

Synthesis and bio-evaluation of alkylaminoaryl phenyl cyclopropyl methanones as antitubercular and antimalarial agents

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Abstract

A series of 4-alkylaminoaryl phenyl cyclopropyl methanones (**6a-6u** and **8a-8c**) were synthesized in good yields from two 4-fluorochalcones (**3a** and **3b**) by cyclopropanation of double bond followed by nucleophilic substitution of F with different amines. The compounds were screened for their antitubercular and antimalarial activities against *M. tuberculosis* H37Rv and *Plasmodium falciparum* 3D7 strains *in vitro* respectively. Several compounds (**6a**, **6d-6h**, **6p**, **6q** and **8a-8c**) exhibited good *in vitro* antitubercular activities with MIC values 3.12-12.5 µg/mL and preferentially inhibited the growth of *Plasmodium falciparum in vitro* (**4a**, **4c**, **6a-6d**, **6f**, **6s**, **8a** and **8c**) with IC₅₀ as low as 0.080 and 0.035 µg/mL and SI values 4975 and 6948 respectively. Molecular docking studies against FAS-II enzymes and their *in vitro* inhibition analysis using reporter gene assays were carried out to elucidate the mode of action of these molecules. The compounds were screened *in vitro* against FAS-II enzymes wherein two of the compounds (**4a** and **6g**) showed significant inhibition at 25 µM concentration of the compound.

Keywords: Cyclopropylphenyl methanones, Antitubercular, Antimalarial, FAS-II, *M. tuberculosis* H37Rv, *Plasmodium falciparum* 3D7.

1. Introduction

The current arsenal of anti-infective agents in our hand for the treatment of tuberculosis and malaria is insufficient to protect us over the long term. With more than 1.6 million deaths and 9.2 million new cases being reported each year, tuberculosis is a leading infectious disease claiming millions of deaths today, mostly in developing countries harboring latent TB infections.^{1,2} HIV epidemics in many countries have led to the emergence of new waves of TB infection as co-infection with HIV and mycobacteria render latent TB to an active condition, often with fatal consequences.^{3,4} The current cocktail of antitubercular drug regimens can achieve more than 99% efficacy but this is often reduced due to alarming growth of MDR and XDR-TB.⁵⁻⁹ Although a number of lead molecules exist today¹⁰⁻¹² to develop new drugs but no new chemical entity has emerged for clinical use over the last 40

years. In conjunction with tuberculosis, malaria is also a devastating infectious disease having high morbidity and mortality rate with 300-500 million clinical cases and 1.5-2.7 million deaths every year.¹³⁻¹⁵ Nearly all the fatal cases are caused by the malaria parasite *Plasmodium falciparum*.¹⁶ The widespread resistance of malaria parasite to most common antimalarials^{17,18} (chloroquine, pyrimethamine), and cross resistance to structurally unrelated drugs makes the current situation more worsening.¹⁹⁻²¹ Artemisinin or their structural analogs are potent antimalarial^{22,23} but these are associated with the problem of high cost²⁴ and recently reported resistance to artesunate in Pailin and Western Cambodia.²⁵ Moreover, the adverse effect of co-infection with HIV and malaria is become increasingly apparent.²⁶ Therefore, new agents that will (i) shorten the current TB treatment, (ii) compatible with HIV drugs and (iii) have novel target with high selectivity for both of diseases are needed.

M. tuberculosis and *P. falciparum* allocate enzymatic components of the type II fatty acid biosynthetic pathway (FAS-II)^{27, 28} for their fatty acid biosynthesis. In *Mycobacterium*, FAS-II pathway comprises a group of enzymes as MabA (FabG), InhA (FabI), FabH as well as Kas A or Kas B which sequentially catalyses the reduction and condensation steps of fatty acid synthesis.^{29,30} Among these enzymes InhA and Kas A or Kas B is the most important target for tuberculosis chemotherapy. For Plasmodium, FAS-II is the only fatty acid pathway whose enzymatic components reside at a unique plastid organelle known as apicoplast and essential for development of the parasite.²⁸ Another most important metabolic pathway, the isoprenoid pathway DOXP is also known in apicoplast.³¹ Both the DOXP and FAS-II pathways are valuable drug targets in anti-infective drug development since these two pathways are absent in the human host.³² Out of several inhibitors of the FAS-II and DOXP, triclosan (**I**)³³ and fosmidomycin³⁴ are very important (**Fig.-1**). Simple acetophenones, benzylideneacetophenones and p-nitro- -acetylamino- -hydroxypropionophenones are reported to have good antitubercular activities.³⁵⁻³⁸ The cyclopropane derivative of fosmidomycin (**II**) (**Fig.-1**) with limited conformational flexibility in the backbone is as active as fosmidomycin.³⁹ Moreover, cyclopropyl ring is a common structural element in the mycobacterial cell wall⁴⁰ and its chemotherapeutic importance is also well known.⁴¹⁻⁴³ Aryl cyclopropyl ketones play a prominent role as intermediates in the synthesis of many other biologically active compounds.⁴⁴ As a part of our ongoing research devoted for the synthesis of anti-infective agents⁴⁵⁻⁵⁰ we have already identified the aryloxyphenyl cyclopropyl methanones (**III**)⁵¹ (**Fig.-1**) as potent antitubercular agent. Keeping in view the above we were prompted to synthesize 4-alkylamino aryl phenyl cyclopropyl methanones and evaluate them for anti-mycobacterial and antimalarial activities.

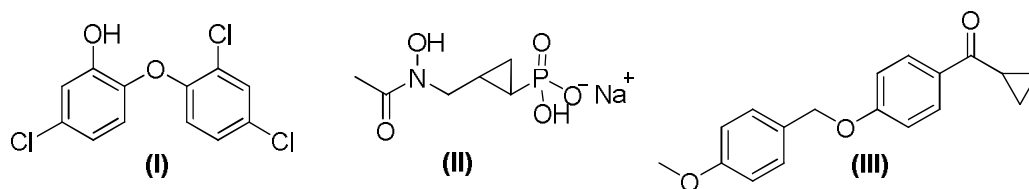


Figure 1 Chemical structures of FAS-II inhibitor and representatives of biologically active molecules with cyclopropane ring.

2. Results and discussion

2.1. Chemistry

The protocol for the synthesis of 4-alkylamino substituted aryl phenyl cyclopropyl methanones begins with the synthesis of chalcones (**3a** and **3b**).^{52,53} The aldol reaction

between 4-fluoro acetophenone (**1**) and substituted aromatic aldehydes (**2a**, **2b**) followed by in situ dehydration, leads the desired chalcones (**3a**, **3b**) in quantitative yields. There are three possible pathways to synthesize the title compounds as shown in **Fig. 2**. The first one we discarded as amines may react with ketones and a complex mixture would be obtained (**Path-1**). The second approach (**Path-2**) involves the *ipso* nucleophilic substitution at 4-fluoro group in one of the phenyl ring of chalcone **3a** by various amines followed by cyclopropanation. The third approach (**Path 3**) involves the cyclopropanation of double bond in chalcones followed by *ipso* nucleophilic substitution at 4-fluoro group in one of the phenyl ring of chalcone **3a** to give the title molecules.

The *ipso* nucleophilic substitution at 4-fluoro group in one of the phenyl ring of chalcone **3a** by various amines in presence of K_2CO_3 /DMF at 120 °C in an inert atmosphere of nitrogen gave the desired alkylamino substituted chalcones (**4a-4e**) in moderate yields only (Scheme 1). The unsatisfactory yield of the products may possibly be due to Michael addition product as we could not isolate the other products of the reaction observed during reaction (TLC). The reaction of the alkylamino substituted chalcones with trimethylsulphoxonium iodide (TMSOI) and 50% aq. NaOH at 80°C in presence of tetrabutylammonium bromide as phase transfer catalyst and CH_2Cl_2 as solvent gave the title compounds (**6a**, **6g**, **6h**, **6j**, **6n**) (Scheme 1). Therefore, the third approach was thought wherein the fluorochalcones **3a** and **3b** were first subjected to cyclopropanation using TMSOI as described above to give the respective cyclopropyl derivatives **5a** and **5b** in good yields. The latter on *ipso* nucleophilic substitution of 4-fluoro group using various amines led to the formation of targeted compounds **6b-6f**, **6i**, **6k-6l** and **6o-6u** in quantitative yields (Scheme 1).

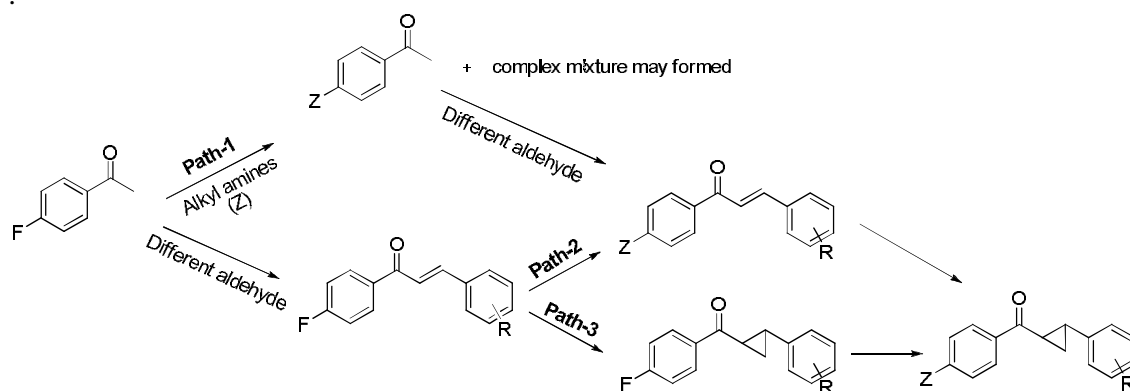
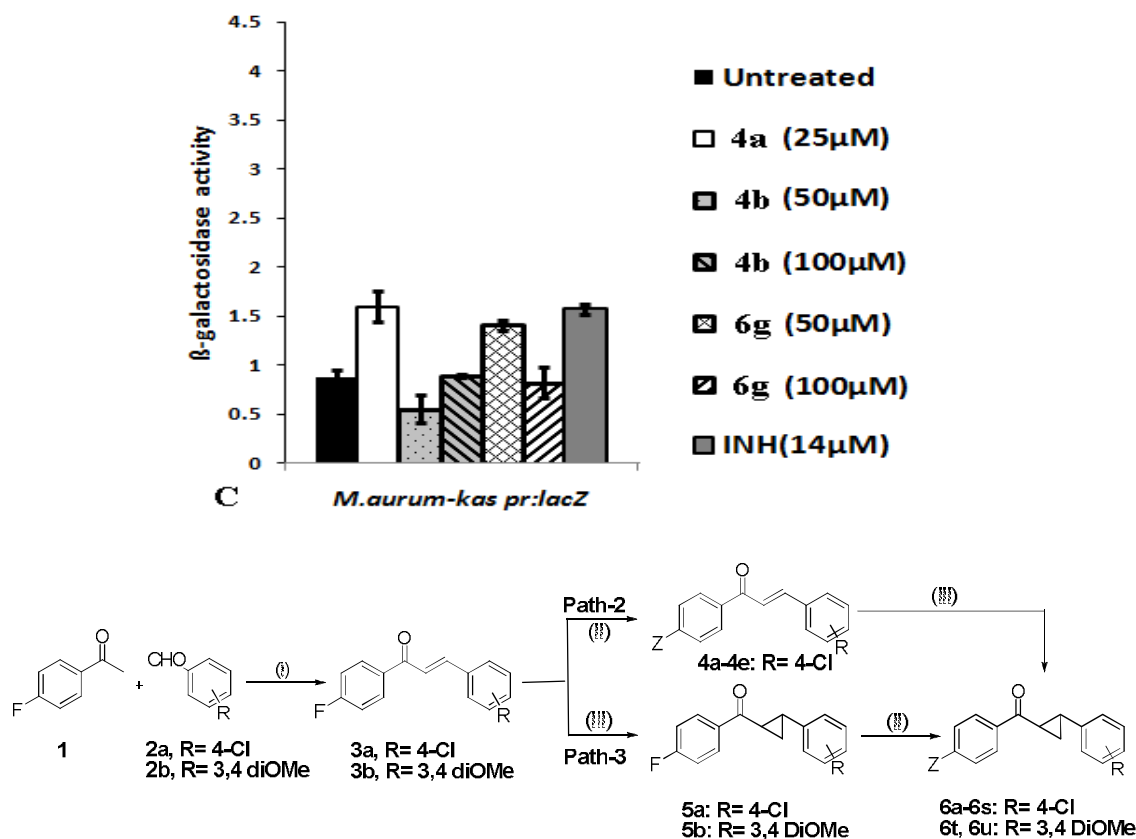


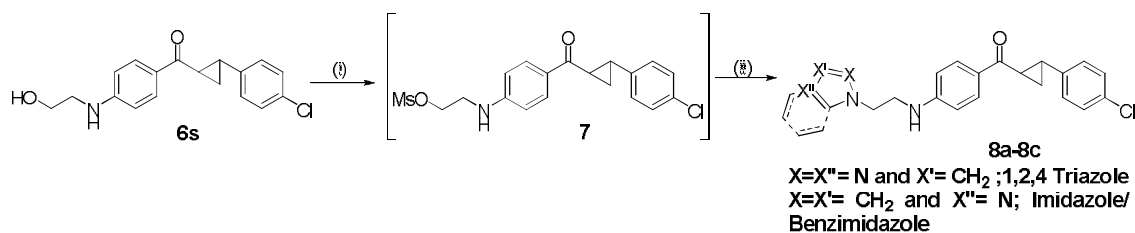
Figure 2: Possible pathways for the formation of alkylaminoaryl phenyl cyclopropyl methanones

In order to see the effect of substituents in the alkylamine chain we have prepared another series of compounds starting from the above compound **6s** having 2-hydroxyethylamino chain at the 4th position of one of the aromatic ring A. Thus **6s** was reacted with $MeSO_2Cl$ in presence of TEA in CH_2Cl_2 at 0°C to give respective 2-methanesulphonyloxyethyl amino derivative (**7**). The nucleophilic substitution of δSO_2Me group in compound **7** with several azoles in presence of NaH/DMF at 0-120°C gave respective 2-(azolyl)-ethylaminoaryl phenyl cyclopropyl methanones (**8a-8c**) in good yields (Scheme 2).

The structures of all the compounds as given in **Table 1** were determined on the basis of their spectroscopic data and microanalyses. The IR spectra of the compounds, in general, exhibited the absorption band at around 3432 cm^{-1} indicating the presence of aromatic amino group, absorption band of carbonyl group at around $1638-1640\text{ cm}^{-1}$. The ESMS (mass spectra) of

the compounds showed $[M+H]^+$ peaks corresponding to their molecular formulae. The ^1H and ^{13}C NMR spectra are consistent with the proposed structures. The ^1H NMR spectrum of a prototype molecule **6g**, displayed exchangeable N-H proton signal as a singlet at 5.39. The four protons of cyclopropyl ring were visible at four different chemical shifts as multiplets at 2.76-2.71, 2.60-2.53, 1.85-1.76 and 1.40-1.34 ppm. The aromatic protons appeared as doublet at 7.83, 7.23, 7.07 and 6.51 ppm with coupling constant in range of 8.28-8.58 Hz. The protons of alkylamino chain were visible at 3.25, 2.42 as triplet and at 1.85-1.76 as multiplet along with one proton of cyclopropyl ring. The protons of the *N,N*-dimethyl group were observed as a singlet at 2.25 ppm. The geometry of the parent chalcone was found to be *trans* from the coupling constant between the olefinic protons of the propenone moiety (compound **4a**, $J = 15.8$ Hz) and the TMSOI mediated cyclopropanation i. e. Corey-Chaykovsky Reaction leads the *trans* product with retained stereochemistry as evident from the literature.⁵⁴⁻⁵⁶ In the ^{13}C NMR spectrum, the only carbonyl carbon appeared at 195.6, while the quaternary aromatic carbon attached to the amine group was observed at 153.0. Three carbons of cyclopropyl ring were visible at 28.7, 28.2 and 18.7 ppm. The other aromatic quaternary carbons (ArC) were observed at their usual chemical shifts of 140.1, 132.3 and 126.8 ppm, while sp^2 aromatic carbons (ArCH) appeared at 131.0, 128.9, 127.9 and 111.7 ppm. Almost similar patterns were observed in ^1H NMR and ^{13}C NMR spectra of other compounds (**8a-8c**) of the series.





Scheme-2: Synthesis of azolyalkylaminoaryl phenyl cyclopropyl methanones (**8a-8c**); **Reagents and conditions** (i) CH₃SO₂Cl, Et₃N, CH₂Cl₂, 0°C-r.t., (ii) Different azoles, NaH, DMF, 0-120°C.

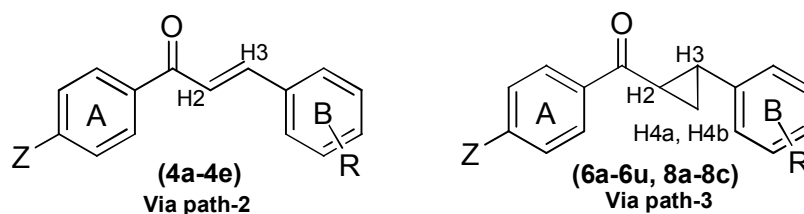


Fig 3. General structure of the synthesized 4-alkylamino substituted chalcones (**4a-4e**) and alkylaminoaryl phenyl cyclopropyl methanones (**6a-6u, 8a-8c**).

Table 1. Synthesis of alkylamino substituted chalcones (**4a-4e**) and alkylaminoaryl phenyl cyclopropyl methanones (**6a-6u** and **8a-8c**)

Compd No.	R	Z (Amine)	M. P. (° C)	% Yield
4a	4-Cl	<i>N,N</i> -Dimethyl aminopropylamine	63-65	35
4b	4-Cl	<i>N</i> -Me Piperazine	100-102	54
4c	4-Cl	Furfuryl amine	72-74	40
4d	4-Cl	Morpholine	98-100	45
4e	4-Cl	Pipridine	111-113	60
6a	4-Cl	<i>N</i> -Me Piperazine	112-114	80
6b	4-Cl	Cyclohexyl amine	98-100	84
6c	4-Cl	Heptyl amine	97-99	82
6d	4-Cl	Butyl amine	128-130	81
6e	4-Cl	4-(2,3-dichlorophenyl) Piperazin	134-136	77
6f	4-Cl	Dibutyl amine	152-154	78
6g	4-Cl	<i>N,N</i> -Dimethyl aminopropylamine	94-96	77
6h	4-Cl	Furfuryl amine	96-98	85
6i	4-Cl	1,5,DiMe hexyl amine	128-130	80
6j	4-Cl	Morpholine	115-117	83
6k	4-Cl	Dodecyl amine	92-94	80
6l	4-Cl	Hexadecyl amine	122-124	85
6m	4-Cl	Octyl amine	154-156	87
6n	4-Cl	Pipridine	118-120	79
6o	4-Cl	<i>N,N</i> -Dimethylamine	132-134	86

6p	4-Cl	Pyrolidine	138-140	86
6q	4-Cl	Oleyl amine	153-155	80
6r	4-Cl	Veratryl amine	140-142	76
6s	4-Cl	Ethanolamine	166-168	73
6t	3,4 Di-OMe	<i>N</i> -Me Piprazine	156-158	81
6u	3,4 Di-OMe	Heptyl amine	147-149	79
8a	4-Cl	(1 <i>H</i> -imidazol-1-yl)ethylamine	110-112	67
8b	4-Cl	(1 <i>H</i> -1,2,4-triazol-1-yl)ethylamine	117-119	68
8c	4-Cl	(1 <i>H</i> -benzo[d]imidazol-1-yl)ethylamine	134-136	70

2.2. Biological activities:

All of the above synthesized compounds including few of the intermediate chalcones were screened for their antitubercular and antimalarial activities against *M. tuberculosis* H37Rv and *P. falciparum* 3D7 strains *in vitro*. The antitubercular screening was carried out *in vitro* using agar microdilution method⁵⁷ while the antimalarial activity was assayed as per earlier reported protocols^{58,61}.

2.2.1. Antitubercular Activity:

Antitubercular screening results of all the synthesized compounds and few of the intermediate chalcones are listed in **Table 2**. As evident from the results out of all the alkylaminoaryl phenyl cyclopropyl methanones screened, compounds **6a**, **6d-6h**, **6p**, **6q** and **8a-8c** showed minimum inhibitory concentration in the range of 3.12-12.5 µg/mL. Among compounds having 4-chloro and 3,4-dimethoxy substituent in aromatic ring B, compounds with 4-chloro substituent (**6a-6s** and **8a-8c**) were more active than the compounds with 3,4-dimethoxy substituents (**6t** and **6u**). The most active compound of the series was found to be compound **6h** having a furfuryl amine substituent with MIC value 3.12 µg/mL. The compound **6a** and **6g** having dialkylamino chain as 4-substituent in one of the phenyl rings (ring A), whether it is cyclic or acyclic have almost similar antitubercular activity with MIC 6.25 µg/mL. On the other hand compounds **6d**, **6f** and **6p** having monoalkylamine chain, dialkylamine chain and cyclic amine with C-chain length of four carbon atoms with comparable lipophilicity displayed only moderate activity with the same MIC value 12.5 µg/mL. However, in contrast compounds with the oleylamine substituent also displayed only moderate antitubercular activity (MIC 12.5 µg/mL). In the second series of compounds having 2-(azolyl)ethylamino substituent at 4-position in benzene ring A showed promising to moderate antitubercular activities. Compound **8a** with imidazolyl ethylamino substituent has MIC value 6.25 µg/mL. However, introduction of any lipophilic moiety to the imidazole unit reduces the activity as compounds **8b** and **8c** have MIC values 12.5 µg/mL each. Similar observation was made with the above compounds having piperazinyl substituents. Replacement of *N*-methyl group (compound **6a**, MIC 6.25 µg/mL) in piprazine by aryl group (compound **6e**) resulted in loss of activity with 12.5 µg/mL.

Encouraged by recent report of antimalarial and antitubercular activities in acetylenic chalcones by Kelly's group⁵⁹ we were prompted to see the effect of 4-alkylamino chalcones (**4a-4e**) against malaria parasite and *M. tuberculosis* with a view to find out the correlation between the unsaturated system and cyclopropanated system. The antitubercular screening results did not offer any definite correlation between activities with the unsaturated or cyclopropanated compounds. It is evident by the fact that, in compound **4a** and **4c** (chalcones having double bond, MIC value 12.5 µg/mL each) the activity is less as compared to their cyclopropanated analogue **6g** and **6h** (MICs 6.25 and 3.12 µg/mL) respectively. On the contrary, the chalcones **4b** and **4d** with MIC value 3.12 and 6.25 µg/mL are better

antitubercular as compared to their cyclopropanated analogues **6a** and **6j** with MICs values 6.25 and >12.5 $\mu\text{g/mL}$ respectively.

Table 2. Antitubercular activity of alkylamino substituted chalcones (**4a-4e**) and alkylamino aryl phenyl cyclopropyl methanones (**6a-6u** and **8a-8c**)

S. No.	Compound No.	Antitubercular activity (MIC $\mu\text{g/mL}$)	S. No.	Compound No.	Antitubercular activity (MIC $\mu\text{g/mL}$)
1	4a	12.5	16	6k	In*
2	4b	3.12	17	6l	In
3	4c	12.5	18	6m	>12.5
4	4d	6.25	19	6n	>12.5
5	4e	>12.5	20	6o	In
6	6a	6.25	21	6p	12.5
7	6b	>12.5	22	6q	12.5
8	6c	>12.5	23	6r	>12.5
9	6d	12.5	24	6s	>12.5
10	6e	12.5	25	6t	>12.5
11	6f	12.5	26	6u	>12.5
12	6g	6.25	27	8a	6.25
13	6h	3.12	28	8b	12.5
14	6i	>12.5	29	8c	12.5
15	6j	>12.5		Inh*	0.025

In* = Insoluble, Inh* = Isoniazid

2.2.2. Antimalarial Activity:

The above alkylamino chalcones and their cyclopropyl derivatives were also screened against malaria parasite *P. falciparum* using the earlier reported protocols.^{58,60,61} Most of the compounds showed moderate to high antimalarial activity. Compounds **4a**, **4c**, **6a-6d**, **6f**, **6s**, **8a** and **8c** were active against the parasite with IC_{50} in the range of 0.035-0.76 $\mu\text{g/mL}$. Among all the screened compounds, compound **8a** was the most potent antimalarial agent with MIC and IC_{50} values of 0.062 $\mu\text{g/mL}$ and 0.035 $\mu\text{g/mL}$ respectively. Compound **6c** was also found to be a potent antimalarial agent with IC_{50} 0.08 $\mu\text{g/mL}$ and very low MIC (0.3 $\mu\text{g/mL}$). Majority of the active compounds have shown good correlation between their MIC and IC_{50} values (**Table 3**). A closure look into structure activity relationship of the compounds revealed that compounds with lipophilic alkylamino substituent (**6b**, **6c**, **6d** and **6f**) showed good antimalarial activity indicating that the lipophilicity has significant impact on antimalarial activity. Similarly compounds with basic alkylamino chain (compounds **4a** and **6a**) orazole moiety (compounds **8a** and **8c**) also exhibited potent antimalarial activities. It seems that basic alkylamino chain and lipophilicity are important factors governing the biological activities.

Table 3. Antimalarial activity and selectivity index of alkylamino substituted chalcones (**4a-4e**) and alkylamino aryl phenyl cyclopropyl methanones (**6a-6u** and **8a-8c**).

S. No.	Compound No.	MIC ($\mu\text{g/mL}$)	IC_{50} ($\mu\text{g/mL}$)	CC_{50} ($\mu\text{g/mL}$)	SI
1	4a	1.56	0.64	5.92	9.25
2	4b	50	11.78	102.46	8.69
3	4c	10	0.76	404.03	531.62

4	4d	50	4.86	35.64	7.33
5	4e	50	8.92	234.59	26.3
6	6a	10	0.54	82.06	151.96
7	6b	10	0.75	196.18	261.57
8	6c	0.3	0.08	398	4975
9	6d	0.6	0.33	316.37	958.69
10	6e	50	8.82	382.61	43.38
11	6f	10	0.73	430.73	590.04
12	6g	50	2.86	15.83	5.53
13	6h	10	1.71	255.89	149.64
14	6i	ND*	ND	ND	ND
15	6j	10	3.02	452.76	149.92
16	6k	>50	10.4	385.7	37.08
17	6l	50	4.57	409.83	89.68
18	6m	10	2.13	214.14	100.53
19	6n	10	6.05	284.28	46.99
20	6o	10	1.43	343.78	240.4
21	6p	>50	9.75	431.49	44.26
22	6q	>50	75.13	393.35	5.24
23	6r	10	8	421.86	52.73
24	6s	2	0.6	305	508.33
25	6t	50	2.09	77.81	37.23
26	6u	10	2.24	272.54	121.67
27	8a	0.062	0.035	243.2	6948.57
28	8b	10	3.41	39.9	11.7
29	8c	2	0.74	285.23	385.45
	Chloroquine	0.05	0.008		

ND*= Not determined

2.2.3. Cytotoxicity:

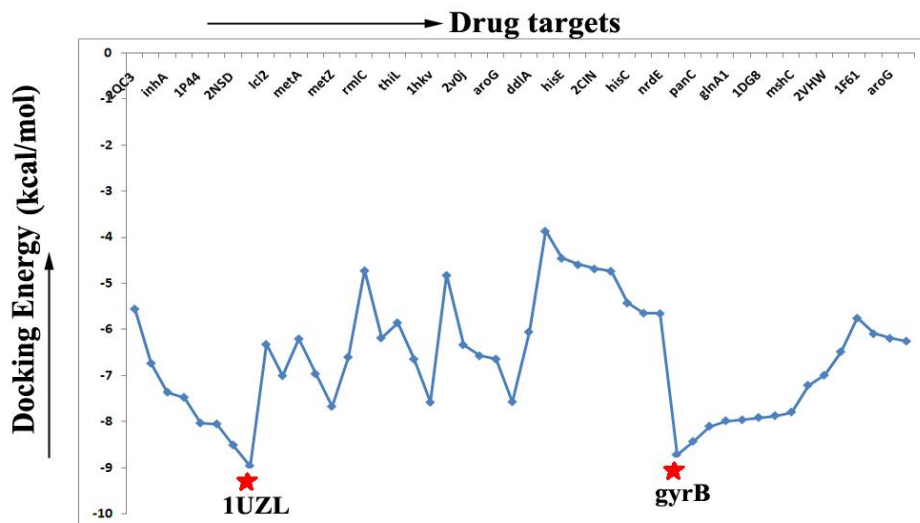
The cytotoxic evaluation of these compounds was carried out on VERO cell line as per earlier reported protocol⁶² and the results are shown in **Table 3**. On the basis of antimalarial/antitubercular and cytotoxic activities, the calculated selectivity indices (SI values) (CC_{50}/IC_{50}) have shown that all the compounds active against malaria and tuberculosis have moderate to high degree of safety. The compound **8a** has shown the best selectivity index (6948.57) as well as potent antimalarial activity. Out of ten antimalarial compounds, nine have shown median cytotoxic concentration to be 82.00-404.03 $\mu\text{g/mL}$ (**Table 3**).

2.2.4. FAS-II inhibitory activities:

2.2.4.1 In silico docking studies:

To identify the drug target of the synthesized compounds first the intermediate chalcone **4a** was selected for *in silico* docking studies^{63,64}. Furthermore to characterize the proteinaceous drug target which is most likely to interact with compound **4a**, the ligand file was uploaded into *Inhibitor Identification Tool (Is-it?)*: (unpublished and implemented in the host laboratory) server and docking jobs were queued by selecting all the 85 drug targets from *M. tuberculosis* enlisted in the server. The top candidates identified by *Is-it?*: server, ranked by interaction energies, included 10 out of the 12 targets from FasII pathway (**Fig. 4**). It is thus likely that aryl amino derivatives may interact with multiple proteins including fabG or

1UZL (star marked in the **Fig. 4**), Enoyl-acyl reductase (1P44, 2NSD, inhA) and fabD (2QC3) from FAS-II pathway. The compound also showed high affinity with another protein Gyrase or gyrB (another red star marked in the **Fig. 4**). The target proteins so identified are enlisted in supplementary **Table 4**. The top potential binding partner identified is MabA, which is also known as FabG1, a part of FAS-II enzyme, catalyzing the NADPH-specific reduction of long chain beta-ketoacyl derivatives. The compound **4a** is predicted to be interacting with most of the reported important residues of the NADP binding site with an estimated inhibition constant, K_i value of 261.41 nM. The chlorine atom of the compound **4a** is present in the vicinity of TYR 153 which is reported to be the part of catalytic triad residues. The molecular interactions of the compound **4a** with MabA are shown in **Fig. 5**.



CDRI_4a13c1fbc916 fasII pathway

Figure 4: The plot of docking energy calculated by Autodock for aryl amino derivative and the protein targets. Only acceptable scores are used for plot. The most potential binders are 1UZL (a fasII pathway enzyme) and gyrB (Gyrase B) and are denoted by asterisk

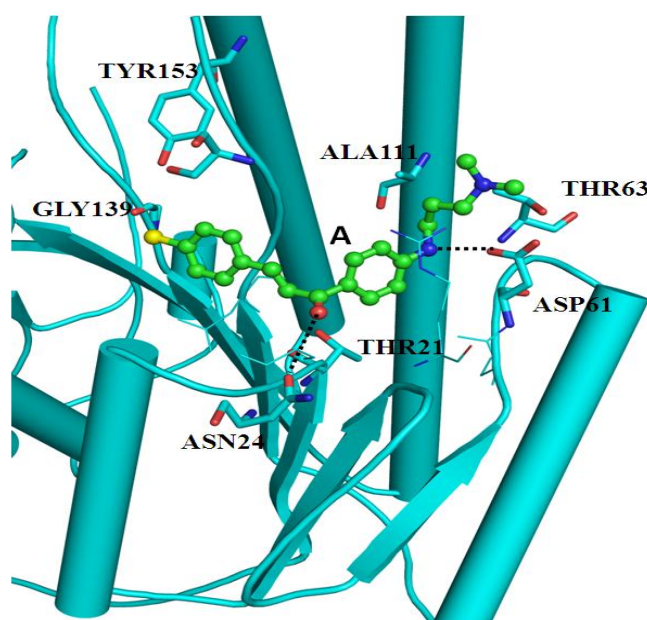


Figure 5. The molecular interactions of Compound **4a** within the MabA binding pocket of the enzyme. The interacting residues are labeled and hydrogen bonds are marked by the dashed lines.

Supplementary Table 4

Drug Target Gene ID/PDB ID	Docking Energy(kcal/mol)	Drug Target Gene ID/PDB ID	Docking Energy(kcal/mol)
2QC3	-5.55	ddlA	-6.05
1HZP	-6.73	fbpA	-3.86
inhA	-7.36	hisE	-4.45
2B35	-7.47	ideR	-4.59
1P44	-8.03	2CIN	-4.68
2H7L	-8.05	trpC	-4.73
2NSD	-8.51	hisC	-5.42
mabA	-8.96	2O2E	-5.64
icl2	-6.32	nrdE	-5.65
ligA	-7.0	gyrB	-8.72
metA	-6.2	panC	-8.43
metC	-6.96	fbpC	-8.1
metZ	-7.67	glnA1	-7.98
murD	-6.6	fbpB	-7.96
rmlC	-4.72	1DG8	-7.91
thiE	-6.18	cyp51	-7.87
thiL	-5.85	mshC	-7.79
1h0r	-6.64	trpA	-7.21
1hkv	-7.58	2VHW	-6.99
2cjh	-4.82	folP2	-6.48
2v0j	-6.33	1F61	-5.75
Alr	-6.57	2QHF	-6.09
aroG	-6.64	aroG	-6.19
Cysk2	-7.57	pncA	-6.25

As no ligand variation was done in the final molecule compared to their parent chalcones, the synthesized final molecules directly undergoes to biological assay against FAS-II pathway.

2.2.4.2. FAS-II inhibition assay:

FAS-II inhibitory activity was assessed using a recombinant non-pathogenic mycobacterial strain, *M. aurum*, which contains *M. tuberculosis kas* operon promoter in fusion with *lacZ* reporter gene⁶⁵. The strain shows continued expression of reporter gene under the influence of *kas* operon promoter during basal conditions, while an increased expression of the reporter gene is noticed only after treatment with FAS-II pathway inhibitors. The preliminary screening of the compounds shows FAS-II inhibitory activity at two different concentration, 25 μ M and 50 μ M and results are listed in **Table-5**. In present studies molecule **4a** was

selected for the assessment, the critical concentration of compound at which it inhibited the growth of *M. aurum* culture by $\geq 85\%$ was determined by cfu analysis and then β -gal enzyme assay was performed to assess the inducibility under treated condition. We observed an induced level of β -gal enzyme activity after treatment with compound **4a** with respect to untreated control (**Fig. 6 A**). The activity gradually increased with increasing concentration of compound and declined at higher concentration owing to killing of cells at exceeding dose of drugs. It may be noted that we repeated the same experiment using another *M. aurum* recombinant strain carrying *hsp60* promoter wherein no inducibility was observed under similar conditions, rather a decline in reporter gene activity was noticed in line with diminishing viability of bacterial cells (**Fig. 6 B**) as the treatment dose increased. Isoniazid (INH), a known FAS-II pathway inhibitor was used as a positive control in both conditions. All the ongoing results of screening suggest that FAS-II is a viable target of the intermediate chalcone **4a**. The assessment of the FAS-II activity of the cyclopropylated analogue of **4a** i. e. **6g** was determined by the same protocol and compound shows 57.37% inhibition against *M. aurum* at 50 μ M concentration of the compound. Moreover β -gal enzyme assay shows induced level of β -gal enzyme activity after treatment with compound **6g** with respect to untreated control (**Fig. 6 C**). To determine the ligand efficacy against FAS-II pathway some active chalcones and cyclopropyl methanones were screened against the culture of *M. aurum*. Among the screened molecules some compound (**4d**, **6f**) shows moderate inhibition (41-47%) of FAS-II pathway and few of the compounds (**4a**, **6d**, **6g**, **8a**) shows good inhibition in the range of 50-88%. While profiling the activity of synthesized compounds several ligands were identified having good affinity towards the enzymes of FAS-II pathway. In general, the aminoalkylamine chain has prominent role in FAS-II inhibitory activity as evidenced by comparing the effect of compounds **4a**, **4b** and **6g** (**Fig. 6 C**) wherein **4a** and **6g** having *N,N*-dimethylaminopropyl chain have prominent inhibitory effect while **4b** has no significant inhibition. Thus, the double bond or the cyclopropyl moiety has no significant role in eliciting the FAS-inhibitory response. Some of the active compounds which did not respond towards the FAS-II pathway might have some other target and needs further investigation. Moreover, the FAS-II pathway being absent in human may be targeted with such small synthetic molecules to get new chemotherapeutics for tuberculosis and malaria.

Table 5. FAS-II inhibitory activities of selected compounds at two different concentrations.

S. No.	Compound No.	% inhibition (25 μ M)	% inhibition (50 μ M)
1	4a	83.40 \pm 3.0	88.1 \pm 0.077
2	4b	13.61 \pm 0.64	14.65 \pm 0.86
3	4d	47.14 \pm 3.19	42.85 \pm 7.99
4	6a	0	0.17 \pm 0.24
5	6b	0	0
6	6c	0	0
7	6d	39.49 \pm 2.37	60.86 \pm 1.15
8	6e	15.66 \pm 8.52	31.82 \pm 2.12
9	6f	41.52 \pm 5.16	37.55 \pm 10.79
10	6g	46.32 \pm 7.75	57.37 \pm 3.48
11	6h	16.48 \pm 9.34	27.45 \pm 6.04
12	6i	17.5 \pm 24.74	27.45 \pm 6.04
13	6j	29.82 \pm 4.2	3.81 \pm 4.2
14	6k	26.99 \pm 6.7	32.71 \pm 6.9

15	6l	36.0 ±10.17	36.72 ±3.2
16	6m	30.25 ±7.9	23.9 ±4.6
17	8a	48.63 ± 2.94	50.22 ± 2.31
18	8b	17.87 ±2.02	30.1 ±2.57
19	8c	1.03 ±1.46	16.93 ±0.54

Table-5. FAS-II inhibitory activities of selected compounds at two different concentrations

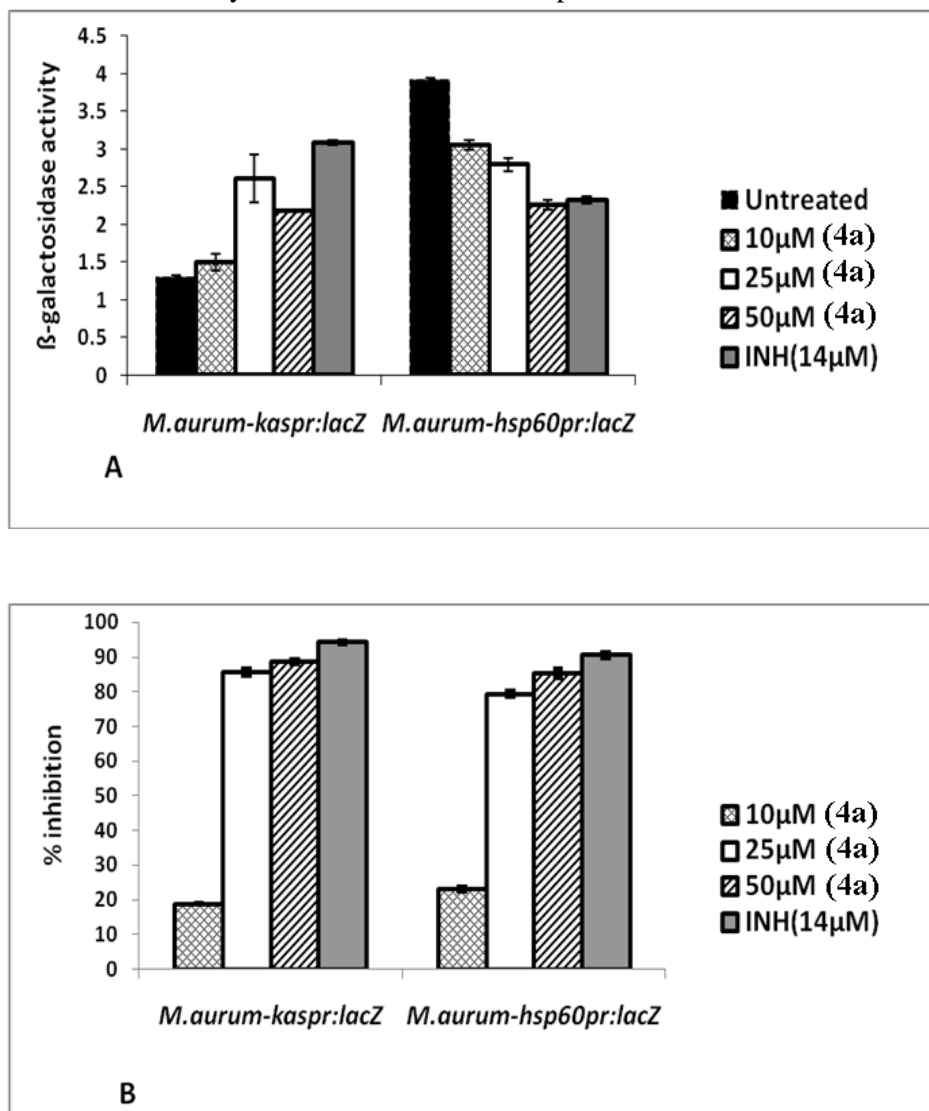


Fig 6. Reporter gene expression and viability assay after treatment with compound **4a**. **(A)** Note the induced level of β -gal activity in treated sample in recombinant strain *M.aurum-kaspr:lacZ* with respect to untreated control. INH as a positive control shows maximum inducibility. Recombinant *M.aurum* strain with hsp60 promoter, *M.aurum-hsp60pr:lacZ*, shows decline in β -gal activity in all treated samples including INH with respect to untreated control. **(B)** Both recombinant strains exhibit similar decline in the viability of bacterial cells; greater inhibition is observed with increasing concentration of compounds. **(C)** Induced level of β -gal activity in treated cyclopropylated analogue of **4a** i. e. **6g** in recombinant strain

M.aurum-kaspr:lacZ with respect to untreated control. Recombinant *M.aurum* strain with hsp60 promoter, *M.aurum-hsp60pr:lacZ*, shows decreased in -gal activity.

3. Conclusion

In conclusion, we have synthesized and evaluated a series of alkylaminoaryl phenyl cyclopropyl chalcones and methanones in good yields. The compounds were evaluated against *M. tuberculosis* and *P. falciparum*. Few of the compounds showed moderate to significant antimalarial and antitubercular activities. We have also shown one of the possible mode of actions of these compounds may be FAS-II inhibition by in silico screen and *in vitro* FAS-II enzyme inhibitory activities. Further exploration of this study is currently underway.

4. Experimental

4.1 Chemistry:

All glasswares were dried in an open flame before use in connection with an inert atmosphere. Solvents were evaporated under reduced pressure. Thin layer chromatography was performed using silica gel 60 F254 plates with detecting agent iodine vapors or by spraying with dragendorff reagent. Silica gel (606120 mesh) was used for column chromatography. Tetramethylsilane (0.0 ppm) was used as an internal standard in ¹H NMR and CDCl₃ (77.0 ppm) was used in ¹³C NMR. The abbreviations used to indicate the peak multiplicity were; s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; Hz, Hertz. FAB MS was recorded on Jeol (Japan)/SX-102. Infrared spectrum was taken with KBr on Perkin-Elmer RX-1. Melting points were determined on a Buchi 535 digital melting point apparatus and were uncorrected. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within ±0.4% of the calculated values.

4.1.1 General procedure for the synthesis of substituted (*E*)-1-phenyl-3-phenylprop-2-en-1-one (**3a**, **3b**):

To a stirring mixture of 4-fluoroacetophenone (8.6 ml, 54.5 mmol) and 4-chlorobenzaldehyde (10 g, 71.13 mmol) or 3,4-dimethoxy benzaldehyde (11.7 g, 70.40 mmol) in ethanol was added KOH (1.98 g, 3.5 mmol) and stirred continued for 10-30 minutes at room temperature till the disappearance of starting materials. The light green/yellow solid was precipitated, filtered washed with water and dried to get the substituted chalcones (**3a** and **3b**) in good yields.

4.1.2 General procedure for the synthesis of substituted (*E*)-3-(4-chlorophenyl)-1-(4-alkylamino)prop-2-en-1-one (**4a-4e**):

A mixture of (*E*)-3-(4-chlorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one (**3a**) (1 g, 3.84 mmol), K₂CO₃ (0.637 g, 4.60 mmol) and desired amine (1.2 eq.) in DMF (5 ml) was stirred magnetically at 100-120 °C for 16-18 hours under inert atmosphere of nitrogen. After the completion of the reaction (TLC), the reaction mixture was cooled to room temperature and poured in water and extracted with ethyl acetate. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product. The latter was purified by column chromatography (SiO₂, 100-200 mesh) using gradient of hexane:ethyl acetate (9:1 6:4)/ 0.2:9.8 0.6:9.4 % methanol:chloroform to give the desired compounds (**4a-4e**, yield 40-56%).

4.1.2.1 (*E*)-3-(4-Chlorophenyl)-1-(4-(3-(dimethylamino) propylamino)phenyl)prop-2-en-1-one (**4a**):

Yellow solid, mp 60-62 °C; Yield 46 %; *R*_f 0.43 (0.2:9.8, MeOH: CHCl₃); IR (KBr) max cm^{-1} : 3426 (N-H), 2928 (C-H), 1605 (C=O). ¹H NMR (200 MHz, CDCl₃): δ 7.92 (2H, d, *J* = 8.84

Hz, ArH), 7.71 (1H, d, $J = 15.8$ Hz, CH=CH), 7.64-7.45 (3H, m, ArH and CH=CH), 7.36 (2H, d, $J = 8.90$ Hz, ArH), 6.56 (2H, d, $J = 8.86$ Hz, ArH), 5.53 (1H, bs, NH), 3.27 (2H, q, $J = 3.12$ Hz NHCH₂), 2.44 (2H, t, $J = 6.24$ Hz, CH₂), 2.24 (6H, s, N(CH₃)₂), 1.81 (2H, m, CH₂). ¹³C NMR (50 MHz, CDCl₃): δ 187.5 (ArCO), 153.1, 141.3 (ArC), 136.1 (CH=CH), 134.4, 131.0, 129.7, (ArCH), 131.0, 128.9, 127.9 (ArCH), 128.7 (ArC), 127.0 (CH=CH), 111.8 (ArCH), 58.4 (NHCH₂), 45.0 ((CH₃)₂NCH₂), 42.9 (NCH₂), 26.3 (CH₂). ESMS (m/z): 343 (M+H)⁺. Analysis Calcd. for C₂₀H₂₃ClN₂O: C, 70.06; H, 6.76; N, 8.17. Found: C, 70.13; H, 6.89; N, 8.26.

4.1.2.2 (E)-3-(4-Chlorophenyl)-1-(4-(4-methylpiperazin-1-yl)phenyl)prop-2-en-1-one (4b):

Yellow solid, mp 84-86 °C; Yield 56 %; R_f 0.40 (0.1:9.9, MeOH:CHCl₃); IR (KBr) \max cm⁻¹: 2938 (C-H), 1610 (C=O); ¹H NMR (200 MHz, CDCl₃): δ 7.92 (2H, d, $J = 8.86$ Hz, ArH), 7.73 (1H, d, $J = 15.54$ Hz, CH=CH), 7.54 (2H, d, $J = 8.76$ Hz, ArH), 7.30-7.16 (3H, m, ArH and CH=CH), 6.85 (2H, d, $J = 8.84$ Hz, ArH), 3.36-3.30 (4H, m, 2xCH₂), 2.58-2.53 (4H, m, 2xCH₂), 2.30 (3H, s, NCH₃). ¹³C NMR (50 MHz, CDCl₃): δ 187.8 (ArCO), 154.6 (ArC), 142.1 (CH=CH), 136.4, 134.2 (ArC), 131.5, 129.8, 129.5 (ArCH), 128.8 (ArC), 122.7 (CH=CH), 113.8 (ArCH), 51.4, 48.8 (2xCH₂), 45.1 (NCH₃). ESMS (m/z): 341 (M+H)⁺. Analysis Calcd. for C₂₀H₂₁ClN₂O: C, 70.48; H, 6.21; N, 8.22. Found: C, 70.38; H, 6.12; N, 8.14.

4.1.2.3 (E)-3-(4-Chlorophenyl)-1-(4-(furan-2-ylmethylamino)phenyl)prop-2-en-1-one (4c):

White solid, mp 82-84 °C; Yield 40 %; R_f 0.37 (0.1:9.9, MeOH:CHCl₃); IR (KBr) \max cm⁻¹: 3445 (N-H), 2932 (C-H), 1600 (C=O). ¹H NMR (200 MHz, CDCl₃): δ 8.03 (2H, d, $J = 8.80$ Hz, ArH), 7.76 (1H, d, $J = 15.60$ Hz, CH=CH), 7.57-7.25 (6H, m, ArH, CH=CH and Ar_F-H), 7.06 (2H, d, $J = 8.86$ Hz, ArH), 6.46-6.37 (2H, m, Ar_F-H), 5.06 (2H, s, CH₂). ¹³C NMR (50 MHz, CDCl₃): δ 188.2 (ArCO), 162.5 (ArC), 149.8(CH=CH), 143.6, 142.8 (ArCH), 136.6, 134.0 (ArC), 131.7 (ArC), 131.1, 129.8, 129.6, 122.6, 115.0, 111.0, 110.8 (ArCH), 62.7 (CH₂). ESMS (m/z): 351 (M+H)⁺. Analysis Calcd. for C₂₁H₁₈ClNO₂: C, 71.69; H, 5.16; N, 3.98. Found: C, 71.58; H, 5.10; N, 3.86.

4.1.2.4 (E)-3-(4-Chlorophenyl)-1-(4-morpholinophenyl)prop-2-en-1-one (4d):

Light green, mp 82-84 °C; Yield 42 %; R_f 0.45 (4:6, EtOAc:Hexane); IR (KBr) \max cm⁻¹: 2932 (C-H), 1608 (C=O); ¹H NMR (200 MHz, CDCl₃): δ 7.99 (2H, d, $J = 9.26$ Hz, ArH), 7.76 (1H, d, $J = 15.62$ Hz, CH=CH), 7.58 (2H, d, $J = 8.72$ Hz, ArH), 7.39-7.25 (3H, m, ArH and CH=CH), 6.91 (2H, d, $J = 9.00$ Hz), 3.88 (4H, t, $J = 4.72$ Hz, 2xCH₂), 3.35 (4H, t, $J = 5.06$ Hz, 2xCH₂). ¹³C NMR (50 MHz, CDCl₃): δ 187.8 (ArCO), 154.6 (ArC), 142.1 (CH=CH), 136.4, 134.2 (ArC), 131.5, 129.8, 129.5 (ArCH), 128.8 (ArC), 122.7 (CH=CH), 113.8 (ArCH), 66.9, 47.9 (2xCH₂). ESMS (m/z): 328 (M+H)⁺. Analysis Calcd. for C₁₉H₁₈ClNO₂: C, 69.62; H, 5.53; N, 4.27. Found: C, 69.56; H, 5.48; N, 4.14.

4.1.2.5 (E)-3-(4-Chlorophenyl)-1-(4-(piperidin-1-yl)phenyl)prop-2-en-1-one (4e):

White solid, mp 116-118 °C; Yield 54 %; R_f 0.55 (10 %, EtOAc:Hexane); IR (KBr) \max cm⁻¹: 2921 (C-H), 1610 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.82 (2H, d, $J = 8.76$ Hz, ArH), 7.74 (1H, d, $J = 15.34$ Hz, CH=CH), 7.24 (2H, d, $J = 8.72$ Hz, ArH), 7.21-7.15 (3H, m, ArH and CH=CH), 6.88 (2H, d, $J = 8.78$ Hz), 3.67 (4H, bs, 2xNCH₂), 1.78 (6H, bs, 3xCH₂). ¹³C NMR (50 MHz, CDCl₃): δ 185.5 (ArCO), 151.6 (ArC), 143.4 (CH=CH), 136.2, 133.2 (ArC), 131.8, 128.8, 128.3 (ArCH), 127.9 (ArC), 122.3 (CH=CH), 113.1 (ArCH), 54.3 (2xCH₂), 23.7 (2x CH₂), 22.8 (CH₂). ESMS (m/z): 326 (M+H)⁺. Analysis Calcd. for C₂₀H₂₀ClNO: C, 73.72; H, 6.19; N, 4.30. Found: C, 73.76; H, 6.09; N, 4.26.

4.1.3 General procedure for the synthesis of substituted aryl phenyl cyclopropyl methanone (5a, 5b) and alkylamino substituted aryl phenyl cyclopropyl methanone (6a, 6g, 6h, 6j, 6n) from 4a-4e:

A mixture of compound **3a** or **3b** (1eq), TMSOI (trimethyl sulphoxonium iodide, 2eq.) and TBAB (tetra butylammonium bromide, 20 mol%) in CH₂Cl₂ (10 ml) was stirred magnetically at ambient temperature for 15 minutes. 50% aq. NaOH (10 ml) solution was subsequently added dropwise and the reaction mixture was refluxed at 80°C, till the disappearance of chalcone (**3a** or **3b**). After the completion of the reaction (TLC), the reaction mixture was cooled to room temperature, excess of dichloromethane was added and the organic layer was extracted. The organic layer was dried (anhyd Na₂SO₄) and concentrated under reduced pressure to get a crude product. The latter was purified by column chromatography (SiO₂, 100-200 mesh) using gradient of hexane:ethylacetate/chloroform:methanol to give the desired compounds (**5a**, **5b** and **6a**, **6g**, **6h**, **6j** and **6n**) in good yields.

General procedure for the synthesis of alkylamino substituted aryl phenyl cyclopropyl methanone (6b-6f, 6i, 6k-6l and 6o-6u) from 5a and 5b:

A mixture of **5a** or **5b** (1 eq.), K₂CO₃ (1.2 eq) and desired amine (1.2 eq.) in DMF (5 ml) was stirred magnetically at 100-120 °C for 16-18 hours under inert atmosphere of nitrogen. After the completion of the reaction (TLC), the reaction mixture was cooled to room temperature and poured in water and extracted with ethyl acetate. The organic layer was dried (anhyd Na₂SO₄) and concentrated under reduced pressure to give a crude product. The latter was purified by column chromatography (SiO₂, 100-200 mesh) using gradient of hexane:ethyl acetate (9:1 6:4)/ 0.2:9.8 0.6:9.4 % methanol:chloroform to give the desired compounds (**6b-6f**, **6i**, **6k-6l** and **6o-6u**) yield 78-86%.

4.1.3.1 (2-(4-chlorophenyl)cyclopropyl)(4-fluorophenyl)methanone (5a).

White solid, mp 118-120 °C; Yield 84 %; *R_f* 0.40 (1.0:9.0, EtOAc:Hexanae); IR (KBr) max cm^{-1} : 2938 (C-H), 1657 (C=O); ¹H NMR (200 MHz, CDCl₃): δ 8.03-7.95 (2H, m, ArH), 7.28-7.23 (2H, m, ArH), 7.18-7.06 (4H, m, ArH), 2.82-2.73 (1H, m, H-2), 2.69-2.60 (1H, m, H-3), 1.95-1.86 (1H, m, H-4a), 1.55-1.45 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 188.7 (ArCO), 165.8, 136.8, 134.5 133.3, (ArC), 131.1, 129.2, 128.4, 115.3 (ArCH), 28.1, 27.3 (C-2 and C-3), 18.2 (C-4). ESMS (m/z): 275 (M+H)⁺. Analysis Calcd. for C₁₆H₁₂ClFO: C, 69.95; H, 4.40; Found: C, 69.97; H, 4.42.

4.1.3.2 (2-(3,4-dimethoxyphenyl)cyclopropyl)(4-fluorophenyl)methanone (5b).

White solid, mp 78-80 °C; Yield 90 %; *R_f* 0.55 (1.5:8.5, EtOAc:Hexanae); IR (KBr) max cm^{-1} : 2934 (C-H), 1652 (C=O); ¹H NMR (200 MHz, CDCl₃): δ 8.04-7.97 (2H, m, ArH), 7.15-7.07 (2H, m, ArH), 6.80 (1H, d, *J* = 8.8 Hz, ArH), 6.69-6.65 (2H, m, ArH), 3.86 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 2.76-2.70 (1H, m, H-2), 2.64-2.60 (1H, m, H-3), 1.91-1.84 (1H, m, H-4a), 1.55-1.48 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 188.9 (ArCO), 165.5, 148.5, 146.7, 136.8, 133.5 (ArC), 131.7, 129.1, 127.2, 126.4, 113.3 (ArCH), 28.3, 27.2 (C-2 and C-3), 18.4 (C-4). ESMS (m/z): 301 (M+H)⁺. Analysis Calcd. for C₁₈H₁₇FO₃: C, 71.99; H, 5.71; Found: C, 72.06; H, 5.74.

4.1.3.3 (2-(4-chlorophenyl)cyclopropyl)(4-(4-methylpiperazin-1-yl)phenyl)methanone (6a).

Pale white solid, mp 98-100 °C; Yield 84 %; *R_f* 0.38 (0.1:9.9, MeOH:CHCl₃); IR (KBr) max cm^{-1} : 2936 (C-H), 1647 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.90 (2H, d, *J* = 8.67 Hz, ArH), 7.27 (2H, d, *J* = 8.28 Hz, ArH), 7.11 (2H, d, *J* = 8.19 Hz, ArH), 6.86 (2H, d, *J* = 8.73 Hz, ArH), 3.38-3.35 (4H, m, 2xCH₂), 2.78-2.72 (1H, m, H-2), 2.62-2.53 (5H, m, 2xCH₂ and H-3), 2.34 (3H, s, NCH₃), 1.89-1.83 (1H, m, H-4a), 1.44-1.38 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.7 (ArCO), 152.5, 139.8, 131.9 (ArC), 130.7, 128.4, 127.2 (ArCH), 126.5 (ArC), 111.3 (ArCH), 51.1, 48.6 (2xCH₂), 45.3 (NCH₃), 28.3, 27.5 (C-2 and C-3), 18.6 (C-4). ESMS (m/z): 355 (M+H)⁺. Analysis Calcd. for C₂₁H₂₃ClN₂O: C, 71.07; H, 6.53; N, 7.89. Found: C, 71.05; H, 6.58; N, 7.83.

4.1.3.4 (2-(4-chlorophenyl)cyclopropyl)(4-(cyclohexylamino)phenyl)methanone (6b).

White solid, mp 128-130 °C; Yield 81 %; R_f 0.50 (15%, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3348 (N-H), 2927 (C-H), 1651 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.83 (2H, d, $J = 8.70$ Hz, ArH), 7.24 (2H, d, $J = 8.31$ Hz, ArH), 7.08 (2H, d, $J = 8.13$ Hz, ArH), 6.52 (2H, d, $J = 8.52$ Hz, ArH), 4.11 (1H, bs, NH), 3.33 (1H, bs, NHCH), 2.74-2.70 (1H, m, H-2), 2.59-2.56 (1H, m, H-3), 2.06-2.02 (2H, m, CH_2), 1.84-1.64 (4H, m, CH_2 , H-4a and H-4b), 1.40-1.16 (6H, m, CH_2). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 195.6 (ArCO), 151.6, 140.1, 132.4 (ArC), 131.0, 128.9, 127.9 (ArCH), 126.8 (ArC), 112.0 (ArCH), 51.6 (NHCH), 33.5 (CH_2) 28.8, 28.3 (C-2 and C-3), 26.1 (CH_2), 25.2 (CH_2), 18.2 (C-4). ESMS (m/z): 354 (M+H)⁺. Analysis Calcd. for $\text{C}_{22}\text{H}_{24}\text{ClNO}$: C, 74.67; H, 6.84; N, 3.96. Found: C, 74.63; H, 6.81 N, 3.94.

4.1.3.5 (2-(4-chlorophenyl)cyclopropyl)(4-(heptylamino)phenyl)methanone (6c).

White solid, mp 134-136 °C; Yield 77 %; R_f 0.50 (1:4, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3353 (N-H), 2924 (C-H), 1569 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.88 (2H, d, $J = 8.61$ Hz, ArH), 7.27 (2H, d, $J = 8.40$ Hz, ArH), 7.11 (2H, d, $J = 8.28$ Hz, ArH), 6.56 (2H, d, $J = 8.61$ Hz, ArH), 4.20 (1H, bs, NH), 3.18 (2H, t, $J = 6.96$ Hz, NHCH_2), 2.79-2.74 (1H, m, H-2), 2.63-2.56 (1H, m, H-3), 1.89-1.83 (1H, m, H-4a), 1.67-1.63 (2H, m, CH_2), 1.44-1.32 (9H, m, 4x CH_2 and H-4b), 0.91 (3H, t, CH_3). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 195.7 (ArCO), 152.6, 140.1, 132.4 (ArC), 131.0, 128.9, 127.9 (ArCH), 127.2 (ArC), 111.7 (ArCH), 43.7, 32.2, 29.7, 29.5 (NHCH_2 and 3x CH_2), 28.8, 28.4 (C-2 and C-3), 27.4, 23.0 (2x CH_2), 18.7 (C-4), 14.5 (CH_3). ESMS (m/z): 370 (M+H)⁺. Analysis Calcd. for $\text{C}_{23}\text{H}_{28}\text{ClNO}$: C, 74.68; H, 7.63; N, 3.79. Found: C, 74.71; H, 7.65; N, 3.76.

4.1.3.6 (4-(butylamino)phenyl)(2-(4-chlorophenyl)cyclopropyl)methanone (6d).

White solid, mp 97-99 °C; Yield 82 %; R_f 0.48 (15%, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3436 (N-H), 3020 (C-H), 1597 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.85 (2H, d, $J = 8.22$ Hz, ArH), 7.26 (2H, d, $J = 7.74$ Hz, ArH), 7.11 (2H, d, $J = 7.83$ Hz, ArH), 6.54 (2H, d, $J = 8.04$ Hz, ArH), 4.11 (1H, bs, NH), 3.21 (2H, d, $J = 8.04$ Hz, NHCH_2), 2.73 (1H, m, H-2), 2.58 (1H, m, H-3), 1.86-1.84 (1H, m, H-4a), 1.68-1.63 (2H, m, CH_2), 1.51-1.39 (3H, m, CH_2 and H-4b), 1.00 (3H, t, $J = 7.11$ Hz, CH_3). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 194.7 (ArCO), 152.1, 139.6, 132.0 (ArC), 130.5, 128.5, 127.5 (ArCH), 126.8 (ArC), 111.2 (ArCH), 42.9 (NHCH_2), 31.4 (CH_2) 28.2, 27.7 (C-2 and C-3), 20.2 (CH_2), 18.2 (C-4), 13.9 (CH_3). ESMS (m/z): 328 (M+H)⁺. Analysis Calcd. for $\text{C}_{20}\text{H}_{22}\text{ClNO}$: C, 73.27; H, 6.76; N, 4.27. Found: C, 73.21; H, 6.73; N, 4.24.

4.1.3.7 (2-(4-chlorophenyl)cyclopropyl)(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)phenyl)methanone (6e).

Light green solid, mp 152-154 °C; Yield 78 %; R_f 0.50 (2 %, MeOH: CHCl_3); IR (KBr) max cm^{-1} : 2936 (C-H), 1593 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.93 (2H, d, $J = 8.70$ Hz, ArH), 7.25 (2H, d, $J = 8.34$ Hz, ArH), 7.17 (2H, d, $J = 7.44$, ArH), 7.09 (2H, d, $J = 8.37$ Hz, ArH), 6.96-6.89 (3H, m, ArH), 3.52-3.49 (4H, m, 2x CH_2), 3.19-3.16 (4H, m, 2x CH_2), 2.80-2.74 (1H, m, H-2), 2.63-2.57 (1H, m, H-3), 1.88-1.82 (1H, m, H-4a), 1.45-1.25 (1H, m, H-4b). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 196.0 (ArCO), 154.5, 151.1, 139.9, 134.7, 132.5 (ArC), 130.5, 129.0 (ArCH), 128.7, 128.2 (ArC), 128.0, 127.9, 125.5, 118.9, 114.2 (ArCH), 51.4, 48.2 (2x CH_2), 28.9, 28.6 (C-2 and C-3), 19.0 (C-4). ESMS (m/z): 485 (M+H)⁺. Analysis Calcd. for $\text{C}_{26}\text{H}_{23}\text{Cl}_3\text{N}_2\text{O}$: C, 64.28; H, 4.77; N, 5.77. Found: C, 64.25; H, 4.79; N, 5.73.

4.1.3.8 (2-(4-chlorophenyl)cyclopropyl)(4-(dibutylamino)phenyl)methanone (6f):

Light green solid, mp 94-96 °C; Yield 77 %; R_f 0.55 (10%, EtOAc:Hexane); IR (KBr) max cm^{-1} : 2959 (C-H), 1594 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.87 (2H, d, $J = 9.00$ Hz, ArH), 7.24 (2H, d, $J = 8.46$ Hz, ArH), 7.08 (2H, d, $J = 8.43$ Hz, ArH), 6.59 (2H, d, $J = 9.03$ Hz, ArH), 3.31 (4H, m, $\text{N}(\text{CH}_2)_2$), 2.77-2.71 (1H, m, H-2), 2.58-2.51 (1H, m, H-3), 1.87-1.81 (1H, m, H-4a), 1.63-1.53 (4H, m, 2x CH_2), 1.41-1.29 (5H, m, 2x CH_2 and H-4b), 0.93 (6H, t, 2x CH_3). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 195.3 (ArCO), 151.6, 140.2, 132.3 (ArC), 130.9, 128.9, 127.9 (ArCH), 125.6 (ArC), 111.0 (ArCH), 51.3 ($\text{N}(\text{CH}_2)_2$), 30.0 (2x CH_2) 28.7, 28.2

(C-2 and C-3), 20.7 (CH₂), 18.4 (C-4), 14.3 (CH₃). ESMS (m/z): 384 (M+H)⁺. Analysis Calcd. for C₂₄H₃₀ClNO: C, 75.08; H, 7.88; N, 3.65. Found: C, 75.01; H, 7.90; N, 3.62.

4.1.3.9 (2-(4-chlorophenyl)cyclopropyl)(4-(3-(dimethylamino)propylamino)phenyl)methanone (6g):

White solid, mp 96-98 °C; Yield 83 %; *R_f* 0.33 (0.1:9.9, MeOH:CHCl₃); IR (KBr) max cm^{-1} : 3432 (N-H), 2928 (C-H), 1690 (C=O). ¹H NMR (300 MHz, CDCl₃): δ 7.83 (2H, d, *J* = 8.58 Hz, ArH), 7.23 (2H, d, *J* = 8.34 Hz, ArH), 7.07 (2H, d, *J* = 8.28 Hz, ArH), 6.51 (2H, d, *J* = 8.58 Hz, ArH), 5.39 (1H, s, NH), 3.25 (2H, bs, NHCH₂), 2.76-2.71 (1H, m, H-2), 2.60-2.53 (1H, m, H-3), 2.42 (2H, t, *J* = 6.24 Hz, NCH₂), 2.25 (6H, s, N(CH₃)₂), 1.85-1.76 (3H, m, CH₂ and H-4a), 1.40-1.34 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.6 (ArCO), 153.0, 140.1, 132.3 (ArC), 131.0, 128.9, 127.9 (ArCH), 126.8 (ArC), 111.7 (ArCH), 58.8 (NHCH₂), 45.7 ((CH₃)₂NCH₂), 43.0 (NCH₂), 28.7, 28.2 (C-2 and C-3), 26.4 (CH₂), 18.7 (C-4). ESMS (m/z): 357 (M+H)⁺. Analysis Calcd. for C₂₁H₂₅ClN₂O: C, 70.67; H, 7.06; N, 7.85. Found: C, 70.59; H, 7.02; N, 7.81.

4.1.3.10 (2-(4-chlorophenyl)cyclopropyl)(4-(furan-2-ylmethylamino)phenyl)methanone (6h):

White solid, mp 96-98 °C; Yield 85 %; *R_f* 0.55 (20%, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3445 (N-H), 2928 (C-H), 1600 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.96 (2H, d, *J* = 8.88 Hz, ArH), 7.43 (1H, m, Furyl-H), 7.25 (2H, d, *J* = 8.46 Hz, ArH), 7.07 (2H, d, *J* = 8.46 Hz, ArH), 6.99 (2H, d, *J* = 8.91 Hz, ArH), 6.42 (1H, m, Furyl-H), 6.36 (1H, m, Furyl-H) 5.04 (2H, s, NHCH₂), 2.80-2.74 (1H, m, H-2), 2.64-2.57 (1H, m, H-3), 1.89-1.83 (1H, m, H-4a), 1.47-1.41 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.7 (ArCO), 151.8, 151.7, 142.5, 140.0, 132.4 (ArC), 130.9, 128.9, 128.0, 127.9, 112.2, 110.8, 107.8 (ArCH), 41.1 (CH₂), 28.8, 28.4 (C-2 and C-3), 18.7 (C-4). ESMS (m/z): 352 (M+H)⁺. Analysis Calcd. for C₂₁H₁₈ClNO₂: C, 71.69; H, 5.16; N, 3.98. Found: C, 71.63; H, 5.13; N, 3.94.

4.1.3.11 (2-(4-chlorophenyl)cyclopropyl)(4-(6-methylheptan-2-ylamino)phenyl)methanone (6i):

White solid, mp 128-130 °C; Yield 80 %; *R_f* 0.30 (5 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3422 (N-H), 2926 (C-H), 1596 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.82 (2H, d, *J* = 8.70 Hz, ArH), 7.24 (2H, d, *J* = 8.37 Hz, ArH), 7.08 (2H, d, *J* = 8.37 Hz, ArH), 6.49 (2H, d, *J* = 8.67 Hz, ArH), 3.95 (1H, bs, NH), 3.53-3.51 (1H, m, NHCH), 2.73-2.67 (1H, m, H-2), 2.57-2.51 (1H, m, H-3), 1.85-1.79 (1H, m, H-4a), 1.56-1.21 (12H, m, 3xCH₂, CH₃, 2xCH and H-4b), 0.87 (6H, d, *J* = 6.6 Hz (CH₃)₂). ¹³C NMR (50 MHz, CDCl₃): δ 195.0 (ArCO), 151.6, 140.1, 132.4 (ArC), 131.0, 128.9, 127.9 (ArCH), 127.1 (ArC), 112.1 (ArCH), 48.7 (CH), 39.3, 37.7 (2xCH₂), 28.7 (CH), 28.3, 28.2 (C-2 and C-3), 24.3, (CH₂), 23.1, 21.1 (CH₃), 18.5 (CH₂). ESMS (m/z): 384 (M+H)⁺. Analysis Calcd. for C₂₄H₃₀ClNO: C, 75.08; H, 7.88; N, 3.65. Found: C, 74.98; H, 7.84; N, 3.61.

4.1.3.12 (2-(4-chlorophenyl)cyclopropyl)(4-morpholinophenyl)methanone (6j):

Light green solid, mp 115-117 °C; Yield 83 %; *R_f* 0.50 (2 %, MeOH:CHCl₃); IR (KBr) max cm^{-1} : 3019 (C-H), 1649 (C=O). ¹H NMR (300 MHz, CDCl₃): δ 7.89 (2H, d, *J* = 9.09 Hz, ArH), 7.24 (2H, d, *J* = 8.46 Hz, ArH), 7.08 (2H, d, *J* = 8.40 Hz, ArH), 6.84 (2H, d, *J* = 8.91 Hz, ArH), 3.83-3.80 (4H, m, 2xCH₂), 3.28-3.25 (m, 4H, 2xCH₂), 2.76-2.70 (1H, m, H-2), 2.60-2.54 (1H, m, H-3), 1.88-1.73 (1H, m, H-4a), 1.42-1.36 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.7 (ArCO), 153.3, 140.1, 132.4 (ArC), 130.4, 129.0, 127.9 (ArCH), 126.9 (ArC), 113.8 (ArCH), 66.7 (2xCH₂), 48.1 (2xCH₂), 28.8, 28.5 (C-2 and C-3), 18.8 (C-4). ESMS (m/z): 342 (M+H)⁺. Analysis Calcd. for C₂₀H₂₀ClNO₂: C, 70.27; H, 5.90; N, 4.10. Found: C, 70.25; H, 5.89; N, 4.07.

4.1.3.13 (2-(4-chlorophenyl)cyclopropyl)(4-(dodecylamino)phenyl)methanone (6k):

White solid, mp 122-124 °C; Yield 85 %; *R_f* 0.50 (3 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3355 (N-H), 2921 (C-H), 1639 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.82 (2H, d, *J* = 8.61

Hz, ArH), 7.23 (2H, d, $J = 8.34$ Hz, ArH), 7.07 (2H, d, $J = 8.31$ Hz, ArH), 6.50 (2H, d, $J = 8.61$ Hz, ArH), 4.13 (1H, bs, NH), 3.15 (2H, t, $J = 6.87$ Hz, NHCH_2), 2.77-2.68 (1H, m, H-2), 2.61-2.52 (1H, m, H-3), 1.84-1.78 (1H, m, H-4a), 1.67-1.57 (2H, m, CH_2), 1.38-1.25 (19H, m, $9 \times \text{CH}_2$ and H-4b), 0.88 (3H, t, $J = 6.85$ Hz, CH_3). ^{13}C NMR (50 MHz, CDCl_3): δ 195.2 (ArCO), 152.4, 140.1, 132.4 (ArC), 130.9, 128.9, 127.9 (ArCH), 127.3 (ArC), 111.8 (ArCH), 43.8, 32.3, 32.2, 30.0, 30.0, 29.8, 29.7 (CH_2), 28.7, 28.2 (C-2 and C-3), 27.5, 27.4, 23.1, 18.6 (CH_2), 18.5 (C-4), 14.6 (CH_3). ESMS (m/z): 440 ($\text{M}+\text{H}$)⁺. Analysis Calcd. for $\text{C}_{28}\text{H}_{38}\text{ClNO}$: C, 76.42; H, 8.70; N, 3.18. Found: C, 76.38; H, 8.68; N, 3.14.

4.1.3.14 (2-(4-chlorophenyl)cyclopropyl)(4-(hexadecylamino)phenyl)methanone (6l):

White solid, mp 154-156 °C; Yield 87 %; R_f 0.55 (2 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3459 (N-H), 2890 (C-H), 1693 (C=O); ^1H NMR (300 MHz, CDCl_3): δ 7.82 (2H, d, $J = 8.64$ Hz, ArH), 7.23 (2H, d, $J = 8.34$ Hz, ArH), 7.08 (2H, d, $J = 8.37$ Hz, ArH), 6.50 (2H, d, $J = 8.67$ Hz, ArH), 4.13 (1H, bs, NH), 3.15 (2H, t, $J = 6.96$ Hz, NHCH_2), 2.71-2.7 (1H, m, H-2), 2.56-2.53 (1H, m, H-3), 1.83-1.80 (1H, m, H-4a), 1.64-1.60 (2H, m, CH_2), 1.38-1.25 (27H, m, $13 \times \text{CH}_2$ and H-4b), 0.90-0.86 (3H, m, CH_3). ^{13}C NMR (50 MHz, CDCl_3): δ 195.0 (ArCO), 152.3, 140.1, 132.4 (ArC), 130.9, 128.9, 127.9 (ArCH), 127.4 (ArC), 111.7 (ArCH), 43.8, 32.3, 30.0, 30.0, 29.7, (CH₂), 28.6, 28.1 (C-2 and C-3), 27.5, 23.1, (CH₂), 18.5 (C-4), 14.5 (CH_3). ESMS (m/z): 497 ($\text{M}+\text{H}$)⁺. Analysis Calcd. for $\text{C}_{32}\text{H}_{46}\text{ClNO}$: C, 77.46; H, 9.34; N, 2.82. Found: C, 77.40; H, 9.29; N, 2.79.

4.1.3.15 (2-(4-chlorophenyl)cyclopropyl)(4-(octylamino)phenyl)methanone (6m):

White solid, mp 134-136 °C; Yield 83 %; R_f 0.50 (5 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3354 (N-H), 2922 (C-H), 1588 (C=O); ^1H NMR (300 MHz, CDCl_3): δ 7.82 (2H, d, $J = 8.67$ Hz, ArH), 7.24 (2H, d, $J = 8.40$ Hz, ArH), 7.07 (2H, d, $J = 8.40$ Hz, ArH), 6.50 (2H, d, $J = 8.67$ Hz, ArH), 4.16 (1H, bs, NH), 3.15 (2H, t, $J = 7.02$ Hz, NHCH_2), 2.73-2.67 (1H, m, H-2), 2.57-2.51 (1H, m, H-3), 1.84-1.78 (1H, m, H-4a), 1.64-1.57 (2H, m, CH_2), 1.38-1.28 (11H, m, $5 \times \text{CH}_2$ and H-4b), 0.95-0.86 (3H, t, CH_3). ^{13}C NMR (50 MHz, CDCl_3): δ 195.0 (ArCO), 152.4, 140.1, 132.4 (ArC), 130.9, 128.9, 127.9 (ArCH), 127.3 (ArC), 111.7 (ArCH), 43.7, 32.2, 29.8, 29.6 (NHCH_2 and $3 \times \text{CH}_2$), 28.6, 28.1 (C-2 and C-3), 27.5, 23.0 ($2 \times \text{CH}_2$), 18.5 (C-4), 14.5 (CH_3). ESMS (m/z): 384 ($\text{M}+\text{H}$)⁺. Analysis Calcd. for $\text{C}_{24}\text{H}_{30}\text{ClNO}$: C, 75.08; H, 7.88; N, 3.65. Found: C, 75.06; H, 7.86; N, 3.67.

4.1.3.16 (2-(4-chlorophenyl)cyclopropyl)(4-(piperidin-1-yl)phenyl)methanone (6n):

Pale white solid, mp 134-136 °C; Yield 85 %; R_f 0.50 (5 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 2921 (C-H), 1596 (C=O); ^1H NMR (300 MHz, CDCl_3): δ 7.78 (2H, d, $J = 8.76$ Hz, ArH), 7.16 (2H, d, $J = 8.28$ Hz, ArH), 7.01 (2H, d, $J = 8.28$ Hz, ArH), 6.74 (2H, d, $J = 8.79$ Hz, ArH), 3.26 (4H, bs, $2 \times \text{NCH}_2$), 2.68-2.62 (1H, m, H-2), 2.51-2.45 (1H, m, H-3), 1.78-1.72 (1H, m, H-4a), 1.58 (6H, bs, $3 \times \text{CH}_2$), 1.32-1.26 (1H, m, H-4b). ^{13}C NMR (50 MHz, CDCl_3): δ 195.5 (ArCO), 152.4, 140.0, 132.1 (ArC), 130.6, 128.8, 127.3 (ArCH), 127.1 (ArC), 111.5 (ArCH), 51.2 ($2 \times \text{NCH}_2$), 28.6, 28.4 (C-2 and C-3), 23.8 ($2 \times \text{CH}_2$), 23.3 (CH_2), 18.7 (C-4). ESMS (m/z): 341 ($\text{M}+\text{H}$)⁺. Analysis Calcd. for $\text{C}_{21}\text{H}_{22}\text{ClNO}$: C, 74.21; H, 6.52; N, 4.12. Found: C, 74.17; H, 6.49; N, 4.14.

4.1.3.17 (2-(4-chlorophenyl)cyclopropyl)(4-(dimethylamino)phenyl)methanone (6o):

White solid, mp 92-94 °C; Yield 80 %; R_f 0.45 (15 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3020 (C-H), 1596 (C=O). ^1H NMR (300 MHz, CDCl_3): δ 7.87 (2H, d, $J = 8.97$ Hz, ArH), 7.24 (2H, d, $J = 8.43$ Hz, ArH), 7.08 (2H, d, $J = 8.37$ Hz, ArH), 6.63 (2H, d, $J = 8.97$ Hz, ArH), 3.05 (6H, s, NHCH_2), 2.76-2.70 (1H, m, H-2), 2.58-2.52 (1H, m, H-3), 1.85-1.79 (1H, m, H-4a), 1.39-1.32 (1H, m, H-4b). ^{13}C NMR (50 MHz, CDCl_3): δ 195.6 (ArCO), 150.8, 139.5, 132.6 (ArC), 130.6 (ArCH), 129.8 (ArC), 129.0, 127.8, 115.5 (ArCH), 43.0 ($\text{N}(\text{CH}_3)_2$), 29.5, 28.9 (C-2 and C-3), 19.5 (C-4). ESMS (m/z): 300 ($\text{M}+\text{H}$)⁺. Analysis Calcd. for $\text{C}_{18}\text{H}_{18}\text{ClNO}$: C, 72.11; H, 6.05; N, 4.67. Found: C, 72.08; H, 6.01; N, 4.65.

4.1.3.18 (2-(4-chlorophenyl)cyclopropyl)(4-(pyrrolidin-1-yl)phenyl)methanone (6p):

White solid, mp 134-136 °C; Yield 81 %; R_f 0.40 (5 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 2960 (C-H), 1607 (C=O); $^1\text{H NMR}$ (200 MHz, CDCl_3): δ 7.91 (2H, d, $J = 8.90$ Hz, ArH), 7.27 (2H, d, $J = 8.48$ Hz, ArH), 7.11 (2H, d, $J = 8.48$ Hz, ArH), 6.52 (2H, d, $J = 8.94$ Hz, ArH), 3.39-3.33 (4H, m, $2 \times \text{NCH}_2$), 2.79-2.73 (1H, m, H-2), 2.63-2.50 (1H, m, H-3), 2.10-1.98 (4H, m, $2 \times \text{CH}_2$), 1.89-1.80 (1H, m, H-4a), 1.43-1.39 (1H, m, H-4b). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 195.7 (ArCO), 151.1, 140.2, 132.3 (ArC), 130.8, 128.9, 127.9 (ArCH), 125.9 (ArC), 111.5 (ArCH), 48.2 ($2 \times \text{NCH}_2$), 28.8, 28.4 (C-2 and C-3), 25.8 ($2 \times \text{CH}_2$), 18.7 (C-4). ESMS (m/z): 326 (M+H) $^+$. Analysis Calcd. for $\text{C}_{20}\text{H}_{20}\text{ClNO}$: C, 73.72; H, 6.19; N, 4.30. Found: C, 73.68; H, 6.17; N, 4.32.

4.1.3.19 (Z)-(2-(4-chlorophenyl)cyclopropyl)(4-(octadec-9-enylamino)phenyl)methanone (6q):

White solid, mp 137-139 °C; Yield 80 %; R_f 0.50 (10 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3353 (N-H), 2923 (C-H), 1590 (C=O); $^1\text{H NMR}$ (200 MHz, CDCl_3): δ 7.86 (2H, d, $J = 8.72$ Hz, ArH), 7.26 (2H, d, $J = 8.32$ Hz, ArH), 7.10 (2H, d, $J = 8.48$ Hz, ArH), 6.54 (2H, d, $J = 8.74$ Hz, ArH), 5.35-5.32 (2H, m, CH=CH), 4.20 (1H, bs, NH), 3.18-3.15 (2H, m, NHCH_2), 2.81-2.69 (1H, m, H-2), 2.63-2.51 (1H, m, H-3), 2.10-1.89 (3H, m, 1H, H-4a + CH_2), 1.91-1.78 (1H, m, H-4b), 1.73-1.56 (2H, m, CH_2), 1.47-1.21 (24H, m, $12 \times \text{CH}_2$), 0.92 (3H, t, $J = 6.64$ Hz, CH_3). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 195.6 (ArCO), 152.6, 140.1, 132.4 (ArC), 131.0 (ArCH), 130.4, 130.0 (CH=CH), 128.9, 127.9 (ArCH), 127.1 (ArC), 111.7 (ArCH), 43.7, 33.04, 32.3, 30.1, 30.0, 29.7, 29.6 (NHCH_2 and $6 \times \text{CH}_2$), 28.7, 28.3 (C-2 and C-3), 27.6, 27.6, 27.5, 23.1 ($4 \times \text{CH}_2$), 18.7 (C-4), 14.5 (CH_3). ESMS (m/z): 522 (M+H) $^+$. Analysis Calcd. for $\text{C}_{34}\text{H}_{48}\text{ClNO}$: C, 78.20; H, 9.26; N, 2.68. Found: C, 78.11; H, 9.21; N, 2.65.

4.1.3.20 (2-(4-chlorophenyl)cyclopropyl)(4-(3,4-dimethoxybenzylamino)phenyl)methanone (6r):

White solid, mp 135-137 °C; Yield 73 %; R_f 0.50 (20 %, EtOAc:Hexane); IR (KBr) max cm^{-1} : 3350 (N-H), 2938 (C-H), 1587 (C=O); $^1\text{H NMR}$ (200 MHz, CDCl_3): δ 7.74 (2H, d, $J = 8.74$ Hz, ArH), 7.26-7.19 (3H, m, ArH), 7.10 (2H, d, $J = 8.48$ Hz, ArH), 6.89-6.79 (2H, m, ArH), 6.61 (2H, d, $J = 8.72$ Hz, ArH), 4.46 (1H, bs, NH), 4.32 (2H, s, NHCH_2), 3.87 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 2.76-2.70 (1H, m, H-2), 2.63-2.55 (1H, m, H-3), 1.89-1.80 (1H, m, H-4a), 1.42-1.34 (1H, m, H-4b). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 195.7 (ArCO), 152.6, 148.6, 148.1, 140.1, 133.1, 132.4 (ArC), 130.9, 128.9, 127.9 (ArCH), 127.2 (ArC), 120.0, 112.1, 111.7, 111.0 (ArCH), 56.2 ($2 \times \text{OCH}_3$), 48.0 (NHCH_2), 28.8, 28.4 (C-2 and C-3), 18.7 (C-4). ESMS (m/z): 422 (M+H) $^+$. Analysis Calcd. for $\text{C}_{25}\text{H}_{24}\text{ClNO}_3$: C, 71.17; H, 5.73; N, 3.32. Found: C, 71.11; H, 5.70; N, 3.28.

4.1.3.21 (2-(4-chlorophenyl)cyclopropyl)(4-(2-hydroxyethylamino)phenyl)methanone (6s):

White solid, mp 144-146 °C; Yield 75 %; R_f 0.50 (5 %, MeOH: CHCl_3); IR (KBr) max cm^{-1} : 3419 (O-H), 3330 (N-H), 2930 (C-H), 1588 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 7.83 (2H, d, $J = 8.58$ Hz, ArH), 7.25 (2H, d, $J = 8.46$ Hz, ArH), 7.09 (2H, d, $J = 8.28$ Hz, ArH), 6.59 (2H, d, $J = 8.43$ Hz, ArH), 3.87-3.71 (2H, m, CH_2), 3.46 (1H, bs, NH), 3.33-3.32 (2H, m, CH_2), 2.79-2.74 (1H, m, H-2), 2.63-2.56 (1H, m, H-3), 1.89-1.83 (1H, m, H-4a), 1.46-1.36 (1H, m, H-4b). $^{13}\text{C NMR}$ (50 MHz, CDCl_3): δ 195.9 (ArCO), 152.6, 140.1, 132.4 (ArC), 131.0, 128.9, 127.9 (ArCH), 127.2 (ArC), 111.7 (ArCH), 58.7 (CH_2OH), 43.3 (NHCH_2), 28.8, 28.4 (C-2 and C-3), 18.7 (C-4). ESMS (m/z): 316 (M+H) $^+$. Analysis Calcd. for $\text{C}_{18}\text{H}_{18}\text{ClNO}_2$: C, 68.46; H, 5.75; N, 4.44. Found: C, 68.39; H, 5.73; N, 4.41.

4.1.3.22 (2-(3,4-dimethoxyphenyl)cyclopropyl)(4-(4-methylpiperazin-1-yl)phenyl)methanone (6t):

White solid, mp 112-114 °C; Yield 81 %; R_f 0.30 (0.1:9.9, MeOH: CHCl_3); IR (KBr) max cm^{-1} : 2939 (C-H), 1651 (C=O); $^1\text{H NMR}$ (200 MHz, CDCl_3): δ 7.89 (2H, d, 8.70, $J = 8.67$ Hz, ArH), 6.87-6.69 (5H, m, ArH), 3.83 (6H, s, DiOMe), 3.36 (4H, bs, $2 \times \text{CH}_2$), 2.57-2.32 (9H,

m, NCH₃, 2xCH₂ and H-2, H-3), 1.80-1.76 (1H, m, H-4a), 1.46-1.40 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.5 (ArCO), 152.3, 144.7, 139.9, 131.7 (ArC), 128.1, 127.5 (ArCH), 126.7 (ArC), 111.8 (ArCH), 56.3, 56.9 (2xOMe), 50.5, 48.1 (2xCH₂), 45.7 (NCH₃), 28.3, 27.6 (C-2 and C-3), 18.3 (C-4). ESMS (m/z): 381 (M+H)⁺. Analysis Calcd. for C₂₃H₂₈N₂O₃: C, 72.60; H, 7.42; N, 7.36. Found: C, 72.57; H, 7.39; N, 7.33.

4.1.3.23 (2-(3,4-dimethoxyphenyl)cyclopropyl)(4-(heptylamino)phenyl)methanone (6u):

White solid, mp 144-146 °C; Yield 79 %; *R_f* 0.40 (1:4, EtOAc:Hexane); IR (KBr) _{max} cm⁻¹: 3357 (N-H), 2921 (C-H), 1556 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.88 (2H, d, *J* = 8.70 Hz, ArH), 6.79-6.66 (3H, m, ArH), 6.54 (2H, d, *J* = 8.73 Hz, ArH), 4.27 (1H, bs, NH), 3.86 (6H, s, DiOMe), 3.17 (2H, m, NHCH₂), 2.74-2.71 (1H, m, H-2), 2.57-2.52 (1H, m, H-3), 1.82-1.79 (1H, m, H-4a), 1.65-1.60 (2H, m, CH₂), 1.43-1.30 (9H, m, 4xCH₂ and H-4b), 0.92 (3H, t, *J* = 6.27, Hz CH₃). ¹³C NMR (50 MHz, CDCl₃): δ 195.5 (ArCO), 152.3, 146.3, 140.3, 132.7 (ArC), 128.3, 127.5 (ArCH), 127.1 (ArC), 111.9 (ArCH), 56.4, 56.5 (2xOMe), 43.5, 32.0, 29.6, 29.3 (NHCH₂ and 3xCH₂), 28.7, 28.3 (C-2 and C-3), 27.1, 23.2 (2xCH₂), 18.6 (C-4), 14.4 (CH₃). ESMS (m/z): 396 (M+H)⁺. Analysis Calcd. for C₂₅H₃₃NO₃: C, 75.91; H, 8.41; N, 3.54. Found: C, 75.36; H, 8.38; N, 3.57.

4.1.4 General procedure for the synthesis of 2-(azolyl)-ethylaminoaryl phenyl cyclopropyl methanones (8a-8c):

A mixture of 2-(4-chlorophenyl)cyclopropyl-4-(2-hydroxyethylamino)phenyl methanone (6s) (5.0 gm, 15.87 mmol), TEA (triethylamine, 2.65 ml, 19.0 mmol) in CH₂Cl₂ (20 ml) was stirred magnetically at 0 °C for 15-20 minutes. A solution of methanesulphonyl chloride (3.17 ml, 31.7 mmol in 5 ml CH₂Cl₂) was subsequently added dropwise and the reaction mixture was stirred at 0 °C, for 4 hours. After completion of the reaction (TLC), the reaction mixture was diluted with excess of dichloromethane and extracted with water. The organic layer was separated and dried (anhyd Na₂SO₄) and concentrated under reduced pressure to get the intermediate 2-(4-(2-(4-chlorophenyl)cyclopropanecarbonyl)phenylamino)ethyl methanesulfonate (7) (4.80 g) and it was used as such without further purification in the subsequent reaction with azoles.

To stirring slurry of NaH (0.11 g, 4.58 mmol) in DMF (4 mL) at 0 °C, the desired azole (1.2 eq) was added and the reaction mixture was stirred at 0 °C for 15-20 minutes. The above obtained 2-(4-(2-(4-chlorophenyl)cyclopropanecarbonyl) phenylamino) ethyl methanesulfonate (7) was added and stirring continued at 100-120 °C till the disappearance of starting material (TLC). The reaction mixture was cooled to room temperature, quenched with ethyl acetate and extracted with water. The organic layer was dried (anhyd Na₂SO₄) and concentrated under reduced pressure to give a crude mass which was purified by column chromatography (SiO₂, 100-200 mesh) using gradient of hexane:ethyl acetate (9:1 6:4)/0.2:9.8 0.6:9.4 % methanol:chloroform to give the desired compound (8a-8c) in varying yields.

4.1.4.1 (4-(2-(1H-imidazol-1-yl)ethylamino)phenyl)(2-(4-chlorophenyl)cyclopropyl)methanone (8a):

White solid, mp 110-112 °C; Yield 67 %; *R_f* 0.40 (5 %, MeOH:CHCl₃); IR (KBr) _{max} cm⁻¹: 3334 (N-H), 2930 (C-H), 1588 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.84 (2H, d, *J* = 8.82 Hz, ArH), 7.50 (1H, s, imidazole-H), 7.27 (2H, d, *J* = 8.46 Hz, ArH), 7.11 (2H, d, *J* = 8.46 Hz, ArH), 6.97 (2H, s, imidazole-H), 6.57 (2H, d, *J* = 8.79 Hz, ArH), 4.19 (2H, t, *J* = 5.61 Hz, CH₂), 3.58 (2H, t, *J* = 5.67 Hz, CH₂), 3.35 (1H, m, NH), 2.81-2.75 (1H, m, H-2), 2.59-2.53 (1H, m, H-3), 1.85-1.79 (1H, m, H-4a), 1.48-1.42 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.7 (ArCO), 152.6, 140.1 (ArC), 138.1 (imidazole-CH), 132.4 (ArC), 131.0, (ArCH), 128.5 (imidazole-CH), 128.9, 127.9 (ArCH), 127.2 (ArC), 121.2 (imidazole-CH), 111.7 (ArCH), 46.3 (N_{imid}CH₂), 43.3 (NHCH₂), 28.8, 28.4 (C-2 and C-3), 18.7 (C-4). ESMS

(m/z): 366 (M+H)⁺. Analysis Calcd. for C₂₁H₂₀ClN₃O: C, 68.94; H, 5.51; N, 11.49. Found: C, 68.87; H, 5.49; N, 11.53.

4.1.4.2 (4-(2-(1H-1,2,4-triazol-1-yl)ethylamino)phenyl)(2-(4-chlorophenyl)cyclopropyl) methanone (8b):

White solid, mp 117-119 °C; Yield 68 %; R_f 0.45 (2 %, MeOH:CHCl₃); IR (KBr) _{max} cm⁻¹: 3331 (N-H), 2934 (C-H), 1593 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 8.17 (1H, s, triazole-H), 7.92 (1H, s, triazole-H), 7.81 (2H, d, J = 8.79 Hz, ArH), 7.25 (2H, d, J = 8.43 Hz, ArH), 7.10 (2H, d, J = 8.43 Hz, ArH), 6.56 (2H, d, J = 8.79 Hz, ArH), 4.40 (2H, t, J = 5.64 Hz, CH₂), 3.58 (2H, t, J = 5.88 Hz, CH₂), 3.34 (1H, m, NH), 2.79-2.74 (1H, m, H-2), 2.57-2.51 (1H, m, H-3), 1.83-1.78 (1H, m, H-4a), 1.46-1.39 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.3 (ArCO), 152.6 (ArC), 150.9, 144.2 (triazole CH), 140.1, 132.4 (ArC), 131.0, 128.9, 127.9 (ArCH), 127.2 (ArC), 111.7 (ArCH), 48.7 (N_{Triazole}CH₂), 43.3 (NHCH₂), 28.8, 28.4 (C-2 and C-3), 18.7 (C-4). ESMS (m/z): 367 (M+H)⁺. Analysis Calcd. for C₂₀H₁₉ClN₄O: C, 65.48; H, 5.22; N, 15.27. Found: C, 65.54; H, 5.20; N, 15.30.

4.1.4.3 (4-(2-(1H-benzo[d]imidazol-1-yl)ethylamino)phenyl)(2-(4-chlorophenyl)cyclopropyl) methanone (8c):

White solid, mp 134-136 °C; Yield 70 %; R_f 0.45 (3 %, MeOH:CHCl₃); IR (KBr) _{max} cm⁻¹: 3337 (N-H), 2932 (C-H), 1585 (C=O); ¹H NMR (300 MHz, CDCl₃): δ 7.89-7.86 (3H, m, ArH), 7.80-7.65 (2H, m, ArH), 7.56-7.50 (1H, m, ArH), 7.36-7.25 (4H, m, ArH), 7.12 (2H, d, J = 8.43 Hz, ArH), 6.58 (2H, d, J = 8.79 Hz, ArH), 4.43 (2H, t, J = 5.31 Hz, CH₂), 3.71 (2H, t, J = 5.70 Hz, CH₂), 3.39 (1H, m, NH), 2.86-2.80 (1H, m, H-2), 2.59-2.53 (1H, m, H-3), 1.85-1.79 (1H, m, H-4a), 1.48-1.42 (1H, m, H-4b). ¹³C NMR (50 MHz, CDCl₃): δ 195.6 (ArCO), 152.3 (ArC), 144.4 (Ar_{Bz}C), 143.6 (Benzimidazole CH), 140.5 (ArC), 134.1 (Ar_{Bz}C), 132.2 (ArC), 131.2, 128.7, 127.5 (ArCH), 127.1 (ArC), 123.6, 123.2, 118.7, 109.9 (Ar_{Bz}CH), 111.4 (ArCH), 48.7 (N_{Benzimid}CH₂), 43.3 (NHCH₂), 28.8, 28.4 (C-2 and C-3), 18.7 (C-4). ESMS (m/z): 416 (M+H)⁺. Analysis Calcd. for C₂₅H₂₂ClN₃O: C, 72.19; H, 5.33; N, 10.10. Found: C, 72.23; H, 5.36; N, 10.13.

4.2. Biology (Assay methods)

4.2.1 Antimycobacterial assay:

Determination of antitubercular activity against *M. tuberculosis* H₃₇Rv strain (Agar microdilution method): Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H₃₇Rv was done by agar microdilution method⁵⁷. The MIC of the test compounds was determined by incorporating two fold dilution of this suspension were added to (in tubes) 7H10 middle brook medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentration of the test compounds keeping the volume constant i.e. 0.1 mL. Medium was allowed to cool keeping the tubes in slanting position. A culture of *M. tuberculosis* H₃₇Rv growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 µg/mL concentration of extracts/compounds was prepared in dimethyl sulphoxide (DMSO). These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H₃₇Rv (5 × 10⁵ bacilli per tube). The tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H₃₇Rv. The lowest concentration of the compound at which complete inhibition of colonies occurred was taken as minimum inhibitory concentration (MIC) of test compound.

4.2.2.1 Antimalarial assay:

The antimalarial assay was performed *in vitro* against chloroquine sensitive *Plasmodium falciparum* 3D7 strains reported previously⁵⁸. Culture was maintained *in-vitro* as described by Trager and Jansen⁶⁰ with some modifications L asynchronous culture of *P. falciparum*

3D7 was added. The parasitaemia was maintained to ~0.5% and hematocrit was adjusted to 1.5%. Plates were incubated in CO₂ incubator maintained at 37°C for 24 hours. Radiolabelled hypoxanthine solution containing 0.5 Ci/well was added. These plates were further incubated for another 48 hrs. Cells were harvested on Whatmann filter papers, transferred in scintillation vials, dried overnight. Scintillation cocktail was added in these vials after 24 hours, radioactivity was counted under scintillation counter. IC₅₀ values of tested compounds were calculated on the basis of radiolabelled hypoxanthine uptake by the parasites.

4.2.2.2 Antimalarial assay using Minimum Inhibitory Concentration (MIC):

MICs of compounds were determined as described by Rieckmann *et al* (1978)⁶¹ with some modifications. Briefly, stock solution of the compounds were prepared at 10mg/ml in DMSO and stored at 0°C until use. The test was performed in 96 well microtiter plates, two fold serial drug dilutions were prepared in complete RPMI 1640 and 50 μ L of each dilution was used in each well. Parasitized RBCs (50 μ L) were added to each well. The final culture suspension had a hematocrit of 364% with 1.062.0% infection (>95% rings). Micro culture plates were incubated for 24-32 hours at 37°C in an incubator supplied with 5% CO₂ to allow the development of malaria parasites, culture plates were taken out and maximum supernatant medium was removed, thin blood smear of each well content were made and stained with 15% Giemsa stain. These smears were checked for the maturation of schizonts relative to their controls.

4.2.3 Cell cytotoxicity assay

The cytotoxic assay was carried out against monkey kidney cell line C1008 (Vero cells). The cells were cultivated in 25cm² tissue culture flask supplemented with MEM- medium (9.7g MEM, 2.2g/l NaHCO₃, 6g HEPES, Gentamycin sulphate 50 mg, amphotericin B 2.5 mg, TDW-1000ml)+15%FBS provided with 5% CO₂ at 37 °C. The culture medium was changed on alternate days. The growth rate was determined as described⁶². For the cytotoxicity assay the cells were washed with PBS, trypsinized with 0.25% trypsin and a cell suspension was made in culture medium. The cells were counted in Neubaur chamber and appropriate dilution was made (1x10⁵ cells/ml). Vero cell suspension (100 μ L) was added to the microtiter plates and allowed to adhere overnight. Serial dilutions of test compounds were prepared in these plates and Vero cells were incubated with these compounds for 72 hours. Resazurin was added in wells and after 4 hours these plates were read under fluorescence reader (Biotek). Cytotoxic concentration (CC₅₀) was determined using MS-EXEL.

4.2.4 FAS-II inhibitory studies:

4.2.4.1 In Silico Studies:

The three-dimensional structure of the Compound **4a** was built and optimized using the Builder module of Insight II (M/s Accelrys Inc.). The compound was then taken as probe and submitted to an in house developed web-based tool called *Inhibitor Identification Tool (IS-IT)* (unpublished). The tool offers docking against 85 potential drug targets specifically chosen from *Mycobacterium tuberculosis*. Missing residues and atoms of each protein structure were repaired using the biopolymer module of Sybyl 6.8 (Tripos Associates), and Kollman^{63,64} charges were assigned to the protein. The grid for docking calculations was centered on the binding site of each protein. Docking simulations were carried out by using autodock 4.0, and interaction energies between compounds and the proteins were calculated using the scoring function of autodock 4.0. The protein target with interaction energies greater than the control docking energies was selected for further enzymatic assays.

4.2.4.2 Bacterial strains and viability assay. The generation of recombinant *M. aurum* strains was described earlier⁶⁵. *M. aurum* cultures were grown in Sauton's medium

supplemented with 0.05% Tween-80 and kanamycin (25 g/mL) and were plated on Nutrient-agar plates with 0.05% Tween-80 (NAT) supplemented with kanamycin. For post treatment viability assay, *M. aurum* was grown in Sauton's medium up to 0.6 OD₆₀₀ and the culture was diluted to 0.05 OD with fresh medium. From these diluents, $\sim 1 \times 10^5$ cells were inoculated into different tubes containing 5 ml fresh medium and varying concentration of compounds. The cultures were allowed to grow for 12 hrs. at 37°C with continuous shaking at 180 rpm. The treated and untreated cultures were plated on NAT-Km plates using 10-fold serial dilution to count the number of viable cells. % inhibition was scored considering the number of bacterial colonies in untreated condition as 100%. Under these experimental conditions we obtained nearly 18.8%, 85.43%, 88.72% and 99.64% inhibition at 10 M, 25 M, 50 M and 75 M of **4a** treatment respectively.

4.2.4.3 Reporter gene expression analysis.

Recombinant *M. aurum* strains were grown in Sauton's medium with Kanamycin at 37°C to 0.5 OD₆₀₀ after which culture was diluted to 0.04 - 0.05 OD with fresh medium. 10 ml diluted culture were distributed to separate tubes, equilibrated for 2 hrs. at 37°C and then varying concentrations (10 M, 25 M, 50 M and 75 M) of compounds were added to different tubes. Following 12 hours incubation at 37°C, 5 ml cultures from each tube were pelleted, washed and resuspended in PBS (Phosphate Buffer Saline, pH 7.2), sonicated at 4°C and supernatant was collected by centrifugation at 13000 rpm for 10 minutes at 4°C. Protein contents were quantified using Bradford Assay reagent (Sigma B6916) as per manufacturer protocol. -gal assay was performed from total cellular protein as described earlier⁶⁵. Briefly, same amount of protein were mixed with 200 µL of ONPG (4mg/ ml) and incubated for 30 min at 37°C. Reaction was stopped by adding 500 µL of 1M Na₂CO₃ and optical density was measured at 410 nm. Experiments were carried out in triplicates for each treatment and -galactosidase units were calculated for each set individually. The culture at each point was also plated to confirm the decline in viability of cells after drug treatment. The whole experiment was repeated twice and similar trends in results were obtained. Mean value and standard deviation were calculated and plotted for each set of data.

4.2.5 Data Analysis

All data were expressed as mean \pm SD with at least three separate experiments. IC₅₀ were determined with linear regression analysis using Microsoft Excel. Statistically significant comparison was calculated using student's *t*-test for unpaired variants. Values of *p* < 0.05 were regarded as statistically significant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:.

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