NJC

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: K. Saravana Mani, W. Kaminsky and S. P. Rajendran, *New J. Chem.*, 2017, DOI: 10.1039/C7NJ02993D.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/njc

View Article Online
DOI: 10.1039/C7NJ02993D

A facile atom economic one pot multicomponent synthesis of bioactive spiro-indenoquinoxaline pyrrolizines as potent antioxidant and anti cancer agents

Kailasam Saravana Mani[a], Werner Kaminsky[b] and Subramaniam Parameswaran Rajendran[a]*

[a]Department of Chemistry, Bharathiar University, Tamil Nadu, India
[b]Department of Chemistry, University of Washington, Seatle, WA 98195, USA
Corresponding author: chemsaran.mani@gmail.com, rajendransp@yahoo.com

Abstract

An competent and highly discriminating one-pot synthesis of biologically active novel spiro-indenoquinoxaline pyrrolizines accumulating three pharmocophoric cores, heterocyclic quinoline scaffold, pyrrolizines and indeno-quinoxaline in a single molecular framework by means of four-component reaction between ninhydrin, *o*-phenylenediamine, L-proline and quinolinyl chalcones in methanol *via* [3+2]cycloaddition. The structures of the compounds were well characterized by FT-IR, NMR, ESI-MS, XRD and elemental analysis. The synthesized compounds were screened for *in vitro* antioxidant activity using DPPH, nitric oxide, super oxide radical and *in vitro* cytotoxic activity against MCF-7 and A-549 cancer cell lines and were visualized using Fluorescent microscopic technique. The newly synthesized compounds exhibited excellent antioxidant activity when compared to the standard molecule *ie*., BHT. In case of cytotoxic activity compounds **5c**, **5e**, **6c** and **6e** were found to be admirable activity when compared to standard doxorubicin against MCF-7 and A-549 cancer cells by MTT assay method. From the cell morphology analysis we clearly indicate that induced cell death by apoptosis and necrosis pathway. Molecular docking studies were performed out using EGFR inhibitor to determine the molecular interactions between compounds and proteins.

Keywords

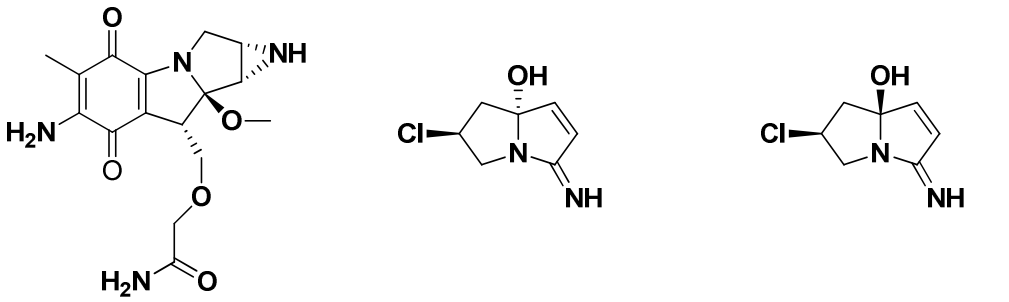
Spiro-indenoquinoxaline pyrrolizines, quinoline dipolarophile, L-proline, antioxidants and cytotoxic activity

View Article Online
DOI: 10.1039/C7NJ02993D

1. Introduction

In current trends, it is still a great challenge to our synthetic chemist for the development of novel bioactive molecules for the treatment of cancer urging with paramount less side effects and safety so far. However chemotherapy is the leading cancer treatment approach, its side effects, including death on normal cells and time duration or dose level, shrink its clinical applications[1]. Nitrogen containing heterocycles constructed through 1,3-dipolar cycloaddition of azomethine ylides[2-5] have been documented as an important cluster of significant bioactive compounds. It is a major class of heterocyclic systems plays an essential role in the field of anticancer drug enlargement and it holds several applications in medicinal chemistry including antibacterial,[6] antimalarial,[7] anti-inflammatory,[8] apoptosis,[9] DNA repair and tyrosine kinases(TK) inhibitor.[10] Tetracyclic Indeno Quinoxalines are also an another important class of heterocycles used as intermediates in the synthesis of several types of spiro compounds[11, 12] which possess potent α-glycosidase inhibitors.

Pyrrolizine derivatives represent a class of novel heterocycles which serves as promising scaffolds for anticancer drugs. The unique antitumor properties of mitocin C inspired chemists to develop different pyrrolizine systems and assess their potential antitumor activities against a wide variety of cancer types.[13]



Mitocin C **Clazamycin A** **Clazamycin B**

Fig. 1: Some important pyrrolizine antitumor candidates

Mitocin C isolated from *Streptomyces caespitosus* or *Streptomyces laevendulae* is used to treat upper esophageal carcinoma, anal, breast cancers and superficial bladder tumors. [14-16] Further it is believed to act as a good DNA alkylator which can cross-link DNA with high efficiency and specificity.[17,18] Clazamycin A and Clazamycin B are naturally occurring pyrrolizines with quite

View Article Online
DOI: 10.1039/C7NJ02993D

interesting anti-tumor properties.[19]**(Fig. 1)**. Recently Multicomponent reactions is a powerful tool to construct molecules of structural diversity and complexity with minimum waste generation, useful widely in both combinatorial and medicinal chemistry arena.[20] The above mentioned tremendous biological evaluation of quinolines, indeno quinoxaline and pyrrolizines prompted us to develop a cluster of all three heterocyclic scaffold together by multicomponent reaction and finding their biological evaluation.

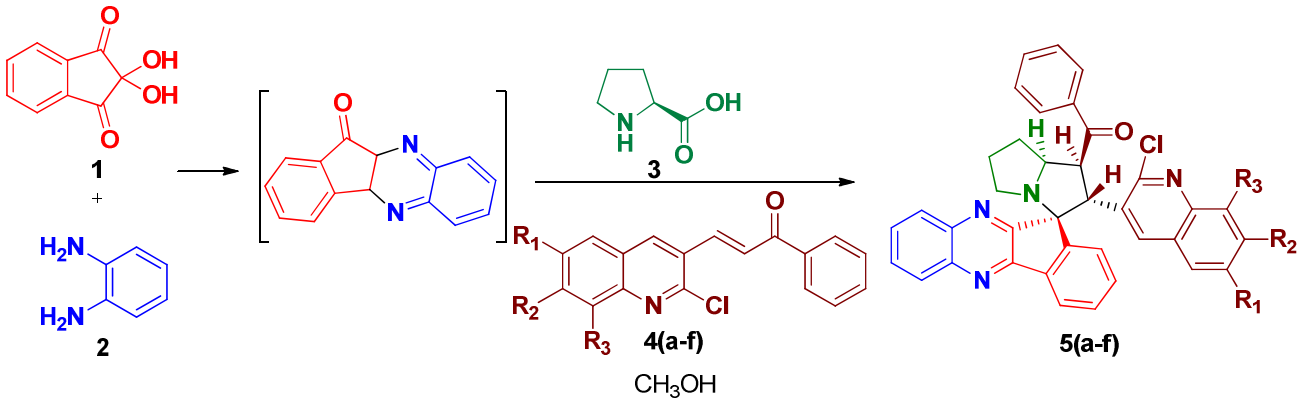
In this paper, we report a new synthetic methodology for the synthesis of novel bioactive spiro-indenoquinoxaline pyrrolizine molecules through multicomponent reactions, Herein we would like to report an efficient, atom economic, regioselective and high yielding MCR protocol for one-pot facile synthesis of bioactive functionalized spiro-indenoquinoxaline pyrrolizine heterocyclic scaffold by the reaction of ninhydrin, *o*-phenylenediamine, proline, quinoline bearing chalcones on refluxing in methanol and the evaluation of their *in vitro* antioxidant and *in vitro* anti tumor activity.

2. Results and Discussion

2.1. Chemistry

The synthesis of spiro [indeno[1,2-*b*] quinoxaline pyrrolizines **5(a-f)** and **6(a-f)** were achieved through a 1,3-dipolar cycloaddition reaction protocol[21]. Initially, quinoline based dipolarophiles **4(a-f)** were synthesized by the Claizen Schmidt condensation reaction between 2-chloro-3-formyl quinoline derivatives and acetophenone under alcoholic KOH condition. An equimolar mixture of ninhydrin **1**, *o*-phenylenediamine **2**, L-proline **3** and dipolarophile **4a** in methanol was refluxed for 2 to 3 hours **(Scheme 1)**. After completion of reaction was monitored by TLC, a single product spiro-indenoquinoxaline pyrrolizines **5a** was separated by evaporation of solvent in good yields. The optimized reaction condition, time and yields of **5(a-f)** were mentioned in **Table 1**. The FT-IR spectrum of **5a** displayed characteristic band at 1680 cm^{-1}, 1588 cm^{-1} and 759 cm^{-1} corresponding to C=O, -C=N and C-Cl in quinoline dipolarophile moiety. ^{1}H NMR spectrum of product **5a**, showed the characteristic multiplets at δ 1.89-2.64 corresponding to pyrrolizine ring.

View Article Online
DOI: 10.1039/C7NJ02993D



Scheme 1: Synthesis of spiro-indenoquinoxaline pyrrolizines 5(a-f)

The proton attached to benzoyl group appeared at δ 4.99 as triplet with coupling constant (J = 9.6 Hz) and pyrrolizine ring proton attached to quinoline moiety appeared at δ 5.70 as doublet with coupling constant (J = 11.6 Hz). The proton attached to benzoyl moiety consistent with *trans* disposition with proton attached to quinoline moiety. Similarly, the –NCH proton attached to pyrrolizine ring is in consistent with *cis* position to the proton attached to benzoyl group as shown XRD structure in **Fig. 2** whereas the signals for all other protons in aromatic region are in good agreement with proposed structure.

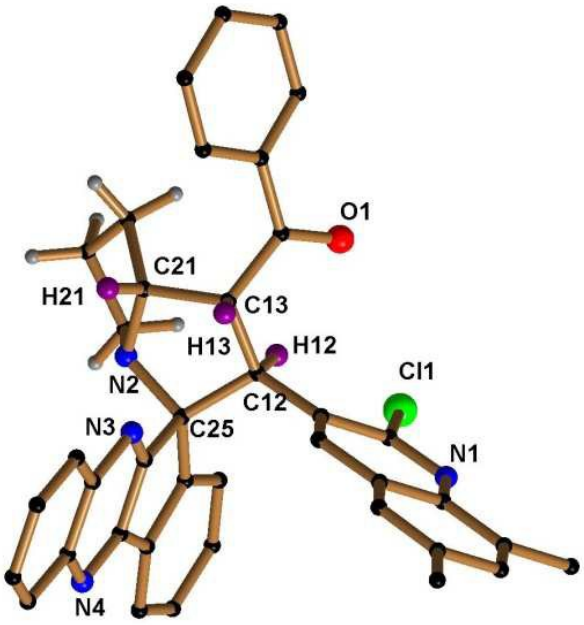
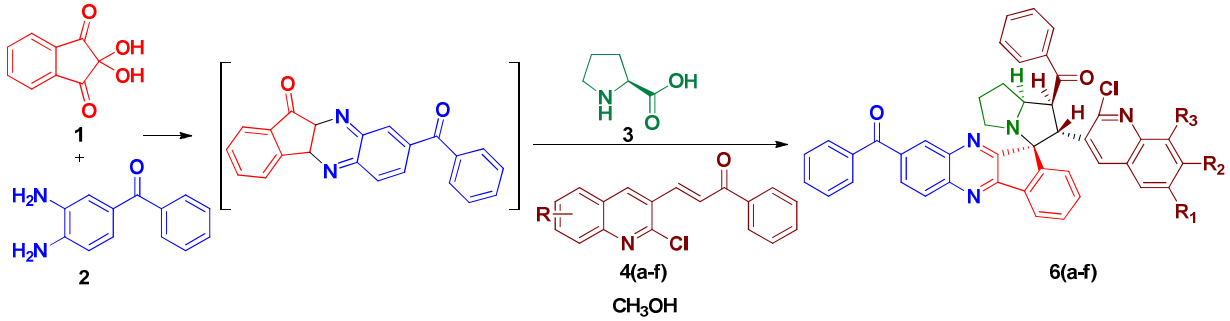


Fig. 2.Molecular structure of compound 5e, except hydrogen atoms in pyrrolizine ring are omitted for clarity. (CCDC 1569306)

View Article Online
DOI: 10.1039/C7NJ02993D

In ^{13}C NMR spectrum, the quaternary spiro carbon appears at δ 75.12 and carbonyl carbon attached to phenyl ring is appeared at δ 197.34. Whereas, the signals for all other carbons are located at appropriate chemical shifts were in perfect agreement with proposed structure. In DEPT-135 NMR, the appearance of three negative signals corresponding to methylene protons in pyrrolizine core further strongly attested the formation of product. The observed mass spectrum of the product HRMS (TOF ES+) *m/z* 593.2092 further confirmed the formation of compound **5a**.

In order to introduce the electron withdrawing substituent benzoyl group in quinoxaline core, we bring in 3,4-diaminobenzophenone instead of *o*-phenylenediamine. With the optimized condition in hand, we then explored the scope of this cycloaddition reaction using ninhydrin **1**, 3, 4-diaminobenzophenone **2a**, L-proline **3** and quinoline dipolarophile **4(a-f)** in refluxing methanol **(Scheme 2)**. After the completion of reaction, monitored by TLC, the precipitate obtained was filtered and washed with water to afford the single product **6(a-f)**. In IR spectrum of compound **6a**, characteristic peak at 1659 cm^{-1}, 1586 cm^{-1} and 701 cm^{-1} attributed to carbonyl stretching, quinoline –C=N and C-Cl respectively. The yield of compounds **6(a-f)** will be slightly high when compared to compounds **5(a-f)**.



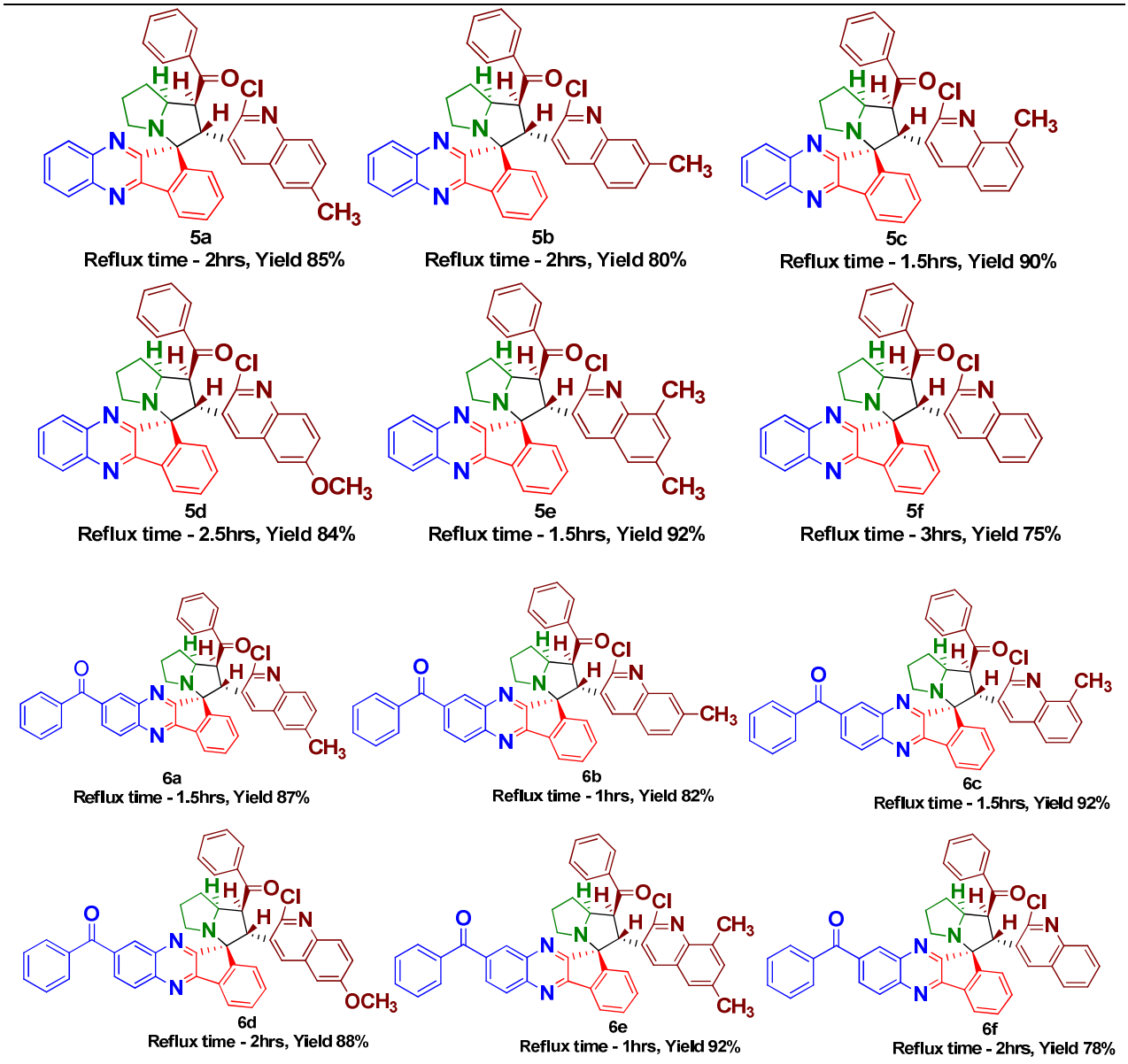
Scheme 2: Synthesis of benzoyl substituted spiro-indenoquinoxaline pyrrolizines 6(a-f)

In ^{1}H NMR spectrum of compound **6a**, the sharp singlet appeared at δ 2.50 attributed to $–CH_3$ group in quinoline scaffold. Multiplets of the pyrrolizine ring proton appeared in the range between δ 1.97 - δ 2.71. The proton attached to quinoline moiety will be slightly deshielded to δ 5.69 as doublet (J = 11.6 Hz) and the proton attached to benzoyl group resonated at δ 4.96 as triplet (J = 9.6 Hz). The chemical shifts at aromatic regions are in good agreement with proposed

View Article Online
DOI: 10.1039/C7NJ02993D

structure. The observed mass spectrum of the product HRMS (TOF ES+) *m/z* 697.2482 further confirmed the formation of compound **6b**.

Table 1: Reaction conditions for the synthesis of spiro indeno-quinoxaline 5, 6(a-f)



[a]Isolated yield, Reaction condition: ninhydrin **1** (1mmol), *o*-phenylenediamine **2** or 3,4-diamino benzophenone **2a**(1mmol), L-proline **3**(1mmol)quinoline chalcones**4**(1mmol), methanol(10ml)

View Article Online
DOI: 10.1039/C7NJ02993D

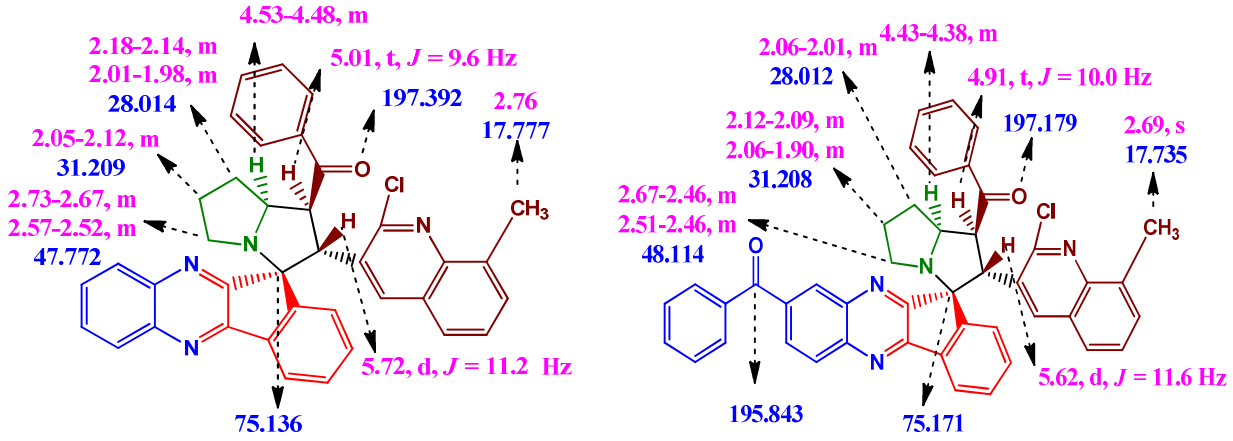


Fig. 3. ^{1}H and ^{13}C NMR chemical shift values of products 5c and 6c

In ^{13}C NMR spectrum, the peak at δ 197.116 and δ 195.863 which is due to two carbonyl carbons and peak at δ 75.181 attributed to quaternary spiro carbon. These observed chemical shift values are in good agreement with the structure of the compound **6a**. In DEPT-135 NMR spectrum the negative signals corresponding to three –CH_2 protons in pyrrolizine core. Furthermore, the presence of molecular ion peak at *m/z* 697.2476 in the mass spectrum confirmed the formation of product **6c**. The formation of spiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizines scaffold probably involves a complex multistep sequence. Initially the cyclocondensation reaction takes place between ninhydrin **1** and *o*-phenylenediamine **2** forms indeno-quinoxaline-11-one which further condensed with proline **3** that undergoes decaboxylation to give azomethine ylide **7** subsequently through cycloaddition reaction with dipolarophile **4** to give final product. **(Scheme 3)**

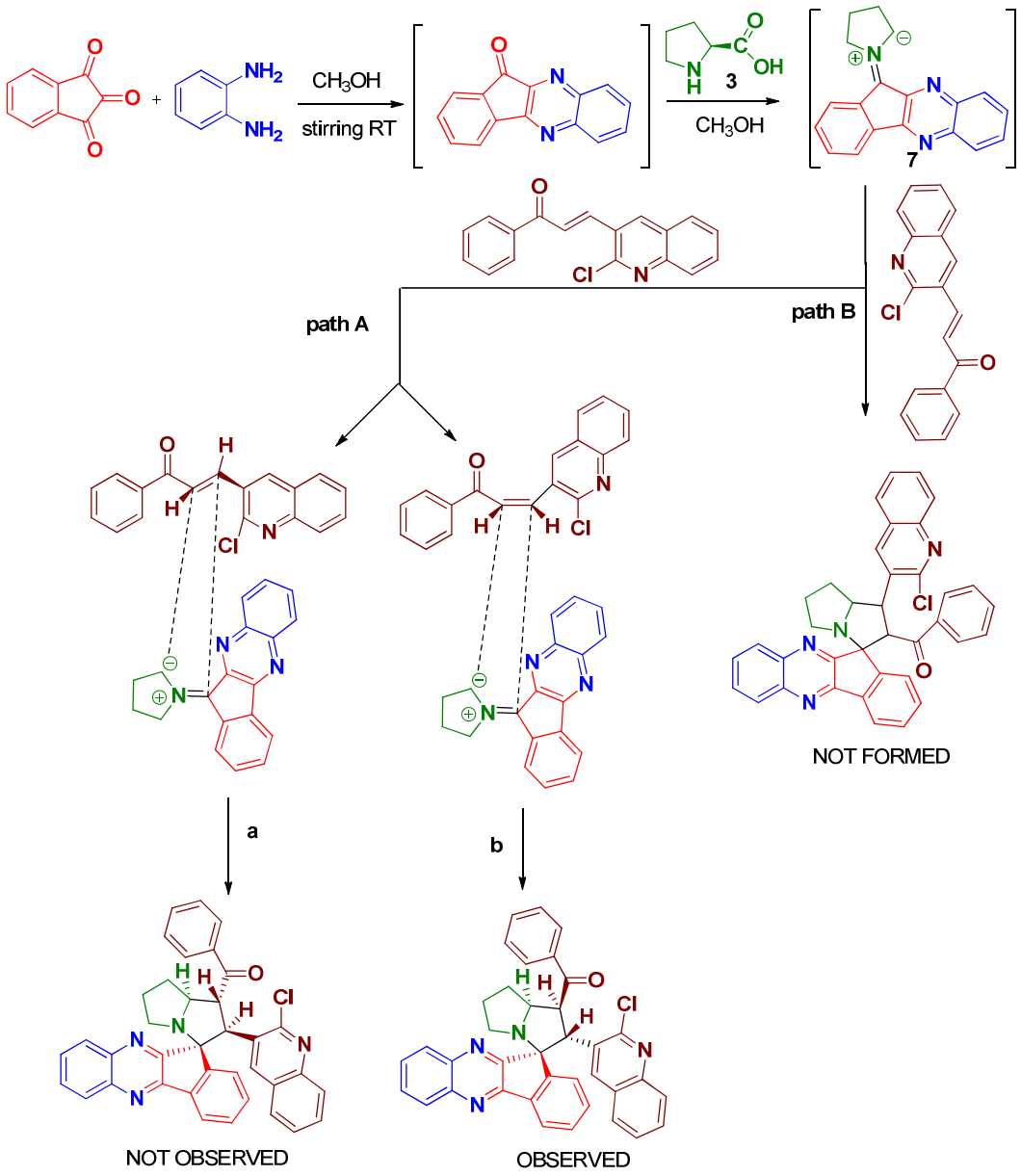
2. Biological Evaluation of Synthesized Compounds

2.1. *In Vitro* Antioxidant Activity

The antioxidant effectiveness mainly involves scavenging radicals and inhibiting oxidations of biological species. A number of quinoline derivatives were tested for anti oxidant activity as reflected the ability to inhibit lipid per-oxidation in rat brain and kidney homogenates. Free radical scavenging capacity can be well-known by the neutralization of electrons or hydrogens. Compounds having low IC_{50} values having high hydrogen donating ability lead more

View Article Online
DOI: 10.1039/C7NJ02993D

antioxidant activity. The radical scavenging capacity of compounds **5(a-f)** and **6(a-f)** along with standard BHT were tested against DPPH, nitric oxide and super oxide radicals and the results were summarized in **(Table 2)**.



Scheme 3: Proposed mechanism for the formation of spiro indeno-quinoxaline pyrrolizines

View Article Online
DOI: 10.1039/C7NJ02993D

Table 2. Radical scavenging activity of compounds **5(a-f)** and **6(a-f)** in **IC_{50} µg/mL**

Compounds	DPPH	Nitric oxide	Super oxide
5a	6.13	5.87	4.43
5b	5.82	4.56	5.69
5c	**3.42**	**2.68**	**4.12**
5d	**3.32**	**5.51**	**3.45**
5e	**2.96**	**1.34**	**4.01**
5f	7.23	9.52	8.54
6a	8.4	6.95	10.11
6b	6.71	5.98	8.65
6c	**3.6**	**5.24**	**3.4**
6d	**3.2**	**5.43**	**3.9**
6e	**3.56**	**3.43**	**3.8**
6f	8.24	6.32	9.54
BHT	7.93	7.19	9.30

From the results obtained all the synthesized compounds **(Fig. 4)** exhibited excellent activity when compared to that of the standard BHT. Interestingly compound **5e** possess most promising activity with the IC_{50} values of 2.96, 1.34 and 4.01 against DPPH, Nitric oxide and super oxide radicals which outperformed standard BHT with the IC_{50} values of 7.93, 7.19 and 9.30 respectively. Next in the series, compounds **5c**, **5d**, **6c, 6d** and **6e** showed excellent activity in all three radical scavenging assays. The compounds **5a**, **5b**, **5f**, **6a**, **6b** and **6f** displayed equipotent activity with standard molecule BHT. The reason for high scavenging capacity of the compounds may be nature of substituents in quinoline core. From the results, we clearly suggest that compounds having electron donating groups[22, 23] at in 6th and 8th position of quinoline scaffold will be significantly more active than that of compound having electron donating group at 7th position. The presence of $–OCH_3$[24] in 6th position will be more active when compared to –

View Article Online
DOI: 10.1039/C7NJ02993D

CH_3 group in 6th position. From the structural analysis, the absence of electron donating group in quinoline moiety showed less activity when compared to rest of the compounds. **(Scheme 4)**

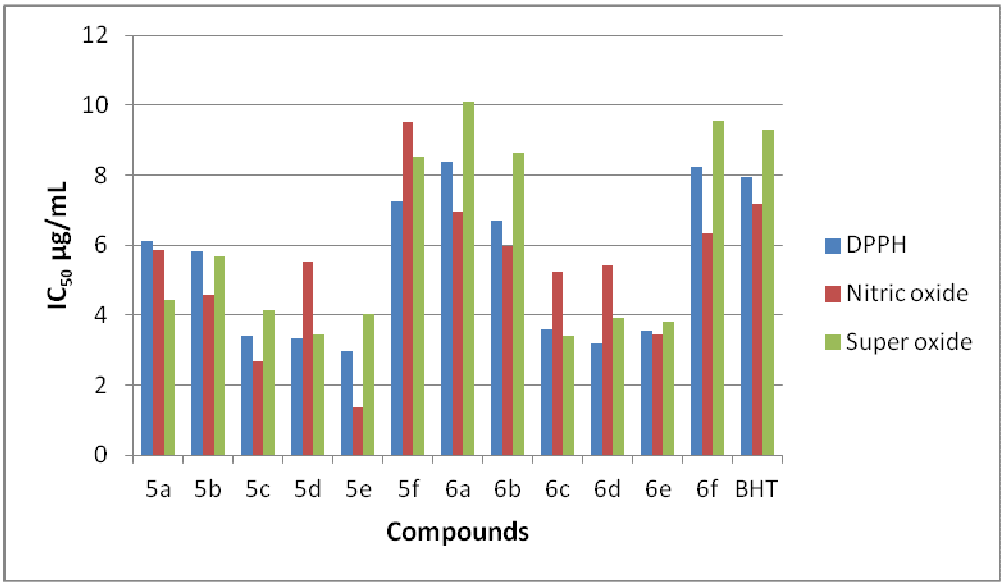


Fig. 4. Flow chart representing antioxidant activity of synthesized compounds

2.2. *In Vitro* Cytotoxic Activity

The use of drugs in different target, often discrete with multiple biological targets, with the expectation of synergistic effects and low toxicity, safety becomes an ideal approach of increasing interest in nowadays research. The anticancer properties of synthesized compounds **5(a-f)** and **6(a-f)** were screened against MCF-7 and A-549 cancer cell lines by MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphentltetrazolium bromide] assay method. Doxorubicin can act as a positive control for this study. Inhibition of cells was measured in terms of IC_{50} values after 48hrs of cell exposure. The results obtained were summarized in **Table 3**.

As listed in **Table 3**, the entire synthesized compounds exhibited good to moderate activity against two cell lines while compared to Doxorubicin standard. Among the synthesized compounds, compounds **5e** and **6e** were showed equipotent activity with IC_{50} values of **15±1.6** and **16±0.5** against MCF-7 cell line and **16±1.8** and **17±1.8** against A-549 cell lines when compared to that of positive control Doxorubicin **14±1.0** (MCF-7) and **15±1.4** (A-549) respectively. Increase in cytotoxic values of **5e** and **6e** suggests that electron donating methyl group may be favorable at 6th and 8th position of quinoline core.

View Article Online
DOI: 10.1039/C7NJ02993D

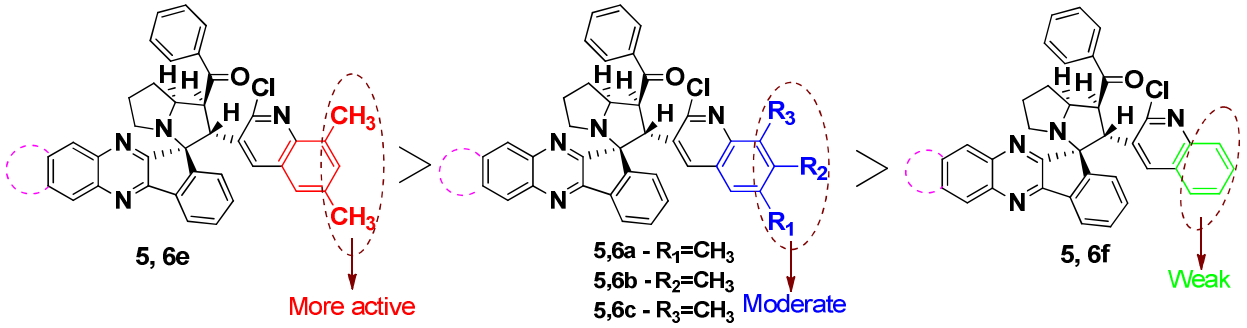
Table 3: Cytotoxic activity of spiro indeno-quinoxaline pyrrolizines (5a-f) & (6a-f) in IC_{50} (µM)

Samples	MCF-7[a]	A-549[b]
5a	33± 1.4	24± 1.0
5b	23±0.5	36± 1.5
5c	**17±1.6**	**19± 0.2**
5d	28± 1.5	24± 1.5
5e	**15± 1.9**	**16±1.8**
5f	35±1.5	37±1.7
6a	25± 1.4	32 ± 1.0
6b	24± 1.5	32± 0.9
6c	**18± 1.3**	**20±1.5**
6d	26± 1.3	24± 0.5
6e	**16± 0.5**	**17± 1.8**
6f	30±0.7	33±1.8
Doxorubucin	**14± 1.0**	**15± 1.4**

[a]MCF-7 (breast cancer cell line)
[b]A-549(lung cancer cell line)

On further investigation on methyl group in the position of quinoline core, surprisingly methyl group at 8th position possess good activity with IC_{50} values of **17±1.6** and **18±1.3 (Compound 5c)** against MCF-7 and **19±0.2** and **20±1.8 (Compound 6c)** against A-549 cancer cell lines. Meanwhile rests of the compounds with electron donating groups at 6th and 7th position showed moderate activity against both cancer cell lines. This clearly indicates that electron donating group at 8th position increase the anticancer potency rather than other positions. Absence of electron donating group in any of the position in quinoline core displayed week activity towards MCF-7 and A-549 cell lines. **(Scheme 4)**

View Article Online
DOI: 10.1039/C7NJ02993D



Scheme 4-Role of electron donation substituents in quinoline core

2.3. Cell Morphology Analysis

To monitor the effect of synthesized compounds on cell morphology, treated cancer cells were examined by inverted light microscopy and compared with untreated cells. Treated cells showed significant changes compared with untreated cells. Cytological investigations elucidate the anticancer effect routed through membrane blebbing, membrane instability and disturbing the cytoskeleton of the cells by the compounds.

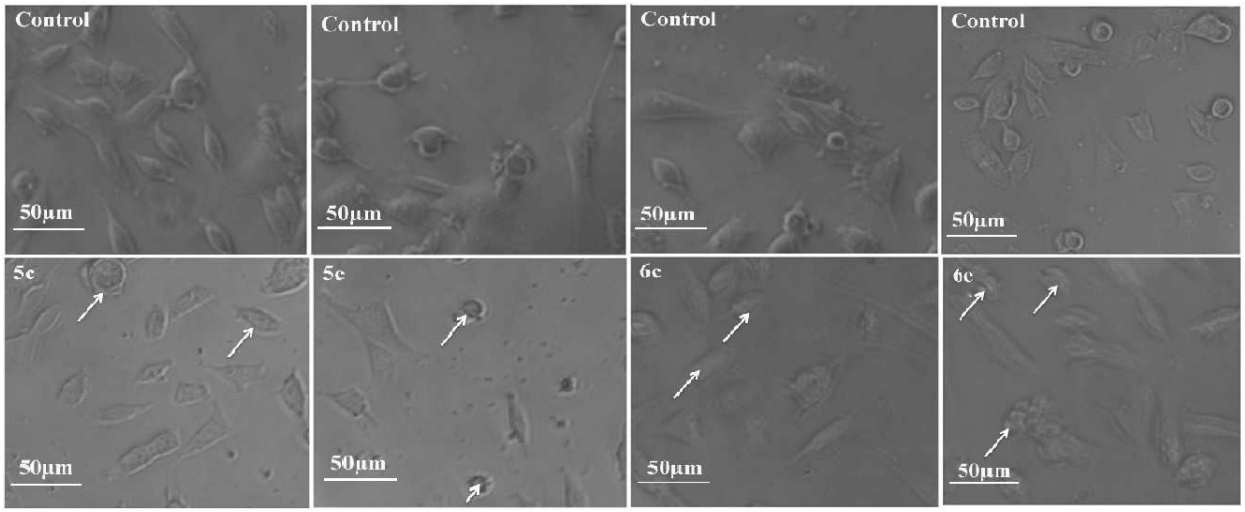


Fig. 5: Morphological analysis of treated MCF-7 cancer cells and the arrow mark indicates the formation of floating cells and appearance of membrane blebbing

Fig. 5 and **Fig. 6** reveal the morphological changes in MCF-7 and A-549 after treatment with compounds with their respective inhibitory concentrations for 24 hrs. Phase contrast micrographs revealed that the compounds **5c**, **5e**, **6c** and **6e** induced increase cell shrinkage,

View Article Online
DOI: 10.1039/C7NJ02993D

membrane blebbing and form floating cells in a dose independent manner.

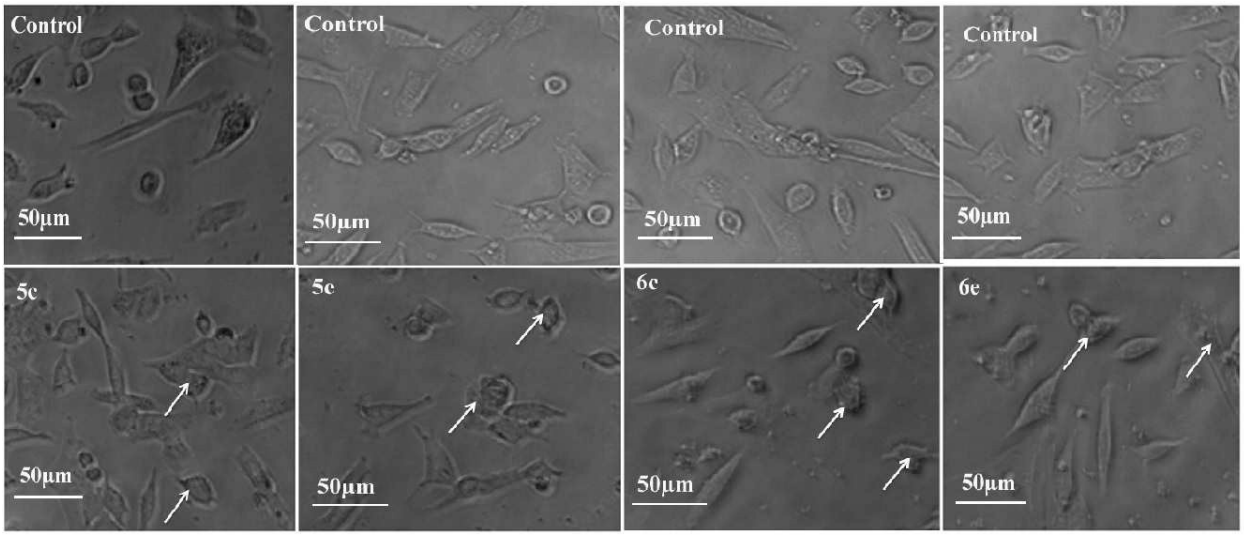


Fig 6: Morphological analysis of treated A-549 cells and the arrow mark indicates the formation of floating cells and appearance of membrane blebbing

2.4. Acridine Orange-Ethidium Bromide (AO-EB) Staining

Acridine orange/ Ethidium bromide (AO/EB) fluorescent staining assay were performed to distinguish the pathway *i.e* apoptotic, necrotic and live cells. [25] AO permeates the intact cell membrane and stains the nuclei green, whereas EB permeates only cells with loss in membrane integrity and stain the nucleus orange. The compounds **5c**, **5e**, **6c** and **6e** untreated and treated MCF-7 and A-549 cells were stained with AO-EB and analyzed under fluorescent microscope. **Fig.7** and **Fig. 8** shows that the untreated cancer cells (control) did not show any significant adverse effects compared to the compounds treated cancer cells. It can be observed that with the addition of compounds to the cancer cells, the green color of cells are converted into orange /red color cells which is due to induced apoptosis and the nuclear condensation effects on the cells. **Fig.7** and **Fig. 8** also shows that the compounds were significantly inducing the apoptosis in performed cancer lines.

View Article Online
DOI: 10.1039/C7NJ02993D

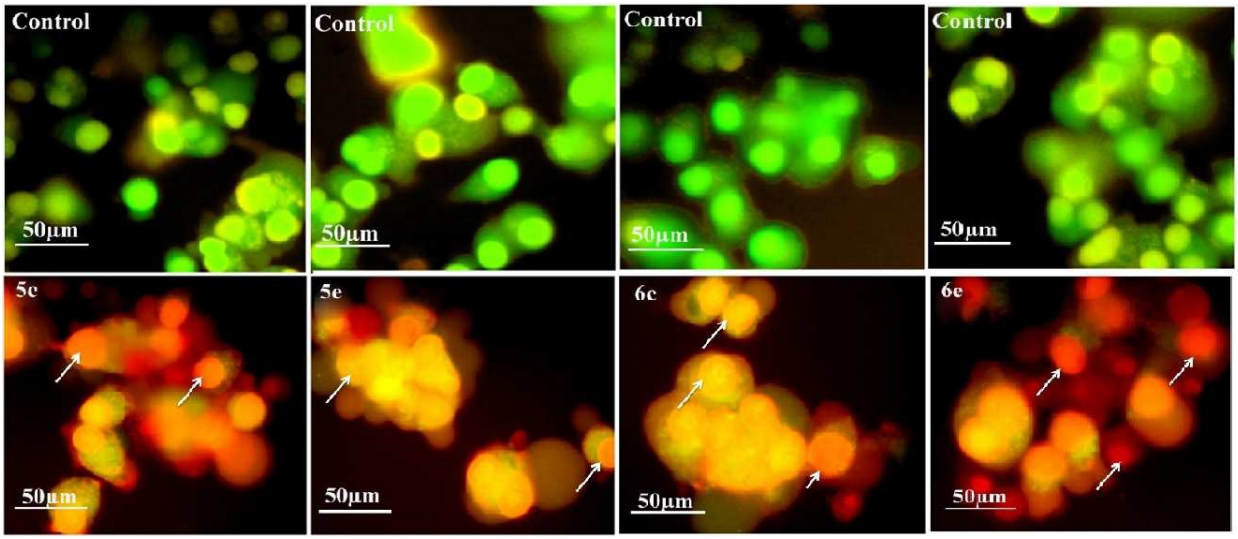


Fig. 7: AO/EB apoptotic analysis of treated MCF-7 cells and arrows indicate apoptotic cells

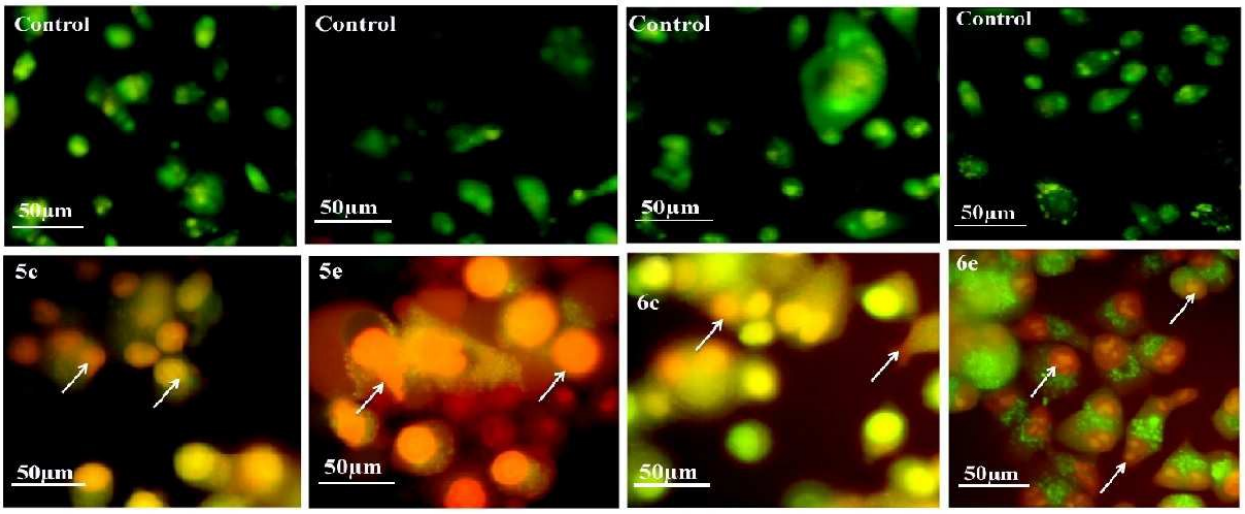


Fig. 8: AO/EB apoptotic analysis of treated A-549 cells and arrows indicate apoptotic cells

2.5. DAPI Straining method

4′,6-Diamidino-2-phenylindole (DAPI) is a nuclear stain which strongly binds to adenine-thymine clusters of the minor groove of double-stranded DNA and detect the chromatin condensation or nuclear damage. Binding of DAPI to DNA produces a ~20-fold enhanced fluorescence, apparently due to the displacement of water molecules from both DAPI and the minor groove.[26] DAPI stains the apoptotic cells as bright colored due to the condensed nucleus

View Article Online
DOI: 10.1039/C7NJ02993D

which is a typical apoptotic feature. Fluorescence microscopy images of lung and breast cancer cells after 24 hrs stained with DAPI in the absence and presence of compounds are shown in **Fig. 9** and **Fig. 10**, which reveals that the untreated cells didn't show any significant changes whereas compounds treated cancer cells shows bright fetches which indicates the condensed chromatins and nuclear fragmentations in the cancer cells. Thus, the results from MTT assay and Fluorescence microscopy analysis, we confidently report here that the compounds may be used as potent therapeutic agents.

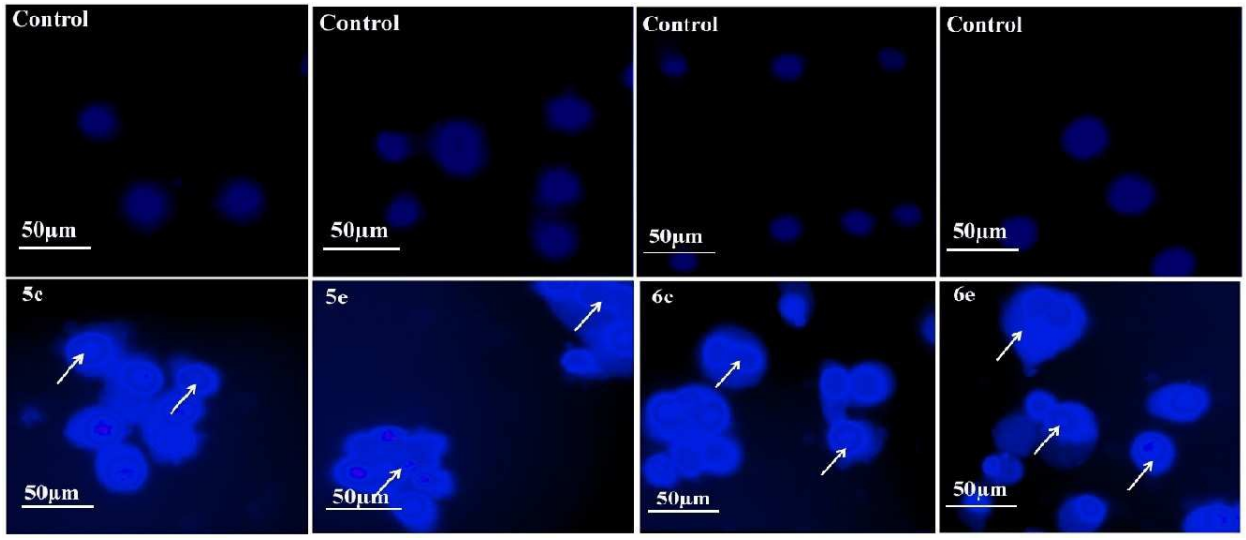


Fig. 9: DAPI apoptotic analysis of treated MCF-7 cells and arrows indicate apoptotic cells

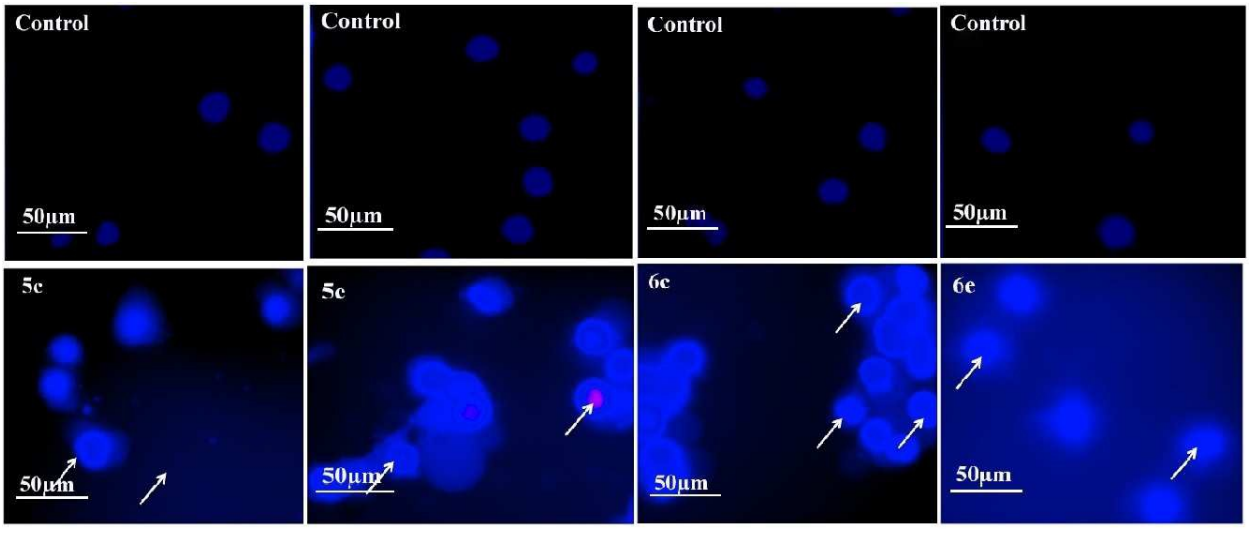


Fig. 10: DAPI apoptotic analysis of treated A-549 cells and arrows indicate apoptotic cells

View Article Online
DOI: 10.1039/C7NJ02993D

2.6. Molecular Docking

Docking studies predicted the interaction of inhibitors with protein and residues involved in this complex. The orientation and conformation of inhibitors bound in the active site of the protein and formed protein–ligand complex with the highest binding energy value are the most important requirement for docking studies. The inhibition potency of the newly synthesized compounds **5e** and **6e** were subjected for further docking studies to explore the binding pattern against Epidermal growth factor receptor (EGFR)(PDB code: 1M17)[28] using Autodock 4.2 software.[28] As depicted from **Fig. 11**, compound **5e** is surrounded by hydrophobic amino acids such as GLY 695, CYS 773, TYR 777, GLY 772, LEU 694, VAL 693, LYS 704 and LYS 692. The key amino acid CYS 773 is considered to play a crucial role in the stabilization of the receptor by compound **5e** which can be observed in hydrophobic enclosure where nitrogen atom in quinoxaline ring is mounted towards the **CYS 773** hydrophobic amino acid (1.8 Å**)** with glide score (-5.54 kcal/mol). Similarly the compound **6e** is surrounded by hydrophobic amino acids ARG 817, LEU 820, GLY 772, CYS 773, LYS 692, PHE 699 *etc*. As like observed in compound **5e**, compound **6e** too observed that key amino acid CYS 773 is considered to play a crucial role in the stabilization of receptor through hydrophobic enclosure where nitrogen atom in

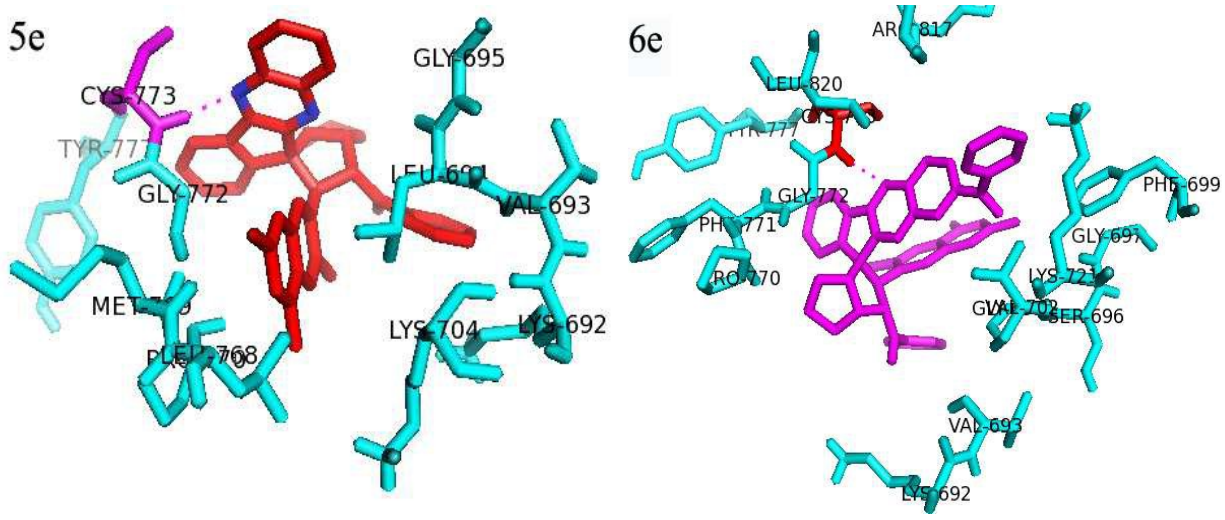


Fig.11. Docking studies on compounds 5e and 6e with EGF receptor and dotted lines shows hydrogen bond

quinoxaline ring is hydrogen bonded with **CYS 773** hydrophobic amino acid (2.2 Å**)** with glide score (-6.05 kcal/mol). So it is assumed that hydrophobicity is one of the important

View Article Online
DOI: 10.1039/C7NJ02993D

physicochemical properties which impart lipophilicity to the molecule as well as membrane permeability and their absorption in biological system.

3. Conclusion

In summary, we have developed a highly atom economic synthesis of spiro indeno-quinoxaline derivatives *via* one pot four component 1,3-dipolar cycloaddition reactions of azomethine ylide and quinoline derived dipolarophile in successful manner. The pure products were isolated by recrystallization. The structures of the synthesized compounds were well characterized by using single crystal XRD, spectral and elemental analysis. All the synthesized compounds were successfully screened them against antioxidants and cytotoxic activity for two cancer cell lines (MCF-7 and A-549). Interestingly all the compounds exhibited excellent activity against DPPH, nitric oxide and super oxide free radicals. Compounds **5c**, **5e**, **6c** and **6e** found to be significantly active in this series and also induced cell death by apoptosis and necrosis. The substituents of electron donating group in 6^{th} and 8^{th} positions turns out to be the best candidates in this series screened.

Acknowledgement

We would like to thank SAIF, CUSAT, Cochin for NMR spectral analysis and University of Washington, Seatle, US for Single crystal XRD data. We thank Department of Botany and Zoology, Bharathiar University for evaluating antioxidant and anticancer studies.

Electronic Supplementary Information

X-ray crystal data and Spectral data of all the compounds associated with article will be available as Supporting Information.

References

1. S. Dhar, N. Kolishetti, S. J. Lippard, O. C. Farokhzad, *Nati Acad Sci U S A*, 2011, 108, 1850.
2. A. M. Akondi, S. Mekala, M. L. Kantam, R. Trivedi, L. Raju Chowhane, A. Dasbc, *New J. Chem*., 2017, 41, 873.
3. R. Sakhuja, S. S. Panda, L. Khanna, S. Khurana, S. C. Jain, *Bioorg. Med. Chem.*, 2011, 21, 5465.

View Article Online
DOI: 10.1039/C7NJ02993D

4. A. A. Raj, R. Raghunathan, M. R. Sridevi Kumari, N. Raman, *Bioorg. Med. Chem.*, 2003, 11, 407.
5. B. V. Subba Reddy, G. Karthik, T. Rajasekaran, A. Antony, B. Sridhar, *Tet. Lett.*, 2012, 53, 2396.
6. N. Sun, R. L. Du, Y. Y. Zheng, B. H. Huang, Q. Guo, R. F. Zhang, K. Y. Wong, Y. J. Lu, *Eur. J. Med. Chem.*, 2017, 135, 1.
7. K. Kaur, M. Jain, R. P. Reddy, R. Jain, *Eur. J. Med. Chem.*, 2010, 45, 8.
8. E. Rajanarendar, M. Nagi Reddy, S. Rama Krishna, K. Rama Murthy, Y. N. Reddy, M. V. Rajam, *Eur. J. Med. Chem.*, 2012, 55, 273.
9. O. Afzal, S. Kumar, M. R. Haider, M. R. Ali, R. Kumar, M. Jaggi, S. Bawa, *Eur. J. Med. Chem.*, 2015, 97, 871.
10. K. M. Amin, H. H. Georgey, F. M. Awadallah, *Med. Chem. Res.*, 2011, 20, 1042.
11. M. T. Maghsoodlu, S. M. Habibi-Khorassani, A. Morani, N. Hazeri, A. Davodi, S. S. Sajadikhah, *Tetrahedron*, 2011, 67, 8492.
12. M. Sahar Kahn, M. A. Munawar, M. Ashraf, *Biorg. Med. Chem. Lett.*, 2014, 22, 1195.
13. A. Belal, B. El-Dien, M. El-Gendy, *Biorg. Med. Chem.*, 2014, 22, 46.
14. S. K. Carter, S. T. Crooke, *Mitocin C: Current status and new developments*; Eds., Academic Press: 1979.
15. H. Schwartz, J. E. Sodergren, F. S. Philips, *Science*, 1963, 142, 1181.
16. W. T. Bradner, *Cancer Treat. Rev.*, 2001, 27, 35.
17. M. Tomasz, R. Lipman, D. Chowdary, J. Pawlak, G. L. Verdine, K. Nakanishi, *Science*, 1987, 235, 1204.
18. M. Tomasz, *Chem. Biol.*, 1995, 2, 575.
19. D. D. Buechter, D. E. Thurston, *J. Nat. Prod.*, 1987, 50, 360.
20. (a). A. Domling, I. Ugi, *Angew. Chem. Int. Ed.*, 2000, 39, 3168. (b). A. Domling, I. Ugi, *Chem. Rev.*, 2006, 106, 17. (c). R. V. A. Orru, M. de Greef, *Synthesis*, 2003, 10, 1471. (d). J. D. Sunderhaus, S. F. Martin, *Chem. Eur. J.*, 2009, 15, 1300. (e). N. Isambert, R. Lavilla, *Chem. Eur. J.*, 2008, 14, 8444. (f). D. J. Ramon, M. Yus, *Angew. Chem. Int. Ed.*, 2005, 44, 1602.
21. (a). A. Dandia, V. Parewa, S. Kumari, S. Bansal, A. Sharma, *Green Chem.*, 2016, 18, 2488. (b). A. Dandia, A. K. Jain, Laxkar. A. K, Bhati. D. S, *Tetrahedron Letts.*, 2013,

View Article Online
DOI: 10.1039/C7NJ02993D

54(12), 3180. (c). A. Dandia, A. K. Jain, A. K. Laxkar, *RSC Adv.*, 2013, 3, 8422. (d). A. Dandia, A. K. Jain, Laxkar. A. K, Bhati. D. S, *Tetrahedron*, 2013, 69(8), 2062.

22. H. Y. Zhang, *Curr. Comput. Aided Drugs Des.*, 2005, 1, 257.
23. M. Sankaran, K. Chandraprakash, C. Uvarani, P. S. Mohan, *Bioorg. Med. Chem. Lett.*, 2010, 20, 7147.
24. K. P. Rakesh, H. M. Manukumar, D. C. Gowda, *Bioorg. Med. Chem. Lett.*, 2015, 25, 1072.
25. Y. Chang, Y. Li, N. Ye, X. Guo, Z. Li, G. Sun, Y. Sun, *Apoptosis*, 2016, 21, 977.
26. T. S. Reddy, H. Kulhari, V. G. Reddy, A. V. S. Rao, V. Bansal, A. Kamal, R. Shukla, *Org. Biomol. Chem.*, 2015, 13, 10136.
27. J. Stamos, M. X. Sliwkowski, C. Eigenbrot, J Biol Chem., 2002, 277, 46265.
28. E. M. Pique, R. Huey, The Autodock 4.2 molecular graphics system. http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page; 2010

View Article Online
DOI: 10.1039/C7NJ02993D

A facile atom economic one pot multicomponent synthesis of bioactive spiro-indenoquinoxaline pyrrolizines as potent antioxidant and anti cancer agents

Kailasam Saravana Mani[a], Werner Kaminsky[b] and Subramaniam Parameshwaran Rajendran[a]*

[a]Department of Chemistry, Bharathiar University, Tamil Nadu, India
[b]Department of Chemistry, University of Washington, Seatle, WA 98195, USA
Corresponding author: chemsaran.mani@gmail.com, rajendransp@yahoo.com

GRAPHICAL ABSTRACT