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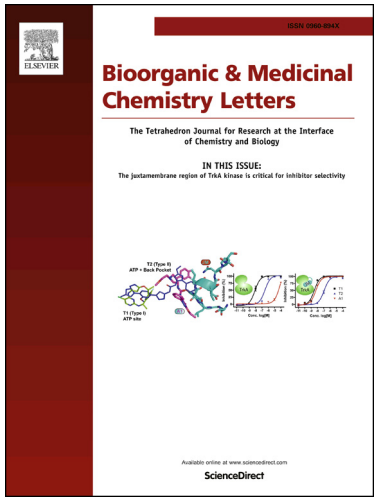

Novel quinoxalinyl chalcone hybrid scaffolds as enoyl ACP reductase inhibitors: Synthesis, molecular docking and biological evaluation

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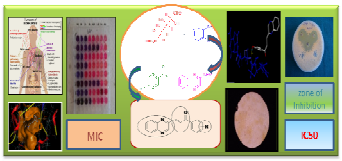
Vidya Desai[a,*], Sulaksha Desai[a], Sonia NaikGaonkar[a], Uddesh Palyekar[a], Shrinivas D. Joshi[b] and Sheshagiri K. Dixit[b]



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Vidya Desai[a,*], Sulaksha Desai[a] , Sonia Naik Gaonkar[a] , Uddesh Palyekar[a], Shrinivas D. Joshi[b] and Sheshagiri K. Dixit[b]

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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 cytoxicity 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, air-borne 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]. 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 i*n 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[28]. In his PhD thesis, Mohan et al has reported work on synthesis and reactions of quinoxalines involving preparation of quinoxaline-2-carbaldehyde[29]. 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**.)

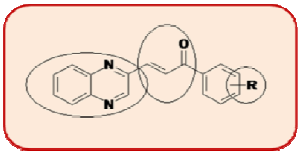
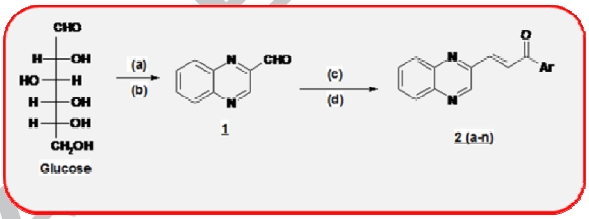


Fig. 1. Molecular hybridisation of quinoxaline and chalcones having drug potency

Quinoxaline-2-carbaldehyde was first synthesized by literature known procedure from glucose, phenylenediamine, hydrazine to form an intermediate, followed by oxidation using sodium metaperiodate[29]. This was then reacted with substituted aromatic acetophenones under Claisen Schmidt condensation basic reaction conditions[30-31] to give corresponding chalcone derivatives 2a-n in moderate to good yields (Scheme 1, Table 1). In all 14 quinoxalinyl chalcone derivatives were synthesized.



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 hours, cool in icebath; (b) H_2O, $NaIO_4$, glacial CH_3COOH, stir, r.t. 16 hours; (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 **2 a-n**

Compound	Time[a]	Yield[b] in %	Melting Point[o]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.

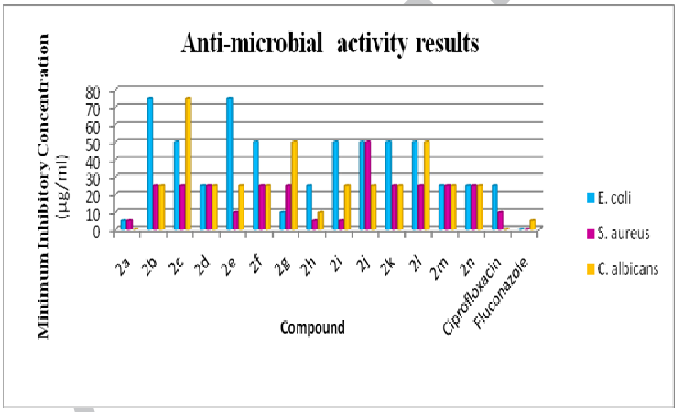
The first six compounds **2a-f** have been reported by us[27], but their molecular docking studies and biological activities have been performed now. The acetophenones (1 equivalent) was treated with aqueous ethanolic solution of sodium hydroxide, stirred at room temperature for 10 minutes for enolate 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}HNMR and ^{13}CNMR 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 1.0mL/min. The detector wavelength was set at 370nm.The run time was set at 20min and retention time of all chalcones was between 16-22 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 synthesised quinoxalinyl chalcone derivatives 2a-n were determined using agar well disc diffusion procedure[32]. 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 plate and 75µL, 50µL, 25µL, 10µL and 5µL of compound were added into the respective wells. Plates were incubated for 18- 24 hrs at 37°C in incubator. Diameter of inhibition zone to nearest whole millimeter was measured by holding the measuring device. Ciprofloxacin and fluconazole was used as standard drug. (**Table 2**)

Table 2 Anti-microbial activity of synthesized quinoxalinyl chalcones **2a-n**

Compound	Ar	Minimum inhibitory concentration-MIC (µg/mL)		
		S. aureus	*E. coli*	*C. albicans*
2a	4-Hydroxyphenyl	5	5	
2b	4-Aminophenyl	50	25	25
2c	4-Bromophenyl	75	25	75
2d	4-Methoxyphenyl	50	25	25
2e	4-Chlorophenyl	25	10	25
2f	4-Fluorophenyl	75	25	25
2g	4-Pyridinyl	50	25	50
2h	3-Hydroxyphenyl	10	5	10
2i	3-Bromophenyl	25	5	25
2j	Phenyl	50	50	25
2k	3-aminophenyl	50	25	25
2l	4-Nitrophenyl	50	25	50
2m	3-Nitrophenyl	50	25	25
2n	Naphthyl	25	25	25
	Ciprofloxacin	2	2	-
	Fluconazole	-	-	16



From the outcomes of the antimicrobial activity of the synthesized quinoxalinyl chalcone derivatives, the following structure activity relationships (SAR) can be established. Overall, the quinoxalinyl chalcones **2a-n** exhibit better antibacterial activity against gram-ve bacterial strains as compared to gram+ve bacterial strains. The best activity against gram-ve and gram +ve bacterial strains for compounds **2a** and **2h** indicates that presence of –OH substituent, is necessary for enhanced antibacterial activity. The position of OH substituent also decides the specificity. In case of compound **2a** (4-OH) exhibits anti-bacterial but no antifungal activity, while compound **2h** (3-OH) exhibits both antibacterial as well as antifungal activity. This was also evident with the comparison of activity of **2c** and **2i** which contains (p-Br) and (m-Br) substituents respectively. Significant antibacterial and antifungal activity for **2n,** containing naphthyl group as compared to **2j** containing phenyl group, indicates that increase in hydrophobicity has positive effect on activity. Replacement of phenyl ring **2j** by a heterocyclic pyridinyl ring **2g** does not show change in activity.

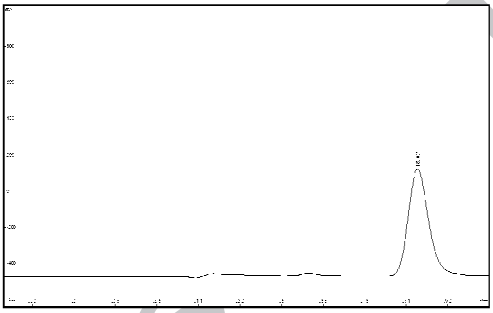


Fig.2. Representative Chromatogram of chalcone

The cytotoxicity of selected compounds **2a, 2b** and **2f** (Table 3) was evaluated using standard MTT assay[33]. In this assay, the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide was measured by mitochondrial succinate dehydrogenase. The cell line used was MCF-7 cell line, a Michigan cancer foundation-7 cell line, a Human mammary gland adenocarcinoma, which was procured from National Centre for Cell Science (NCCS), Pune, India. IC50 is Half maximal inhibitory concentration, at which 50% of cells were undergoing cytotoxic cell death due to synthesized compounds treatment. Cis-Platin and Doxorubicin was used as standard drugs. Absorbance is measured at wavelength of 570nm by using LISA plus.

Table 3 Preliminary cytotoxicity screening of quinoxalinyl chalcones **2a, 2b and 2f**

Sample	**Conc. µg/mL**	**Absorbance[a]**	**Result**	**IC50 µg**
2a	10	0.584	No lysis	
	20	0.581	No lysis	
	30	0.420	No lysis	-
	40	0.399	No lysis	
	50	0.386	No lysis	
2b	10	1.772	Partial lysis	
	20	1.772	Partial lysis	
	30	1.631	50% lysis	25
	40	1.631	>50% lysis	
	50	1.585	>50% lysis	
2f	10	0.836	No lysis	
	20	0.644	No lysis	
	30	0.505	No lysis	50
	40	0.477	No lysis	
	50	0.380	No lysis	

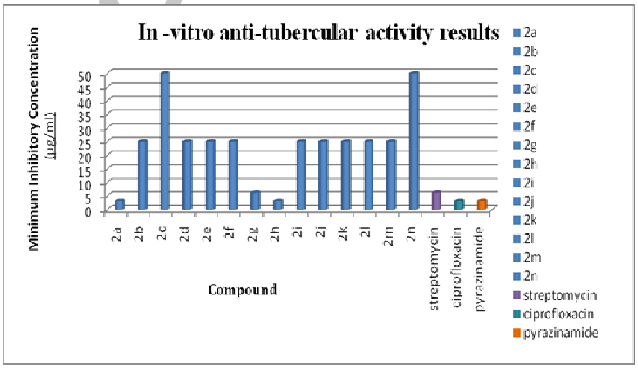
Cis-platin[e]	50% lysis	7.5
Doxorubicin[e]	50% lysis	5-7.5

The **IC50** (half maximal inhibitory concentration) value was determined in case of each compound. The least concentration to show 50% inhibition of cell line was found to be 25µg in case of compound **2b** containing $-NH_2$ substituent and 50µg in case of compound **2f** containing halogen substituent whereas, compound **2a** was found to be inactive against MCF-7 cell lines. Compound **2a** containing OH substituent showed no activity.

The newly synthesized chalcones **2a-n** has been screened for their *in vitro* anti-tubercular activity using Alamar Blue Dye method[34], this methodology is non-toxic, uses thermally stable reagents and showed good correlation with the proportional and BACTEC radiometric method. The *mycobacterium tuberculosis* strain used was MtbH37Rv, while the standard drugs were Streptomycin, Pyrazinamide, and Ciprofloxacin. (**Table 4**)

Table 4 In vitro antitubercular activity of synthesized quinoxalinyl chalcones **2a-n**

Sample	Ar	**Minimum inhibitory concentration-MIC (µg/mL) *MtbH37RV***
2a	4-Hydroxyphenyl	3.12
2b	4-Aminophenyl	25
2c	4-Bromophenyl	50
2d	4-Methoxyphenyl	25
2e	4-Chlorophenyl	25
2f	4-Fluorophenyl	25
2g	4-Pyridinyl	6.25
2h	3-Hydroxyphenyl	3.12
2i	3-Bromophenyl	25
2j	Phenyl	25
2k	3-aminophenyl	25
2l	4-Nitrophenyl	25
2m	3-Nitrophenyl	25
2n	Naphthyl	25
Streptomycin[d]		6.25
Ciprofloxacin[d]		3.12
Pyrazinamide[d]		3.12



A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. (**Fig.3**.)

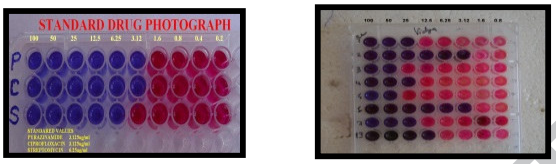


Fig. 3. To the left shows the MIC values for the standard drugs, Streptomycin, Pyrazinamide and Ciprofloxacin, while that to the right shows the MIC values for the synthesized quinoxalinyl chalcones; MIC value 3.12µg/mL (2 and 6) is for chalcones 2a and 2h, containing the –OH substituent.

From the observations of *in vitro* anti-tubercular results for the synthesized quinoxalinyl chalcones, following structure-activity relationships (SAR) can be predicted. The maximal activity exhibited by compounds **2a** and **2h**, is comparable to the standard drugs Pyrazinamide and Ciprofloxacin, indicates that presence of –OH substituent increases the anti-tubercular activity. Replacement of phenyl ring in **2j** by heterocyclic pyridinyl ring in **2g** increases the potency of the compound for anti-tubercular activity. Presence of halogens, methoxy, amino, nitro groups had no significant effect on the anti-tubercular activity. Replacement of phenyl ring in **2j** by naphthyl ring in **2n** decreases the potency of the compound for anti-tubercular activity, indicating that lipophilicity has adverse effect on the activity.

To generalize the innovative approaches to drug targets, the study related to biology of *Mycobacterium tuberculosis* becomes a key component. Among the several drug targets known for tuberculosis [35-36], the cell wall biosynthesis related drug target is the most promising one, since their biosynthetic enzymes do not have homologues in the mammalian system. One of the major drug targets for *Mycobacterium tuberculosis* has also been mycolic acid [37]. Mycolic acid, forms an important fatty acid, and a main constituent of the mycobacterial cell wall, which is present in fatty acid synthase system of *Mycobacterium tuberculosis*. The mycolic acid biosynthesis has been carried out by many successsive enzyme catalyzed cycles equivalent to Fatty Acid Synthase (FAS) systems. The enzyme InhA, which is an enoyl acyl carrier protein reductase from *Mycobacterium tuberculosis* is the key enzyme for the synthesis of type II fatty acid system, which catalyzes NADH-dependent reduction of 2-trans-enoyl-ACP (acyl carrier protein) which is an acyl carrier protein, to yield NAD and reduced enoyl thioester-ACP substrate, which is responsible for the synthesis of mycolic acid. This has been the drug targets for well known anti-Tb drugs like Isoniazid (INH) and Ethionamide [38]. Chalcones derived enoyl ACP reductase inhibitors have been studied and proved to have high binding affinity to the enzymes wherein the main influencing factors of molecular interaction between ENR and chalcone derivatives determined by this study were H-bond, hydrophobic and electrostatic interaction. [39] So, we chose enzyme enoyl ACP reductase for our molecular docking studies on quinoxalinyl chalcones. The crystal structures used were *Mycobacterium tuberculosis* enoyl reductase (InhA) complexed with 1-cyclohexyl-N-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide (PDB ID: 4TZK) for the docking studies, obtained from the Protein Data Bank. The protein was prepared for docking by adding polar hydrogen atom with Gasteiger-Huckel charges [40] and the molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0. [41].

The molecular docking study [42] revealed that **2a and 2h** acts as very good inhibitor of enoyl ACP reductase enzyme. As depicted in **fig. 4**, compound **2a**, makes one hydrogen bonding interaction at the active site of the enzyme (PDB ID: 4TZK), Hydrogen atom of hydroxyl group at 4th position of aromatic ring makes an hydrogen bonding interaction with oxygen of NAD500 (O-H ----- O-NAD500, 1.86 Å). **Table 5** depicts the results of docking studies. As depicted in the **fig. 5.,** compound **2h** makes five hydrogen bonding interaction at the active site of the enzyme (PDB ID: 4TZK), among them three interactions are came from the hydrogen of the hydroxyl group present at 3rd position of aromatic ring, with oxygen of NAD500 and THR196 (O-H ----- O-NAD500, 1.81 & 2.71 Å; HG-THR196, 2.34 Å) and remaining two interactions raised from the oxygen of carbonyl group with hydrogen of NAD500 & TYR158 (C=O ----- H-NAD500, 1.88 Å; H-TYR158, 2.00 Å).

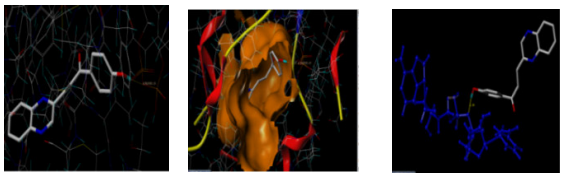


Fig.4. Interaction of 2a at the binding active site of the enzyme enoyl acp reductase (PDB ID: 4TZK). Ligand and key residues are represented as stick models and coloured by atom type, here the proteins are represented by red dotted lines. White: hydrogen atom; red: oxygen atom; dark blue: nitrogen atom; blue: the backbone and carbon atom of compound 2a. Quinoxalinyl moiety of compound 2a embedded in the binding pocket of enoyl ACP reductase. Hydrogen atom of hydroxyl group at 4th position of aromatic ring makes an hydrogen bonding interaction with oxygen of NAD500.

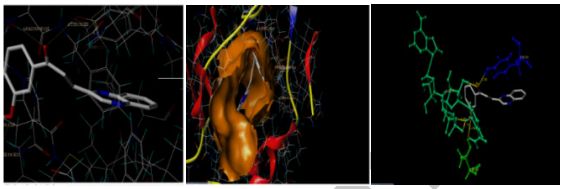


Fig.5. Docked view of compound 2h at the active site of the enzyme PDB ID: 4TZK. Compound 2h makes five hydrogen bonding interaction at the active site of the enzyme (PDB ID: 4TZK), among them three interactions are came from the hydrogen of the hydroxyl group present at 3rd position of aromatic ring.

Table 5 Surflex Docking score (kcal/mol) of the lists of quinoxalinyl chalcones **(2a-n)**

Code	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
2a	6.97	-1.28	3.03	-140.48	-74.20	-222.05	-45.17
2b	4.08	-0.62	0.00	-110.13	-53.35	-161.40	-34.34
2c	5.56	-0.65	1.91	-117.88	-59.66	-134.90	-39.40
2d	6.29	-1.37	2.91	-127.33	-58.88	-205.39	-44.61
2e	5.67	-0.51	1.93	-115.39	-57.18	-131.25	-39.22
2f	5.65	-0.51	1.82	-109.28	-61.87	-125.36	-36.89
2g	5.28	-0.81	0.00	-118.75	-44.64	-184.05	-34.59
2h	8.02	-1.71	2.97	-137.23	-57.94	-218.32	-45.35
2i	4.97	-0.73	1.81	-117.07	-67.96	-143.90	-40.66
2j	5.99	-1.20	1.82	-122.92	-44.00	-168.55	-39.99
2k	5.34	-0.88	0.84	-115.44	-60.01	-175.74	-38.99
2l	4.10	-0.62	0.00	-118.93	-49.13	-173.90	-33.28
2m	4.81	-1.37	0.00	-132.45	-37.36	-209.00	-35.93
2n	6.11	-2.11	1.15	-145.14	-64.81	-225.27	-46.90

[a]C Score (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score;

[b]Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration.

[c]Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds.

[d]D-score for charge and van der Waals interactions between the protein and the ligand.

[e]PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF).

[f]G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies.

[g]Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.

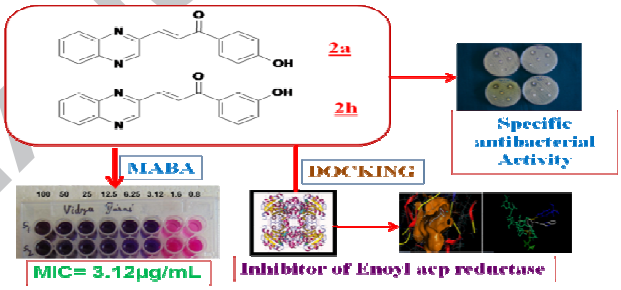


Fig.6. Quinoxalinyl chalcone 2a and 2h, containing –OH substituent shows significant antitubercular activity against MTbH37RV, Chalcone 2a also exhibits specific antibacterial activity against both gram+ve as well as gram –ve bacteria and no activity against antifungal activity, while chalcone 2h showed both antibacterial as well as antifungal activity.

In conclusion, this work on newly synthesized quinoxalinyl chalcones investigates, the role of quinoxalinyl chalcone moiety as a versatile pharmacophoric unit, having range of biological activities. The study tells us about how molecular hybridisation of quinoxaline and chalcone can be exploited to search novel drug targets to treat highly dreaded diseases like tuberculosis and cancer. The MTT assay studies revealed that chalcone **2b**, containing the –NH_2 group, exhibit good anticancer activity. The chalcone **2a,** containing the –OH group showed specifically anti-bacterial and *in vitro* antitubercular activity, but no anticancer activity. The good inhibitory action of quinoxalinyl chalcones **2a** and **2h** towards the enzyme enoyl ACP reductase, has been an additive research on such chalcones. The wide array of bioactivities exhibited by quinoxalinyl chalcones, reflects the importance of such heterocyclic moiety in designing novel drug targets in drug development. This novel findings has thrown light on how a simple quinoxalinyl chalcone hybridisation can lead to a potential moiety as targets to develop new drug entities. Further, such chalcones can be excellent building blocks to heterocycles with great diversity, a heterocyclic scaffold, having varied biological applications.

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31. General procedures for the preparation of chalcone derivatives **(2a-2n):** To a conical flask containing NaOH solution (1.5eq, 10 mL H_2O) was added substituted acetophenones (1mmole) in ethanol (10 mL), and the reaction mixture was stirred for 10 minutes to allow enolate formation, to this was added quinoxaline-2- carbaldehyde **1** (1mmole) and the reaction mixture was stirred till completion. After completion of the reaction, as monitored by TLC the reaction mixture was poured in an ice bath and was acidified using conc. HCl. The solid obtained was then filtered, dried and recrystallized using Ethanol. The quinoxalinyl chalcone **2a-n** were obtained in (60-95% yields.

(2E)-1-(4-hydroxyphenyl)-3-(quinoxalin-2-yl)-prop-2-en-1-one (2a). This chalcone was synthesized from quinoxaline-2-carbaldehyde **1** (200mg, 1.26mmol) and 4-hydroxyacetophenone (171mg, 1.26mmol) to yield **2a**, as pale yellow solid (272 mg, yield 78%) mp 256-260°C; FT-IR (KBr) cm^{-1}: 3250, 3015, 1655, 1606, 1585, 1262; ^{1}HNMR (400MHz, DMSO) δ 10.48 (brs, 1H, OH), 9.52 (s, 1H, N=C-H), 8.25 (d, 2H, j= 8.3Hz, C3 & C5 phenyl ring), 8.1-8.14 (m, 4H, C5-C8 quinoxaline ring), 7.89 (dd, 2H, J=8.29Hz, C2 & C6 phenyl ring), 7.83 (d, 1H, J= 17.7 Hz, H-C=C-C=O), 6.94 (d, 1H, J= 17.7Hz, C=CH-C=O) ^{13}C NMR (75MHz, $CDCl_3$) δ 189.05, 157.82, 150.23, 145.8, 142.25, 141.8, 132.25, 129.97, 129.97, 129.18, 129.05, 128.53, 128.09, 128.09, 123.3, 115.42, 115.42. ESI-HRMS (m/z): calcd. For $C_{17}H_{12}N_2O_2$ $[M+H]^+$: 277.097; found; 277.083.

(2E)-1-(4-aminophenyl)-3-(quinoxalin-2-yl)-prop-2-en-1-one (2b). This chalcone was synthesized from quinoxaline-2-carbaldehyde **1** (200mg, 1.26mmol) and 4-aminoacetophenone (170mg, 1.26mmol) to yield **2b**, as pale yellow solid as orange solid (260 mg, yield 75%) mp 182-185°C; FT-IR (KBr) cm^{-1}: 3354, 3040, 1658, 1609, 1585; ^{1}HNMR (400MHz, $CDCl_3$) δ 9.48 (s, 1H, N=C-H), 7.98-8.30 (m, 4H, C5-8 quinoxaline ring), 7.96-8.01 (dd, 2H, J=8.60Hz, C2 & C6 phenyl ring), 7.81-7.86 (dd, 2H, J=8.62Hz, C3 & C5 phenyl ring), 7.80 (d, 1H, j=17.30Hz, H-C=C-C=O), 6.71(d, 1H, J=17.30Hz, C=CH-C=O), 3.5 (brs, 2H, NH_2) ^{13}C NMR (75MHz, $CDCl_3$) δ 189.05, 150.23, 149.06, 145.8, 142.25, 141.8, 130.08, 130.08, 129.18, 129.05, 128.53, 128.09, 128.09, 127.71, 123.3, 113.17, 113.17. ESI-HRMS (m/z): calcd. For $C_{17}H_{13}N_3O$ $[M+H]^+$: 276.113; found; 276.101.
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42. **Molecular docking procedure**: The crystal structures used were Mycobacterium tuberculosis enoyl reductase (InhA) complexed with 1-cyclohexyl-N-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide (PDB ID: 4TZK) for the docking studies, obtained from the Protein Data Bank. The protein was prepared for docking by adding polar hydrogen atom with Gasteiger-Huckel charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program (Tripos Inc., St. Louis, USA) and its energy-minimized conformation was obtained with the help of the Tripos force field using Gasteiger-Huckel charges and molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0. and other miscellaneous parameters were assigned with the default values given by the software.

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Supplementary Details

Supplementary data associated with this article can be found, in the electronic file.