# *SYNOPSIS*

The work in this thesis entitled "**Development of New Synthetic Methods for Some Biologically Important Heterocycles and Evaluation of Cytotoxic and Antimicrobial Potential**" has been divided into five Chapters. The main aim of this work is the design and synthesis of biologically important heterocycles, such as spiro-pyrazolo-pyridine derivatives, spiro-pyrazolo[3,4-*f*]quinoline derivatives, 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-one derivatives, 4'-phenyl-1'*H*-spiro[indoline-3,2'-quinazolin]-2-ones, quinoxalines and quinoxaline-sulphonamide conjugates, pyrrolo[1,2-*a*]quinoxalines and their evaluation of cytotoxic and antimicrobial activities.

**CHAPTER I:** Describes the "Multicomponent and green synthesis of spirooxindoles and evaluation of their cytotoxicities" and it has been divided into two sections.

**SECTION-A:** This section deals with the "Sulfamic acid promoted one-pot, three-component, green synthesis and cytotoxic evaluation of spiro pyrazolo-pyridine derivatives."

**SECTION-B:** This section deals with the "Amberlite IR-120H catalysed facile, aqueous phase and green synthesis of pyrazolo[3,4-*f*]quinoline-spirooxindole derivatives and their cytotoxic evaluation."

**CHAPTER II:** Explains the "Green synthesis of spiro-quinazoline derivatives and evaluation of their antimicrobial activities" and it has been divided into two sections.

**SECTION-A:** This section describes the "Sulfamic acid catalysed efficient and green synthesis of 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-one derivatives and evaluation of their antimicrobial and anti-biofilm activities."

**SECTION-B:** This section describes the "Catalyst-free, one pot and three-component synthesis of 4'-phenyl-1'*H*-spiro[indoline-3,2'-quinazolin]-2-ones."

**CHAPTER III:** Summarizes the "Green synthesis of quinoxalines and naphtho[2,3-*f*]quinoline derivatives and evaluation of cytotoxic and antimicrobial activity" and it has been divided into two sections.

**SECTION-A:** This section illustrates the "L-Proline mediated synthesis of quinoxalines; Evaluation of cytotoxic and antimicrobial Activity."

**SECTION-B:** This section describes the "Amberlite IR-120H catalyzed MCR: An efficient and green synthesis of Naphtho[2,3-*f*]quinoline derivatives and their cytotoxicity."

**CHAPTER IV:** Explains the "Efficient and green synthesis of pyrrolo[1,2-*a*]quinoxalines."

**SECTION-A:** This section describes the "Sulfamic acid: An efficient and recyclable solid acid catalyst for the synthesis of 4,5-dihydropyrrolo[1,2-*a*]quinoxalines."

**SECTION-B:** This section describes the "Amberlite IR-120H: An efficient and recyclable heterogeneous catalyst for the synthesis of pyrrolo[1,2-*a*]quinoxalines and 5'*H*-spiro[indoline-3,4'-pyrrolo[1,2-*a*]quinoxalin]-2-ones."

**CHAPTER V:** Explains the "Synthesis and Biological evaluation of Triazole-Pyrazole amide conjugates."

**CHAPTER I:** Describes the "Multicomponent and green synthesis of spirooxindoles and evaluation of their cytotoxicities" and it has been divided into two sections.

## Section-A: Sulfamic acid promoted one-pot, three-component, green synthesis and cytotoxic evaluation of spiro pyrazolo-pyridine derivatives

Multicomponent reactions (MCRs) are useful organic reactions in which three or more components react to form a final product in a single reaction vessel. MCRs allow the formation of several bonds in a single operation and are highly advantageous with respect to simplicity, extraction and purification thereby resulting in high atom economy. This approach is economical and non-tedious with broad range of applications in the areas of drug discovery and organic synthesis. Therefore, design of new MCRs with green and recyclable procedures attracted great attention, particularly employing water as a solvent due to its exclusive properties.

The spirooxindole core moiety is of greater biological interest due to the presence of a spirocarbon in these heterocycles by causing structural rigidity due to conformational restrictions and considerably influences the biological activities. Spirooxindole is a privileged heterocyclic core present in large number of natural products such as Spirotryprostatin A and B, pteropodine, elacomine, horsfiline and alstonisine (Figure). Spirotryprostatin A and B are the two natural alkaloids isolated from the fermentation broth of *Aspergillus fumigates*, that are known to inhibit the mammalian cell cycle in the G2/M phase, pteropodine act as positive modulators of muscarinic M2 and 5-HT2 receptors, elacomine which is naturally occurring hemiterpene spirooxindole alkaloids isolated from the roots of the shrub *Elaeagnus commutate*, (-)-horsfiline, found in *Horsfieldia superba* plant which is used as analgesic, Furthermore, spirooxindole system is also a core scaffold of many synthetic pharmaceutical ingredients with wide range of biological applications such as antimicrobial, antitumor, antibiotic and inhibitors of the human NK-1 receptor. Therefore, due to their significant biological activities it is important to find new and simple synthetic methods for the preparation of such spirooxindoles.

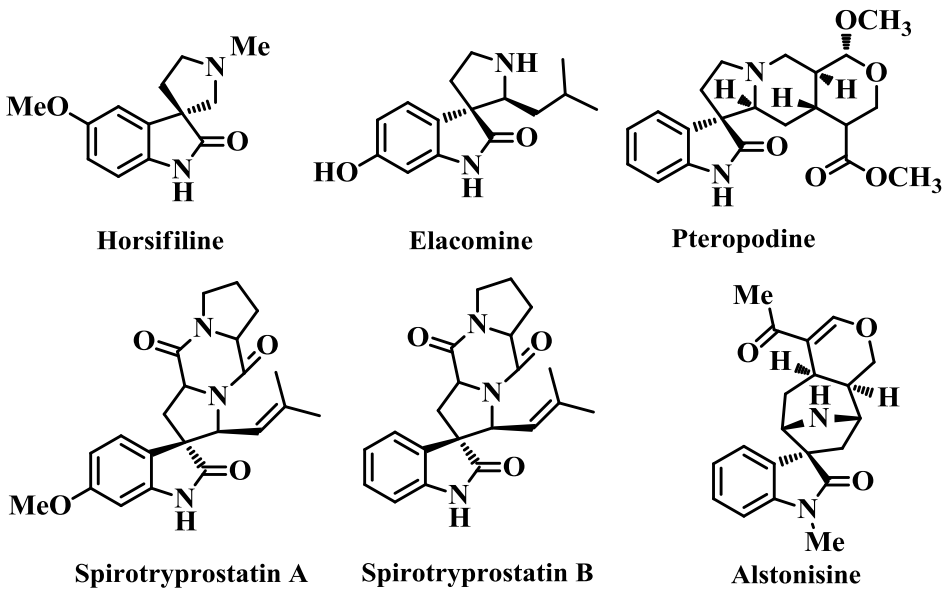


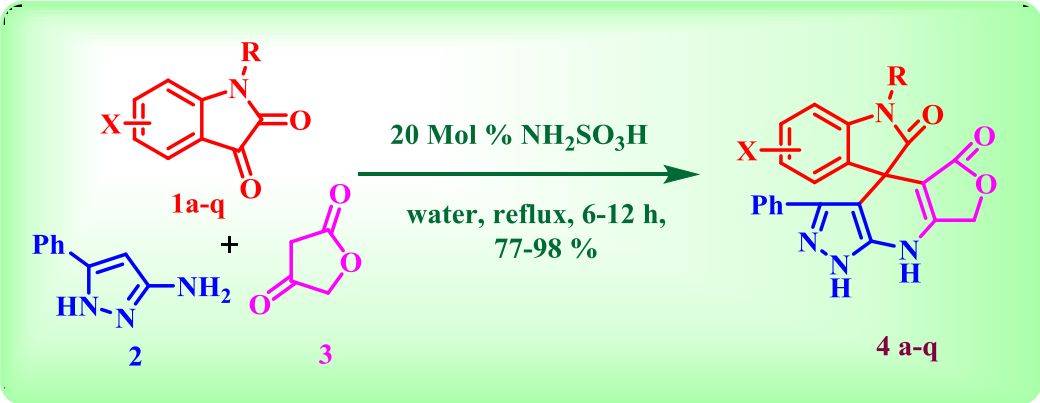

**Figure**: Naturally occurring and biologically active spirocyclic oxindoles.

On the other hand, pyrazolopyridine is a heterocycle that has attracted considerable interest in recent years and its derivatives have been reported for a wide range of biological applications such as antibacterial, antitubercular, anticancer, antiviral, anti-inflammatory, antimalarial and antileishmanial, antioxidant, antidepressant, cardiotonic, antihypertensive and neuroleptic activities.

Presently organic reactions in aqueous phase have attracted the attention of researchers because of the added advantages of water as an environmentally benign and economically affordable solvent. As a part of our ongoing program towards the development of greener chemical approaches for the synthesis of spiroxindoles, we herein report an eco-compatible, atom-economy, multicomponent, facile synthesis and cytotoxic activities of two series of 3-phenyl-7,8 dihydrospiro[furo[3,4-*e*]pyrazolo[3,4-*b*]pyridine-4,3'-indoline]-2',5(1*H*)-dione derivatives and 3'-phenyl-8',9'-dihydrospiro[indoline-3,4'-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyrimidine]-2,5',7'(1'*H*,6'*H*)-trione derivatives *via* a three component reaction employing isatins, 5-phenyl-1*H*-pyrazol-3-amine and 1,3-dicarbonyl compounds using sulfamic acid as a green catalyst in water. Thus in this investigation, isatin containing electron donating substituents afforded products in comparably lower yields than

isatin containing electron withdrawing substituents. Similarly, excellent yields of products were observed in the absence of substituents on isatin.

**Scheme 1:** Sulfamic acid catalysed synthesis of lactone based spirooxindole derivatives




**Scheme 2:** Sulfamic acid catalysed synthesis of uracil based spirooxindole derivatives


All the synthesized compounds were evaluated for their *in vitro* cytotoxic activities against a panel of three human cancer cell lines namely MDA-MB-231 (breast cancer), A549 (lung cancer) and DU-145 (prostate cancer) by using MTT assay and the results are summarized in Table 4. Compounds from both the series **4a-q** and **6a-q** have shown moderate to very good cytotoxicity with $IC_{50}$ values in the range of 0.35-75.20 µM. From the results, it can be concluded that the compounds containing lactone ring (**4k**, **4m**, **4o** and **4p**) exhibited better cytotoxic activities than the compounds containing uracil ring against MDA-MB-231.

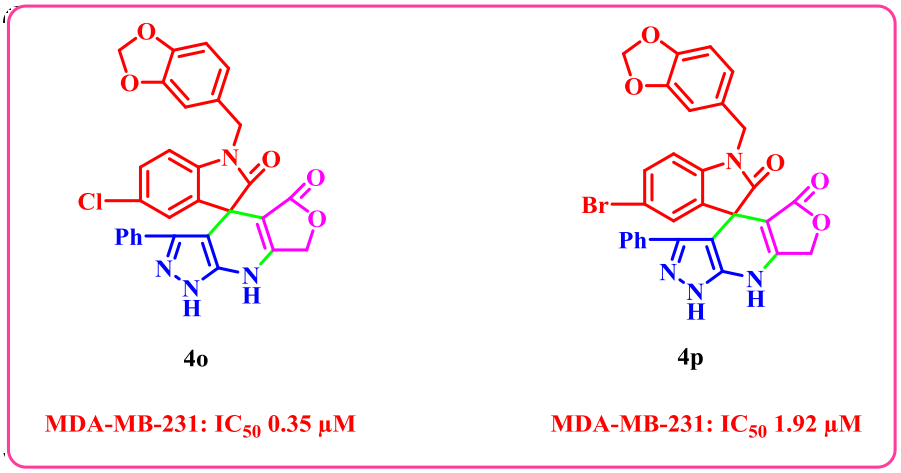



**Figure:** Most active compounds in the series.

In conclusion, a simple, efficient and environmentally benign method for the synthesis of spirooxindoles has been developed employing sulfamic acid as a green catalyst. The advantage of the present method is the use of water as a solvent that avoids environmentally harmful conventional organic solvents. Furthermore, using this method a series comprising of 34 spirooxindoles have been synthesized and screened for their cytotoxicity. The compounds exhibited good to moderate cytotoxicity against the cell lines tested. One of the compounds **4o** exhibited significant cytotoxicity with an $IC_{50}$ of 0.35 µM against MDA-MB-231 cell line.

(*Bioorg. Med. Chem. Lett*. **2015**, *25*, 2199)

## Section-B: Amberlite IR-120H catalysed facile, aqueous phase and green synthesis of pyrazolo[3,4-*f*]quinoline-spirooxindole derivatives and their cytotoxic evaluation

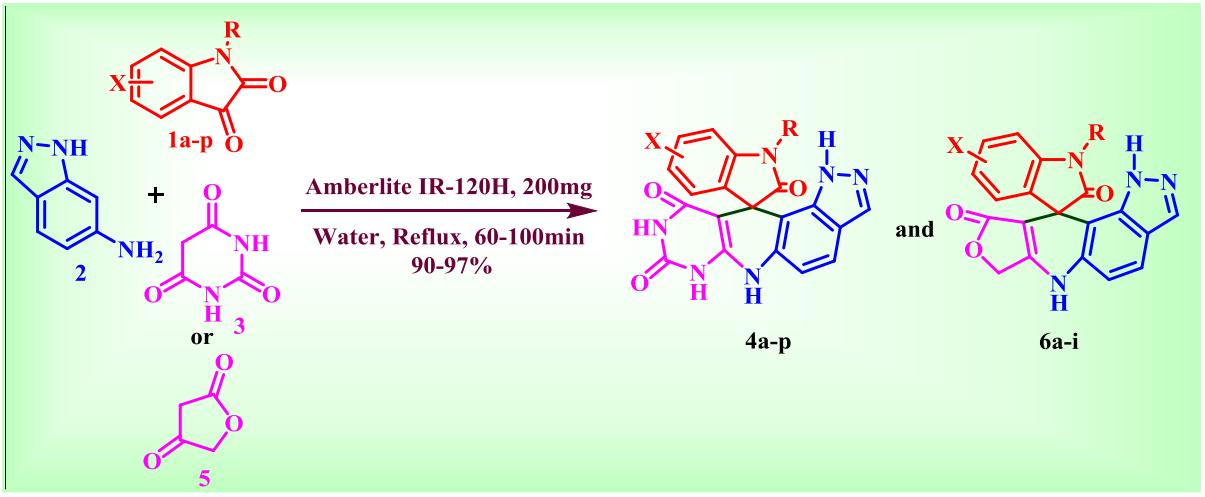
Pyrazolo-quinoline constitute, an important class of tricyclic fused heterocycles, because of broad pharmacology and biological activity, including antimalarial, antibacterial, antifungal, antiallergic, antileishmanial, antiviral, antimicrobial and human $A_3$ adenosine receptor antagonists, immunostimulants, lowering of serum cholesterol, modulators of cytokine biosynthesis and they also act as promising materials for optoelectronic applications.

In continuation of our interest in the development of simple and environmentally benign synthetic methods for the preparation of heterocyclic compounds, herein we wish to report a simple, highly efficient and environmentally friendly method for the synthesis of pyrazoloquinoline based spirooxindoles and cytotoxic activities of these two new series of spirooxindoles via a three component reaction using isatins, 6-aminoindazole and 1,3-dicarbonyl compounds. To the best of our knowledge, synthesis of these pyrazoloquinoline based spirooxindoles has been achieved for the first time.

In due course of methodology development, for the first time various solvents and catalysts were examined. The catalysts such as HCl, $CH_3SO_3H$, *p*-TSA, $ZnCl_2$ and $H_2NSO_3H$ can catalyze this reaction in good yields. However the best results were obtained when Amberlite IR-120H (200 mg) was used, in terms of the yield and the reaction time.

Substitution on the isatins played a crucial role in governing the product yield. Electron-withdrawing groups on isatin gave good yields while electron- donating groups on isatin gave slightly lower yields, in comparison with simple isatin.

**Scheme 3:** Amberlite IR-120H catalysed synthesis of pyrazoloquinoline based spirooxindoles




All the synthesized compounds were evaluated for their cytotoxic activities against three human cancer cell lines namely MCF-7 (breast cancer), A549 (lung cancer) and DU-145 (prostate cancer) by using MTT assay and 5-fluorouracil as a positive control. The results revealed that some of these compounds exhibit promising anticancer activity against

the tested cell lines. Compounds from the uracil series, **4n**, **4o** and **4p** containing *N*-piperonyl isatin exhibited appreciable cytotoxicity with $IC_{50}$ values ranging from 3.37 to 11.23 µM. Particularly compound **4p** showed profound cytotoxicity with $IC_{50}$ values of 3.37, 5.84 and 3.91 µM against MCF-7, A549 and DU-145 respectively. On the other hand from lactone series, the compounds containing isatin without *N*-substitution exhibited significant activity.

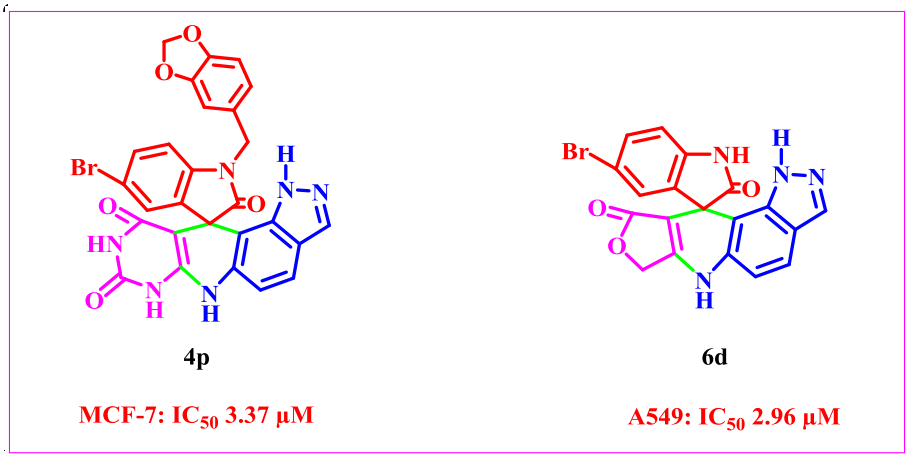



**Figure:** Most active compounds in the series.

In conclusion, we have developed an efficient and environmentally benign synthetic method for the synthesis of pyrazoloquinoline based spirooxindole derivatives using Amberlite IR-120H resin, a commercially available green and recyclable catalyst. The advantages of this method include its simplicity of operation, use of water as a solvent, cleaner reaction, absence of side products and excellent yields. Using this method a series of twenty five spirooxindoles has been synthesized and screened for their cytotoxicity. Two compounds, **4p** and **6d** exhibited significant cytotoxicity against the tested cell lines. Theoretical studies carried out to predict drug like properties of these compounds indicated that these compounds could be potential drug candidates as most of these satisfy Lipinski's rule of five.

*(Under communication)*

**CHAPTER II:** Explains the "Green synthesis of spiro-quinazoline derivatives and evaluation of their antimicrobial activities" and it has been divided into two sections.

## Section-A: Sulfamic acid catalysed efficient and green synthesis of 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-one derivatives and evaluation of their antimicrobial and anti-biofilm activities

Due to growing environmental concerns, development of economical and environmentally benign syntheses is an area of research that is being pursued vigorously for the replacement of highly volatile, environmentally harmful and biologically incompatible conventional organic solvents, since the amounts of solvents used are usually much larger than the amounts of reagents and products, and their recycling is normally difficult. In this context, water is a nature's reaction medium and it is non-hazardous, non-flammable, non-toxic, uniquely redox-stable, inexpensive solvent that is almost available freely.

Benzimidazole is a vital structural motif in medicinal chemistry that is known to exhibit a broad range of biological activities like antimicrobial, antitumor, anti-inflammatory, antiviral, antifungal, anti-hypertensive and antihelminthic. Moreover, benzimidazole is structurally is similar to some naturally occurring compounds such as purine, therefore, they can easily interact with biomolecules of the living systems. It is present in some drugs such as thiabendazole, mebendazole and albendazole which are used as antihelmintic drugs, Nocadazole, Hoechst 33258, and 2-phenylbenzimidazole-4-carboxamides, which possess excellent cytotoxic activity. Benomyl and Chlormidazole which acts antifungal agents, Rabeprazole acts as antiulcer, Astemizole is a receptor antagonist drug and compound is an antibacterial.

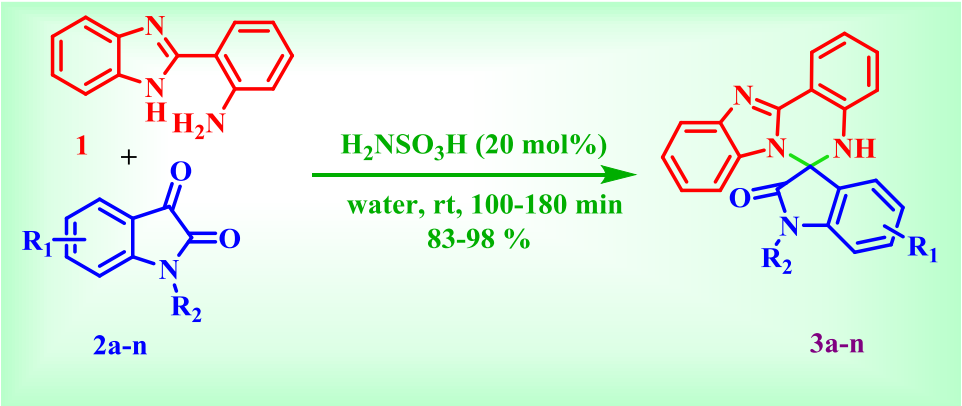
The combined molecule of benzimidazole and quinazoline structures gives benzimidazoquinazolines. They exhibit a wide range of therapeutic activities, such as anticancer, anti-inflammatory, antiviral, antimicrobial and anticonvulsants, antitumor.

As discussed in the previous chapter, spiroxindoles are remarkable for their molecular architecture as well as the prominent biological activities. They are attractive synthetic

targets because of their broad occurrence in various natural products. Spirooxindoles are playing key role particularly in the area of drug discovery and development. Development of new synthetic stratagies for the construction of spirocyclic compounds is an interesting and challenging task in organic synthesis. Considering, their wide spectrum of biological activities, the synthesis of spirooxindoles is of high practical value.

Therefore, continuing our efforts to develop environmentally benign methods for the synthesis of spirooxindoles, we herein report a green, simple and practical approach for the synthesis of 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-one derivatives using 12-(1*H*-benzo[*d*]imidazol-2-yl)aniline and substituted isatins by using SA as catalyst in water. By utilizing this transformation, various isatins (electron-rich and electron-deficient), affording the corresponding products in good to excellent yields.

**Scheme 4:** SA catalysed synthesis of 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-one derivatives.




All the synthesized 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-one derivatives were evaluated for their antimicrobial activity using well diffusion method against both Gram-positive bacterial strains as well as Gram-negative bacterial strains. Most of the compounds were selectively active against Gram-positive bacterial strains. Among the tested compounds, compound **3j** exhibited significant antimicrobial activity with MIC values of 7.8 µg/mL against *Staphylococcus aureus* MTCC 96 and 3.9 µg/mL against *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MLS16 MTCC 2940 and *Micrococcus luteus* MTCC 2470. Compound **3c** showed good antimicrobial activity with MIC 3.9 µg/mL against

*S. aureus* MLS16 MTCC 2940 and 7.8 µg/mL against *S. aureus* MTCC 96, *B. subtilis* MTCC 121 and *M. luteus* MTCC 2470.

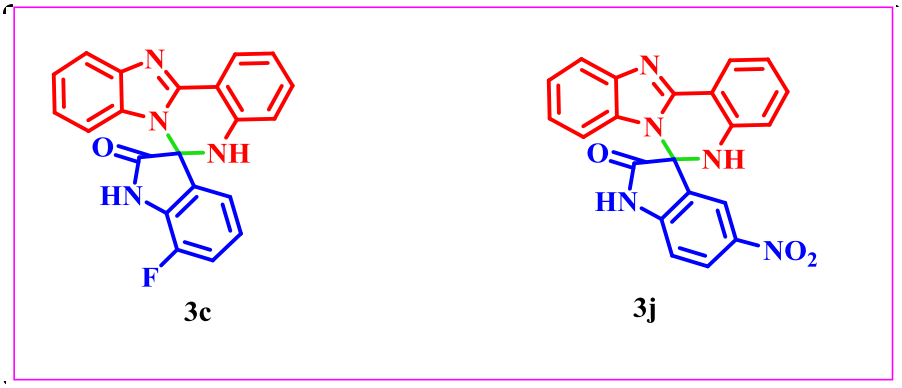



**Figure:** Most active compounds in the series.

In conclusion, we have developed a simple, efficient, convenient and environmentally benign method for the synthesis of 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-ones using water as a solvent and sulfamic acid ($H_2NSO_3H$) as a green and recyclable catalyst under room temperature conditions in quantitative yields. Furthermore, using this method a series of 15 spiroxindoles have been synthesized and screened for their antimicrobial and anti-biofilm activities. Among these, compounds **3b**, **3c** and **3j** showed promising to excellent MIC values ranging between 3.9 and 7.8 µg $mL^{-1}$ selectively active against Gram-positive bacteria and compound **3j** showed very good anti-biofilm activity with $IC_{50}$ value 1.8 µg $mL^{-1}$ against *S. aureus*.

(*Under communication*)

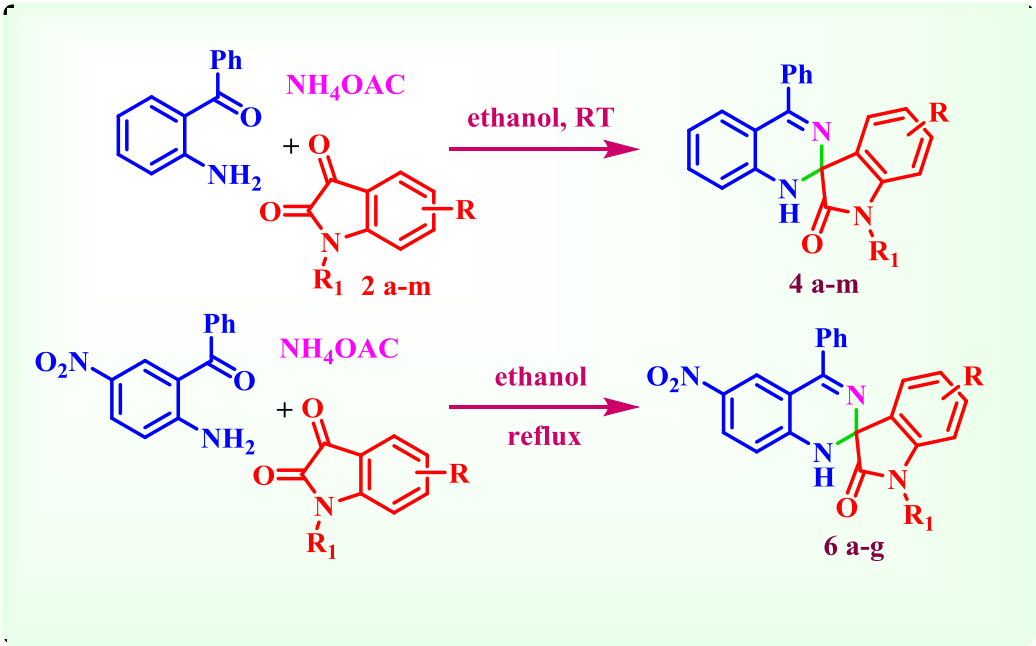
## Section-B: Catalyst-free, one pot and three-component synthesis of 4'-phenyl-1'*H*-spiro[indoline-3,2'-quinazolin]-2-ones

Quinazolines are an important class of heterocycles found in a wide range of natural products and pharmaceuticals and exhibit several biological activities including antibacterial, anticancer, antiplasmodial, anti-inflammatory, antiviral, anti-malarial, anti-tubercular, and anti-oxidant activities, apart from their usage as photo-chemotherapeutic agents, DNA-gyrase, PDE5, EGFR tyrosine kinase inhibitors, T-

type calcium channel blocking and CB2 receptors are familiar targets of various quinazoline derivatives. This scaffold is also the building block for many naturally occurring alkaloids such as *Bacillus cereus, Bouchardatia neurococca*, *Peganum nigellastrum* and *Dichroa febrifuga*.

In the continuation to the interest in the green chemistry research prompted for developing a greener process. In this context, a practical protocol was developed for the synthesis of 4'-phenyl-1'*H*-spiro[indoline-3,2'-quinazolin]-2-ones 2-amino benzophenones, isatins and ammonium acetate in ethanol under catalyst-free conditions.

**Scheme 5:** Synthesis of 4'-phenyl-1'*H*-spiro[indoline-3,2'-quinazolin]-2-one derivatives and 6'-nitro-4'-phenyl-1'*H*-spiro[indoline-3,2'-quinazolin]-2-one derivatives.




The reaction tolerates both electron withdrawing as well as donating substituents on the isatin without any significant deviation in yields. Similarly N-protected isatins also afforded the corresponding products in excellent yields. Furthermore, the presence of an electron withdrawing group at position 5 of the 2-aminobenzophenone, slowed down the reaction resulting in comparably longer reaction time and lower yields.

All the synthesized 5*H*-spiro[benzo[4,5]imidazo[1,2-*c*]quinazoline-6,3'-indolin]-2'-one derivatives were evaluated for their antimicrobial activity using well diffusion method against both Gram-positive bacterial strains as well as Gram-negative bacterial strains. Some of the compounds were selectively active against one Gram-positive bacteria, *Micrococcus luteus* MTCC 2470 and one Gram-negative bacteria, *Klebsiella planticola* MTCC 530. Compounds **4j** and **6c** exhibited potent antimicrobial activity with MIC value 3.9 µg/ml and **4m** and **6g** showed good antimicrobial activity with MIC 7.8 µg/mL against *Klebsiella planticola* MTCC 530.

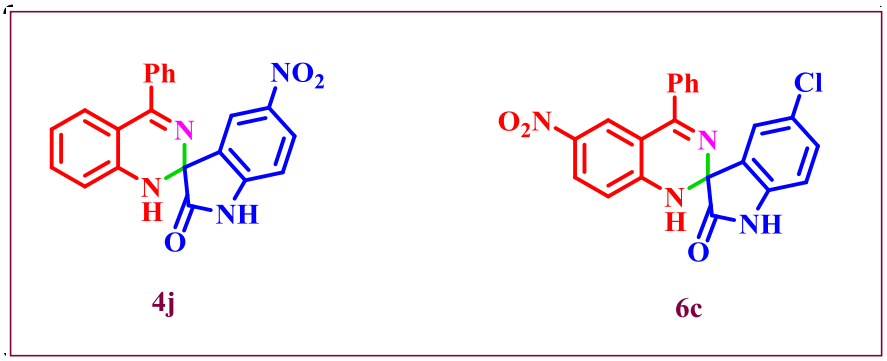



**Figure:** Most active compounds in the series.

In conclusion, a mild, efficient and environmentally benign method for the synthesis of 4'-phenyl-1'*H*-spiro[indoline-3,2'-quinazolin]-2-ones and 2,4-diphenyl-1,2-dihydroquinazolines has been developed without using any catalyst in ethanol. The advantages of this method include its simplicity of operation, cleaner reactions, absence of side products and higher yields. Furthermore, the spiroquinazolines have been screened for their antimicrobial activities. Among these, compounds **4f**, **6a, 6c** and **6g** showed selective activity against Gram-positive bacteria. Whereas compounds **4j**, **4m**, **6c** and **6g** showed potent antimicrobial activity against Gram-negative bacteria.

(*Tetrahedron Lett*. **2015**, *56*, 6373)

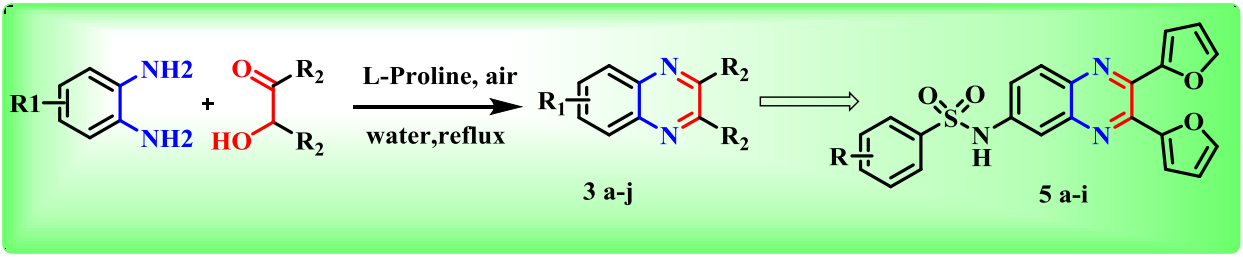
**CHAPTER III:** Summarizes the "Green synthesis of quinoxalines and naphtho[2,3-*f*]quinoline derivatives and evaluation of cytotoxic and antimicrobial activity" and it has been divided into two sections.

## Section-A: L-Proline mediated synthesis of quinoxalines; Evaluation of cytotoxic and antimicrobial activity

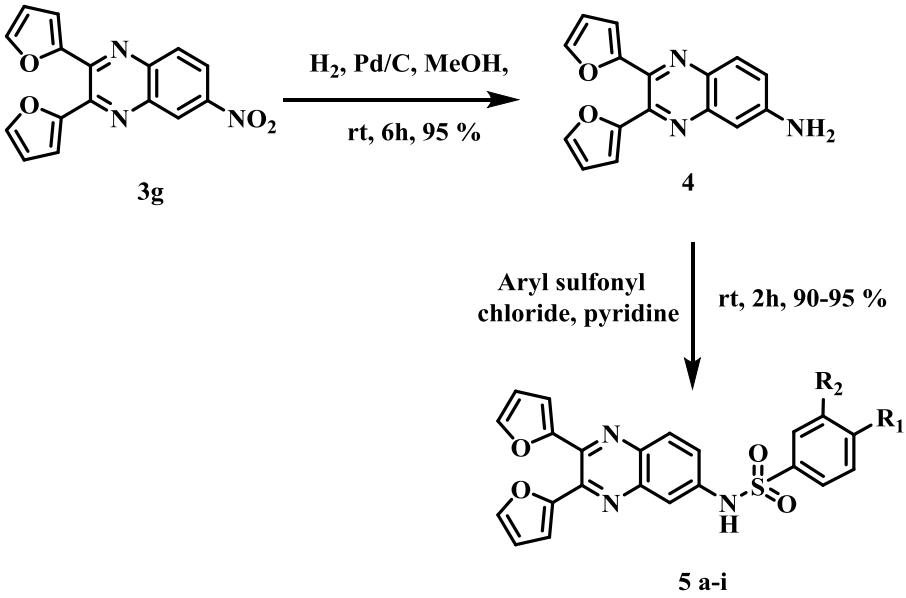
Quinoxalines are one of the important classes of nitrogen containing heterocyclic compounds and are well known for their wide range of biological activities such as antibacterial, anticancer, antiviral, anti-HIV, kinase inhibition etc. This scaffold is an active ingredient of several antitumor agents such as chloroquinoxaline sulphonamide (CQS) **(I)**, NCG555879-01 (**II**), XK469 **(III)** etc that are known to effect by targeting different pathways leading to cancer. Natural products like izumiphenazine C, saphenamycin **(IV)** etc (Fig. 1) also contain this scaffold. Furthermore, quinoxaline structure is found in various antibiotics such as levomycin, echinomycin and actinoleutin, that are known to inhibit the growth of gram positive bacteria. Besides their medicinal significance, quinoxaline derivatives find technical applications in dyes, cavitands, electroluminescent materials, organosemiconductors, dehydroannulenes and serve as suitable ligands in coordination chemistry.

In view of their significance, several useful strategies have been developed for the synthesis of quinoxalines. However, Most of these metal catalyzed reactions involve specially designed ligands or well-defined catalysts/reagents, which may increase the cost and limit the scope of applications. Therefore in order to overcome the disadvantages of previous methods and considering the advantages of L-proline as a catalyst and ecofriendly nature of water as a solvent we have developed a new method to synthesize quinoxalines. We herein report a green, simple and practical method for the synthesis of quinoxaline derivatives from hydroxy ketones and 1,2-diamines catalyzed by L-proline.

**Scheme 6:** L-Proline mediated synthesis of quinoxalines from hydroxy ketone with 1,2-diamines




**Scheme 7:** Synthesis of quinoxalin-sulphonamide conjugates




These quinoxalin-sulphonamide conjugates **5a-i** were investigated for their antiproliferative activity against three human cancer cell lines namely Hela (cervical cancer), DU145 (prostate cancer) and A549 (non-small cell lung cancer). Nocodazole was employed as reference standard. All the compounds (**5a-i**) have exhibited moderate to good cytotoxicity with the $IC_{50}$ values ranging between 5.0 and 38.9 µM. Notably, the compounds **5a** and **5b** have shown significant activity on all the cell lines examined. Furthermore the synthesized quinoxalin-sulphonamide conjugates **5a-i** were also tested for their activity against various Gram positive bacteria like *Bacillus. subtilis* MTCC121, S*taphylococcus. aureus* MTCC 96,

*staphylococcus MLS-16* MTCC2940, *Micrococcus luteus* MTCC2470 and Gram negative bacteria like *Escherichia coli* MTCC739, *Pseudomonas aeruginosa* MTCC2453, *Klebsiella planticola* MTCC530 taking chloramphenicol as a positive control. As evident from the results, most of the conjugates have shown activity against all the strains tested. Interestingly, the compounds have shown selectivity towards Gram positive bacteria. Some of the conjugates **5b**, **5c** and **5d** have exhibited excellent antibacterial activity (MIC 10 µg/ml) comparable to and sometimes even better than the standard against the pathogens *B. Subtilis*, *S. MLS* and *M. luteus*. However, other conjugates have displayed moderate activity against both Gram positive and Gram negative bacteria

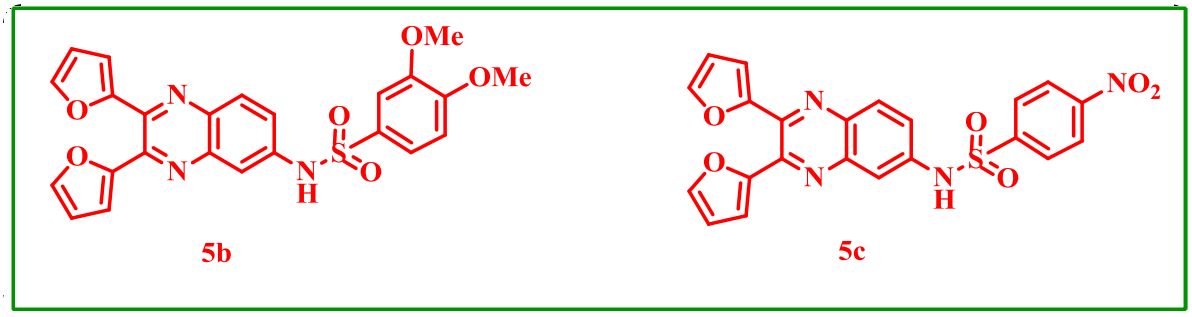



**Figure:** Most active compounds in the series.

In conclusion, a simple, efficient and eco-friendly method was developed for the synthesis of quinoxalines from 1,2- diamines and a-hydroxy ketones using cost-effective and readily available catalyst L-proline. To the best of our knowledge this transformation has not been reported with an organic catalyst. The mild reaction condition makes this methodology an alternative procedure to the conventional acid or base-catalyzed processes for the synthesis of quinoxalines and has practical applicability. Further, using this protocol a short library of quinoxaline–sulphonamide conjugates have been developed. The conjugates were found to be cytotoxic and effectively inhibit the growth of many bacterial strains.

(*RSC. Adv*. **2014**, *4*, 46369)

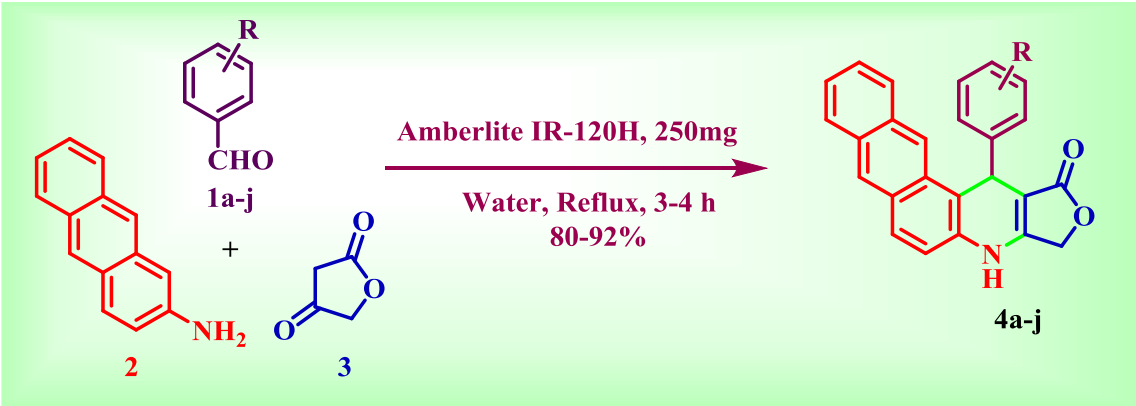
## Section-B: Amberlite IR-120H catalyzed MCR: An efficient and green synthesis of Naphtho[2,3-*f*]quinoline derivatives and their cytotoxicity

Organic reactions, generally involve the use of solvents, which may be toxic, hazardous and inflammable. Environmental pollution, caused by these chemical processes, has become a major issue to be addressed at global level. Apart from the atmospheric pollution, the use of solvent media in an industrial process may cause health hazards. In view of the seriousness of the chemical pollution, the use of a wide range of volatile, nonvolatile, inflammable, non-polar as well as polar aprotic solvents is being re-examined, leading to a search for the design and development of environmentally benign sustainable reaction processes. The green chemistry principles in general envisage the maximum conversion of reactants into products, with lesser generation of waste or by-products, utilizing minimum energy requirements as well as reduction in toxic chemical or solvents use.

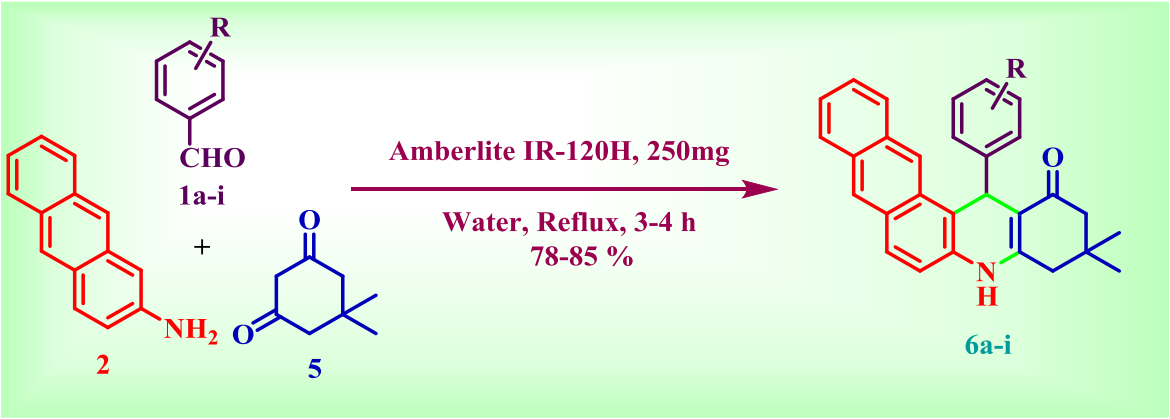
Podophyllotoxin (**I**), is the main component of *podophyllum* resin, a naturally occurring antimitotic cyclolignan which shows strong anticancer activity against various cancer cell lines. It shows cytotoxic properties by acting at the colchicine-binding site on tubulin and is known as an antimicrotubule agent. The versatility of the MCR approach, allowed us to prepare a diverse library of podophyllotoxin mimetics. Among them, the derivatives of 4-azapodophyllotoxin (Figure **1**), were reported as powerful DNA topoisomerase II inhibitors and have recently attracted considerable interest.

Herein, our continued interest in the green chemistry research prompted us for developing a greener process. In this context, we developed a greener protocol for the synthesis of two series of 13-phenyl-4,13-dihydrofuro[3,4-*b*]naphtho[2,3-*f*]quinolin-1(3*H*)-one derivatives and 3,3-dimethyl-14-phenyl-3,4,5,14-tetrahydronaphtho[2,3-*a*]acridin-1(2*H*)-one derivatives *via* a three component reaction employing isatins, 2-aminoanthracene and 1,3-dicarbonyl compounds using Amberlite IR-120H as a green and recyclable catalyst in water.

**Scheme 8:** Amberlite IR-120H catalyzed synthesis of 13-phenyl-4,13-dihydrofuro[3,4-*b*]naphtho[2,3-*f*]quinolin-1(3*H*)-one derivatives.




**Scheme 9:** Amberlite IR-120H catalyzed synthesis of 13-phenyl-4,13-dihydrofuro[3,4-*b*]naphtho[2,3-*f*]quinolin-1(3*H*)-one derivatives.




All the synthesized 13-phenyl-4,13-dihydrofuro[3,4-*b*]naphtho[2,3-*f*]quinolin-1(3*H*)-one (**4a-j)** and 3,3-dimethyl-14-phenyl-3,4,5,14-tetrahydronaphtho[2,3-*a*]acridin-1(2*H*)-one derivatives **(6a-i)** were evaluated for their anti-proliferative activity in a panel of four human cancer cell lines, namely HeLa (cervical), A549 (lung), MCF-7 (breast) and DU-145 (prostate) by employing MTT assay, and Podophyllotoxin was used as the reference drug. It was observed that all the synthesized compounds showed good to excellent anti-proliferative activities against the tested cell lines with $IC_{50}$ values ranging from 0.89 to 4.77 μM.

In conclusion, an efficient and environmentally benign synthetic method was developed for the synthesis of naphtho[2,3-*f*]quinoline derivatives using Amberlite IR-120H resin, a commercially available green and recyclable catalyst. The advantages of this method include its simplicity of operation, use of water as a solvent, cleaner reaction and excellent yields. Moreover, the resin is inexpensive, stable, noncorrosive, easy to handle, and potentially reusable. Using this method, two series of naphtho[2,3-*f*]quinoline derivatives have been synthesized and screened for their cytotoxicity. All these compounds exhibited excellent antiproliferative activities on different cancer cell lines.

**CHAPTER IV:** Explains the "Efficient and green synthesis of pyrrolo[1,2-*a*]quinoxalines."

## Section-A: Sulfamic acid: An efficient and recyclable solid acid catalyst for the synthesis of 4,5-dihydropyrrolo[1,2-*a*]quinoxalines

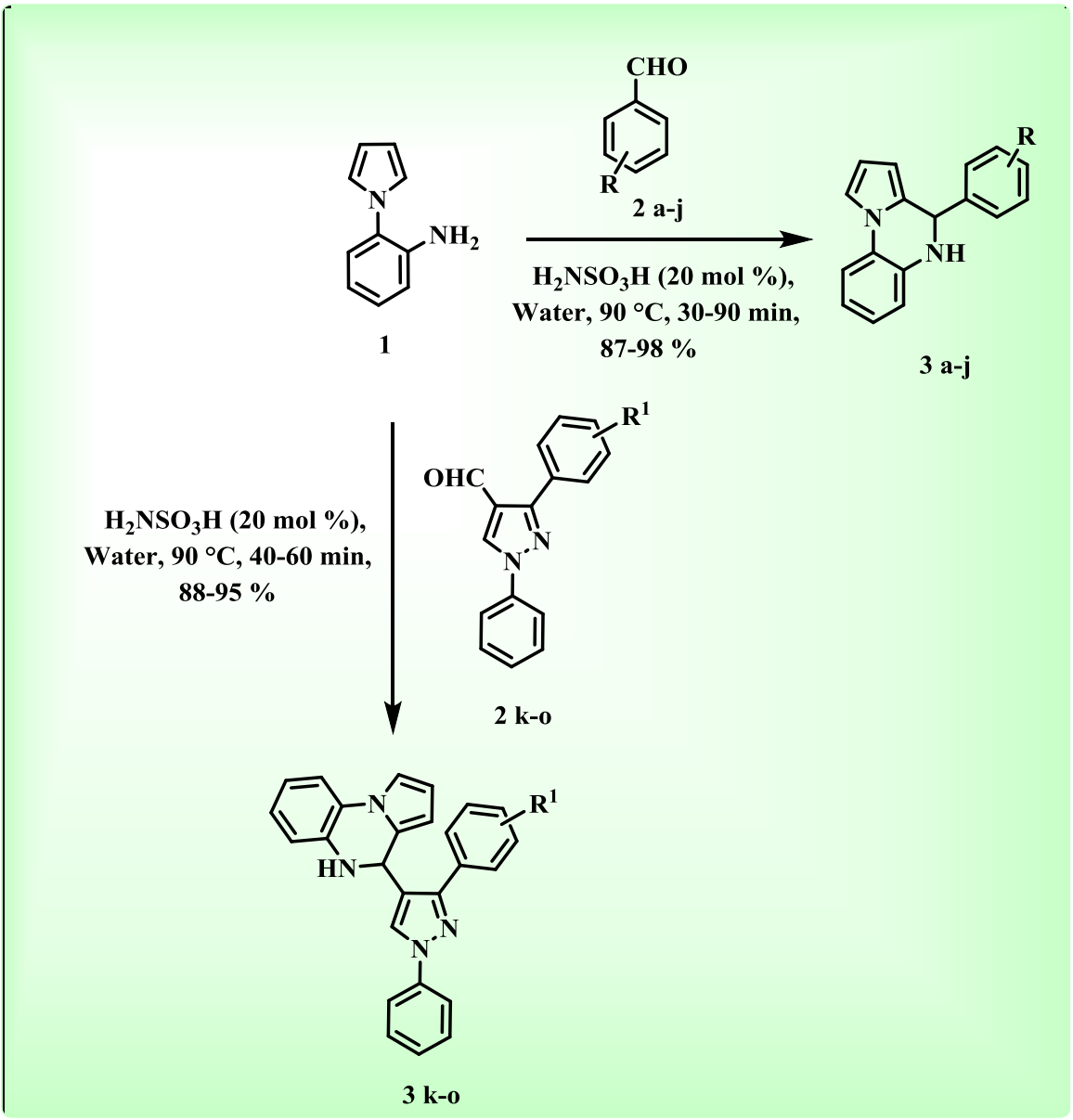
Quinoxalines represent as an important class of biologically active compounds that are known to possess antimicrobial, anticancer, antiviral, antidiabetic, antifungal and antidepressant activities. In these, particularly pyrrolo[1,2-*a*]quinoxaline scaffold is of considerable importance in medicinal chemistry and is present in various biologically and medicinally useful molecules, and act as anticancer **(I)**, as well as anti-HIV agents **(II)**, antimycobacterial agents, **(III)** cannabinoid receptor antagonists (**IV**), PARP-1 inhibitors (**V**) and also important intermediates for the construction of 5-$HT_3$ receptor agonists (**VI**) (Fig. 1).

Among the various C–C bond-forming reactions, the Pictet-Spengler reaction has been widely used for the formation of pyrrolo[1,2-*a*]quinoxalines. According to this protocol, the reaction proceeds with the initial formation of Schiff's base intermediate after the elimination of a water molecule followed by intramolecular cyclization to give dihydro derivative, which subsequently on oxidation affords pyrrolo[1,2-*a*]quinoxalines.

In the continuation to the interest in the green chemistry research to develop eco-friendly synthetic methods for the synthesis of bioactive compounds, we herein report an efficient, environment friendly and greener practical protocol for synthesis of 4,5-

dihydropyrrolo[1,2-*a*]quinoxalines using 1-(2-aminophenyl)pyrrole and substituted aldehydes using sulfamic acid ($H_2NSO_3H$, SA), a green and recyclable catalyst in water.

**Scheme 10:** SA catalysed synthesis of 4,5-dihydropyrrolo[1,2-*a*]quinoxalines and 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4,5-dihydropyrrolo[1,2-*a*]quinoxaline derivatives




The presence of electron-withdrawing groups on the aldehyde afforded the cyclized products in excellent yields when compared to the presence of electron donating substituents on the aldehyde. The reaction was also performed using different 1,3-diaryl pyrazole

carboxaldehydes. All the aldehyde precursors gave very good yields irrespective of their substitution. Overall very good to excellent yields of the desired dihydropyrroloquinoxalines were obtained by employing a greener protocol.

As quinoxalines are well known for their biological activity, it was considered of interest to determine their cytotoxic activities. In this regard, all the synthesized compounds were evaluated for their in vitro cytotoxicity against two human cancer cell lines namely MCF7 (breast cancer) and HepG-2 (liver cancer) employing the MTT assay. Some of the compounds have exhibited moderate cytotoxic activities, compound **3e** showed cytotoxicity with $IC_{50}$ values of 21.67 and 55.21 μM against MCF7 and HepG-2 cell lines, respectively. In addition, compounds such as **3i** and **3l** exhibited significant cytotoxic activities with $IC_{50}$ values of 29.09 and 29.46 μM against MCF7 and HepG-2 cell lines, respectively.

In conclusion, a simple, mild, efficient and environmentally benign method for the synthesis of pyrrolo[1,2-*a*]quinoxalines was developed using sulfamic acid as a green catalyst in water. The advantages of this method include its simplicity of operation, cleaner reaction, selectivity with no side products and higher yields. Furthermore, the synthesized compounds were screened for their cytotoxic activity against two human cancer cell lines and some of these exhibited moderate activity.

(*Tetrahedron Lett*. **2015**, *56*, 4619)

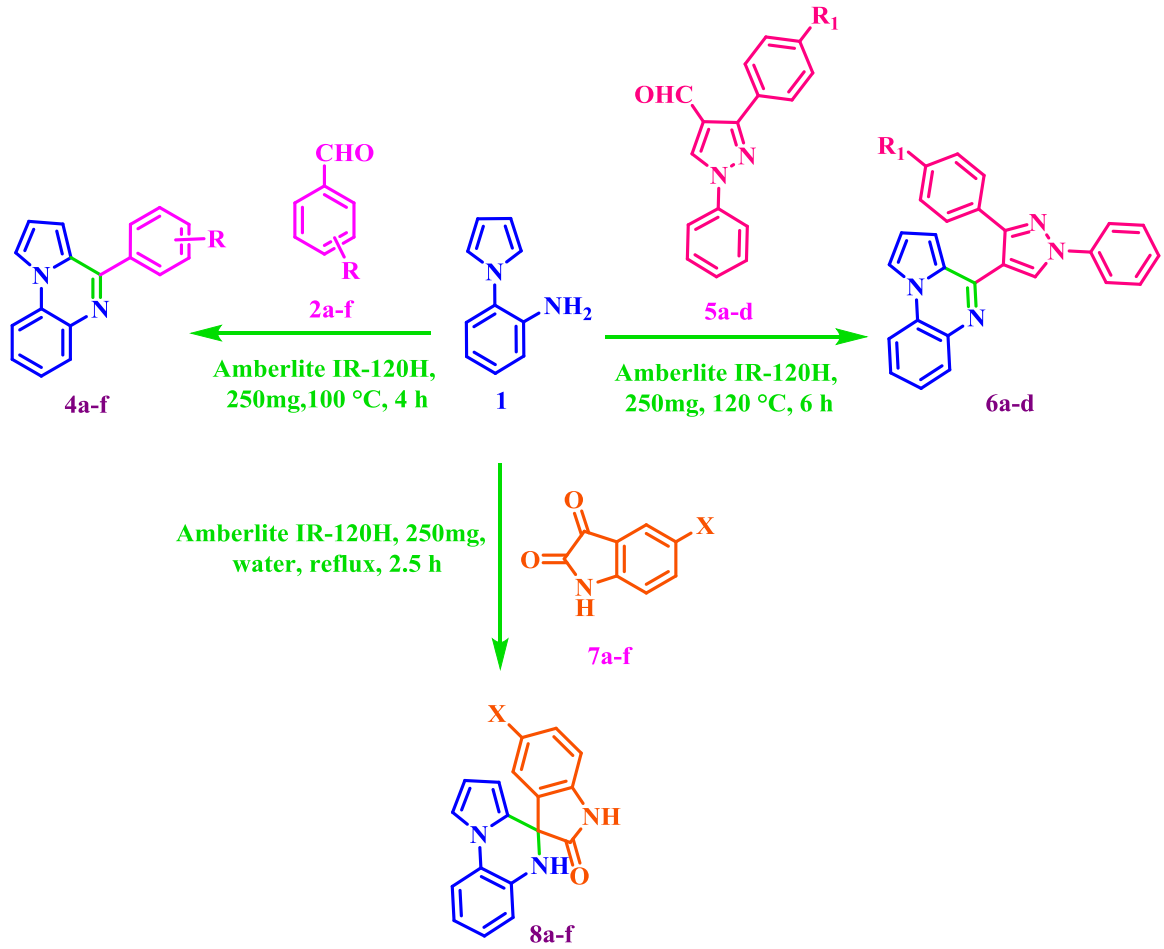
## Section-B: Amberlite IR-120H: An efficient and recyclable heterogeneous catalyst for the synthesis of pyrrolo[1,2-*a*]quinoxalines and 5'*H*-spiro[indoline-3,4'-pyrrolo[1,2-*a*]quinoxalin]-2-ones.

Green chemistry has applied to chemical processes and can be considered as a series of reduction energy, auxiliaries, waste, etc. and it leads to the simplification of the process in terms of the number of chemicals and steps. Because of the increasing concern of the harmful effects of organic solvents on the environment and human body, organic reactions that are operated with green solvents or without conventional organic solvents have provoked the attention of organic and medicinal chemists. Removing of solvent from a chemical process is likely to often be the greatest reduction and simplification in the work-up as well as the

reaction that can be achieved. Solvent-free or solid state reaction may be carried out using the reactants alone or incorporating them in zeolites, clays, silica, alumina or other matrices. Thermal process or irradiation with UV, microwave or ultrasound can be employed to bring about the reaction. Solvent-free reactions obviously reduce pollution and handling costs due to simplification of experimental procedure and work up technique. Here solvent-free procedures are described for the synthesis of compounds of commercial value.

In the previous section A, an efficient, sulfamic acid catalysed green synthesis of of 4,5-dihydropyrrolo[1,2-*a*]quinoxalines was described. In this section, a simple, highly efficient and environmentally benign method for the synthesis of pyrrolo[1,2-*a*]quinoxalines has been developed using a green and recyclable catalyst Amberlite IR-120H resin under solvent-free conditions described.

**Scheme 11:** Amberlite IR-120H catalysed synthesis of 4-phenylpyrrolo[1,2-*a*]quinoxalines, 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyrrolo[1,2-*a*]quinoxalines and 5'*H*-spiro[indoline-3,4'-pyrrolo [1,2-*a*]quinoxalin]-2-one derivatives




In conclusion, a mild, efficient and environmentally benign synthetic method was developed for the synthesis of pyrrolo[1,2-*a*]quinoxalines and 5'*H*-spiro[indoline-3,4'-pyrrolo[1,2-*a*]quinoxalin]-2-ones using Amberlite IR-120H resin, a commercially available green and recyclable catalyst. The advantages of this method over previous reports include its simplicity of operation, mild reaction conditions and no usage of external oxidant. The acidic ion-exchange-resin catalyst could be easily separated and directly reused for five times without any reactivation, while showing no significant loss of activity.

(*Tetrahedron Lett*. **2015**, *56*, 7012)

**CHAPTER V:** Explains the "Synthesis and Biological evaluation of Triazole-Pyrazole amide conjugates."

Cancer is a class of diseases or disorders characterized by uncontrolled/ abnormal division of cells and the ability of these to spread, either by direct growth into adjacent tissue, or by implantation into distant sites by metastasis, in which cancer cells are transported through the bloodstream or lymphatic system.

Over the past two decades, pyrazole derivatives have emerged as attractive scaffolds in medicinal chemistry for the development of potential drug molecules. Molecules with pyrazole nucleus are known to exhibit broad range of medicinal properties such as, antibacterial, insecticidal, anticancer, anti-angiogenic, anti-inflammatory, cyclooxygenase-2 inhibitor, PDE4 inhibitors, antitubercular, antimalarial. In recent years 1,2,3-triazoles have emerged as an attractive scaffolds in medicinal chemistry for the development of potential drug molecules. Molecules with triazole nucleus are known to exhibit broad spectrum of medical properties such as anticancer, antifungal, antibacterial, anticonvulsant, anti-HIV, anti-inflammatory and antitubercular activities.

In recent years molecular hybridization *i.e,* combination of two or more active pharmacophores to produce a single molecule, has become a successful and promising approach in drug discovery. We are also more interested to develop a new pharmacophore by coupling of two active pharmacophores such as pyrazole and triazole clusters. Our approach of hybridizing two pharmacophoric units to design and develop improved anticancer agents. In this context, we concluded that the coupling of pyrazole moiety with triazole scaffold may improve its general anticancer potential and stability.

Considering this fact, a series of twenty one pyrazole linked triazole conjugates (**11a-u**) were designed and synthesized. The joining of these two moieties resulted in a four ring (A, B,C and D) molecular scaffold that comprises of polar heterocyclic rings in the middle associated with rotatable single bonds and substituted aryl rings placed in the opposite directions. The aryl rings were decorated with carefully selected substituents ($OCH_3$, $CH_3$, Cl, $OC_6H_5$, 3,4,5-TriOMe and $CF_3$).

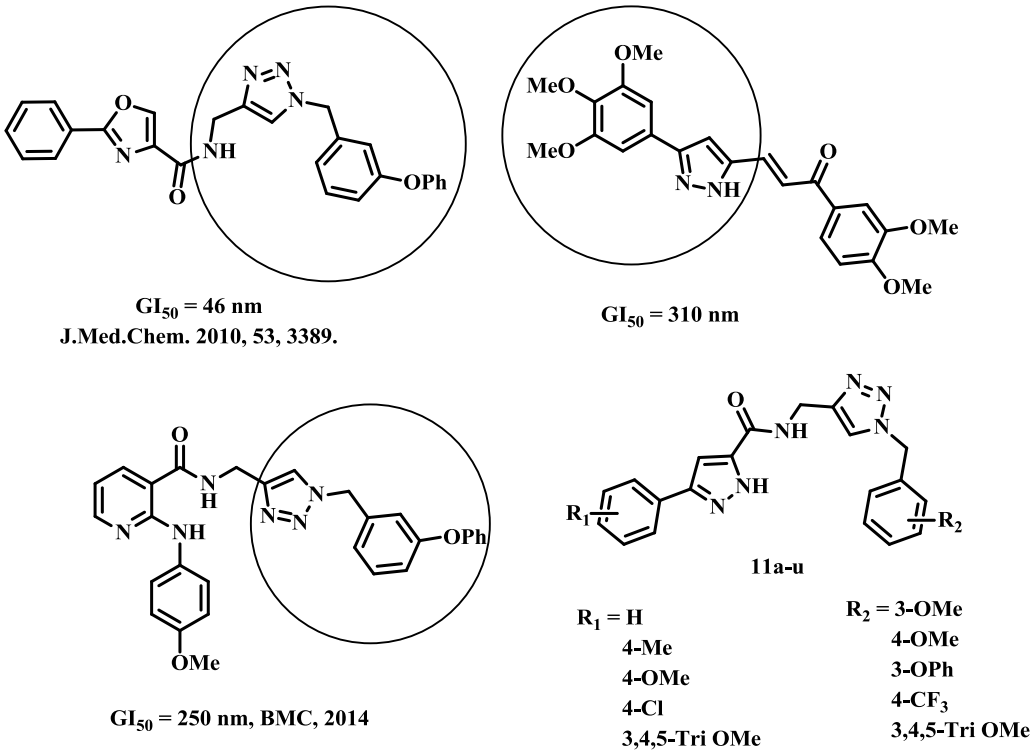


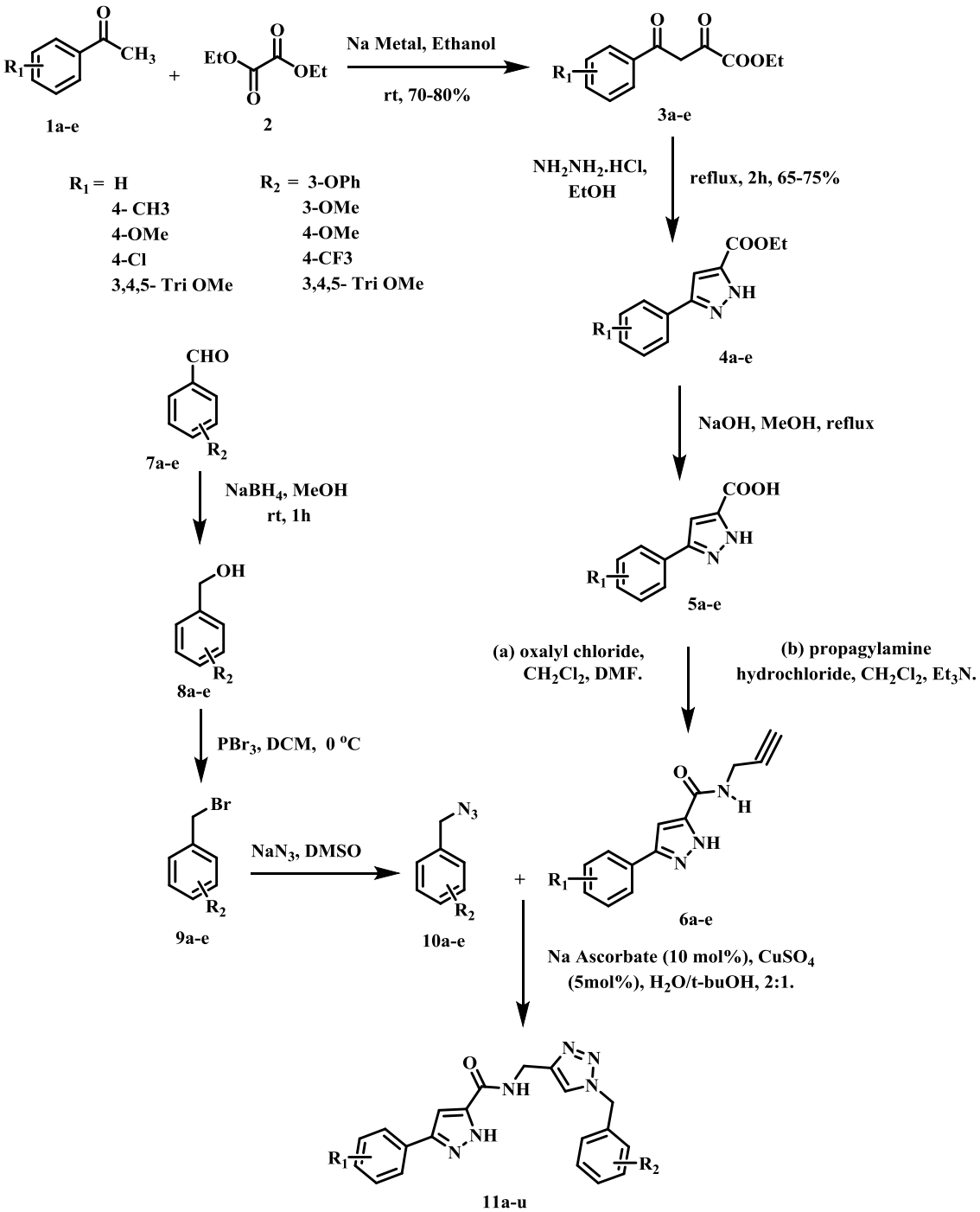

**Figure:** Chemical structures of compounds containing pyrazole and triazole nucleus

All the newly synthesized conjugates of *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-phenyl-1*H*-pyrazole-5-carboxamides were evaluated for their antiproliferative activity against a panel of four different human cancer cell lines cervical cancer cell line (HeLa), liver carcinoma cell line (HepG2), lung carcinoma cell line (A549) and breast cancer cell line (MCF-7) using the MTT assay. The results of growth inhibitory activities ($IC_{50}$ values) are shown (in Table **1**) in micromolar concentrations, nocodazole and doxorubicin were used as the reference compounds. The compounds have shown varied inhibitory effects from excellent to good cytotoxicity against the human cancer cell lines tested.

Some of the compounds in this series such as **11a**, **11e**, **11h**, **11k**, **11s** and **11u** exhibited excellent antiproliferative activity <0.1 μM. Compound **11h**, bearing methyl substituent on ring A and trifluoro substituent on Ring B, exhibited good cytotoxicity with IC50 values 0.058, 0.068 and 0.053 μM against HeLa, HepG2 and MCF-7 cancer cell lines respectively. Compound **11k**, having 4-methoxy substituent on ring A and 3,4,5-trimethoxy

substituents on Ring B, exhibited significant cytotoxicity with IC50 values 0.081, 0.098 and 0.049 μM against HeLa, HepG2 and A549 cancer cell lines respectively.

**Scheme 12**: Sequential synthesis of pyrazole-triazole amide conjugates



**Conclusion**

In conclusion, a series of twenty one compounds has been synthesized by conjugating two biologically active heterocyclic scaffolds, pyrazoles and triazoles. The synthesized conjugates were decorated with diverse array of substituents on both pyrazoles and triazole nucleus to presume the structure activity relationship (SAR). All these synthesized compounds were evaluated for their antiproliferative activity against a panel of four different human cancer cell lines. All these compounds showed excellent to good cytotoxicities with IC50 values ranging from 0.049-5.880 μM. Compounds **11h** and **11k** were found to be the most effective conjugates from the series displaying $IC_{50}$ value of 0.053 and 0.049 μM against MCF-7 cell line and A549 cell lines respectively. Further studies to probe the mode of action of these conjugates are under progress.