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Novel quinoxalinyl chalcone hybrid scaffolds as enoyl ACP reductase inhibitors: Synthesis, molecular docking and biological evaluation

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ABSTRACT

We report herein, first ever synthesis of series of novel differently substituted quinoxalinyl chalcones using Claisen Schmidt condensation, its molecular docking studies, and potential to be good anti-microbial, anti-tubercular and anti-cancer agents. The antimicrobial studies were carried out against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* using disc diffusion procedure. The selected chalcones were tested for anti-cancer and cytotoxicity activity against MCF-7 cancer cell line using MTT assay method. All the synthesized compounds were screened for *in vitro* anti-tubercular screening against MtbH37RV strains by Alamar blue dye method. These results were compared with molecular docking studies carried out on *Mycobacterium tuberculosis* enzyme enoyl ACP reductase using Surflex-Dock program that is interfaced with Sybyl-X 2.0. SAR analysis for antimicrobial and antitubercular activity has also been proposed.

One of the major advances in the field of medicinal chemistry and drug discovery has been the molecular hybridization approach. This has risen from "drug evolution", which drug-drug hybridization is leading to drug molecules of potential bioactivity. Konishi et al. reported hybridization of Benzocaine and Metoclopramide leading to generation of 16 new molecules.[1] The large number of libraries of compounds is an encouragement in the findings for new drug candidates. In the same lines, molecular hybridization is a tool in drug designing, wherein simples molecules can be linked together to construct new hybrid molecules of varied biological interest.[2] This versatile approach of designing new drug entities is the key to achieving large number of hybrid molecules having better affinity and efficacy than the parent molecule from which they are derived. One of the pharmacophoric moieties, which have been more often the target of medicinal chemists has been naturally occurring as well as synthesized. Chalcones are biologically important α,β-unsaturated carbonyl compounds, which as excellent building blocks to heterocycles has attracted organic as well as medicinal chemists.[3] Chalcone derivatives have been demonstrated to have wide range of biological activities.[4,5] Chalcone linked hybrids with heterocycles is a step towards achieving new drug targets,[6] for example coumarinyl-chalcone hybrids as a promising bioactive agents showing wide spectrum of biological properties,[7] novel chalcone-thiazole hybrids as having high antibacterial action against *Staphylococcus aureus,* making it a potential candidate to act as antibiotic.[8] Variety of chalcone hybrids having naphthyl, isoxazolyl and indolyl moieties have been known to show potency as anticancer agents.[9–11] N-heterocyclic chalcones have been known in literature, and have been biologically evaluated for their anti-microbial and anti-tubercular activity.[12–14]

Tuberculosis (TB) is a highly dreaded, chronic, infectious, airborne disease affecting more than two million people all around the world and with more than 8 million cases every year.[15] Due to emergence of multi-drug resistant varieties of *Mycobacterium tuberculosis* and aids epidemic, drugs like isoniazid, rifampicin, pyrazinamide, ethambutol are no more effective.[16] There are several promising clinical trials development programs carried out to conduct evaluation of new anti-TB drugs, such as PA-824, which is a nitroimidazooxazine, an anti-TB drug candidate in the late stage clinical development, showing increased activity against *Mycobacterium tuberculosis*. However, there are few novel compounds known to hit the target. Numbers of chalcones are known to show high inhibitory activity against the growth of *in vitro Mycobacterium tuberculosis* H37Rv strains, when used in low concentrations.[17] Quinoxaline, a bicyclic nitrogen heterocycle is said to have enough potential to be explored for biological evaluation.[18]

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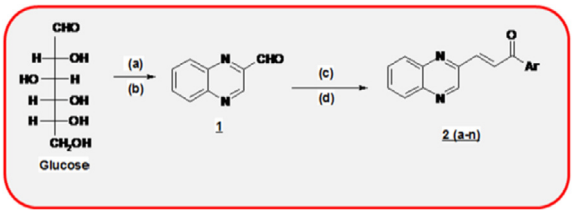
Various derivatives of quinoxaline are known to possess wide range of biological activities ranging from anti-microbials, anti-tubercular, kinase inhibitors, anti-viral, anti-inflammatory, analgesics, anticancer, anxiolytics, antihelmintics, anticonvulsants, antioxidants, antidepressant, anti-hypertensives to antiHIVs.[19–22] Thus, being a part of well-known antibiotics, like echinomycin laevomycin and actinoleutin, the substituted quinoxaline skeleton need to be exploited more in drug discovery.[23]

The combination of these properties of quinoxaline and chalcone in one compound would lead to a drug with potent bioactivity. Molecular hybridization approach from quinoxaline and chalcone include novel 2-acetylquinoxaline derived-chalcones which exhibited *in vitro* glioma cell proliferation activity.[24] Quinoxaline-6-carbaldehyde have also been converted to chalcones using aromatic aldehydes and evaluated for breast cancer.[25] Quinoxaline derived chalcones has been synthesized and biologically evaluated.[26,27] Chalcone derivatives of quinoxaline-1,4-dioxides have been screened as anti-TB agents.[22] In his PhD thesis, Mohan et al. has reported work on synthesis and reactions of quinoxalines involving preparation of quinoxaline-2-carbaldehyde.[28] Interestingly, in literature, quinoxaline-2-carbaldehyde has not been exploited and there are no reports on anti-tubercular activity of quinoxalinyl chalcones. In view of this, an approach was designed to get a quinoxalinyl chalcone hybrid molecule from acetophenones and quinoxaline-2-carbaldehyde and study its potential as antitubercular agents (Fig. 1).

Quinoxaline-2-carbaldehyde was first synthesized by literature known procedure from glucose, phenylenediamine, hydrazine to form an intermediate, followed by oxidation using sodium metaperiodate.[28] This was then reacted with substituted aromatic acetophenones under Claisen Schmidt condensation basic reaction conditions[29,30] to give corresponding chalcone derivatives **2a–n** in moderate to good yields (Scheme 1, Table 1). In all 14 quinoxalinyl chalcone derivatives were synthesized.

The first six compounds **2a–f** have been reported by us,[27] but their molecular docking studies and biological activities have been performed now. The acetophenones (1 equivalent) was treated with aqueous ethanolic solution of sodium hydroxide, stirred at room temperature for 10 min for enolate formation, then to quinoxaline-2-carbaldehyde (1 equivalent) was added and the stirring continued till the completion of the reaction, which was monitored by thin layer chromatography. The reaction was worked up by addition ice and hydrochloric acid. The solid product filtered, was purified by recrystallization using ethanol. All the synthesized compounds have been identified and confirmed by FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy. The purification of the compounds was confirmed by mass spectral analysis and by HPLC measurements on CXTH-3000 Chromatography Data Handling System (Analytical Technologies Limited). Chromatographic separation was achieved at ambient temperature by using mobile phase consisting of methanol and water in the ratio 90:10 (v/v) by 20 min. The mobile phase was pumped at the rate of 1.0 mL/min. The detector wavelength was set at 370 nm. The run time was set at 20 min and retention time of all chalcones was between 16 and 22 min. The purity of all the synthesized compounds was found to be 95% and above. The chromatogram of chalcone is shown in Fig. 2. We were successful in achieving synthesis of new quinoxalinyl chalcone hybrids bearing different substituents. That gave us an opportunity to explore the applications of such chalcones for antimicrobial, anticancer and anti-tubercular activity, as well predict structure activity relationships (SAR analysis).

Antibacterial and antifungal activities of the newly synthesized quinoxalinyl chalcone derivatives **2a–n** were determined using agar well disc diffusion procedure.[31] In brain heart infusion agar, against gram positive strain *Staphylococcus aureus* and gram negative strains *Escherichia coli*, and in sabouraud agar medium against fungal organism *Candida albicans*. Five wells were made on each



Scheme 1. Synthetic route towards quinoxalinyl chalcones from glucose (**2a–n**) Reagents and conditions: (a) H_2O, *o*-phenylene diamine, glacial CH_3COOH, NH_2-NH_2·H_2O, reflux, 5 h, cool in ice bath; (b) H_2O, $NaIO_4$, glacial CH_3COOH, stir, r.t. 16 h; (c) Aromatic ketones, NaOH, H_2O, EtOH, stir, r.t.; (d) Ice, concentrated HCl.

Table 1
Claisen Schmidt condensation of quinoxaline-2-carbaldehyde with various acetophenones to give chalcones **2a–n**.

Compound	Time[a]	Yield[b] in %	Melting Point °C[c]
2a	2	78	256–258
2b	2	75	182–185
2c	2	80	124–126
2d	4	85	98–100
2e	3	75	126–128
2f	2	80	135–138
2g	2	65	150–152
2h	2	80	144–146
2i	2	95	120–122
2j	4	70	136–138
2k	2	75	156–158
2l	2	60	164–167
2m	2	60	205–207
2n	2	90	130–132

[a] Time taken for completion of the reaction monitored by thin layer chromatography.
[b] Calculated from the amount of chalcone obtained after recrystallization.
[c] Determined using thiels tube paraffin method.

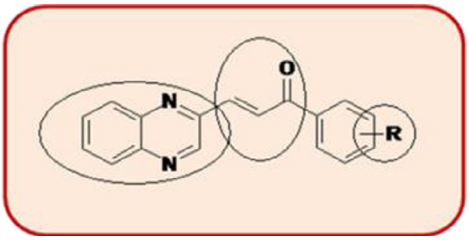


Fig. 1. Molecular hybridisation of quinoxaline and chalcones having drug potency.

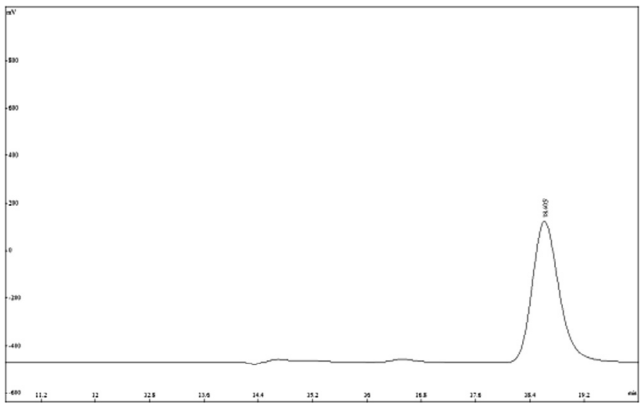


Fig. 2. Representative Chromatogram of chalcone.