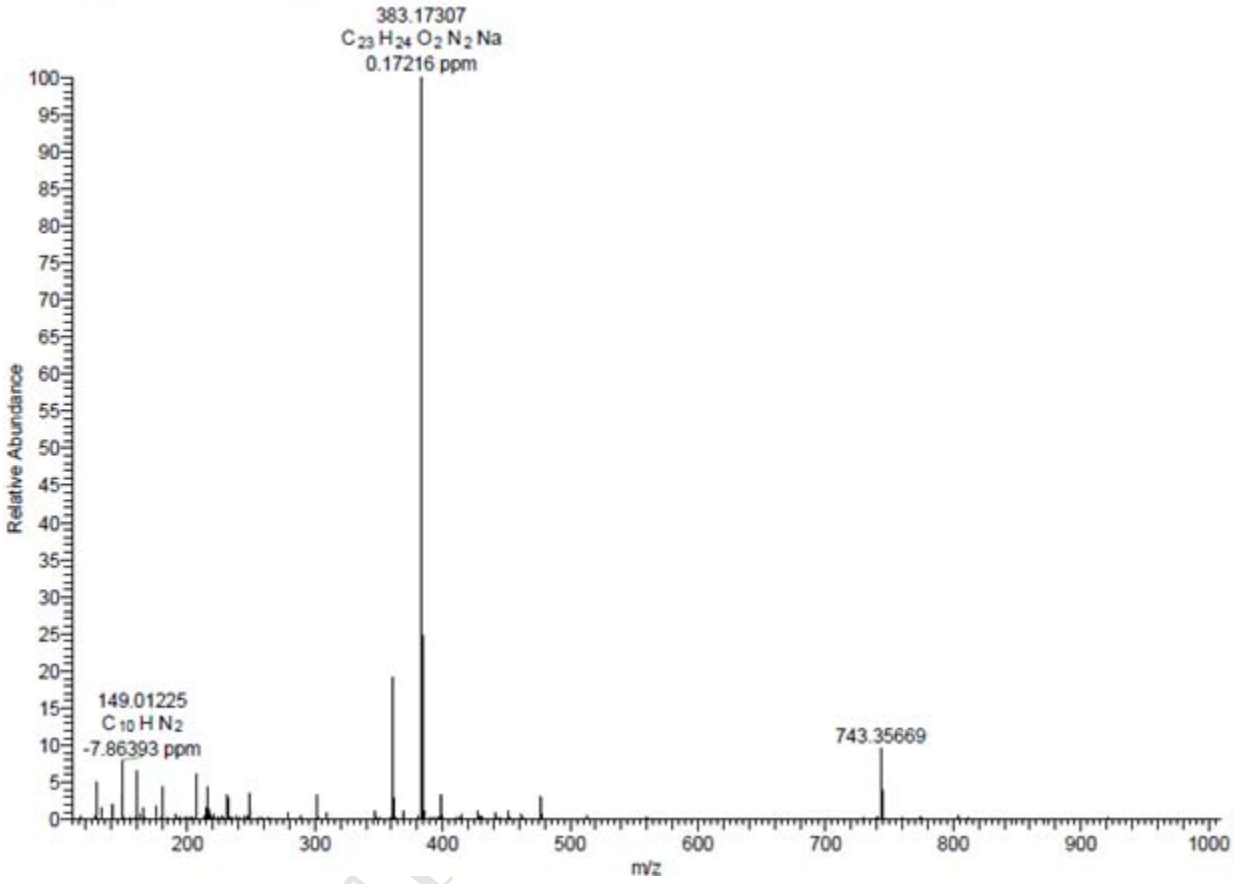
$^{1}$H NMR spectrum of 8a



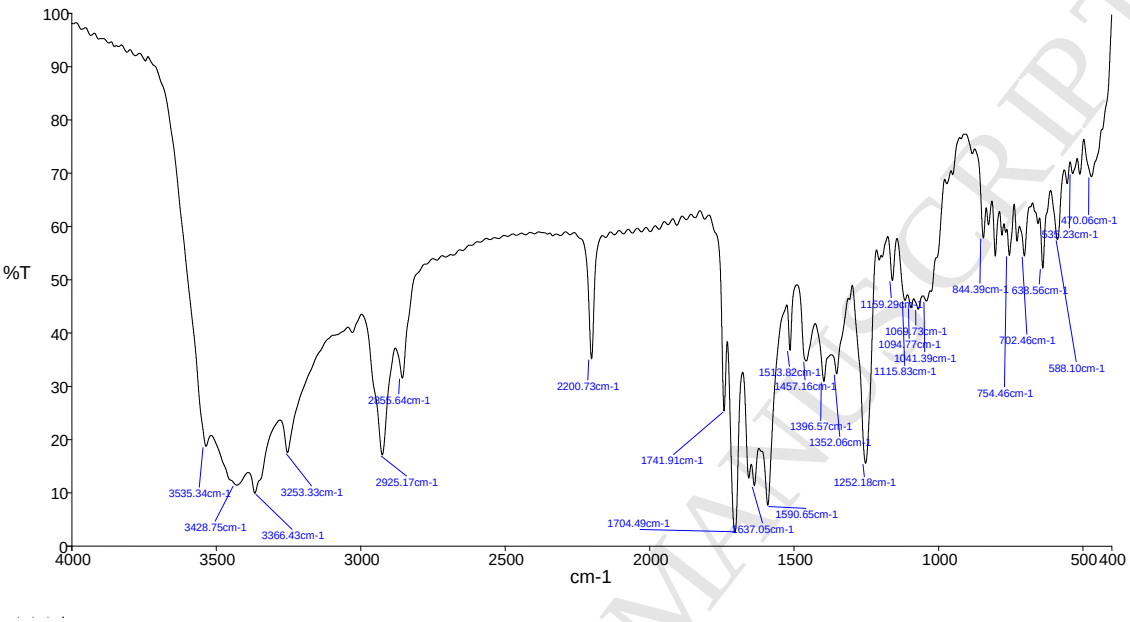
$^{13}$C NMR spectrum of 8a



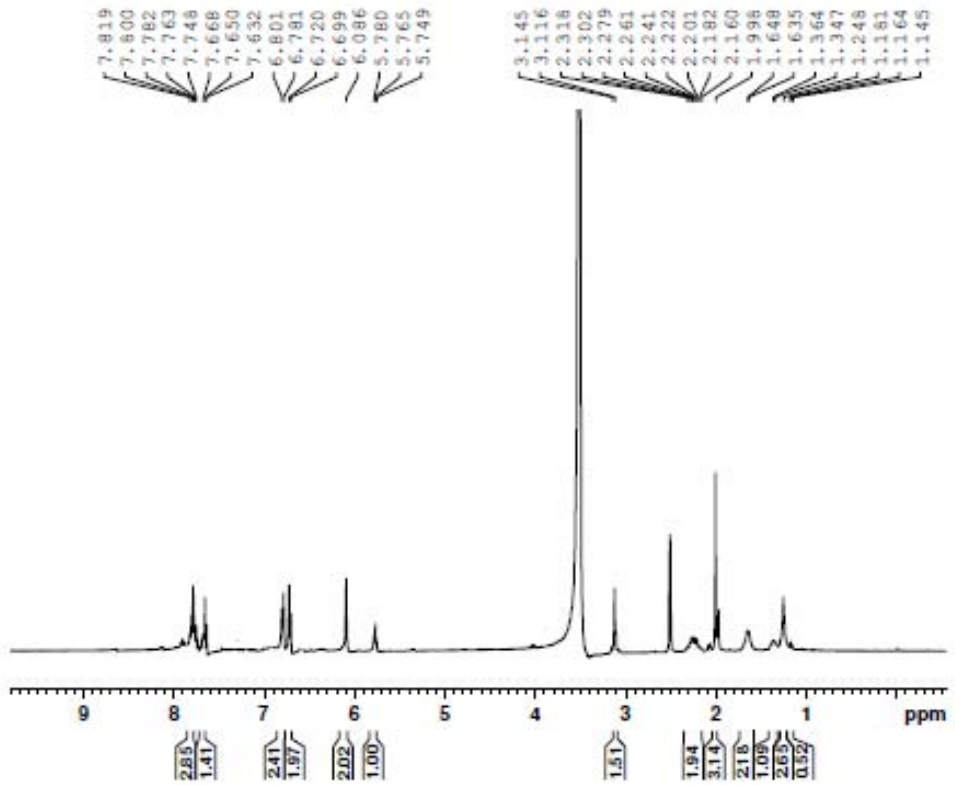
Mass spectrum of 8a



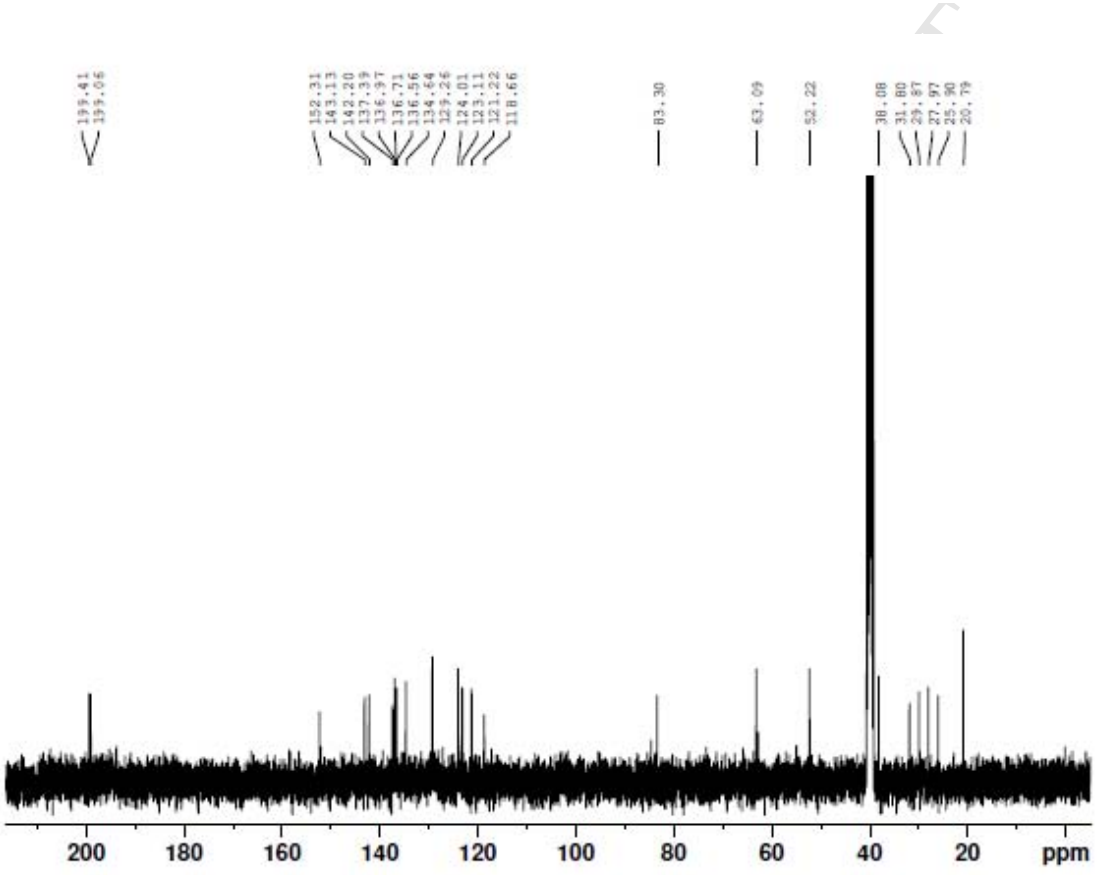
# IR Spectrum of compound 4c



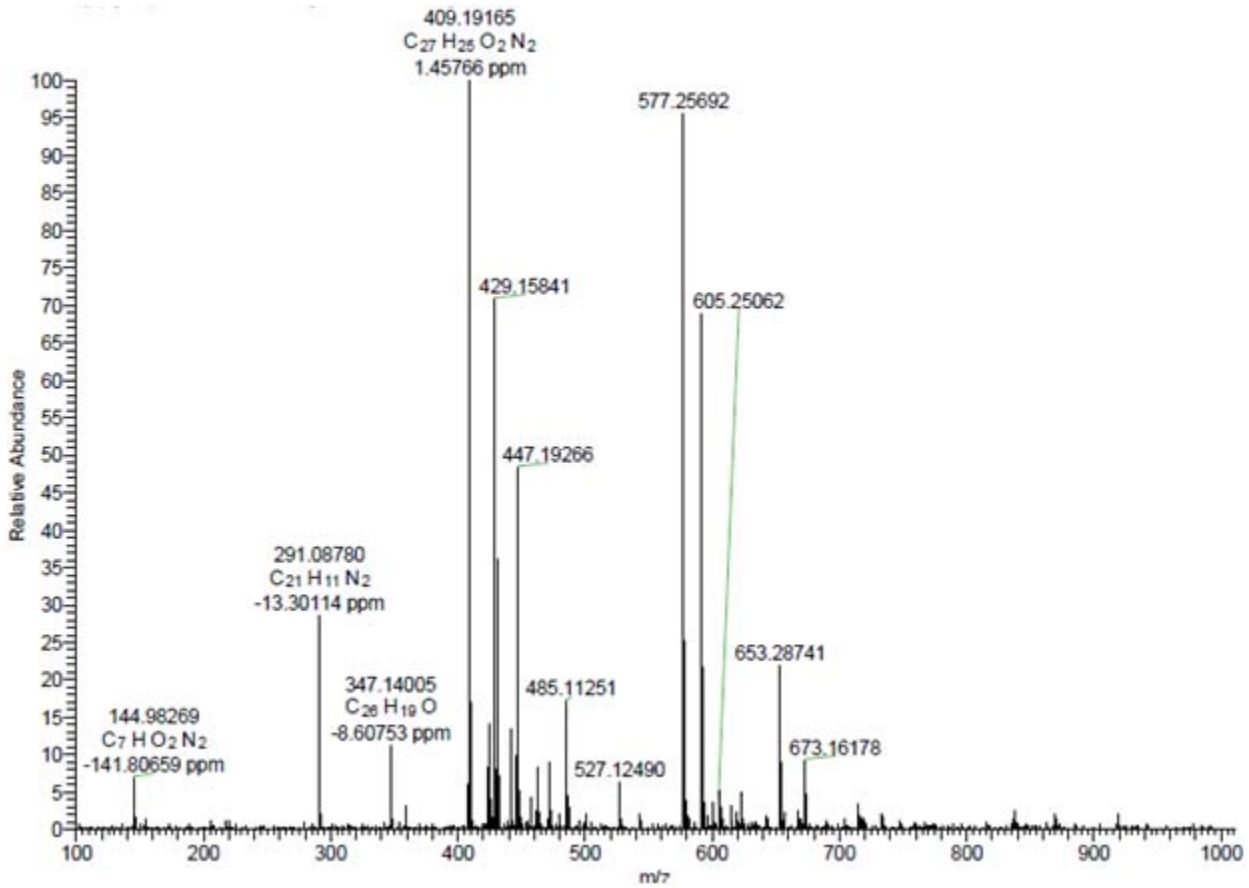
$^{1}$H NMR spectrum of 4c



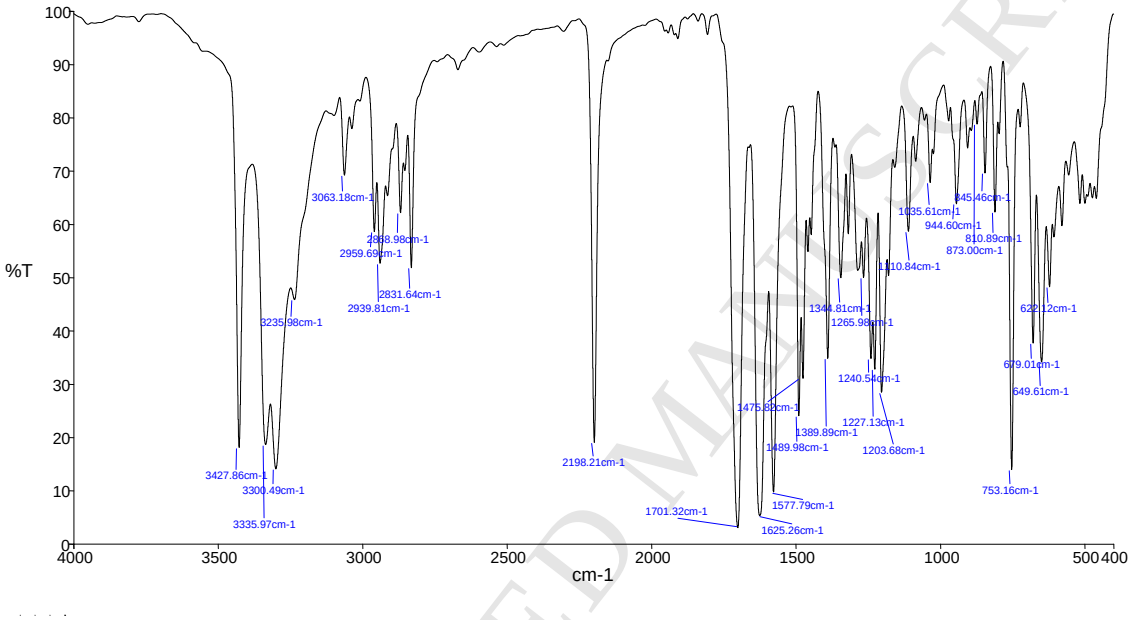
$^{13}$C NMR spectrum of 4c



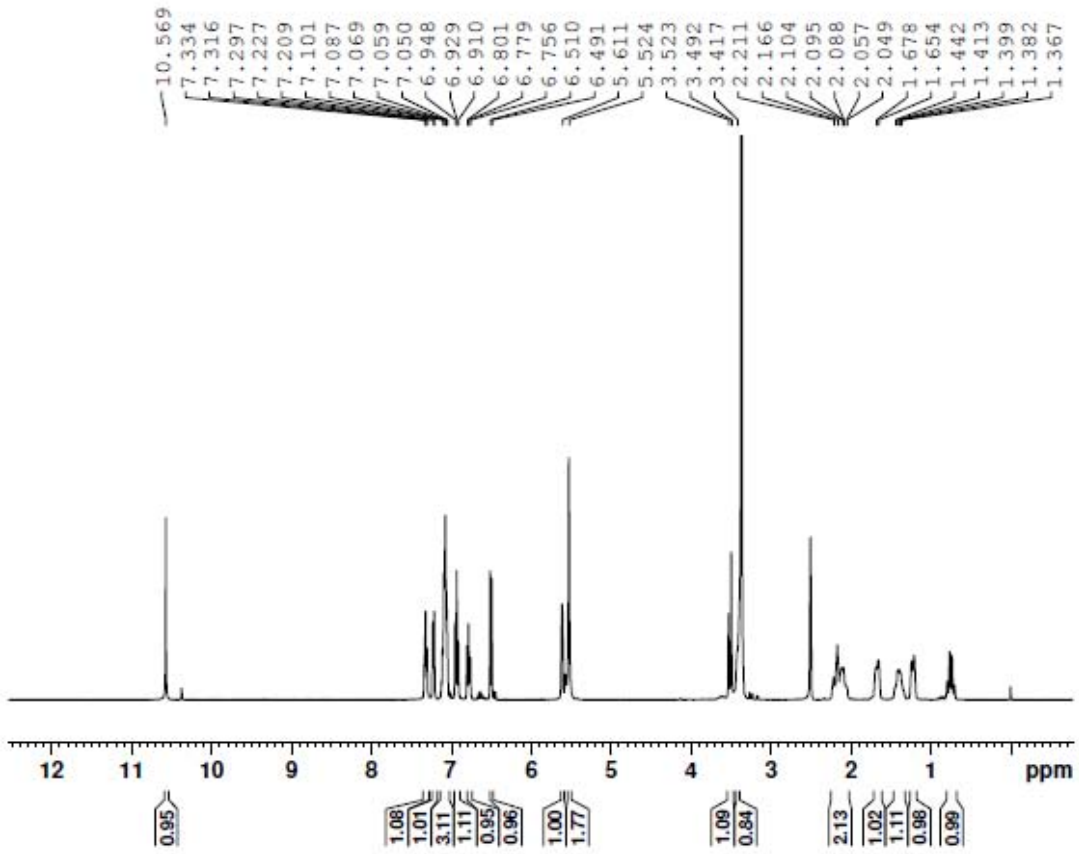
Mass spectrum of 4c



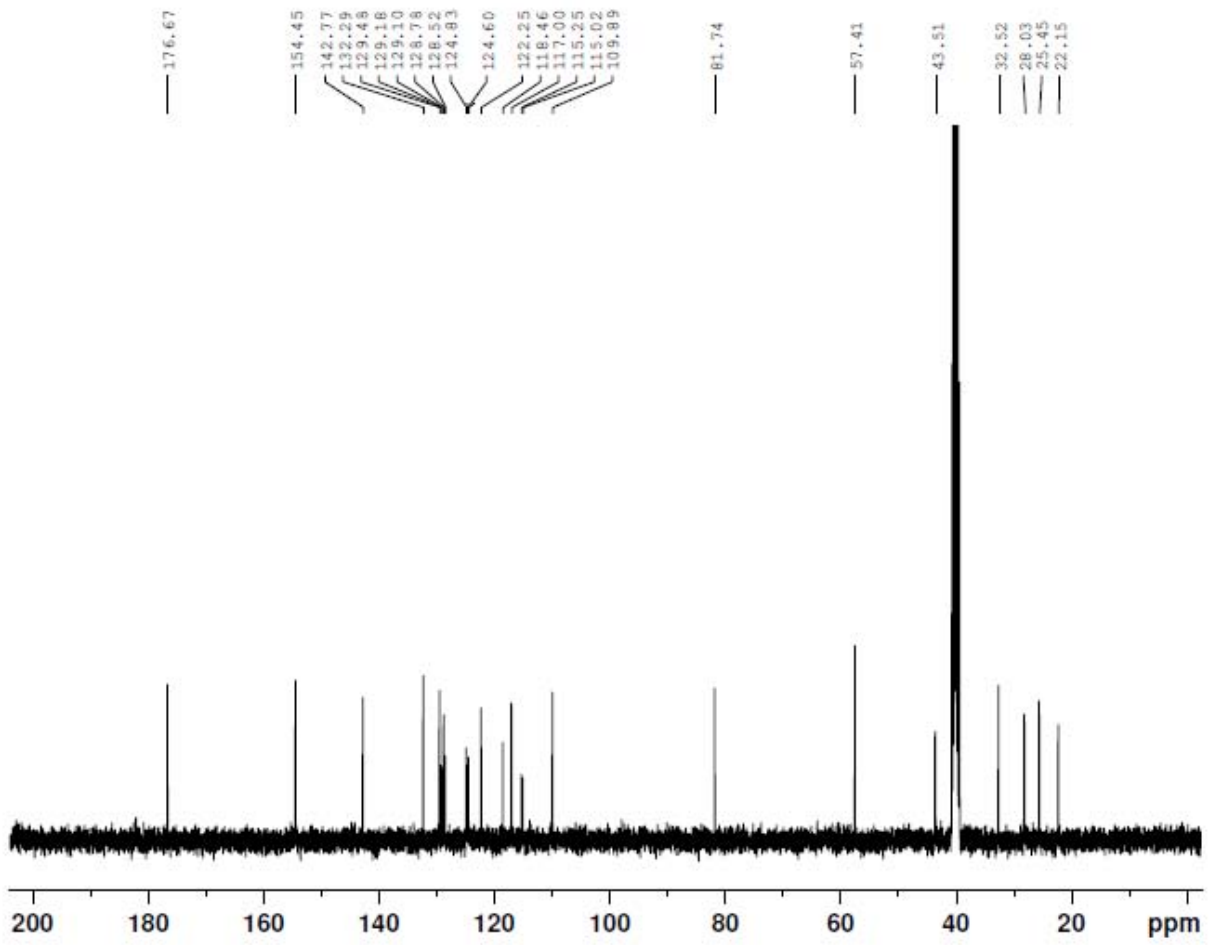
# IR Spectrum of compound 6d



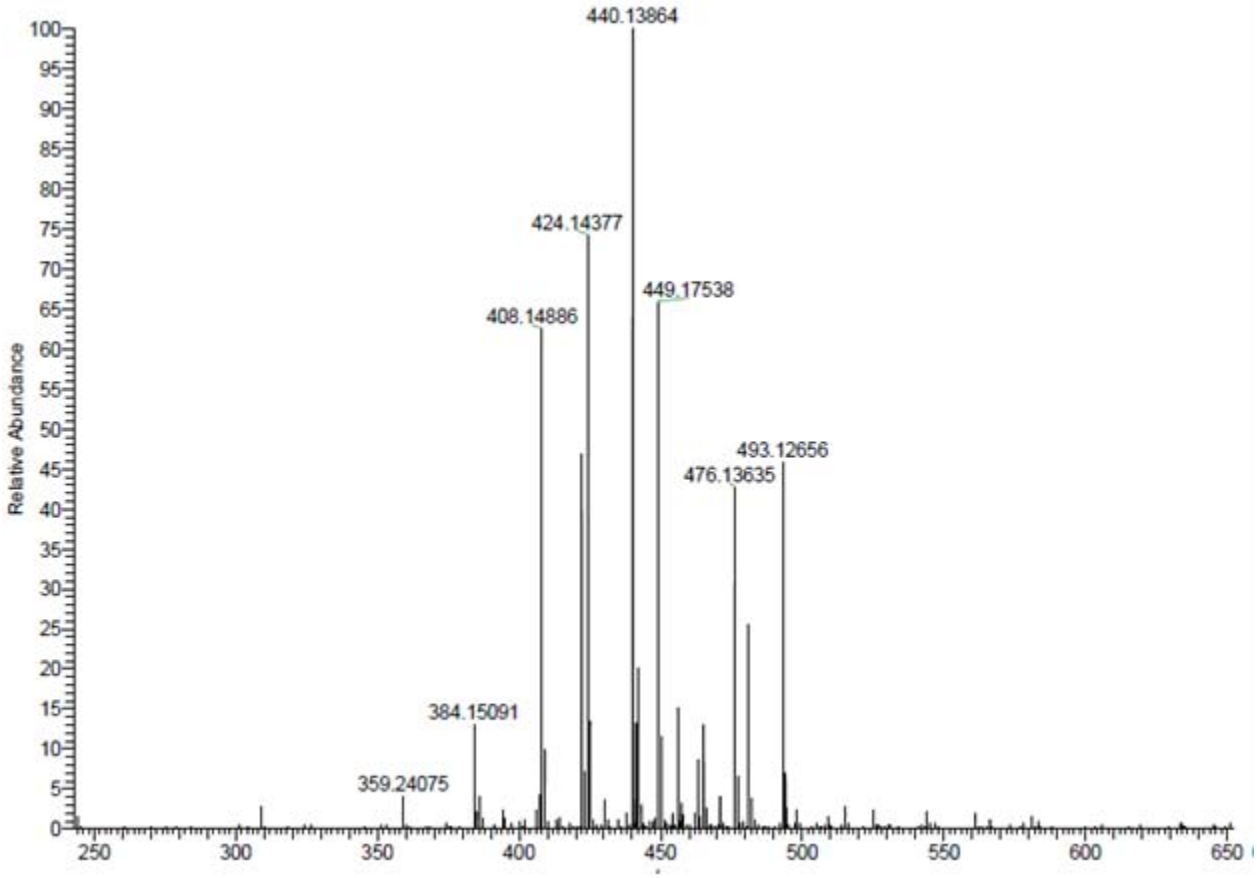
$^{1}$H NMR spectrum of 6d



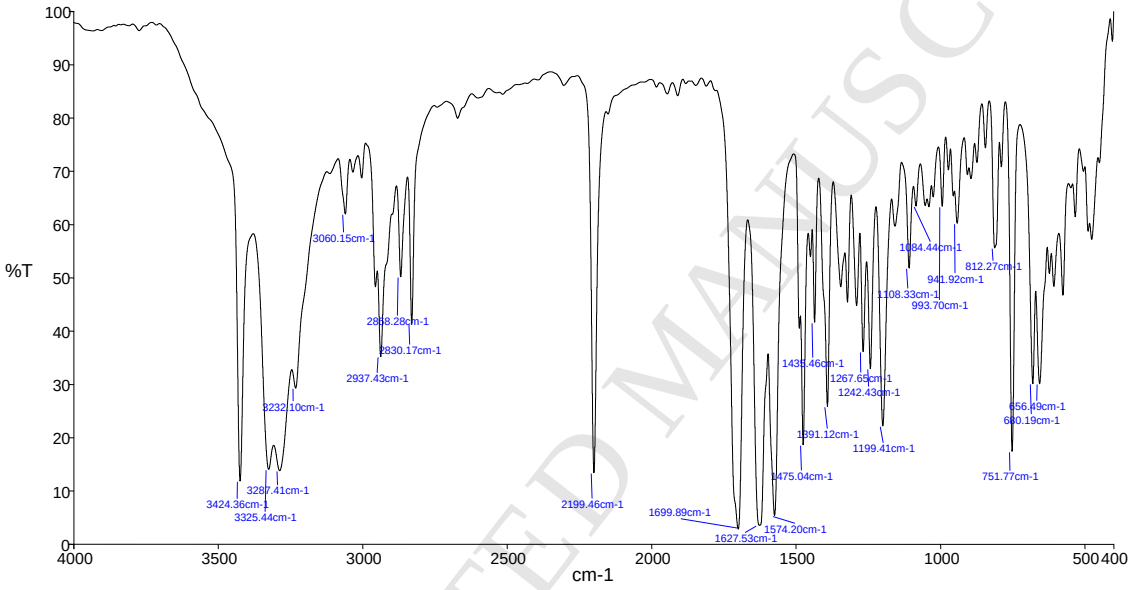
$^{13}$C NMR spectrum of 6d



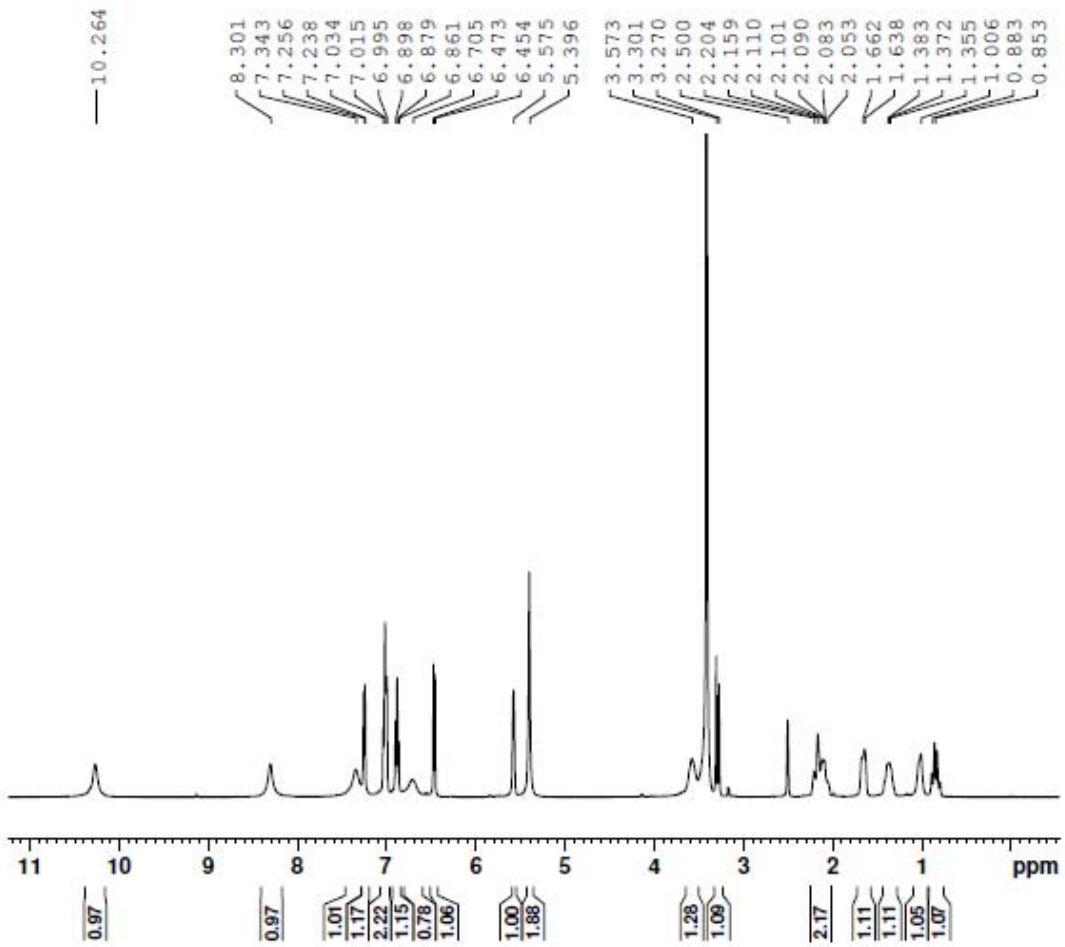
Mass spectrum of 6d



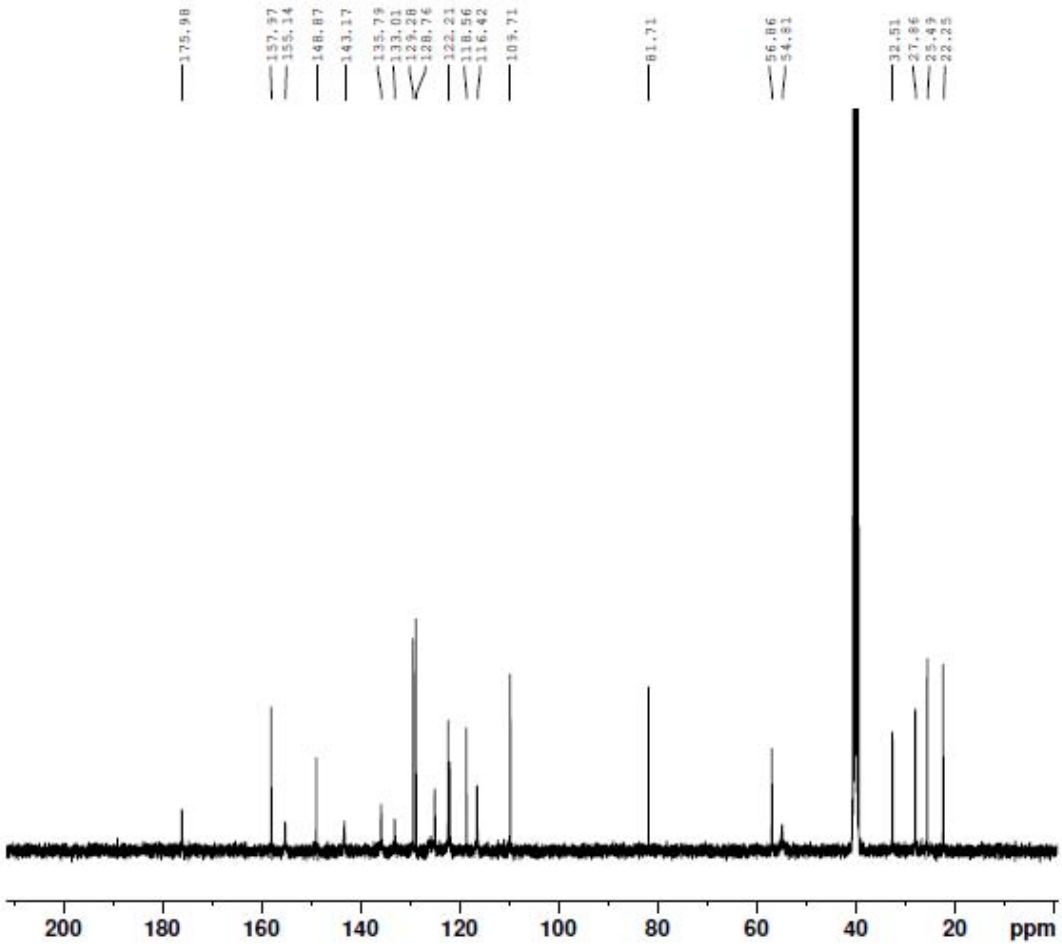
IR Spectrum of compound 6j



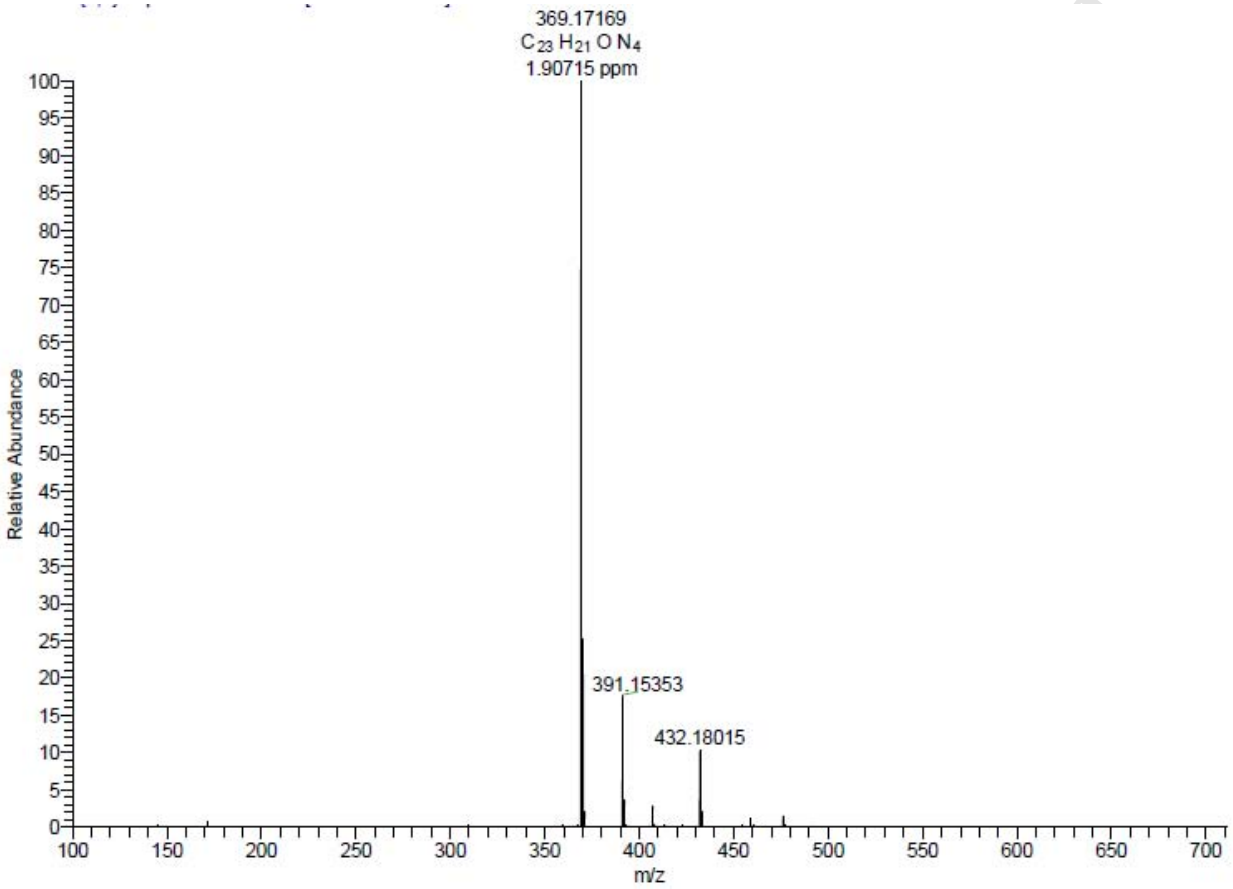
$^{1}$H NMR spectrum of 6j



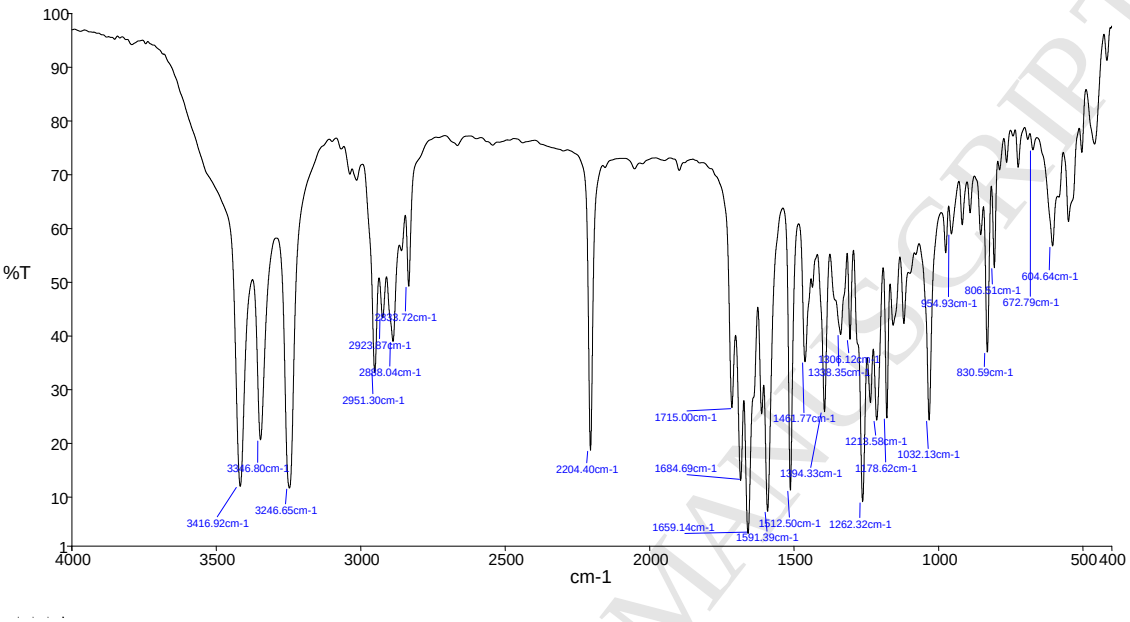
$^{13}$C NMR spectrum of 6j



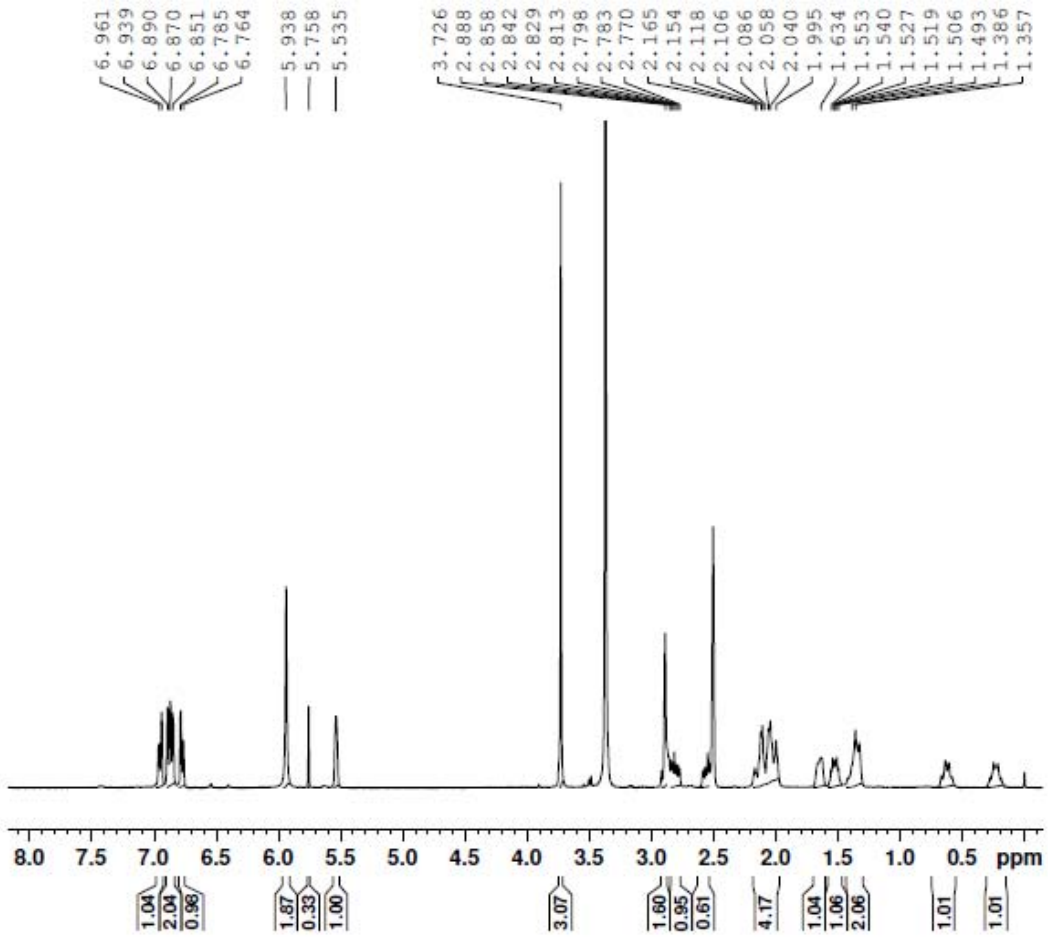
Mass spectrum of 6j



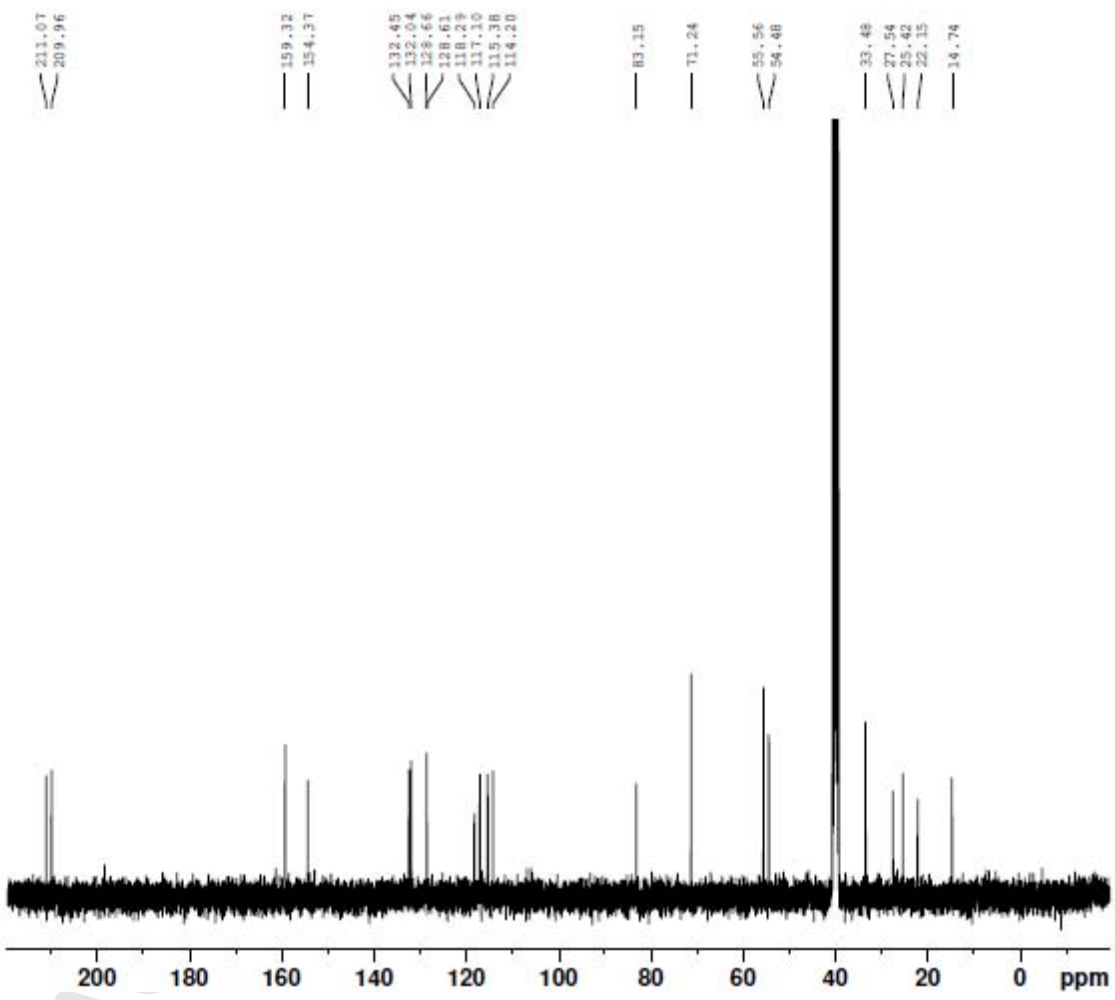
# IR Spectrum of compound 8b



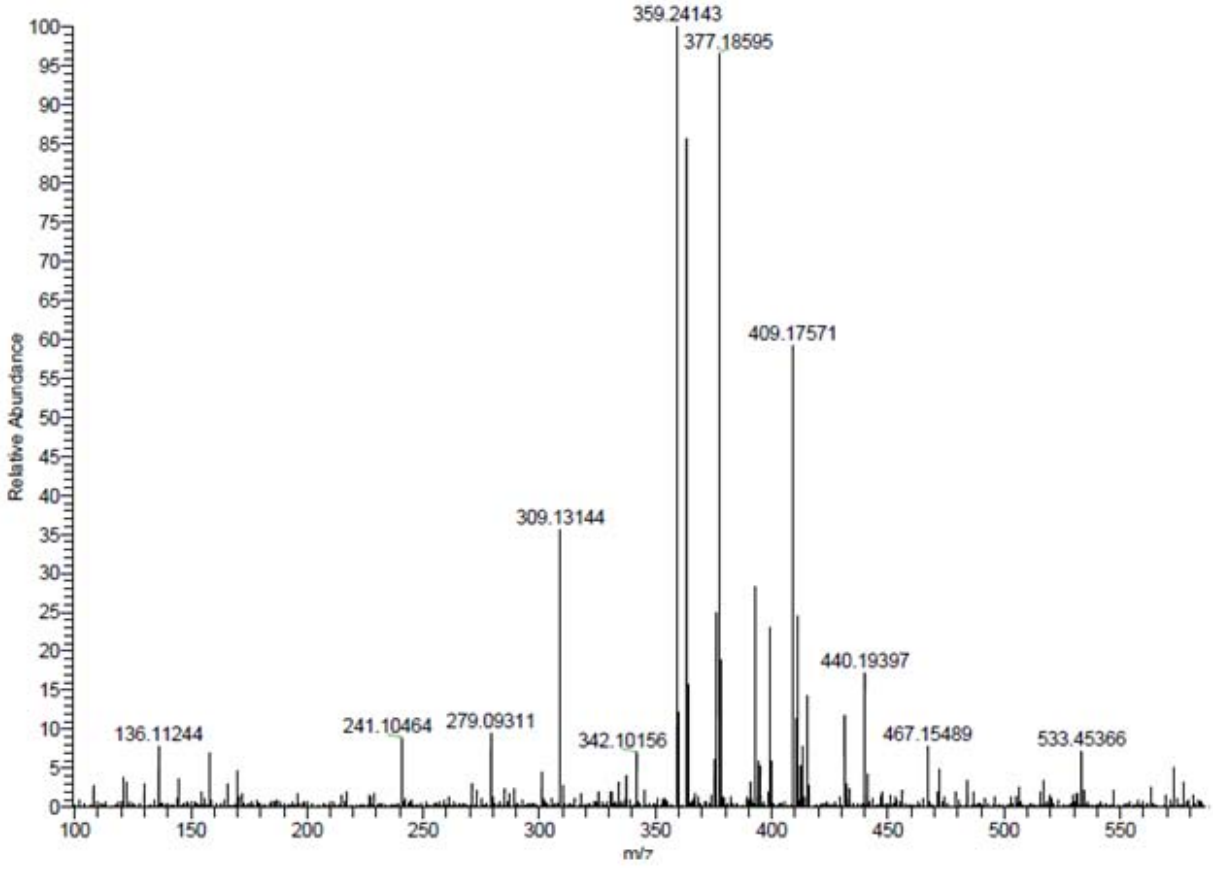
$^{1}$H NMR spectrum of 8b



$^{13}$C NMR spectrum of 8b



Mass spectrum of 8b



Binding energy and the interaction of ligands with the DNA gyrase receptor

| Ligand | Interaction | Free energy of Binding (kcal/mol) |
|---|---|---|
| 4a | ARG 1069 H-acceptor<br>GLY 1072 Pi-H | -10.34 |
| 4b | LYS 1077 (B) H-acceptor<br>ARG 1069 (B) pi-cation | -10.63 |
| 4c | ARG 1069 (B) H-acceptor | -9.90 |
| 4d | ARG 1122 (B) H-acceptor<br>MET 1075 (B) pi-H | -11.52 |
| 4e | ARG 1069 (B) pi-H | -8.33 |
| 4f | GLN 1056 (B) H-donor | -9.49 |
| 4g | ARG 1069 (B) H-acceptor | -8.45 |
| 4h | MET 1121 (B) H-acceptor | -8.33 |
| 4i | ARG 1122 | -11.64 |
| 4j | GLY 1076 (B) pi-H | -10.09 |
| 4k | MET 1075 (B) pi-H<br>ARG 1122 (B) pi-cation | -10.36 |
| 4l | MET 1075 (B) pi-H | -9.86 |
| 4m | GLN 1056 (B) H-donor | -9.51 |
| 6a | ARG 1069 (B) H-acceptor | -11.52 |
| 6b | ARG 1069 (B) H-acceptor | -11.20 |
| 6c | ARG 1122 (B) pi-H | -8.66 |
| 6d | GLY 1072 (B) H-donor | -11.01 |
| 6e | ASP 1073 (B) H-donor<br>LYS 1077 (B) H-acceptor<br>RG 1069 (B) pi-cation | -8.66 |
| 6f | ARG 1069 (B) pi-cation | -9.61 |
| 6g | ARG 1122 (B) pi-H<br>ARG 1122 (B) pi-H | -9.17 |
| 6h | ALA 1068 (B) H-donor | -8.14 |
| 6i | ASP 1073 (B) H-donor<br>LYS 1077 (B) H-acceptor | -10.66 |
| 6j | ARG 1069 (B) H-acceptor<br>ARG 1069 (B) H-acceptor | -10.82 |
| 8a | ARG 1069 (B) H-acceptor | -9.61 |
| 8b | ARG 1069 (B) H-acceptor | -10.52 |

Binding energy and the interaction of ligands with the ALK receptor

| Ligand | Interaction | Free energy of Binding (kcal/mol) |
|---|---|---|
| 4a | ASP 1203 H-donor | -15.20 |
| 4b | ASP 1203 Pi-H | -14.05 |
| 4c | ARG 1253 H-acceptor<br>HIS 1124 H-acceptor | -14.55 |
| 4d | GLU 1210 H-donor | -13.26 |
| 4e | ARG 1253 Pi-cation | -14.16 |
| 4f | ASP 1203 H-donor | -12.85 |
| 4g | MET 1328 H-donor<br>HIS 1124 Pi-H | -12.29 |
| 4h | GLU 1210 H-donor | -12.58 |
| 4i | HIS 1124 H-acceptor | -16.13 |
| 4j | HIS 1124 Pi-H | -11.84 |
| 4k | HIS 1124 H-acceptor | -14.37 |
| 4l | GLU 1210 H-donor | -12.60 |
| 4m | MET 1328 H-donor<br>HIS 1124 Pi-H | -12.29 |
| 6a | GLU 1210 H-donor | -11.57 |
| 6b | GLN 1177 H-acceptor | -11.79 |
| 6c | ARG 1253 H-acceptor | -12.69 |
| 6d | ASP 1249 H-donor<br>GLY 1272 H-acceptor | -12.45 |
| 6e | LYS 1267 H-acceptor<br>GLN 1146 Pi-H | -11.55 |
| 6f | GLU 1197 H-donor | -11.27 |
| 6g | ARG 1253 Pi-cation | -12.72 |
| 6h | ARG 1209 H-acceptor | -11.23 |
| 6i | ASP 1249 H-donor<br>ASN 1254 H-donor<br>GLY 1272 H-acceptor | -18.43 |
| 6j | HIS 1124 Pi-H<br>LYS 1205 Pi-H | -13.13 |
| 8a | LEU 1198 H-acceptor | -11.98 |
| 8b | HIS 1124 H-acceptor<br>HIS 1124 Pi-H | -13.97 |