The thesis entitled

"**Cinnamide/Naphthalimide/Chalcone/Pyrazoline Hybrides as Potential Anticancer Agents and Quinoline Derivatives as Antimicrobial Agents"** has been divided into four chapters.

Chapter-I: General introduction of Cancer, Tubulin and Tuberculosis

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. Small molecules that disrupt microtubule/tubulin dynamics are used widely in cancer treatment. Hence discovery and development of new small molecules useful in cancer chemotherapy has identified tubulin as possible molecular target medicinal chemists for past few years. Microtubules are one of the key structural components of the cytoskeleton in eukaryotic cells comprising of α and β-tubulin heterodimers. They play a crucial role in various cellular processes and have emerged as an attractive and viable target in the development of anticancer drugs mainly due to their indispensability in mitotic cell division.

Tuberculosis (TB) is an ancient, contagious disease caused by the pathogen *Mycobacterium tuberculosis (M. tb)* and is responsible for more human deaths than any other single infectious disease. According to World Health Organization (WHO), TB has become a more prevalent chronic disease causing 2-3 million deaths annually and infecting one third of the world population. Its deadly synergy with HIV has further aggravated the mortality and spread of this disease. In recent years, chemotherapy of TB has evolved in due course of time. Though there are many drugs available for the treatment of TB the emergence of resistance such as multi drug resistance (MDR) and extensive drug resistance (XDR) pose threat to the human life. Advances in research such as genetic engineering of *M. tb* have offered many validated targets to develop chemical libraries that may eventually lead to new drugs to combat TB.

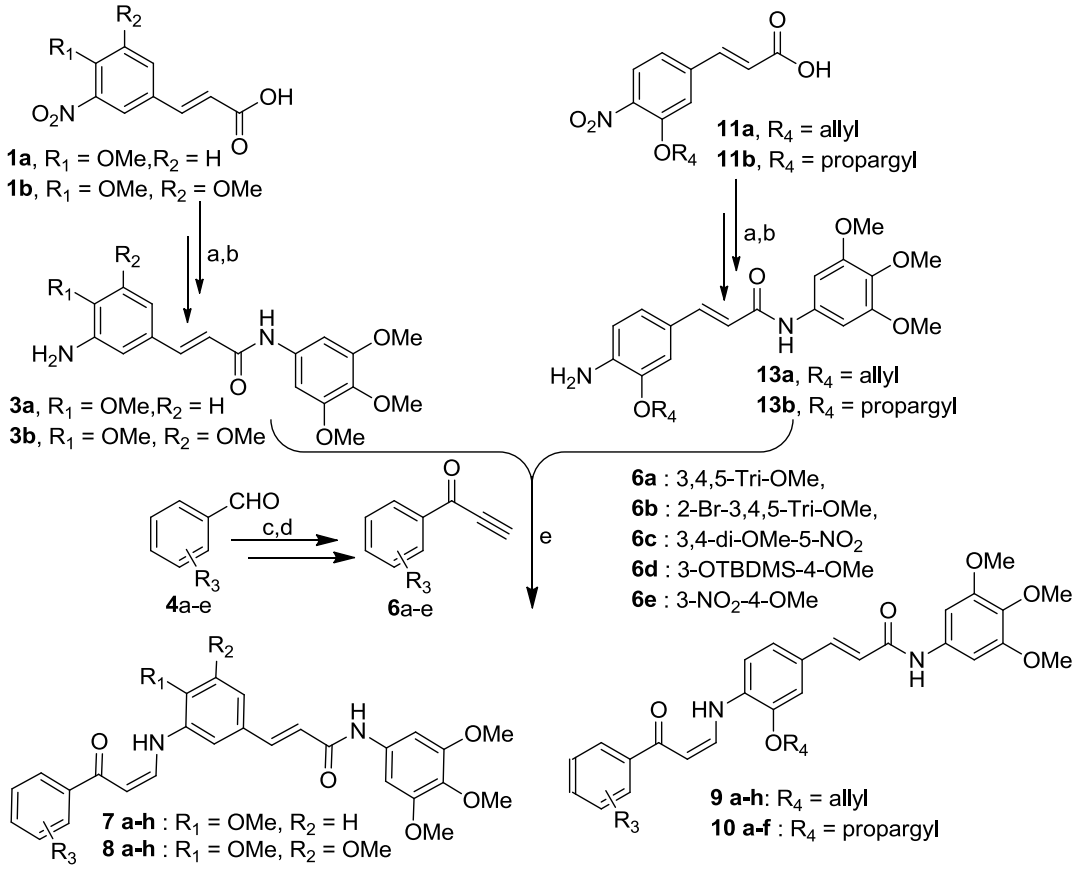
Statement of Problem

Chapter-II: Arylcinnamido-Propionone Hybrids as Tubulin Polymerization Inhibitors and Apoptotic inducers.

Cinnamides are another class of anticancer agents, its natural analogues are known for the treatment of cancer for over centuries. Phenylcinnamides are shown to bind to tubulin,

thereby causing an inhibition of its polymerization and alteration in the tubulin-microtubule equilibrium. They are known to possess an α,β-unsaturated carbonyl moiety, which can be considered as a Michael acceptor, employed as a powerful tool in the design of antimitotic agents. Some of the potent hybrid/conjugate molecules that have been recently developed as new anticancer agents are obtained by the combination of different pharmacophores. Structural features of designed molecules, including the trimethoxyphenyl moiety (found in colchicine and **8H**), suggested that these molecules exerted cytotoxic action through microtubule binding and mitotic arrest. Based on these observations we describe modifications on **8H** scaffold which contain trimethoxyphenyl moiety without disturbance and further congeners were generated by conjugates with substituted arylpropynones to cinnamide scaffolds. In this context, we have designed, synthesized some newer cinnamido-propionone conjugates and evaluated them for their cytotoxic potential apart from their effect on the inhibition of tubulin polymerization.

Scheme: Synthesis of arylcinnamido-propionone hybrids



Reagents and Conditions: (a) (i) $(COCl)_2$, dry DCM, 0 °C to rt, 3 h; (ii) Trimethoxy aniline, Et_3N, dry THF, 3 h; 69−71%; (b) Zn, HCO_2NH_4, MeOH, rt, 6 h; 60−70%; c)

ethynylmagnesium bromide, THF, 0°C−rt, 8−9 h; (d) 2-iodoxybenzoic acid, DMSO, rt, 5 h, 73−88%; (e) EtOH, 3-4 h; 76−88%.

Results and discussions

A series of arylcinnamido-propionone conjugates (**7-9a-h** and **10a-f**) has been designed, synthesized and evaluated for their anticancer potential against the four human cancer cell lines. Among them, conjugates **9d** and **9g** have showed significant cytotoxic activity against prostate cancer cells (DU-145) displaying IC_{50} of 7.48 and 8.91 µM respectively. Studies to understand the mechanism of action of **9d** and **9g** indicates that inhibit the tubulin polymerization thereby arresting the cell cycle in G2/M phase. Furthermore, studies like mitochondrial membrane potential and Annexin V-FITC assay suggested that these conjugates **9d** and **9g** induced cell death by apoptosis. The molecular docking studies suggested that the binding of these conjugates at the colchicine site of the tubulin protein.

Conclusions

Cinnamido-propionone conjugates (**7-9a-h** and **10a-f**) were synthesized and evaluated for their cytotoxic activity. Conjugates **9d** and **9g** showed significant cytotoxic activity against human prostate cancer cell line, (DU-145). The flow cytometric analysis revealed that these conjugates cause cell cycle arrest at G2/M phase. Furthermore, they effectively inhibited microtubule assembly. Moreover, the triggering of the apoptotic cell death after mitotic arrest was investigated by mitochondrial membrane potential and Annexin V FITC assays suggest that these conjugates induced apoptosis. The molecular modeling study carried out on the colchicine binding site of tubulin demonstrated that these molecules are involved in a series of interactions with the protein thereby binding well with the tubulin. Therefore, the work reported herein could be considered of significant importance to provide valuable insights in the development newer leads for the treatment of cancer.

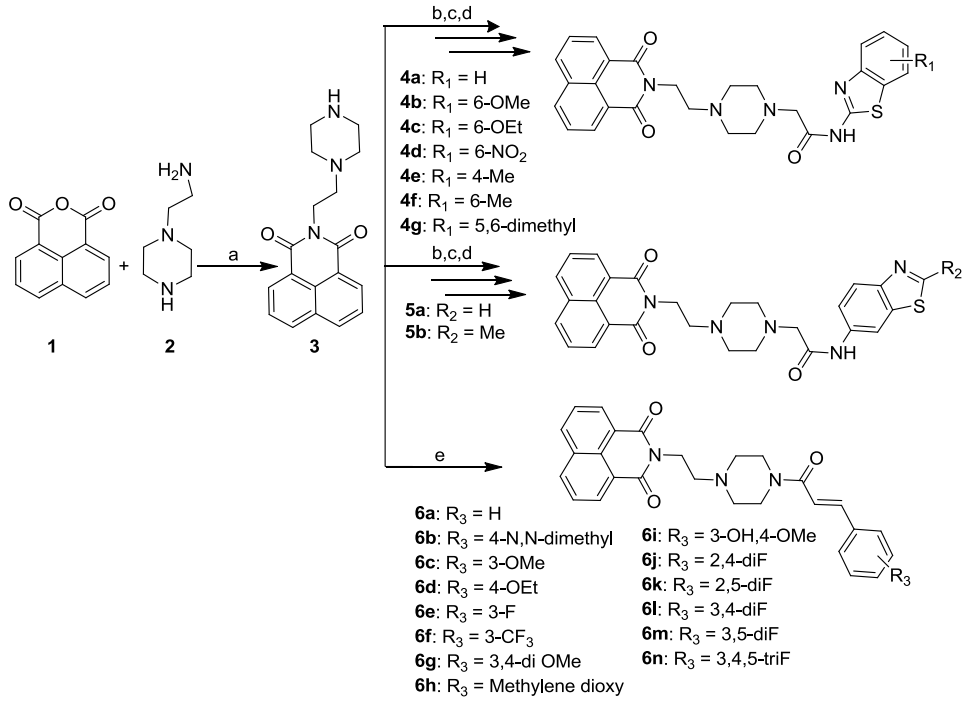
Statement of Problem

Chapter IIIA: Naphthalimide-Amidobenzothiazole and Naphthalimide-Cinnamide Hybrids as Potential Anticancer Agents.

Naphthalimides represent an important chemical class of DNA-intercalating topoisomerase II inhibitors. These agents consist of flat chromophores, generally π-deficient aromatic or hetero aromatic system, which binds to DNA by insertion between the base pairs of the double helix. They are also characterized by the presence of basic side chains attached

to the chromophore. Charge transfer and stacking interactions are the main driving forces for binding. Intercalation causes the base pairs to separate vertically, thereby distorting the sugar phosphate backbone and changing the degree of rotation between successive base pairs, and finally disrupts the cell division. The most potent drugs in this series were amonafide and mitonafide both tested in humans as anticancer agents. Amonafide, a 5-amino substituted naphthalimide is a novel inhibitor of Topo II that intercalates into DNA and induces apoptotic signalling by blocking Topo II binding to DNA. To improve therapeutic properties of naphthalimides extensive efforts have been made including the modification of side chain, aromatic ring system, and the substituents on the ring. The promising biological activity exhibited by these naphthalimides prompted us to develop some newer hybrid molecules by linking the naphthalimide pharmacophore with aminobenzothiazole and cinnamide scaffold with a view to enhance their anticancer activity. Thus a library of twenty three new class of naphthalimide-amidobenzothiazole, naphthalimide-cinnamide hybrids were designed, synthesized and evaluated for their anticancer potential.

Scheme: Synthesis of naphthalimide-amidobenzothiazole and naphthalimide-cinnamide hybrids



Reagents and Conditions: (a) EtOH, Reflux, 60°C, 3h; (b) 2-bromoethyl acetate, K_2CO_3, DMF, 24 h; (c) LiOH, THF, H_2O, 12 h; (d) Substituted benzothiazoles, EDCI, HOBt, CH_2Cl_2, 24 h; (e) Substituted cinnamic acids, EDCI, HOBt, CH_2Cl_2, 24 h.

Results and discussions

These conjugates (**4a-g, 5a-b and 6a-n**) were synthesized and evaluated for their antiproliferative activity against NCI-60 Cell line panel. Among them **5a** and **5b** showed remarkable growth inhibition on all the tested cell lines with GI_{50} values in the range of activity ranging 1.38-43.1 μM and 1.19-14.1 μM respectively. Initial DNA interaction studies of these compounds (by CD, UV/vis, and the fluorescence spectroscopy) expectedly suggested the π-π stacking interactions facilitate DNA intercalation activity. Furthermore, representative compounds were proved to possess strong inhibition against topoisomerase II. Therefore DNA and topoisomerase are the main targets of the agents.

Conclusions

A series of new naphthalimides were designed and synthesized and their cytotoxic activities were evaluated in vitro. Majority of these naphthalimides potentially inhibited the growth of HT-29, A549 cancer cell lines selectively. Interestingly hybrid compounds **5a**, **5b** that have an amide bond at 6-position displayed promising cytotoxic activity than reference drug amonafide. We were also investigated antitumor mechanism of action of **5a** and **5b**. The most active compounds potentially inhibit topo II. Moreover DNA interaction of the representative compounds was studied by CD spectra, UV-Visible Spectra and fluorescence spectra. Studies demonstrating that these compounds intercalate with DNA.

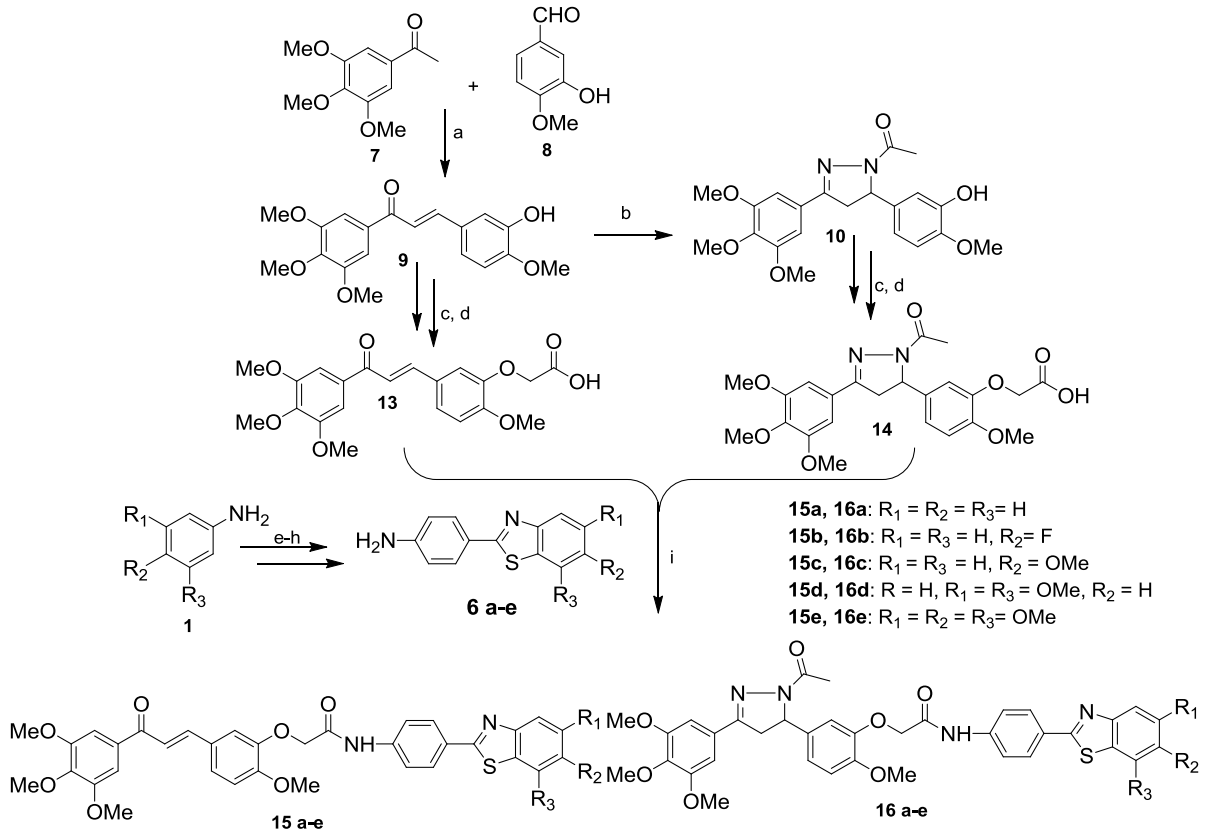
Statement of Problem

Chapter IIIB: Chalcone/Pyrazoline based 2-(4-aminophenyl) benzothiazole Hybrids as Potential Anticancer Agents.

Chalcones (3-diaryl-2-propen-1-ones) are biosynthetic products of the shikimate pathway, belonging to flavanoid family is precursors of open chain flavonoids and isoflavonoids, which are abundant in edible plants. Chalcones are also key precursors in the synthesis of many biologically important heterocycles such as benzothiazepine, pyrazolines, 1,4-diketones, and flavones. Chemically, they consist of open chain flavanoids in which the two aromatic rings are joined by a three carbon α, β- unsaturated carbonyl system. Chalcones and pyrazolines have attracted increasing attention due to numerous pharmacological applications. They have displayed a broad spectrum of pharmacological activities, among which antimalarial, anticancer, antiprotozoal (antileishmanial and antitrypanosomal), anti-inflammatory, antibacterial, antifilarial, antifungal, antimicrobial, anticonvulsant and antioxidant activities have been reported. Similarly 2-(4-aminophenyl)benzothiazoles

constitute a new and simple class of antitumor molecules possessing diverse biological properties. These biologically active conjugates further encouraged to synthesize some newer conjugates that are likely to enhance the anticancer activity. In this context we designed and synthesized some new chalcone/pyrazoline-2-(4-aminophenyl) benzothiazole conjugates **15-16(a-f)** as potential anticancer agents.

Scheme: Synthesis of chalcone/pyrazoline based 2-(4-aminophenyl) benzothiazole hybrids



Reagents and conditions: (a) aq. KOH, ethanol, 6h; (b) $NH_2NH_2.H_2O$, Acetic acid, reflux, 14h; (c) 2-bromoethyl acetate, K_2CO_3, DMF, 12h; (d) LiOH. H_2O, THF, MeOH, H_2O, 14h; (e) Pyridine, reflux, 3 h; (f) Lawesson's reagent, toluene, reflux, 8 h; (g) $K_3Fe(CN)_6$, aq.NaOH, EtOH, 90 °C, 2-3 h; (h) $SnCl_2.2H_2O$, EtOH, 3 h; (i) EDCI/HOBT, DCM, 14-16h.

Conclusions

In conclusion, a series consisting of 10 analogs have been synthesized by conjugating with biologically active heterocyclic scaffold 2-phenylbenzothiazole with chalcone and pyrazoline. The synthesized hybrids were decorated with diverse array of substituents on 2-phenyl benzothiazoles to deduce the structure activity relationship (SAR). These conjugates

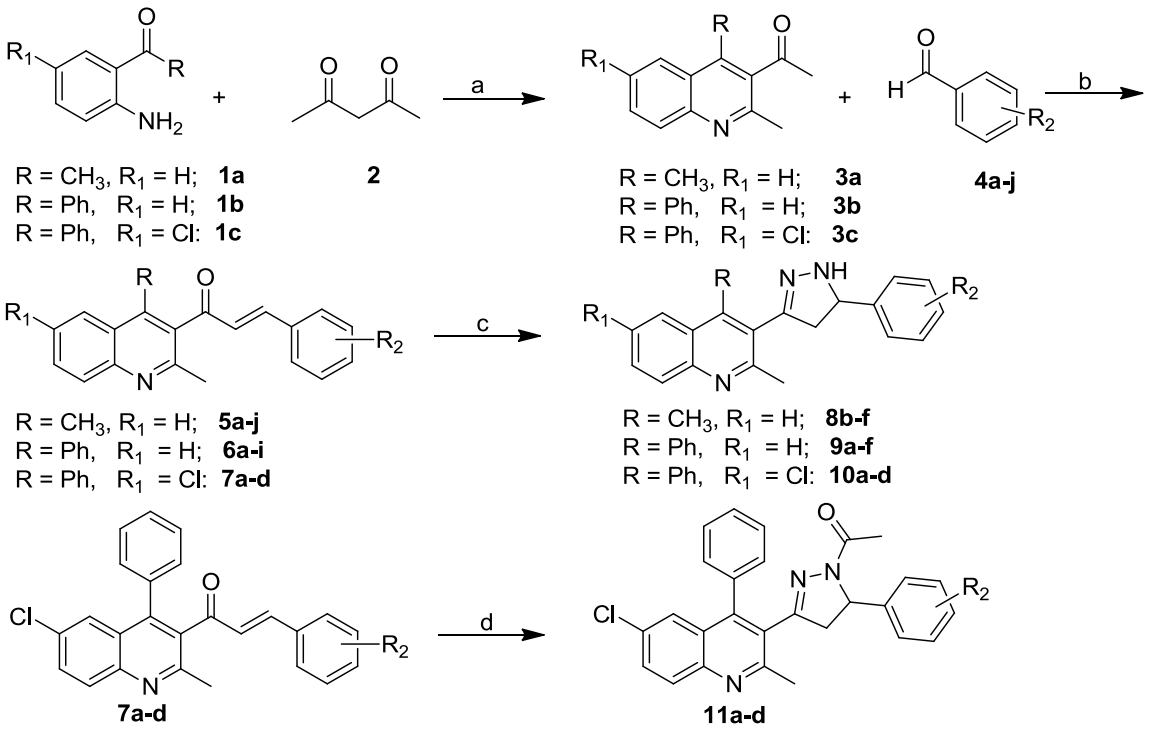
were tested for their anticancer potential against a panel of five different human cancer cell lines. Compounds **16a** was found to be the most effective hybrid from the series displaying IC_{50} value of 0.25 ± 0.01 μM against the lung cancer cell line A549. Further studies to probe the mode of action of this conjugate are under progress. Moreover another two promising compounds **15a** and **15b** have shown potent cytotoxicity in B_{16} cells at 1.29 μM and 1.12 μM.

Statement of Problem

Chapter IV: New Quinoline Linked Chalcone and Pyrazoline Conjugates: Molecular Properties Prediction, Antimicrobial and Antitubercular Activities.

The quinoline nucleus is an important heterocyclic structure found in several natural and synthetic products with a wide range of pharmacological activities such as anti-tuberculosis, antimalarial, anti-inflammatory, anticancer, antibiotic, anti-hypertensive, and anti-HIV functions. Fluoroquinolones, such as gatifloxcin and moxifloxacin, target DNA topoisomerase IV and DNA gyrase and can be used as anti-TB agents, however, they often suffer from resistance. Quinoline-based anti-TB compound (TMC207), bearing a bulky biaryl side chain at position C_3 is a highly potent anti-TB agent and is currently in phase II clinical trials. On the other hand, chalcones, which are important class of natural compounds, have been reported to possess diverse and interesting biological properties such as antioxidant, antifungal, antitumor, analgesic, anti-tuberculosis and anti-HIV. In addition, there have been numerous reports on the synthesis of pyrazoline derivatives owing to their wide ranging biological activities and their associated therapeutic potential as anti-HIV, anti-inflammatory, anticancer and anti-microbial agents. Therefore, considering the antimicrobial potential of quinoline, chalcone and pyrazoline derivatives we have developed a new structural motif containing all these structures to find potent antimicrobial agents. In the present investigation, two new series comprising of forty three conjugates (**5a-j**, **6a-i**, **7a-d**, **8b-f**, **9a-f**, **10a-d** and **11a-d**) have been synthesized by conjugating quinoline scaffold with chalcone as well as pyrazoline moieties.

Scheme: Synthesis of quinoline linked chalcone and pyrazoline conjugates



5a: R = CH_3, R_1 = H, R_2 = 3- OMe, 4-OH
8b, 5b: R = CH_3, R_1 = H, R_2 = 3- OH, 4-OMe
8c, 5c: R = CH_3, R_1 = H, R_2 = 3,4,5-tri-OMe
8d, 5d: R = CH_3, R_1 = H, R_2 = 4-F
8e, 5e: R = CH_3, R_1 = H, R_2 = 3,5-di-F
8f, 5f: R = CH_3, R_1 = H, R_2 = 3-F, 4-OMe
5g: R = CH_3, R_1 = H, R_2 = 2,4,6-tri-OMe
5h: R = CH_3, R_1 = H, R_2 = 3,4-di-Me
5i: R = CH_3, R_1 = H, R_2 = OCF_3
5j: R = CH_3, R_1 = H, R_2 = CF_3

9a, 6a: R = Ph, R_1 = H, R_2 = 3- OMe, 4-OH
9b, 6b: R= Ph, R_1 = H, R_2 = 3- OH, 4-OMe
9c, 6c: R = Ph, R_1 = H, R_2 = 3,4,5-tri-OMe
9d, 6d: R = Ph, R_1 = H, R_2 = 4-F
9e, 6e: R= Ph, R_1 = H, R_2 = 3,5-di-F
9f, 6f: R= Ph, R_1 = H, R_2 = 3-F, 4-OMe
6g: R = Ph,R_1 = H, R_2 = 2,4,6-tri-OMe
6h: R = Ph, R_1 = H, R_2 = 3,4-di-Me
6i: R = Ph, R_1 = H, R_2 = OCF_3

7a,10a,11a: R_2 = 3-OMe, 4-OH
7b,10b,11b: R_2 = 3-OH, 4-OMe
7c,10c,11c: R_2 = 3,4,5-tri-OMe
7d,10d,11d: R_2 = 4-CF_3

Reagents and conditions: (a) CH_3CN, dil. HCl, 3 h, 79-81%; (b) EtOH, KOH, rt, 3 h, 80-93%; (c) $N_2H_4.H_2O$, EtOH, 8 h , reflux, 71-83%; (d) $N_2H_4.H_2O$, AcOH, 8 h, reflux, 77-83%.

Results and discussions

All the synthesized conjugates were tested for their antibacterial, antifungal and antitubercular activities. It is important to note that some of these compounds such as **5e**, **5f**, **5i**, **5j**, **7a**, **8b**, **8c**, **8e**, **8f**, **11b** and **11c** showed potent antibacterial effects against both Gram positive and Gram negative pathogenic strains to variable extents. The conjugates **5j** and **8b**

demonstrated excellent antibacterial activity with the MIC values ranging from 4-8 µg/mL against the Gram positive bacterial strains. The active compounds **5j** and **8b** showed very good inhibitory zone at 20 µg/mL concentration when compared to standard reference drugs. Moreover MIC values of antifungal activity showed similar trend as antibacterial activity. Compound **5j** was found to be the most promising candidate among the tested conjugates demonstrating MIC 4 µg/mL against both $H_{37}R_V$ as well as Rif^R strains.

Conclusions

In conclusion two new series comprising of forty two compounds have been conveniently synthesized by linking the quinoline scaffold with the chalcone, pyrazoline moieties and evaluated them for prediction of their molecular properties through online software in order to find suitable molecules for the antitubercular, antimicrobial and antifungal activities with anticipation of generating new structural leads serving as potent anti-infective agents. Some of these compounds exhibited appreciable antibacterial activity against the tested bacterial strains. Compounds **5j** and **8b** were found to be the promising candidates exhibiting MIC 4 µg/mL against *B. subtilis* MTCC 121 and *M. luteus* MTCC 2470. In addition, several compounds showed good inhibition of certain fungal organisms. Further, some of the compounds displayed good to appreciable anti-tubercular activity against both $H_{37}R_V$ as well as Rif^R strains. Moreover several potent compounds were evaluated for their cytotoxic effect in MRC-5 Lung fibroblast cells and results of this study demonstrated that active compounds showed relatively low level of cytotoxicity. Selectivity index value for compound **5j** was ≥ 5 which indicated that promising antitubercular compound.

The binding modes and effect of the C_4 quinoline substitution on the activity have been studied using molecular modeling. Methyl group was found to be more suitable than the aromatic phenyl group which was supported by the antimicrobial activity data. Therefore, compounds such as **5j** and **8b** could be considered as potential leads to generate chemical libraries that aid the process of development of newer antimicrobial agents to combat antibiotic resistance.