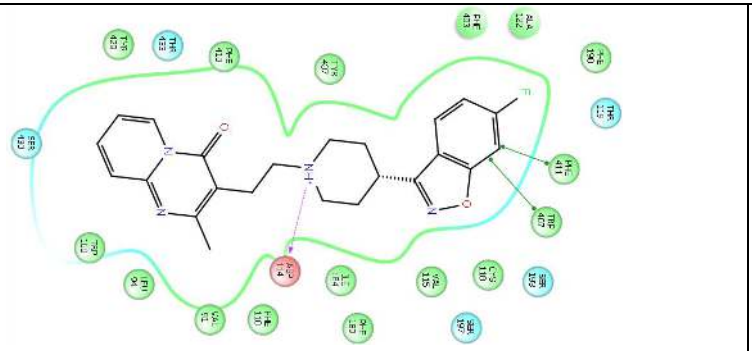
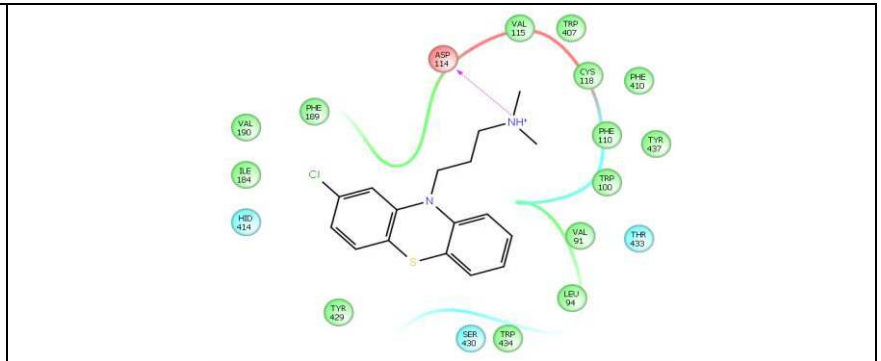
5. Results and Discussion

5.1. Section 1: Preliminary *In-silico* Studies

5.1.1. Off-target virtual screening and filtering of designed molecules

The design strategy was structural modification of neuroleptic phenothiazine drug chlorpromazine (CPZ), for improved antitubercular activity. The molecules were designed using CPZ as a template. Hence, the designed molecules were virtually screened against dopamine D2 (PDB code: 6CM4) and D3 (PDB code: 3PBL) receptors through high-throughput virtual screening module in Schrodinger [154]. Later, we filtered the molecules with cut-off of docking score ≤ 2.0 Kcal/mol. The filtered molecules produced only least interaction with dopamine receptors (D2 & D3) and may produce no/ less neuroleptic side effect. We obtained a total of 78 hit molecules having least interaction with dopamine receptors, out of 550 designed molecules. Risperidone, the co-crystallized ligand and CPZ, the template molecule, produced docking score of -9.0963 and 7.9910 Kcal/mol respectively, against D2 receptor (**Figure 5-1)**. Risperidone and CPZ produced the essential interaction with ASP114, while such interaction was found missing with the hits. Docking poses of some hits obtained from off-target virtual screening and filtering against D2 receptor are depicted in **Figure 5-1**. Eticlopride, the co-crystallized ligand and CPZ produced docking score of -8.6115 and -8.6093 Kcal/mol respectively, against D3 receptor (**Figure 5-2**). Eticlopride and CPZ produced essential interaction with ASP110, while it was found missing with the hits. Docking poses of some hits obtained from this off-target virtual screening and filtering against D3 receptor are depicted in **Figure 5-2**.

	
Risperidone (co-crystallized ligand) Docking Score:-9.0963 Kcal/mol	Chlorpromazine Docking Score:-7.9910 Kcal/mol

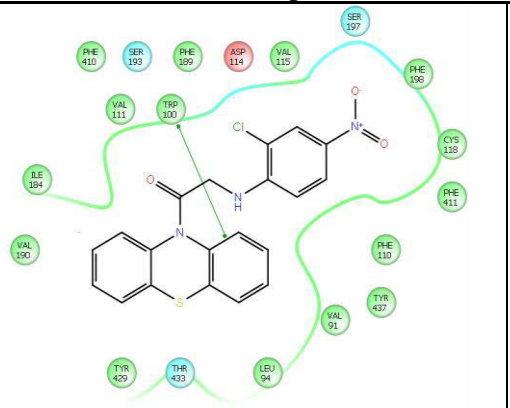
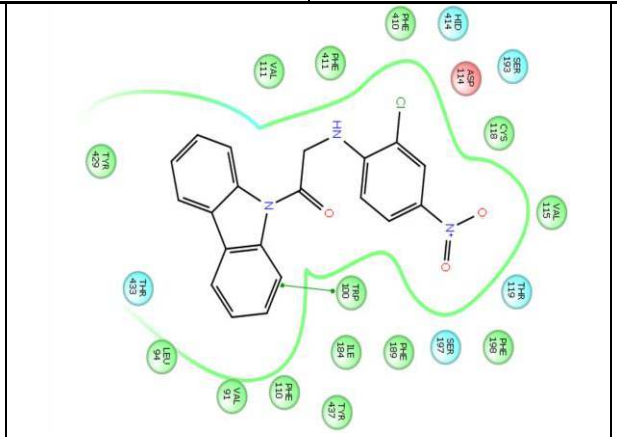
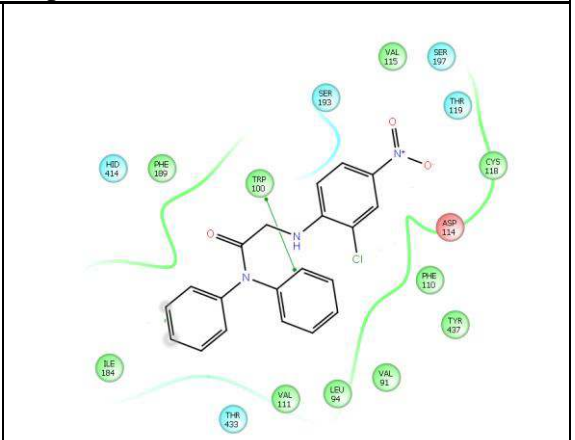
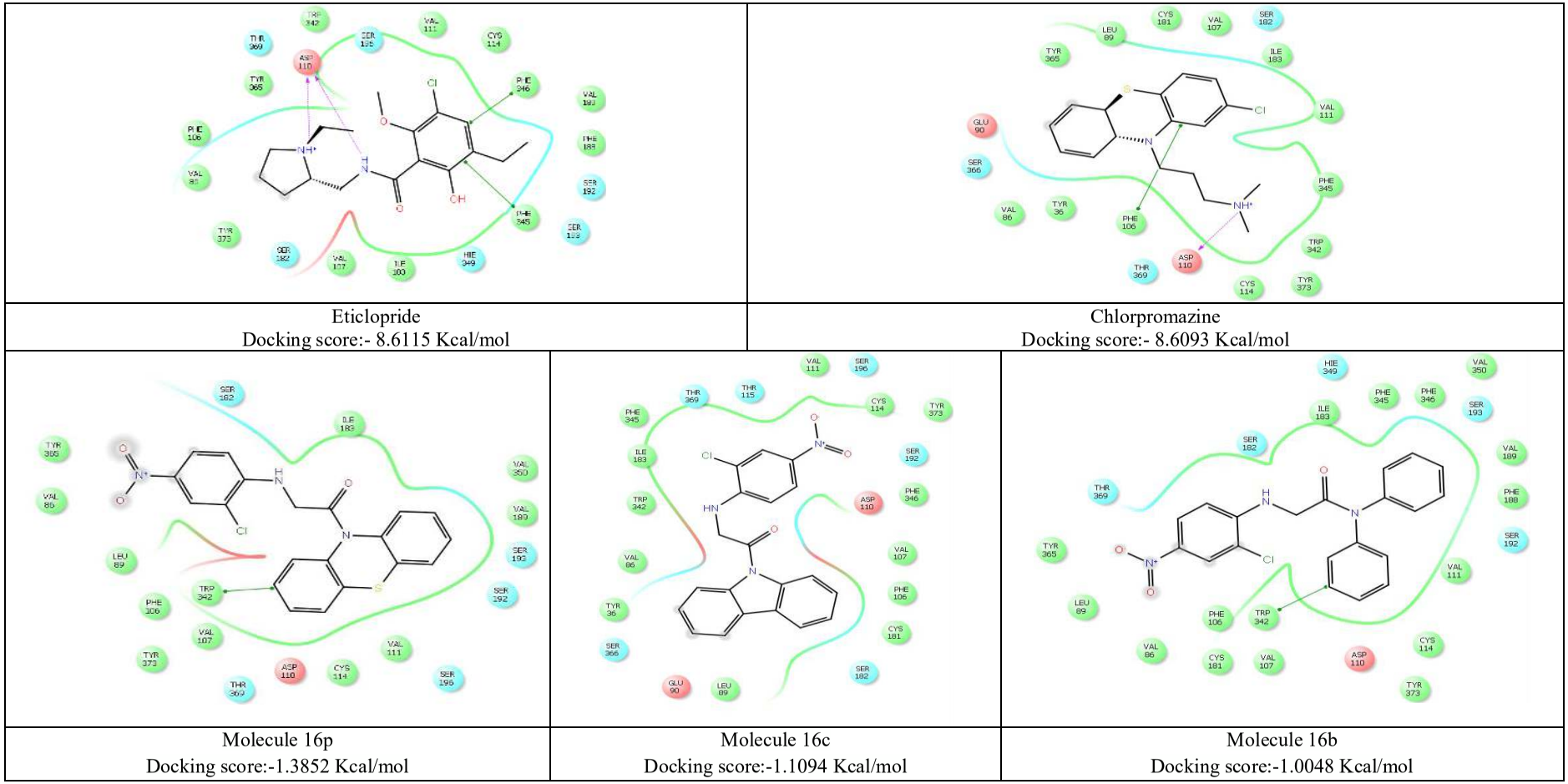
		
Molecule 16p Docking score:-1.8519 Kcal/mol	Molecule 16c Docking score:-1.5181 Kcal/mol	Molecule 16b Docking score:-1.0407 Kcal/mol

Figure 5-1: Docking pose of risperidone, chlorpromazine and designed molecules against D2 receptor (PDB code: 6CM4)



Eticlopride Docking score:- 8.6115 Kcal/mol	Chlorpromazine Docking score:- 8.6093 Kcal/mol
Molecule 16p Docking score:-1.3852 Kcal/mol	Molecule 16c Docking score:-1.1094 Kcal/mol
Molecule 16b Docking score:-1.0048 Kcal/mol	

Figure 5-2: Docking pose of eticlopride, chlorpromazine and designed molecules against D3 receptor (PDB code: 3PBL)

5.1.2. Preliminary in-silico *study against NDH-2*

5.1.2.1. Homology modelling

Phenothiazines *viz.* CPZ, TPZ and TZ were reported to inhibit NDH-2 with IC_{50} of 10 to 30μM [128]. Therefore, we hypothesized that the designed molecules could act by inhibiting NDH-2. It has a catalytic role in oxidation of NADH to NAD^+ with concomitant reduction of quinone to quinol, involved in the energy metabolism for production of ATP. The 3D protein structure of *Mtb* NDH-2 was not available, therefore, we developed a 3D protein structure/model through homology modeling, using the deposited protein sequence of *Mtb* NDH-2 *(strain CDC 1551)* available in uniport database. Homology modeling was performed using ModFOLD, version 6.0 web server. The stereochemical property of the developed model was validated using RAMPAGE and PROCHECK server and found that the model is having more than 90% of residues in the most favoured region **(Table 5-1)**.

Model	**Package**	**Ramachandran Plot Quality (%)**		
		Favored	**Allowed**	**Outlier**
Mtb ndh-2	RAMPAGE	92.2	5.3	2.5
	PROCHECK	88.3	10.3	1.5

Table 5-1: Ramachandran quality parameter check for the developed homology model of *Mtb* NDH-2 using RAMPAGE and PROCHECK.

VERIFY_3D score of the model showed that 87.97% of residues have an average 3D-1D score ≥ 0.2, which is above the cutoff limit of 80%. The overall quality factor was determined by ERRAT and was found 82.24%, indicating good quality-resolution of the structure (2.5-4 Å). The global model quality score was found to be 0.5948, which is greater than 0.4, indicating that the model is more complete and confident. It is also indicative that the model is more similar to the native structure. The p-value of the model was 1.434×10^{-6}, hence there is only 0.0001 % chance of the model being incorrect.

5.1.2.2. Superimposition of protein structures of NDH-2 from different species

The developed protein model of *Mtb* NDH-2 was superimposed against the known protein structures of *S. aureus* NDH-2 (PDB code: 4XDB) and *C. thermarum* NDH-2 (PDB code: 4NWZ) to check the similarity with the native structures **(Figure 5-3)**. The quinone binding site is the critical part of the model so we calculated root mean square deviation (RMSD) for the residues forming the quinone binding motif and was found to be ≤ 0.151 and ≤ 0.558 against *S. aureus* and *C. thermarum* NDH-2, respectively **(Table 5-2 & Table 5-3)**. The RMSD value is very low, which is indicative that the quinone binding motif of *Mtb* NDH-2 is highly conserved. Hence, the model can be used for docking analysis to predict the active binding of the inhibitors.

Type-2 NADH dehydrogenase (NDH-2) of *S. aureus* (PDB code: 4XDB)	**Type-2 NADH dehydrogenase (NDH-2) of *M. tuberculosis* (developed through homology modeling)**	**RMSD**
Ala-319	Ala-333	0.101
Gln-320	Gln-334	0.046
Ile-321	Gly-335	0.114
Ala-322	Ala-336	0.121
Met-323	Ile-337	0.148
Gln-324	Gln-338	0.151

Table 5-2: Superimposition of the developed homology model against the protein structure of *S. aureus* NDH-2

Type-2 NADH dehydrogenase (NDH-2) of *C. thermarum* (PDB code:4NWZ)	**Type-2 NADH dehydrogenase (NDH-2) of *M. tuberculosis* (developed through homology modeling)**	**RMSD**
Ala-316	Ala-333	0.117
Gln-317	Gln-334	0.338
Ile-318	Gly-335	0.254
Ala-319	Ala-336	0.344
Ile-320	Ile-337	0.423
Gln-321	Gln-338	0.558

Table 5-3: Superimposition of the developed homology model against the protein structure of *C. thermarum* NDH-2

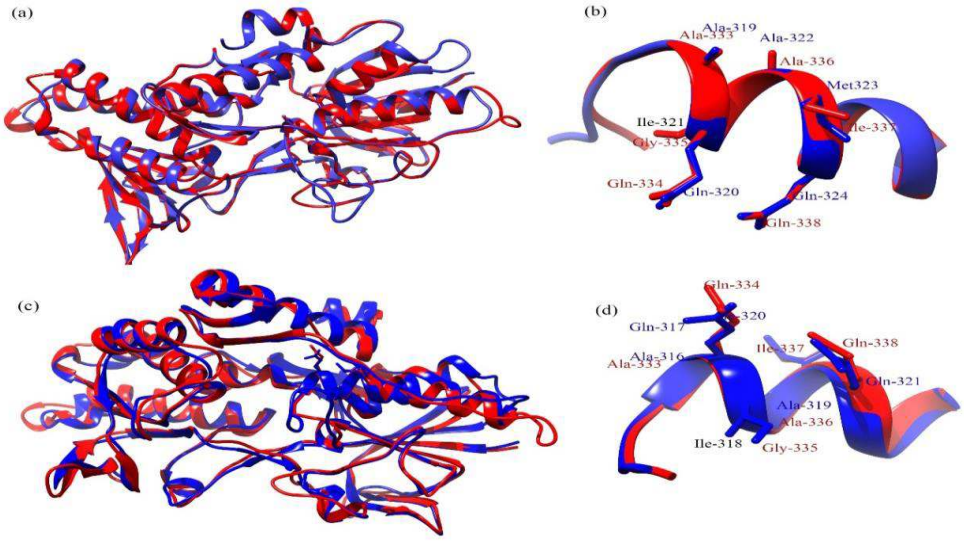


Figure 5-3: Superimposition of *S. aureus* and *C. thermarum* NDH-2 over *Mtb* NDH-2: Complete protein (a) and active motif (b) of *S. aureus* NDH-2 over *Mtb* NDH-2. Followed by, complete protein (c) and active motif (d) of *C. thermarum* NDH-2 over *Mtb* NDH-2.

5.1.3. Preliminary in-silico *study against ATP synthase*

5.1.3.1. Docking of purchasable subset of ZINC database against ATP synthase

The purchasable subset of ZINC Database was screened against Mycobacterial ATP synthase. The bedaquiline co-crystallized protein (PDB code: 4V1F) was used for the purpose. Molecules ZINC39552534, ZINC39490094 and ZINC38959526 produced docking score of -7.05852, -6.65839 and -6.10790 Kcal/mol respectively, while it was -6.067435 Kcal/mol for the standard drug Bedaquiline (**Table 5-4**). Essential hydrogen bond interaction was noticed with Glu65 in all the three molecules. Further, ZINC39552534 showed three hydrogen bond interactions with GLU 65 *i.e.* two from NH groups of tetrazole and one from NH group of piperidine. Similarly, ZINC39490094 also showed three hydrogen bond interactions *i.e.* two from NH groups of tetrazole and one from NH group of tetrahydropyrrole. While, ZINC38959526 showed only two hydrogen bond interactions with GLU 65, from NH and OH group of tetrahydopyrimidine-4-one respectively. Lipophilic interactions were also found associated with all the molecules, but lesser than bedaquiline (**Figure 5-4**).

ZINC38959526 showed structural similarity with our designed molecules and also produced docking score (-6.10790 Kcal/mol) comparable to bedaquiline (-6.067435 Kcal/mol). Based on this pilot study, we hypothesized that our compounds could act by inhibiting ATP synthase. Later, we did extra-precision docking of the designed molecules against ATP synthase (PBD code: 4V1F) to understand the binding energy of the molecules against the target.

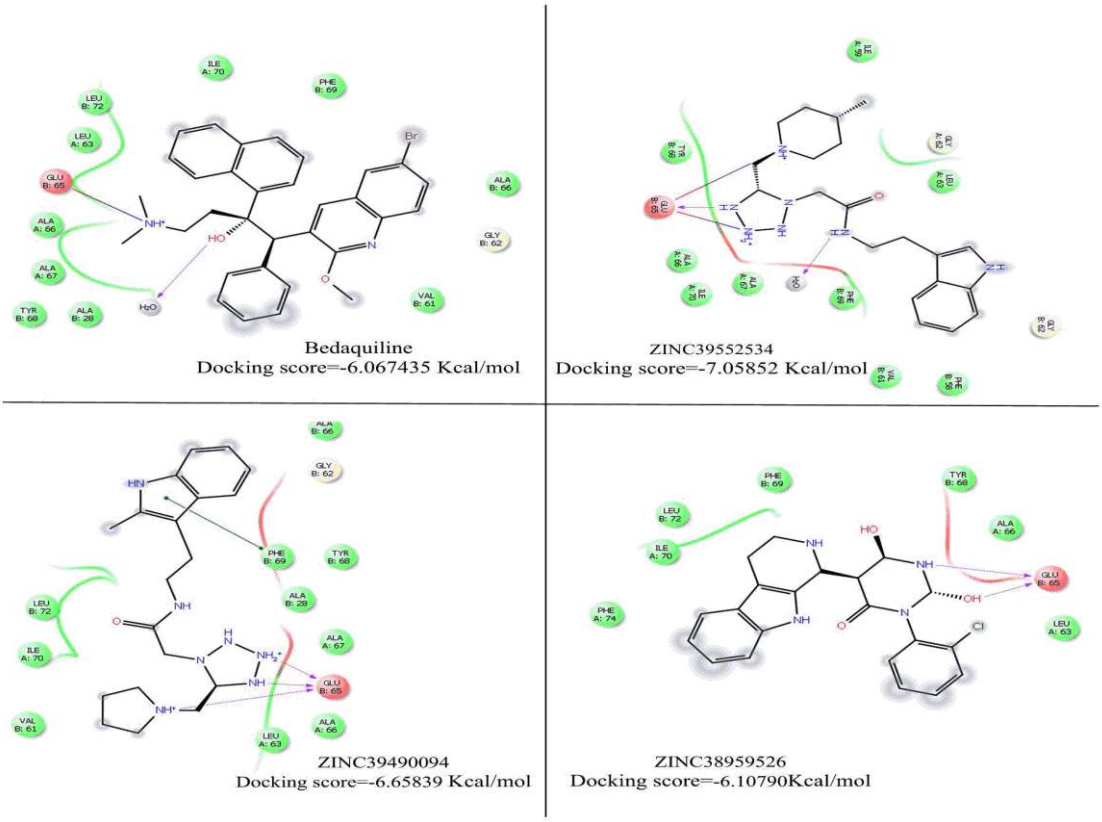


Figure 5-4: Docking of Bedaquiline and ZINC database hits against ATP synthase

5.1.3.2. Alignment of amino acid sequence of different mycobacterial and human ATP synthase c-subunits

Bedaquiline interacts with a stretch of nine residues *viz*. Gly 62, Leu 63, Glu 65, Ala 66, Ala 67, Tyr 68, Phe 69, Ile 70, and Leu 72 in *M. pheli* ATP synthase [157]. All the nine amino acids are conserved in *Mtb* and *M. smegmatis* ATP synthases, however, out of nine residues only three residues (Leu 63, Glu 65, Ala 66) are conserved in human counterpart (**Figure 5-5**). Human ATP synthase is more complex compared to

mycobacterial and shares only 35% similarity with *M. pheli* ATP synthase [157]. The presence of small amino acid Ala 67 at binding site of mycobacterial ATP synthase compared to large residue methionine in human ATP synthase should provide the selectivity to the designed analogues. From these evidences, it is clear that the binding site environment is different at human ATP synthase with respect to *Mtb* enzyme. It can be concluded that the designed compounds have minimum chance to inhibit human ATP synthase, thus they should be safe and efficacious.

Code	RMSD	Docking score	Glide gscore	Glide lipo	Glide hbond	Glide rewards	Glide evdw	Glide ecoul	Glide erotb	Glide energy
Bedaquiline	**-**	**-6.06743**	**-6.07043**	**-1.40308**	**-0.6894**	**-0.53**	**-33.1618**	**-12.7896**	**0.228587**	**-45.9516**
ZINC39552534	0.473579	-7.05852	-7.22152	-1.21507	-0.81965	-1.82689	-26.0328	-15.8154	0.635435	-41.8482
ZINC39371747	0.407618	-6.65839	-6.82619	-1.01802	-0.8151	-1.82314	-24.7965	-14.6966	0.635435	-39.4931
ZINC38959526	0.524091	-6.10790	-6.2271	-0.82628	-0.5331	-2.14753	-27.9053	-12.9004	0.775805	-40.8057
ZINC39420954	0.470915	-6.05566	-6.11376	-1.02976	-0.67287	-1.62904	-28.8449	-12.7164	0.637665	-41.5612
ZINC39371748	0.473438	-6.05496	-6.12276	-0.7535	-0.47783	-1.82689	-26.7398	-14.192	0.635435	-40.9318
ZINC39016160	0.577249	-5.38963	-5.40453	-1.25058	-0.38179	-1.66343	-27.175	-7.51718	0.610848	-34.6922
ZINC39231767	0.414538	-5.24385	-6.11115	-0.87105	-0.53236	-1.92496	-25.5906	-11.0747	0.440477	-36.6653
ZINC71764554	0.669219	-5.22277	-5.23397	-0.877	-0.19281	-1.46098	-24.0847	-11.7383	0.387943	-35.8229
ZINC38975417	0.577237	-5.01698	-5.03138	-0.34204	-0.34561	-2.07741	-15.3821	-11.1073	0.485411	-26.4894

Table 5-4: Docking of purchasable subset of ZINC Database against ATP synthase

```
Human     MFACAKLACTP-SLIRAGSRVAYRPISASVLSRPEASRTGEGSTVFNGAQNGVSQLIQREFQTSAISRDIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM------
M.pheli   ------MELDPNALITAG----------ALI--------G-GGLIMGG---GA----------------------IGAGIGD-GIAGN---------ALISGIARQPEAQGRLFTPFFITVGLVEAA--YFINLAFMALFVFATPGLQ
M.tb.     --------MDP--TIAAG----------ALI--------G-GGLIMAG---GA----------------------IGAGIGD-GVAGN---------ALISGVARQPEAQGRLFTPFFITVGLVEAA--YFINLAFMALFVFATP-VK
M.smeg.   ------MDLDPNAIITAG----------ALI--------G-GGLIMGG---GA----------------------IGAGIGD-GIAGN---------ALISGIARQPEAQGRLFTPFFITVGLVEAA--YFINLAFMALFVFATPGLQ
```

Figure 5-5: Alignment of amino acid sequence of selected ATP synthase c-subunits. Amino acids at the same position but from other species are in blue color. Residues involved in drug coordination are in red color (matched with human counterpart)

5.2. Section 2: Molecular Docking, Synthesis, Characterization and Biological Profiling of Phenothiazine derivatives

5.2.1. Extra-precision molecular docking

Every molecule in the study was docked against the *Mtb* NDH-2 protein developed through homology modeling. The function of the NDH-2 enzyme/ protein can only be arrested, when the inhibitors bind to the quinone binding motif. Interestingly, all the molecules in the study entered the quinone binding tunnel to produce essential interactions with the quinone binding motif (formed by the residues Ala-333, Gln-334, Gly-335, Ala-336, Ile-337 & Gln-338). A critical hydrogen bond interaction was observed against Ala-333, the essential residue for effective binding of the substrate (quinone) to the motif (**Figure 5-6**). Docking score is imperative to understand how effective the inhibitors bind to the motif. The score varies between -5.8194 and -3.4273 Kcal/mol. The molecules **15p, 16p** and **17p** produced very good binding with minimum binding energy of -5.5957, -5.8194 and -5.7346 Kcal/mol, respectively **(Table 5-5)** and a better correlation with antitubercular MIC was noticed. The developed molecules could inhibit NDH-2, thereby the ATP production could be affected to produce antimicrobial activity.

5.2.2. Molecular property and toxicity prediction

The reduced bioavailability on oral administration and the associated toxicity are the prime factors for the failure of drug candidates at clinical development stage. About one-third of drug candidates fail due to their poor pharmacokinetic profiles. Hence, computational study was performed to predict the molecular property and toxicity of the developed compounds. Lipophilicity defines the pharmacokinetics and also pharmacodynamics of a drug molecule.

S.No	Ligand code	Lowest binding energy (Kcal/mol)	Residues forming the quinone binding motif (residues producing H-bonding with the ligand were indicated in bold)
1	1p	-3.9931	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
2	2p	-4.2298	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
3	3p	-4.2714	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
4	4p	-4.2955	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
5	5p	-4.3046	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
6	6p	-4.3003	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
7	7p	-4.3275	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
8	8p	-4.2990	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
9	9p	-4.3284	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
10	10p	-4.6584	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
11	11p	-4.6216	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
12	12p	-4.7313	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
13	13p	-4.6779	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
14	14p	-5.4481	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
15	15p	-5.5957	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
16	16p	-5.8194	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
17	17p	-5.7346	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
18	18p	-4.1018	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
19	19p	-3.4273	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
20	20p	-3.5119	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
21	S1	-2.0127	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
22	S2	-2.3924	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
23	S3	-2.5061	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
24	S4	-2.5033	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
25	S5	-2.5017	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
26	S6	-2.4602	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)

Table 5-5: Docking of designed phenothiazine molecules against NDH-2 of *Mtb*

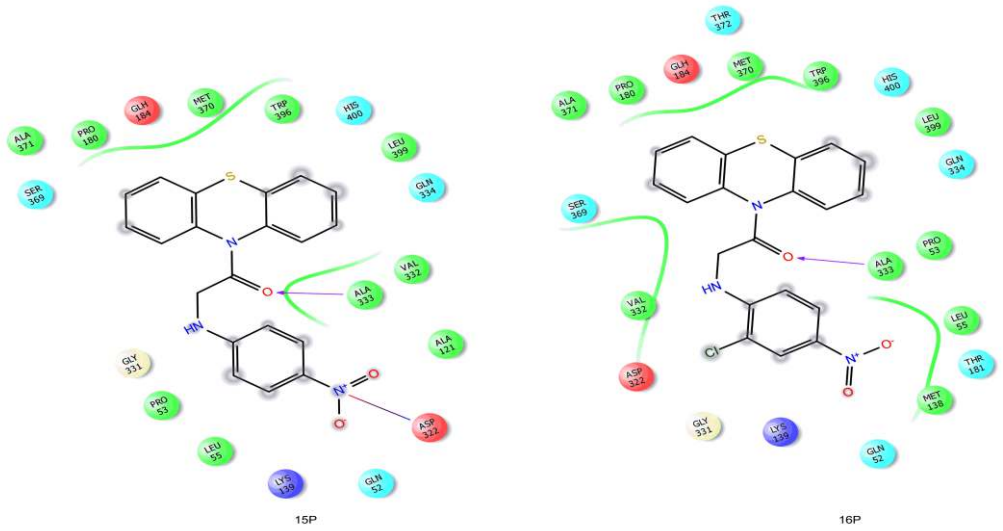


Figure 5-6: Docking poses of molecules 15p and 16p against NDH-2

It was determined by calculating clogP using OSIRIS DataWarrior and was found in the range between 2.92 and 5.93. The clogP of molecules (**14p, 15p & 16p**) was found impressive at 3.71, 3.41 and 4.32, respectively. Topological polar surface area (TPSA) was computed and was found to be 57.64 for compounds **2p** to **13p**, and 103.46 for compounds **14p** to **17p**, while 94.94, 52.09 and 97.91 for the compounds **18p**, **19p** and **20p,** respectively. The good percentage of absorbance could lead to better oral bioavailability. Hence, the percentage absorption was calculated and was found to be over 73 percent for all the compounds. Molecular descriptors like hydrogen bond donor, hydrogen bond acceptor and drug likeness were computed and were found within the desired range. The results of the study are presented in **Table 5-6**. The fragments of a given molecule are the indicators of toxicity risk. Therefore, all the molecules were predicted for mutagenicity, tumorigenicity, and irritancy through OSIRIS DataWarrior. Except **1p**, none of the molecules in the study were predicted to be mutagenic and tumorigenic, but irritancy was noticed. Molecules **10p** and **16p** were indicated with low levels of irritancy and molecules **1p, 19p** and **20p** were indicated with high level of irritancy (**Table 5-6**). The designed molecules were devoid of toxicity risks and further development of this class of molecules might be fruitful.

Code	cLogP	cLogS	HBA	HBD	TSA	TPSA	DL	MU	TU	IR	SI	MF	MC	EA	RB	NR	AR	SA
1p	4.13	-5.05	2	0	211	45.61	1.63	H	H	H	0.44	0.37	0.76	4	1	3	2	6
2p	4.63	-5.64	3	1	278	57.64	2.61	N	N	N	0.5	0.46	0.84	4	3	4	3	8
3p	5.24	-6.38	3	1	293	57.64	2.65	N	N	N	0.48	0.45	0.79	5	3	4	3	6
4p	5.24	-6.38	3	1	288	57.64	2.65	N	N	N	0.48	0.48	0.79	5	3	4	3	6
5p	5.24	-6.38	3	1	280	57.64	2.65	N	N	N	0.52	0.48	0.79	5	3	4	3	8
6p	4.73	-5.96	3	1	267	57.64	1.27	N	N	N	0.48	0.46	0.84	5	3	4	3	6
7p	4.73	-5.96	3	1	266	57.64	1.27	N	N	N	0.52	0.46	0.84	5	3	4	3	8
8p	5.36	-6.48	3	1	285	57.64	0.82	N	N	N	0.48	0.48	0.79	5	3	4	3	6
9p	5.36	-6.48	3	1	295	57.64	0.82	N	N	N	0.52	0.48	0.79	5	3	4	3	8
10p	5.85	-7.11	3	1	305	57.64	2.65	N	N	L	0.46	0.48	0.80	6	3	4	3	9
11p	5.93	-7.31	3	1	309	57.64	0.82	N	N	N	0.46	0.48	0.80	6	3	4	3	9
12p	5.34	-6.69	3	1	289	57.64	1.31	N	N	N	0.50	0.48	0.80	6	3	4	3	6
13p	5.46	-6.79	3	1	289	57.64	-0.51	N	N	N	0.5	0.45	0.81	6	3	4	3	6
14p	3.71	-6.10	6	1	292	103.46	-2.51	N	N	N	0.48	0.48	0.85	7	4	4	3	6
15p	3.71	-6.10	6	1	292	103.46	-2.51	N	N	N	0.51	0.48	0.85	7	4	4	3	8
16p	4.32	-6.84	6	1	304	103.46	-2.45	N	N	L	0.50	0.47	0.83	8	4	4	3	6
17p	4.32	-6.84	6	1	317	103.46	-2.45	N	N	N	0.46	0.48	0.83	8	4	4	3	6
18p	4.12	-5.65	5	2	286	94.94	2.47	N	N	N	0.44	0.46	0.86	6	4	4	3	6
19p	5.37	-5.92	4	0	335	52.09	6.5	N	N	H	0.53	0.48	0.81	6	3	5	3	10
20p	2.92	-5.65	7	0	347	97.91	1.41	N	N	H	0.53	0.48	0.87	8	4	5	3	10
S1	3.46	-4.90	3	1	254	57.64	3.12	N	N	N	0.47	0.43	0.83	4	3	4	2	7
S2	3.80	-5.17	3	1	255	57.64	2.07	N	N	N	0.5	0.43	0.83	4	3	4	2	7
S3	4.14	-5.44	3	1	268	57.64	0.40	N	N	N	0.47	0.42	0.83	4	3	4	2	8
S4	4.48	-5.71	3	1	281	57.64	-2.39	N	N	L	0.5	0.45	0.84	4	3	4	2	8
S5	4.82	-5.98	3	1	285	57.64	-1.96	N	N	N	0.48	0.46	0.84	4	3	4	2	9
S6	5.89	-6.25	3	1	291	57.64	-2.13	N	N	N	0.5	0.48	0.84	4	3	3	2	9

Table 5-6: Molecular properties and predicted toxicity of phenothiazine molecules. HBA= H-Acceptors; HBD= H-Donor; TSA=Total surface area; TPSA= Total Polar surface area; DL= Druglikeness; MU=Mutagenic; TU=Tumorigenic; IR=Irritant; SI=Shape Index; MF=Molecular Flexibility; MC=Molecular Complexity; EA=Electronegative Atoms; RB=Rotatable Bonds; AR=Aromatic Rings; SA=Symmetric atoms; NR=No. of Rings; N=none; H=High; L=low.

5.2.3. Synthesis and characterization

All the designed molecules were synthesized as shown in **Scheme 1** *i.e.* synthesis of phenothiazine **(b)** followed by 2-chloro-1-(10H-phenothiazin-10-yl)ethan-1-one **(1p)**. Later, the final derivatives were synthesized on reaction of **1p** with different phenyl amines/phenyl piperazines/ cyclic amines. The progress of reaction was monitored by thin layer chromatography. Column chromatography was performed to obtain pure novel phenothiazine derivatives. The yield was obtained in the range of 45 to 95 percent. Melting point, elemental composition, ^{1}H NMR, ^{13}C NMR and Mass data of all the compounds in phenothiazine series are as follows,

*5.2.3.1. 10H-phenothiazine (**b**)*

Yield: 90%; MP: 181-183ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.533, 7.517 (d, J = 8 Hz, 2H, C1, C8-phenothiazine), 7.412-7.395 (dd, J = 8.5, 1.1 Hz, 2H, C4, C5-phenothiazine), 7.310-7.295 (td, J = 7.5, 1.1 Hz, 2H, C2, C7-phenothiazine), 7.224-7.195 (td, J = 7.5, 1.1 Hz, 2H, C3, C6-phenothiazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 138.051, 133.405, 128.350, 127.619, 127.526, 126.738 (aromatic carbons); MS (ESI) m/z: 200.7 (100%). Anal. Calcd for $C_{12}H_9NS$: C, 72.33; H, 4.55; N, 7.03; Found: C, 72.54; H, 4.57; N, 7.08 %.

*5.2.3.2. 2-chloro-1-(10H-phenothiazin-10-yl) ethan-1-one (**1p**)*

Yield: 95%; MP: 111-113°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.524, 7.508 (d, J = 8 Hz, 2H, C1, C8-phenothiazine), 7.403-7.386 (dd, J = 7.5, 1.1 Hz, 2H, C4, C5-phenothiazine), 7.301-7.286 (td, J = 7.5, 1.1 Hz, 2H, C2, C7-phenothiazine), 7.215-7.186 (td, J = 7.5, 1.1 Hz, 2H, C3, C6-phenothiazine), 4.111 (s, 2H, methylene-CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 165.717 (C=O), 138.111, 133.405, 128.353, 127.626, 127.529, 126.743 (aromatic carbons), 41.994 (methylene carbon); MS (ESI)

m/z: 278.3 (25%), 276. 5 (75%). Anal. Calcd for $C_{14}H_{10}ClNOS$: C, 60.98; H, 3.66; N, 5.08; Found: C, 61.24; H, 3.69; N, 5.03 %.

*5.2.3.3. 1-(10H-phenothiazin-10-yl)-2-(phenylamino)ethan-1-one (**2p**)*

Yield: 78%; MP: 145-147°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm); 7.612, 7.596 (d, J=7.5 Hz, 2H, C1, C8-phenothiazine), 7.548, 7.533 (d, J=7.5 Hz, 2H, C4, C5-phenothiazine), 7. 483-7.425 (td, J=14, 7.5 Hz, 2H, C2, C7-phenothiazine), 7.381-7.325 (td, J=14, 7.5 Hz, 2H, C3, C6-phenothiazine), 7.105-7.089 (d, J=8 Hz, 2H, C2, C6-phenyl), 6.818-6.802 (t, J=8 Hz, 2H, C3, C5-phenyl), 6.634-6.618 (t, J=8 Hz, 1H, C4-phenyl), 3.966 (s, 2H, methylene-CH_2), 3.497 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.328 (C=O), 145.043, 139.997, 134.651, 130.825, 128.174, 127.079, 126.653, 123.138, 120.367, 115.552 (aromatic carbons), 55.672 (methylene carbon); MS (ESI) *m/z*: 333.2 (42.1%); Anal. Calcd for $C_{20}H_{16}N_2OS$: C, 72.26; H, 4.85; N, 8.43; Found: C, 72.41; H, 4.87; N, 8.47 %.

*5.2.3.4. 2-((2-chlorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**3p**)*

Yield: 75%; MP: 154-156ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.574, 7.558 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.442-7.427 (d, J=7.5 Hz, 2H, C4, C5-phenothiazine), 7.340-7.307 (td, J=7.5, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.253-7.220 (m, 3H, C3, C6-phenothiazine, C3-phenyl), 7.153-7.127 (t, J=6.5 Hz, 1H, C5-phenyl), 6.718, 6.701 (d, J=8.5 Hz, 1H, C6-phenyl, 6.653-6.627 (t, J=6.5 Hz, 1H, C4-phenyl), 4. 175 (s, 2H, methylene-CH_2) 3.864 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.005 (C=O), 145.971, 140.135, 134.883, 131.099, 130.455, 128.238, 127.654, 126.342, 123.214, 121.535, 119.958, 114.822 (aromatic carbons), 53.526 (methylene carbon); MS (ESI) *m/z*: 369.5 (25%), 367.6 (74%); Anal. Calcd for $C_{20}H_{15}ClN_2OS$: C, 65.48; H, 4.12; N, 7.64; Found: C, 65.61; H, 4.13; N, 7.67 %.

*5.2.3.5. 2-((3-chlorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**4p**)*

Yield: 82%; MP: 155-156ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.577, 7.561 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.452-7.433 (dd, J=9.5, 1.5 Hz, 2H, C4, C5-phenothiazine), 7.348-7.314 (td, J=7.5, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.261-7.230 (td, J=6, 1.5 Hz, 2H, C3, C6-phenothiazine), 7.014 (s, 1H, C2-phenyl), 6.718, 6.702 (d, J=8 Hz, 1H, C4-phenyl), 6.411, 6.395 (d, J= 8 Hz, 1H, C6-phenyl), 6.652-6.624 (t, J=7 Hz, 1H, C5-phenyl), 4.177 (s, 2H, methylene CH_2), 3.928 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.011 (C=O), 147.975, 140.141, 134.891, 135.455, 130.457, 128.246, 127.066, 126.336, 123.212, 119.995, 115.902, 112.549 (aromatic carbons), 53.526 (methylene carbon); MS (ESI) *m/z*: 369.5 (25%), 367.4 (75%); Anal. Calcd for $C_{20}H_{15}ClN_2OS$: C, 65.48; H, 4.12; N, 7.64; Found: C, 65.57; H, 4.11; N, 7.68 %.

*5.2.3.6. 2-((4-chlorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**5p**)*

Yield: 79%; MP: 155-157ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.645, 7.629 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.521, 7.506 (d, J=7.5 Hz, 2H, C4, C5-phenothiazine), 7.448-7.415 (td, J=15, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.550-7.517 (td, J=15, 1.5 Hz, 2H, C3, C6-phenothiazine), 7.112, 7.098 (d, J=6.5 Hz, 2H, C3, C5-phenyl), 6.622, 6.604 (d, J=8.5 Hz, 2H, C2, C6-phenyl), 4. 013 (s, 2H, methylene-CH_2) 3.582 (1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.972 (C=O), 144.783, 139.251, 133.918, 130.582, 128.733, 127.915, 126.626, 125.125, 122.288, 115.161 (aromatic carbons), 52.651 (methylene carbon); MS (ESI) *m/z*: 369.5 (24%), 367.6 (75%); Anal. Calcd for $C_{20}H_{15}ClN_2OS$: C, 65.48; H, 4.12; N, 7.64; Found: C, 65.65; H, 4.13; N, 7.65 %.

*5.2.3.7. 2-((3-fluorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**6p**)*

Yield: 67%; MP: 151-153ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.575, 7.559 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.451-7.432 (dd, J=9.5, 1.5 Hz, 2H, C4, C5-phenothiazine),

7.351-7.317 (td, J=7.5, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.258-7.227 (td, J=6, 1.5 Hz, 2H, C3,C6-phenothiazine), 7.009 (s, 1H, C2-phenyl) , 6.720,6.704 (d, J=8 Hz, 1H, C4-phenyl), 6.659-6.631 (t, J=7 Hz, 1H, C5-phenyl), 6.408, 6.392 (d, J= 8 Hz, 1H, C6-phenyl), 4.198 (s, 2H, methylene CH_2), 3.935 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.023 (C=O), 147.805, 141.041, 135.991, 134.432, 130.762, 128.421, 126.973, 126.181, 124.002, 120.086, 116.109, 112.330 (aromatic carbons), 53.842 (methylene carbon); MS (ESI) *m/z*: 351.3 (78%); Anal. Calcd for $C_{20}H_{15}FN_2OS$: C, 68.55; H, 4.31; N, 7.99; Found: C, 68.62; H, 4.33; N, 8.03 %.

*5.2.3.8. 2-((4-fluorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**7p**)*

Yield: 62%; MP: 152-154ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.634, 7.618 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.525, 7.510 (d, J=7.5 Hz, 2H, C4, C5-phenothiazine), 7.452-7.417 (td, J=15, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.547-7.514 (td, J=15, 1.5 Hz, 2H, C3, C6-phenothiazine), 7.152, 7.138 (d, J=7 Hz, 2H, C3, C5-phenyl), 6.628, 6.610 (d, J=8.5 Hz, 2H, C2, C6-phenyl), 4.102 (s, 2H, methylene-CH_2) 3.529 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.041 (C=O), 144.808, 139.346, 133.537, 130.591, 128.905, 127.611, 126.584, 125.222, 122.169, 115.184 (aromatic carbons), 53.563 (methylene carbon); MS (ESI) *m/z*: 351.3 (78%); Anal. Calcd for $C_{20}H_{15}FN_2OS$: C, 68.55; H, 4.31; N, 7.99; Found: C, 68.68; H, 4.25; N, 8.02 %.

*5.2.3.9. 2-((3-bromophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**8p**)*

Yield: 58%; MP: 176-179ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.581, 7.566 (d, J=7.5 Hz, 2H, C1, C8-phenothiazine), 7.470-7.442 (dd, J=6, 1.5 Hz, 2H, C4, C5-phenothiazine), 7.369-7.236 (m, 4H, C2, C3, C6, C7-phenothiazine), 6.991 (s, 1H, C2-phenyl), 6.852-6.835 (d, J=8.5 Hz, 1H, C4-phenyl), 6.798-6.766 (t, J=8 Hz, 1H, C5-phenyl), 6.581-6.565(dd, J=8, 1.5 Hz, 1H, C6-phenyl), 4.178 (s, 2H, methylene-CH_2), 3.923 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.225 (C=O), 148.411, 142.353, 136.262,

134.907, 131.071, 128.542, 127.009, 126.011, 124.134, 120.490, 116.717, 112.926 (aromatic carbons), 54.023 (methylene carbon); MS (ESI) *m/z*: 414.1 (49%), 412.2 (50%); Anal. Calcd for $C_{20}H_{15}BrN_2OS$: C, 58.40; H, 3.68; N, 6.81; Found: C, 58.52; H, 3.64; N, 6.82 %.

*5.2.3.10.2-((4-bromophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**9p**)*

Yield: 55%; MP: 177-189ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.593, 7.578 (d, J=7.5 Hz, 2H, C1, C8-phenothiazine), 7.476-7.458 (dd, J=9 Hz, 2H, C4, C5-phenothiazine), 7.372-7.357 (dd, J=7.5, 1.5 Hz, 2H, C3, C5-phenyl), 7.342-7.214 (m, 4H, C2, C3, C6, C7-phenothiazine), 6.571-6.549 (dd, J=11, 1.5 Hz, 2H, C2,C6-phenyl), 4.183 (s, 2H, methylene CH_2), 3.551 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.331 (C=O), 148.465, 142.341, 136.318, 134.891, 131.127, 128.584, 127.102, 126.018, 124.177, 120.536, 116.520, 112.961 (aromatic carbons), 54.112 (methylene carbon); MS (ESI) *m/z*: 414.4 (49%), 412.3 (50%); Anal. Calcd for $C_{20}H_{15}BrN_2OS$: C, 58.40; H, 3.68; N, 6.81; Found: C, 58.51; H, 3.62; N, 6.80 %.

*5.2.3.11.2-((3,5-dichlorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**10p**)*

Yield: 58%; MP: 185-187ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.592, 7.577 (d, J=7.5 Hz, 2H, C1, C8-phenothiazine), 7.471-7.453 (dd, J=9, 1.5 Hz, 2H, C4, C5-phenothiazine), 7.369-7.336 (td, J=7.5, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.282-7.252 (td, J=7.5, 1.5 Hz, 2H, C3, C6-phenothiazine), 6.824 (s, 2H, C2, C6-phenyl), 6.512 (s, 1H, C4-phenyl), 4.183, (s, 2H, methylene-CH_2), 3.902, (s, 1H,NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 170.115 (C=O), 147.863, 142.231, 136.781, 132.315, 130.848, 128.511, 126.456, 123.447, 121.619, 115.021 (aromatic carbons), 54.724 (methylene carbon); MS (ESI) *m/z*: 406.1 (5%), 404.2 (32%), 402.1 (74%); Anal. Calcd for $C_{20}H_{14}Cl_2N_2OS$: C, 59.86; H, 3.52; N, 6.98; Found: C, 59.91; H, 3.56; N, 6.96 %.

*5.2.3.12.2-((3,5-dibromophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**11p**)*

Yield: 54%; MP: 191-194ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.574-7.529 (dd, J=22.5, 8 Hz, 2H, C1, C8-phenothiazine), 7.474-7.432 (dd, J=21, 7.5 Hz, 2H, C4, C5-phenothiazine), 7.369-7.311 (td, J=14, 7.5 Hz, 2H, C2, C7-phenothiazine), 7.282-7.226 (m, 3H, C3, C6-phenothiazine, C4-phenyl), 6.518 (s, 2H, C2, C6-phenyl), 4.178, (s, 2H, methylene-CH_2), 3.462, (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 170.462 (C=O), 147.281, 142.515, 136.388, 133.407, 130.933, 127.738, 126.102, 123.623, 120.009, 115.648 (aromatic carbons), 54.852 (methylene carbon); MS (ESI) *m/z*: 495.3 (46%), 493.2 (97%), 491.4 (49%); Anal. Calcd for $C_{20}H_{14}Br_2N_2OS$: C, 49.00; H, 2.88; N, 5.71; Found: C, 49.11; H, 2.92; N, 5.74 %.

*5.2.3.13. 2-((3-chloro-4-fluorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**12p**)*

Yield: 59%; MP: 175-177°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm); 7.643, 7.628 (d, J=7.5 Hz, 2H, C1, C8-phenothiazine), 7.524, 7.509 (d, J=7.5 Hz, 2H, C4, C5-phenothiazine), 7.438-7.375 (td, J=14, 7.5 Hz, 2H, C2, C7-phenothiazine), 7.291-7.227 (td, J=14, 7.5 Hz, 2H, C3, C6-phenothiazine), 7.105-6.889 (m, 3H, C2, C5, C6-phenyl), 4.261 (s, 2H, methylene-CH_2), 3.568 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.328 (C=O), 146.438, 142.373, 140.152, 134.651, 130.825, 128.174, 127.079, 126.653, 123.138, 121.647, 120.367, 115.552 (aromatic carbons), 55.672 (methylene carbon); MS (ESI) *m/z*: 387.5 (24%), 385.7 (75%); Anal. Calcd for $C_{20}H_{14}ClFN_2OS$: C, 62.42; H, 3.67; N, 7.28; Found: C, 62.49; H, 3.67; N, 7.31 %.

*5.2.3.14. 2-((2-bromo-4-fluorophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**13p**)*

Yield: 56%; MP: 189-191ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.578, 7.561 (d, J=8.5 Hz, 2H, C1, C8-phenothiazine), 7.448-7.430 (dd, J=9, 1.5 Hz, 2H, C4, C5-phenothiazine), 7.345-7.311 (td, J=17, 7.5 Hz, 2H, C2, C7-phenothiazine) 7.255-7.224 (td, J=15.5, 7.5 Hz, 2H, C3, C6-phenothiazine), 7.135 (s, C3-phenyl), 6.821, 6.805 (d, J=8 Hz, 1H, C5-

phenyl), 6.512, 6.497, (d, J=7.5 Hz, 1H, C6-phenyl), 4.180 (s, 2H, methylene CH_2), 3.707 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.731 (C=O), 146.085, 141.727, 135.325, 132.411, 130.319, 128.324, 127.227, 126.210, 124.463, 122.302, 120.694, 115.049 (aromatic carbons), 53.522 (methylene carbon); MS (ESI) *m/z*: 432.6 (49%), 430.4 (50%); Anal. Calcd for $C_{20}H_{14}BrFN_2OS$: C, 55.96; H, 3.29; N, 6.53; Found: C, 56.03; H, 3.35; N, 6.54 %.

*5.2.3.15.2-((3-nitrophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**14p**)*

Yield: 56%; MP: 195-196ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.669, 7.653 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.581-7.562 (dd, J=9.5, 1.5 Hz, 2H, C4, C5-phenothiazine), 7.466-7.432 (td, J=7.5, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.352-7.321 (td, J=6, 1.5 Hz, 2H, C3,C6-phenothiazine), 7.117 (s, 1H, C2-phenyl), 6.737,6.721 (d, J=8 Hz, 1H, C4-phenyl), 6.658, 6.644 (d, J=7 Hz, 1H, C6-phenyl), 6.432-6.400 (t, J= 8.0 Hz, 1H, C5-phenyl), 4.201 (s, 2H, methylene-CH_2), 3.931 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 171.802 (C=O), 148.012, 141.209, 135.218, 134.164, 131.365, 129.432, 127.321, 126.299, 123.627, 120.613, 116.510, 112.388 (aromatic carbons), 53.258 (methylene carbon); MS (ESI) *m/z*: 378.5 (36%); Anal. Calcd for $C_{20}H_{15}N_3O_3S$: C, 63.65; H, 4.01; N, 11.13; Found: C, 63.71; H, 3.98; N, 11.16 %.

*5.2.3.16.2-((4-nitrophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**15p**)*

Yield: 52%; MP: 192-194ºC; ^{1}H-NMR (500 MHz, DMSO-d6) δ; 7.683, 7.668 (d, J=7.5 Hz, 2H, C1, C8-phenothiazine), 7.551, 7.535 (d, J=8 Hz, 2H, C4, C5-phenothiazine), 7.402-7.371 (t, J=7.5 Hz, 2H, C2, C7-phenothiazine), 7.310-7.280 (t, J=7.5 Hz, 2H, C3, C6-phenothiazine), 7.191, 7.174 (d, J=8.5 Hz, 2H, C3, C5-phenyl), 6.864, 6.846 (d, J=9 Hz, 2H, C2, C6-phenyl), 4.153 (s, 2H, methylene-CH_2) 3.395 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 165.717 (C=O), 149.997, 144.825, 138.111, 133.405, 128.353, 127.623, 127.529, 126.743, 115.353 (aromatic carbons), 51.194 (methylene carbon);

MS (ESI) *m/z*: 378.8 (48%); Anal. Calcd for $C_{20}H_{15}N_3O_3S$: C, 63.65; H, 4.01; N, 11.13; Found: C, 63.72; H, 4.02; N, 11.14 %.

*5.2.3.17. 2-((2-chloro-4-nitrophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**16p**)*

Yield: 56%; MP: 201-205ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.202 (s, 1H, C3-phenyl), 7.984-7.961 (dd, J=11.5, 2.5 Hz, 1H, C5-phenyl), 7.592, 7.577 (d, J=7.5 Hz, 2H, C1, C8-phenothiazine), 7.473-7.455 (dd, J=7.5, 1.5 Hz, 2H, C4, C5-phenothiazine), 7.371-7.337 (td, J=8, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.285-7.252 (td, J=9, 1.5 Hz, 2H, C3, C6-phenothiazine), 6.728, 6.710 (d, J=9 Hz, 1H, C6-phenyl), 4.832 (s, 1H, NH), 4.189 (s, 2H, methylene-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 172.463 (C=O), 147.618, 141.241, 136.553, 132.328, 130.432, 128.263, 127.281, 126.642, 124.518, 122.332, 120.174, 116.155 (aromatic carbons), 54.104 (methylene carbon); MS (ESI) *m/z*: 414.7 (25%), 412.6 (74%); Anal. Calcd for $C_{20}H_{14}ClN_3O_3S$: C, 58.33; H, 3.43; N, 10.20; Found: C, 58.41; H, 3.48; N, 10.24 %.

*5.2.3.18. 2-((4-chloro-2-nitrophenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**17p**)*

Yield: 49%; MP: 199-201ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.112 (s, 1H, C3-phenyl), 7.593, 7.577 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.473, 7.459 (d, J=7 Hz, 2H, C4, C5-phenothiazine), 7.372-7.347, (t, J=6 Hz, 2H, C2, C7-phenothiazine), 7.342-7.255 (m, 3H, C3, C6-phenothiazine, C5-phenyl), 6.774, 6.757 (d, J=8.5 Hz, 1H, C6-phenyl), 6.108 (s, 1H, NH), 4.190 (s, 2H, methylene-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 171.961 (C=O), 147.322, 141.752, 137.025, 132.331, 130.584, 128.626, 127.811, 126.426, 124.733, 122.501, 120.227, 116.513 (aromatic carbons), 54.039 (methylene carbon); ESI-MS *m/z*: 414.2 (25%), 412.5 (75%); Anal. Calcd for $C_{20}H_{14}ClN_3O_3S$: C, 58.33; H, 3.43; N, 10.20; Found: C, 58.38; H, 3.46; N, 10.22 %.

*5.2.3.19.2-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)benzoic acid (**18p**)*

Yield: 45%; MP: 165-167ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 12.283 (s, 1H, OH), 7.837, 7.821 (d, J=8 Hz, 1H, C3-phenyl), 7.622, 7.606 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.531-7.516 (d, J=7.5 Hz, 2H, C4, C5-phenothiazine), 7.441-7.426 (td, J=7.5, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.355-7.340 (td, J=7.5, 1.5 Hz, 2H, C3, C6-phenothiazine), 7.244-7.218 (t, J=6.5 Hz, 1H, C5-phenyl), 7.001 -6.918 (m, 2H, C4, C6-phenyl), 4.246 (s, 2H, methylene-CH_2) 3.935 (1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 184.128 (COOH), 170.132 (C=O), 146.626, 141.427, 135.628, 132.521, 130.629, 128.238, 127.442, 126.164, 123.426, 121.621, 119.895, 115.926 (aromatic carbons), 53.811 (methylene carbon); MS (ESI) *m/z*: 377.9 (38%); Anal. Calcd for $C_{21}H_{16}N_2O_3S$: C, 67.01; H, 4.28; N, 7.44; Found: C, 67.10; H, 4.31; N, 7.47 %.

*5.2.3.20. 2-(4-(4-chlorophenyl)piperazin-1-yl)-1-(10H-phenothiazin-10-yl)ethan-1-one (**19p**)*

Yield: 85%; MP: 177-180ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.671, 7.655 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.518, 7.503 (d, J=7.5 Hz, 2H, C4, C5-phenothiazine), 7.434-7.403 (td, J=15.5, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.253-7.220 (td, J=15, 1.5 Hz, 2H, C3, C6-phenothiazine), 7.121, 7.108 (d, J=6.5 Hz, 2H, C3, C5-phenyl), 6.631, 6.613 (d, J=9 Hz, 2H, C2, C6-phenyl), 4.135 (s, 2H, methylene-CH_2) 3.212-3.188 (m, 4H, piperazine); 2.712-2.689 (m, 4H, piperazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.731 (C=O), 145.549, 139.438, 134.726, 131.461, 129.612, 127.742, 126.514, 125.843, 122.472, 115.857 (aromatic carbons), 53.948 (methylene carbon); 52.482, 50.791 (piperazine carbons); MS (ESI) *m/z*: 438.6 (25%), 436.3 (74%); Anal. Calcd for $C_{24}H_{22}ClN_3OS$: C, 66.12; H, 5.09; N, 9.64; Found: C, 66.19; H, 5.15; N, 9.67 %.

*5.2.3.21. 2-(4-(4-nitrophenyl)piperazin-1-yl)-1-(10H-phenothiazin-10-yl)ethan-1-one (**20p**)*

Yield: 77%; MP: 181-182ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.690, 7.674 (d, J=8 Hz, 2H, C1, C8-phenothiazine), 7.527, 7.510 (d, J=8.5 Hz, 2H, C4, C5-phenothiazine), 7.421-7.389 (td, J=16, 1.5 Hz, 2H, C2, C7-phenothiazine), 7.234-7.204 (td, J=15, 1.5 Hz, 2H, C3, C6-phenothiazine), 7.160, 7.146 (d, J=7 Hz, 2H, C3, C5-phenyl), 6.625, 6.607 (d, J=9 Hz, 2H, C2, C6-phenyl), 4. 182 (s, 2H, methylene-CH_2) 3.225-3.201 (m, 4H, piperazine); 2.715-2.692 (m, 4H, piperazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.769 (C=O), 146.128, 140.432, 135.583, 131.728, 129.835, 127.934, 126.623, 125.782, 122.681, 116.125 (aromatic carbons), 54.263 (methylene carbon); 53.573, 51.382 (piperazine carbons); MS (ESI) *m/z*: 447.71 (42%); Anal. Calcd for $C_{24}H_{22}N_4O_3S$: C, 64.56; H, 4.97; N, 12.55; Found: C, 64.65; H, 5.00; N, 12.57 %.

*5.2.3.22. 2-(cyclopropylamino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**S1**)*

Yield: 82%; MP:115-117ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 7.562, 7.547 (d, J = 7.5 Hz, 2H, Ar-CH), 7.434, 7.418 (d, J = 8 Hz, 2H, Ar-CH), 7.303-7.268 (t, J = 8 Hz, 2H, Ar-CH), 7.222-7.192 (t, J = 7.5 Hz, 2H, Ar-CH), 3.705 (s, 2H, methylene-CH_2), 3.102 (s, 1H, NH), 1.512-0.998 (m, 5H, cyclopropyl); ^{13}C NMR (125 MHz, DMSO-d6) δ (ppm): 169.191 (C=O), 138.154, 133.031, 127.802, 127.175, 126.925 (aromatic carbons), 55.316 (methylene carbon), 35.076, 7.111 (cycloproyl carbons); ESI-MS (m/z) = 297.5 (26%). Anal. Calcd for $C_{17}H_{16}N_2OS$: C, 68.89; H, 5.44; N, 9.45; Found: C, 69.07; H, 5.47; N, 9.43 %.

*5.2.3.23. 2-(cyclobutylamino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**S2**)*

Yield: 75%; MP: 119-121ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 7.566,7.550 (d, J=8 Hz, 2H, Ar-CH), 7.531,7.513 (dd, J=8, 1.5 Hz, 2H, Ar-CH), 7.370-7.339 (t, J=8 Hz, 2H, Ar-CH), 7.293-7.261 (td, J= 7.5, 1 Hz, 2H, Ar-CH), 3.986 (s, 2H, methylene-CH_2), 3.398 (s, 1H, NH), 1.638-1.214 (m, 7H, cyclobutyl); ^{13}C NMR (125 MHz,

DMSO-d6) δ (ppm): 169.191 (C=O), 138.154, 132.032, 127.802, 127.175, 126.925, (aromatic carbons), 55.316 (methylene carbon), 35.076, 14.192, 7.111 (cyclobutyl carbons); ESI-MS (m/z) = 311.8 (25%). Anal. Calcd for $C_{18}H_{18}N_2OS$: C, 69.65; H, 5.85; N, 9.02; Found:C, 69.78; H, 5.87; N, 8.98 %.

*5.2.3.24. 2-(cyclopentylamino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**S3**)*

Yield: 78%; MP:124-126ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm):7.712,7.684 (d, J=15, 2H, Ar-CH), 7.654,7.638 (d, J=8, 2H, Ar-CH), 7.488-7.458 (t, J=7.5, 2H, Ar-CH), 7.412-7.382 (t, J=8, 2H, Ar-CH), 3.957 (s, 2H, methylene CH_2), 3.450 (s, 1H, NH), 1.842-1.145 (m, 9H, cyclopentyl); ^{13}C-NMR (125 MHz, DMSO-d6) δ (ppm): 170.213 (C=O), 141.287, 134.011, 130.416, 129.752, 129.614 (aromatic carbons), 62.981 (methylene carbon), 51.467, 32.371, 27.213 (cyclopentyl carbons); ESI-MS (m/z) = 325.4 (25%). Anal. Calcd for $C_{19}H_{20}N_2OS$: C, 70.34; H, 6.21; N, 8.63; Found: C, 70.58; H, 6.22; N, 8.65 %.

*5.2.3.25. 2-(cyclohexylamino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**S4**)*

Yield: 85%; MP: 127-128ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.554, 7.539 (d, J = 7.5 Hz, 2H, Ar-CH), 7.426, 7.410 (d, J = 8 Hz, 2H, Ar-CH), 7.295-7.260 (t, J = 10 Hz, 2H, Ar-CH), 7.214-7.184 (t, J = 10 Hz, 2H, Ar-CH), 3.685 (s, 2H, methylene-CH_2), 3.245 (s, 1H, NH), 1.942-1.036 (m, 11H, cyclohexyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 168.713 (C=O), 137.111, 133.841, 126.336, 125.648, 125.492 (aromatic carbons), 58.251 (methylene carbon), 48.152, 31.474, 24.598, 22.111 (cyclohexyl carbons); ESI-MS (m/z) = 339.2 (26%). Anal. Calcd for $C_{20}H_{22}N_2OS$: C, 70.97; H, 6.55; N, 8.28; Found: C, 71.22; H, 6.56; N, 8.24 %.

*5.2.3.26.2-(cycloheptylamino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**S5**)*

Yield: 71%; MP: 132-135ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 7.678,7.662 (d, J=8 Hz, 2H, Ar-CH), 7.643,7.625 (dd, J=8, 3.5 Hz, 2H, Ar-CH), 7.482-7.451 (t, J=8

Hz, 2H, Ar-CH), 7.405-7.373 (td, J= 7.5, 1 Hz, 2H, Ar-CH), 3.898 (s, 2H, methylene-CH_2), 3.510 (s, 1H, NH), 2.138-1.088 (m, 13H, cycloheptyl); ^{13}C NMR (125 MHz, DMSO-d6) δ (ppm): 171.038 (C=O), 139.726, 134.001, 128.961, 128.305, 128.068 (aromatic carbons), 54.735 (methylene carbon), 46.572, 31.118, 25.829, 21.324 (cycloheptyl carbons); ESI-MS (m/z) = 353.1 (25%). Anal. Calcd for $C_{21}H_{24}N_2OS$: C, 71.56; H, 6.86; N, 7.95; Found: C, 71.68; H, 6.88; N, 7.92 %.

*5.2.3.27.2-(cyclooctylamino)-1-(10H-phenothiazin-10-yl)ethan-1-one (**S6**)*

Yield: 66%; MP:139-141ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 7.632, 7.617 (d, J = 7.5 Hz, 2H, Ar-CH), 7.504, 7.488 (d, J = 8 Hz, 2H, Ar-CH), 7.373-7.338 (t, J = 10 Hz, 2H, Ar-CH), 7.292-7.262 (t, J = 10 Hz, 2H, Ar-CH), 3.986 (s, 2H, methylene-CH_2), 3.672 (s, 1H, NH), 2.356-1.108 (m, 15H, cyclooctyl); ^{13}C NMR (125 MHz, DMSO-d6) δ (ppm): 171.691 (C=O), 140.032, 133.893, 130.010, 129.297, 129.008 (aromatic carbons), 57.868 (methylene carbon), 49.692, 35.165, 25.790, 24.137, 21.234 (cyclooctyl carbons); ESI-MS (m/z) = 367.3 (26%). Anal. Calcd for $C_{22}H_{26}N_2OS$: C, 72.09; H, 7.15; N, 7.64; Found: C, 72.24; H, 7.16; N, 7.62 %.

5.2.4. Antitubercular screening

All the synthesized compounds were screened against *Mtb* (H37Rv) by using Micro-plate Alamar Blue assay (MABA) [165] and the result is presented in **Table 5-7**. Structure-activity relationship (SAR) was established from the results of antitubercular screening. The nitro compounds were found to be more active among all the derivatives. Further, the *meta*- and *para*-substituted nitro compounds **(14p, 15p, 16p)** showed better activity (1.56µg/ml) in comparison to *ortho*-substituted compound **(17p)** (3.13µg/ml). The *meta*-substituted halogen compounds (**10p-12p)** were found to show better activity in comparison to *ortho*- or *para*-substituted halogen compound **(13p)**. The number of halogen substitution seems to play a significant role, as the di-

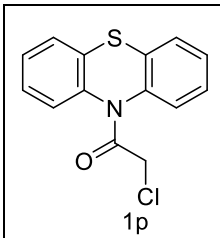
substituted halogen compounds **(10p to 13p)** produced better activity than mono substituted. The electron withdrawing nature of halogen and nitro groups seems to be important for the activity of the compounds. In compounds **19p** and **20p**, the acyl linker was replaced with piperazinyl linker that caused reduction in activity. Further, the compound with nitro substitution at *para*-position (**19p)** was found more active in comparison to chloro substitution (**20p)**. It indicated that the piperazinyl linker might not have major role in improving the activity.

5.2.5. Antibacterial screening

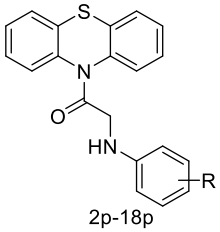
The compounds were also screened for antibacterial activity by disc diffusion method [166]. The compounds **(14p, 15p, 16p)** displayed maximum inhibition against *S. aureus* and *E. coli* at MIC of 0.98µg/ml and 3.91µg/ml, respectively (**Table 5-7**). The activity was better against *S. aureus* in comparison to *E. coli* and *Mtb*. The nitro substituted compounds **(14p, 15p, 16p)** were more active in comparison to halogen substituted compounds **(3p to 13p)** and also the *para*- and *meta*-substitution was found essential to produce antibacterial activity.

5.2.6. BBB permeability screening

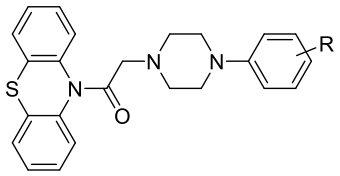
BBB permeability determines the possible CNS effect of the compounds and all the newly designed phenothiazine derivatives were screened for BBB permeability by Parallel artificial membrane permeability assay (PAMPA). The permeability of the compounds was compared with commercial drugs and classified as high permeable (CNS+), low permeable (CNS-) and permeable uncertain (CNS+/-). The chlorpromazine and diazepam produced effective permeability (Pe) of 6.1 $x10^{-6}$ and 12.4 $x10^{-6}$ cm/s respectively and were classified as high permeable drugs, whereas, atenolol, verapamil and levofloxacin produced effective permeability of 1.1 $x10^{-6}$, 0.0 and 0.0 cm/s respectively and classified as low permeable drugs.



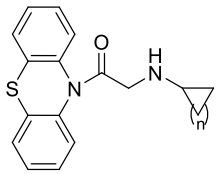
1p



2p-18p



19p, 20p



n =1,2,3,4,5,6
S1-S6

Code	R/n	MIC in µg/mL (µM) [a]	MIC in µg/mL (µM) [b]	MIC in µg/mL (µM) [c]
1p	-	62.50 (226.65)	62.50 (226.65)	50 (181.32)
2p	H	15.63 (47.01)	31.25 (94.00)	25 (75.20)
3p	2-Cl	15.63 (46.39)	31.25 (92.76)	25 (74.21)
4p	3-Cl	7.81 (23.18)	31.25 (92.76)	12.5 (37.10)
5p	4-Cl	7.81 (23.18)	31.25 (92.76)	12.5 (37.10)
6p	3-F	7.81 (22.28)	31.25 (89.18)	12.5 (35.67)
7p	4-F	7.81 (22.28)	31.25 (89.18)	12.5 (35.67)
8p	3-Br	7.81 (18.98)	31.25 (75.97)	12.5 (30.38)
9p	4-Br	7.81 (18.98)	31.25 (75.97)	12.5 (30.38)
10p	3,5-diCl	3.91 (9.74)	7.81 (19.46)	6.25 (15.57)
11p	3,5-diBr	3.91 (7.97)	7.81 (15.93)	6.25 (12.74)
12p	3-Cl,4-F	3.91 (10.15)	7.81 (20.29)	6.25 (16.24)
13p	2-Br,4-F	3.91 (9.10)	15.63 (36.40)	6.25 (14.55)
14p	3-NO_2	0.98 (2.59)	3.91 (10.35)	1.56 (4.13)
15p	4-NO_2	0.98 (2.59)	3.91 (10.35)	1.56 (4.13)
16p	2-Cl,4-NO_2	0.98 (2.37)	3.91 (9.49)	1.56 (3.78)
17p	2-NO_2,4-Cl	1.96 (4.75)	15.63 (37.94)	3.13 (7.59)
18p	2-COOH	7.81 (20.74)	15.63 (41.52)	12.5 (33.20)
19p	4-Cl	7.81 (17.91)	15.63 (35.85)	12.5 (28.67)
20p	4-NO_2	3.91 (8.75)	7.81 (17.49)	3.13 (7.00)
S1	1	31.25 (105.43)	125 (421.74)	25 (84.34)
S2	2	15.63 (50.35)	62.50 (201.34)	25 (80.53)
S3	3	7.81 (24.07)	62.50 (192.63)	12.5 (38.52)
S4	4	3.90 (11.52)	31.25 (92.32)	12.5 (36.93)
S5	5	7.81 (22.15)	62.50 (117.30)	25 (70.92)
S6	6	15.63 (42.64)	125 (341.04)	50 (136.41)
Chlorpromazine	-	7.81 (24.49)	15.63 (49.01)	12.5 (39.20)
Ciprofloxacin	-	1.95 (5.88)	3.91 (11.80)	3.13 (9.44)
Pyrazinamide	-	-	-	3.13 (25.42)

Table 5-7: Antitubercular and antibacterial activities of phenothiazine derivatives

[a] Minimum Inhibitory Concentration (MIC) against *S. aureus* (ATCC 25323)

[b] Minimum Inhibitory Concentration (MIC) against *E. coli* (ATCC35218)

[c] Minimum Inhibitory Concentration (MIC) against *M. tuberculosis* H37Rv (ATCC 27294)

The effective permeability (Pe) of the screened compounds (**1p to 18p**) was found in the range of 2.8 $x10^{-6}$ to 3.8 $x10^{-6}$ cm/s and were classified as permeability uncertain. The compounds **19p** and **20p** produced effective permeability (Pe) of 4.5 $x10^{-6}$ and 4.2 $x10^{-6}$ cm/s respectively and were classified as permeability high (**Table 5-8**). The reduced BBB permeability could reduce the CNS effect of the developed compounds (**1p to 18p**) in comparison to chlorpromazine.

5.2.7. In-vitro *cytotoxicity screening*

The antimicrobial drugs should be free from toxicity towards normal mammalian cells. Therefore, all the compounds were screened against VERO (monkey kidney epithelial) cells to check their toxicity. The concentration required to produce 50% inhibition (CC_{50}) was determined and was found in the range of 84.7 to 201.8μg/mL (**Table 5-9**). All the compounds were found to be non-toxic to mammalian cells. The selectivity of drug molecules towards a desired activity is a challenging task in drug discovery. The compounds should be toxic only towards *Mtb* and other microbial species rather than the normal human cells. The selectivity index (SI) was calculated from the obtained mammalian cell cytotoxicity (CC_{50}) and tubercular cytotoxicity (MIC). The potent compounds (**14p, 15p & 16p**) from antitubercular screening showed SI more than 57, indicating their selectivity towards *Mtb* rather against normal human cells.

Compound Code	P_e x10^{-6} cm/s	Classification
Chlorpromazine	6.1±0.059	CNS+
Atenolol	1.1±0.021	CNS-
Verapamil	0.0	CNS-
Diazepam	12.4±0.263	CNS+
Levofloxacin	0.0	CNS-
1p	2.8±0.038	CNS+/-
2p	3.0±0.041	CNS+/-
3p	3.2±0.167	CNS+/-
4p	3.3±0.062	CNS+/-
5p	3.2±0.251	CNS+/-
6p	3.0±0.040	CNS+/-
7p	3.0±0.174	CNS+/-
8p	3.5±0.262	CNS+/-
9p	3.5±0.386	CNS+/-
10p	3.6±0.158	CNS+/-
11p	3.8±0.053	CNS+/-
12p	3.6±0.326	CNS+/-
13p	3.8±0.213	CNS+/-
14p	3.4±0.091	CNS+/-
15p	3.4±0.023	CNS+/-
16p	3.7±0.291	CNS+/-
17p	3.7±0.082	CNS+/-
18p	3.2±0.109	CNS+/-
19p	4.5±0.080	CNS+
20p	4.2±0.315	CNS+
S3	4.9±0.117	CNS+
S4	5.2±0.382	CNS+

Table 5-8: BBB permeability of commercial drugs and phenothiazine derivatives

Data are expressed as mean ± SEM (n =3)

CNS+ = high BBB permeation compounds, *i.e.* Pe= > 4.0 * 10^{-6} cm s^{-1}

CNS- =low BBB permeation compounds, *i.e.* Pe= < 2.0 * 10^{-6} cm s^{-1}

CNS+/- =BBB permeation uncertain compounds, *i.e.* Pe =4.0 - 2.0 *10^{-6} cm s^{-1}

Compound Code	CC_{50} (µg/ml) [a]	Selectivity Index [b]
1p	201.82±2.476	4.0
2p	164.35±2.891	6.6
3p	166.91±9.739	6.7
4p	154.43±1.924	12.3
5p	155.07±1.563	12.4
6p	160.97±10.615	12.9
7p	157.52±6.291	12.6
8p	153.28±10.860	12.3
9p	124.75±2.132	10.0
10p	129.20±2.728	20.7
11p	117.64±4.945	18.8
12p	120.12±2.974	19.2
13p	115.85±1.846	18.5
14p	95.91±1.663	61.47
15p	90.18±2.781	57.8
16p	97.34±5.126	62.4
17p	92.76±5.370	29.6
18p	108.94±4.212	8.7
19p	89.52±2.618	7.1
20p	84.71±1.331	27.0
S3	117.80±2.471	9.4
S4	102.33±4.057	16.4

Table 5-9: Cytotoxicity of phenothiazine derivatives against VERO cells; [a] Data are expressed as mean ± SEM (n =3); [b] Selectivity index is ratio of cytotoxicity (CC_{50}) to *Mtb* MIC.

5.2.8. NDH-2 inhibitory screening

The compounds (**10p** to **17p**) with strong growth inhibition against the whole *Mtb* H37Rv were screened for NDH-2 inhibitory action to establish their molecular mechanism. NDH-2 inhibition screening was performed through NADH:menadione oxidoreduction assay. Percentage inhibition of residual NADH oxidation activity was recorded at 50µM concentrations. Compounds **16p, 15p, 13p** and **17p** produced 30.09, 28.42, 26.85 and 24.56 percent inhibitions respectively. Rest of the compounds produced ≤20 percent inhibitions at that concentration (**Table 5-10**). Percentage inhibition of all the test compounds was found to be less than 50 percent at 50µM concentrations. Hence, the IC_{50} was recorded as greater than 50 micromolar (>50µM).

Compound Code	% inhibition of residual NADH oxidation activity at 50µM concentration
10p	7.42±0.602
11p	15.43±1.326
12p	8.28±0.101
13p	26.85±2.232
14p	20.71±1.119
15p	28.42±1.916
16p	30.09±1.925
17p	24.56±1.028
HQNO	$82.8\pm0.524^{x,y,z,@,\#,\$,\%,\&}$

Table 5-10: NDH-2 inhibitory study of phenothiazine derivatives. Data expressed as a Mean ±SEM (n=3). $^{x}P< 0.05$ compared to 10p, $^{y}P< 0.05$ compared to 11p, $^{z}P< 0.05$ compared to 12p, $^{@}P< 0.05$ compared to 13p, $^{\#}P< 0.05$ compared to 14p, $^{\$}P< 0.05$ compared to 15p, $^{\%}P< 0.05$ compared to 16p, $^{\&}P< 0.05$ compared to 17p [One-way ANOVA followed by Newmann-Keuls test].

5.3. Section 3: Molecular Docking, Synthesis, Characterization and Biological Profiling of Carbazole derivatives

5.3.1. Extra precision molecular docking

Docking was performed on the protein structure of *Mtb* NDH-2 that was obtained through homology modeling. The inhibitors can cease the function of NDH-2 when bind only to the quinone binding motif. All the molecules in the study reached the quinone binding tunnel and produced the essential interactions with the amino acid residues forming the quinone binding motif "333-AQxAxQ-338". Hydrogen bond interaction was observed with Ala-333, a critical residue in quinone binding (**Figure 5-7, Table 5-11**). The docking scores, observed in the range of -6.1925 to -3.6134 Kcal/mol, are indicator of effective binding of inhibitors to the binding motif. The best binding was observed with the molecules **15c**, **16c** and **17c** with minimum binding energy of -6.1925, -6.1912 and -6.0253 Kcal/mol, respectively. A better correlation between the docking score and antitubercular MIC was observed, which indicates that the antitubercular activity may be due to the arrest of ATP synthesis by the inhibition of NDH-2.

5.3.2. Molecular property and toxicity prediction

The molecular property and toxicity of designed molecules were predicted by OSIRIS DataWarrior. The clogP of the compounds was found in the range of 2.19 to 5.36. The clogP of the potent compounds (**14c, 15c, 16c, 17c**) was impressive at 2.99, 2.99, 3.59 and 3.59, respectively and this optimum clogP could have increased biological activities of the compounds. Topological polar surface area (TPSA) was found to be 34.03 for compounds **2c** to **13c**, 79.85 for compounds **14c** to **17c,** and 71.33, 28.48 and 74.3 for the compounds **18c**, **19c** and **20c,** respectively.

Ligand code	**Lowest binding energy (Kcal/mol)**	**Residues forming the quinone binding motif (residues producing H-bonding with the ligand were indicated in bold)**
1c	-4.0123	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
2c	-4.3882	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
3c	-4.2916	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
4c	-4.3974	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
5c	-4.5590	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
6c	-4.3718	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
7c	-4.5326	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
8c	-4.3183	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
9c	-4.5679	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
10c	-4.9368	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
11c	-4.9734	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
12c	-5.0003	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
13c	-4.9668	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
14c	-5.8362	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
15c	-6.1925	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
16c	-6.1912	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
17c	-6.0253	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
18c	-4.6294	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
19c	-3.6134	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
20c	-3.9251	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S13	-1.9614	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S14	-1.9938	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S15	-2.0168	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S16	-2.0411	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S17	-2.0035	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S18	-1.9471	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)

Table 5-11: Docking of designed carbazole molecules against NDH-2 of *Mtb*

All the compounds showed above 83 percent of absorption, which is an indicator of their good oral bioavailability. The number of hydrogen bond donor, hydrogen bond acceptor and drug likeness value were also found within the limit **(Table 5-12)**.

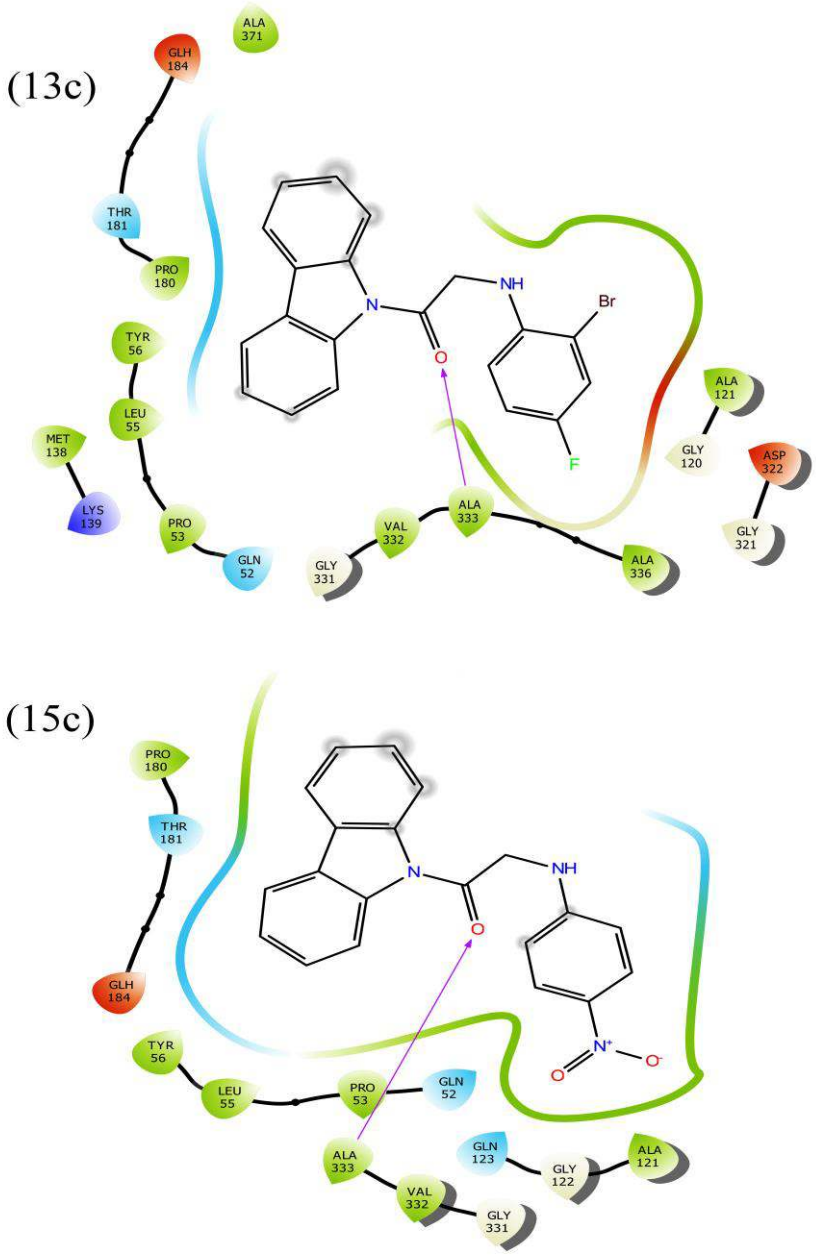


Figure 5-7: Docking poses of molecules 13c and 15c against NDH-2

Code	cLogP	cLogS	HBA	HBD	TSA	TPSA	DL	MU	TU	IR	SI	MF	MC	EA	RB	NR	AR	SA
1c	3.40	-4.12	2	0	211	22	1.25	H	H	H	0.47	0.33	0.78	3	2	3	3	6
2c	3.91	-4.71	3	1	258	34.03	2.28	N	N	N	0.52	0.44	0.79	3	4	4	4	8
3c	4.51	-5.45	3	1	271	34.03	2.33	N	N	N	0.5	0.43	0.81	4	4	4	4	6
4c	4.51	-5.45	3	1	287	34.03	2.33	N	N	N	0.5	0.46	0.80	4	4	4	4	6
5c	4.51	-5.45	3	1	300	34.03	2.33	N	N	N	0.54	0.46	0.80	4	4	4	4	8
6c	4.01	-5.03	3	1	269	34.03	0.94	N	N	N	0.5	0.46	0.80	4	4	4	4	6
7c	4.01	-5.03	3	1	283	34.03	0.94	N	N	N	0.54	0.46	0.80	4	4	4	4	8
8c	4.63	-5.55	3	1	295	34.03	0.49	N	N	N	0.5	0.46	0.80	4	4	4	4	6
9c	4.63	-5.55	3	1	286	34.03	0.49	N	N	N	0.54	0.46	0.80	4	4	4	4	8
10c	5.12	-6.18	3	1	294	34.03	2.33	N	N	N	0.48	0.46	0.81	5	4	4	4	9
11c	5.36	-6.38	3	1	315	34.03	0.49	N	N	N	0.48	0.46	0.81	5	4	4	4	9
12c	4.62	-5.76	3	1	298	34.03	0.99	N	N	N	0.52	0.46	0.82	5	4	4	4	6
13c	4.73	-5.86	3	1	285	34.03	-0.84	N	N	N	0.52	0.3	0.82	5	4	4	4	6
14c	2.99	-5.17	6	1	286	79.85	-2.87	N	N	N	0.5	0.49	0.82	6	5	4	4	6
15c	2.99	-5.17	6	1	290	79.85	-2.87	N	N	N	0.53	0.49	0.81	6	5	4	4	8
16c	3.59	-5.91	6	1	321	79.85	-2.81	N	N	N	0.51	0.45	0.84	7	5	4	4	6
17c	3.59	-5.91	6	1	286	79.85	-2.81	N	N	N	0.48	0.46	0.84	7	5	4	4	6
18c	3.39	-4.73	5	2	280	71.33	2.12	N	N	N	0.46	0.46	0.82	5	5	4	4	6
19c	4.65	-4.99	4	0	338	28.48	6.43	N	N	H	0.55	0.46	0.82	5	4	5	4	10
20c	2.19	-4.72	7	0	346	74.3	1.23	N	N	H	0.54	0.48	0.83	7	5	5	4	10
S13	2.73	-3.97	3	1	252	34.03	2.80	N	N	N	0.5	0.41	0.78	3	4	4	3	7
S14	3.07	-4.24	3	1	261	34.03	1.76	N	N	N	0.52	0.41	0.79	3	4	4	3	7
S15	3.41	-4.51	3	1	251	34.03	0.09	N	N	N	0.5	0.40	0.79	3	4	4	3	8
S16	3.76	-4.78	3	1	268	34.03	-2.74	N	N	L	0.52	0.43	0.79	3	4	4	3	8
S17	4.10	-5.05	3	1	285	34.03	-2.27	N	N	N	0.5	0.44	0.79	3	4	4	3	9
S18	5.17	-5.32	3	1	292	34.03	-2.44	N	N	N	0.52	0.46	0.79	3	4	3	3	9

Table 5-12: Molecular properties and predicted toxicity of carbazole molecules; HBA= H-Acceptors; HBD= H-Donor; TSA=Total surface area; TPSA= Total Polar surface area; DL= Drug likeness; MU=Mutagenic; TU=Tumorigenic; IR=Irritant; SI=Shape Index; MF=Molecular Flexibility; MC=Molecular Complexity; EA=Electronegative Atoms; RB=Rotatable Bonds; AR=Aromatic Rings; SA=Symmetric atoms; NR=No. of Rings; N=none; H=High; L=low

5.3.3. Synthesis and Characterization

The designed carbazole molecules were synthesized as shown in **Scheme 2**, involving the synthesis of first intermediate carbazole **(c)** followed by the second intermediate 1-(9H-carbazol-9-yl)-2-chloroethan-1-one **(1c)**. The reaction of second intermediate (**1c**) with different phenyl amines/phenyl piperazines/ cyclic amines afforded the final derivatives of carbazole. The progress of reaction was closely monitored and completion was established by thin layer chromatography. Column chromatography was performed to get pure derivatives of carbazole. The yield of the compounds was found in the range of 45 to 79 percent. Melting point, elemental composition, ^{1}H NMR, ^{13}C NMR and Mass data of all the compounds in carbazole series are as follows,

*5.3.3.1. Synthesis of Carbazole (**c**)*

Yield: 75%; MP: 245-246°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.127, 8.111 (d, J = 8 Hz, 2H, C1, C8-carbazole), 7.569, 7.753 (dd, J = 8, 3.5 Hz, 2H, C4, C5- carbazole), 7.471-7.456 (td, J=7.5, 1.5 Hz, 2H, C2, C7- carbazole), 7.132-7.118 (td, J=7, 1 Hz, 2H, C3, C6- carbazole); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 139.755, 126.055, 123.631, 120.545, 119.681, 110.792; MS (ESI) m/z: 168.3 (100%). Anal. Calcd for $C_{12}H_9N$: C, 86.20; H, 5.43; N, 8.38; Found: C, 86.48; H, 5.44; N, 8.35 %.

*5.3.3.2. Synthesis of 1-(9H-Carbazol-9-yl)-2-chloroethan-1-one (**1c**)*

Yield: 40%; MP: 102-103°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.183 (d, J = 8.4 Hz, 2H, C1, C8-carbazole), 7.523-7.501 (dd, J =11, 1.5 Hz, 2H, C4, C5-carbazole), 7.436-7.390 (td, J =11.5, 1.5 Hz, 2H, C2, C7-carbazole), 7.213-7.184 (td, J=7.5, 1.1 Hz, 2H, C3, C6-carbazole), 4.855 (s, 2H, methylene CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.68, 138.02, 127.71, 126.73, 124.43, 120.03, 116.24 (aromatic carbons) 44.85 (methylene carbon); MS (ESI) m/z: 246.5 (25%), 244.5 (75%). Anal. Calcd for $C_{14}H_{10}ClNO$: C, 69.00; H, 4.14; N, 5.75; Found: C, 69.23; H, 4.16; N, 5.69 %.

*5.3.3.3. 1-(9H-carbazol-9-yl)-2-(phenylamino)ethan-1-one (**2c**)*

Yield: 64%; MP: 151-152°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm); 7.927, 7.911 (d, J=7.5 Hz, 2H, C1, C8-carbazole), 7.816, 7.801 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7. 329-7.271 (td, J=14, 7.5 Hz, 2H, C2, C7- carbazole), 7.194-7.138 (td, J=14, 7.5 Hz, 2H, C3, C6- carbazole), 7.109-7.093 (t, J=8 Hz, 2H, C3, C5-phenyl), 6.815-6.799 (t, J=8 Hz, 2H, C2, C6-phenyl), 6.631-6.615 (t, J=8 Hz, 1H, C4-phenyl), 4.121 (s, 2H, methylene-CH_2), 3.491 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.422 (C=O), 145.128, 138.691, 135.748, 130.914, 128.216, 127.134, 126.659, 123.205, 120.358, 115.425 (aromatic carbons), 54.351 (methylene carbon); MS (ESI) *m/z*: 301.1 (39.4%); Anal. Calcd for $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33; Found: C, 80.11; H, 5.41; N, 9.37 %.

*5.3.3.4. 1-(9H-carbazol-9-yl)-2-((2-chlorophenyl)amino)ethan-1-one (**3c**)*

Yield: 62%; MP: 149-151ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.941, 7.925 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.826,7.801 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7.344-7.311 (td, J=7.5, 1.5 Hz, 2H, C2, C7- carbazole), 7.142-7.109 (m, 3H, C3, C6- carbazole, C3-phenyl), 7.155-7.129 (t, J=6.5 Hz, 1H, C4-phenyl), 6.721, 6.704 (d, J=8.5 Hz, 1H, C6-phenyl), 6.661-6.635 (t, J=6.5 Hz, 1H, C5-phenyl), 4. 123 (s, 2H, methylene-CH_2) 3.526 (s, 1H, NH) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.595 (C=O), 145.436, 139.961, 135.761, 130.926, 130.164, 128.221, 127.523, 126.421, 123.270, 121.617, 119.862, 114.438 (aromatic carbons), 54.415 (methylene carbon); MS (ESI) *m/z*: 337.2 (25%), 335.1 (74%); Anal. Calcd for $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 8.37; Found: C, 71.81; H, 4.55; N, 8.37 %.

*5.3.3.5. 1-(9H-carbazol-9-yl)-2-((3-chlorophenyl)amino)ethan-1-one (**4c**)*

Yield: 65%; MP: 154-156ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.136, 8.120 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.931-7.912 (dd, J=9.5, 1.5 Hz, 2H, C4, C5- carbazole), 7.326-7.292 (td, J=7.5, 1.5 Hz, 2H, C2, C7- carbazole), 7.159-7.128 (td, J=6, 1.5 Hz, 2H, C3,C6- carbazole), 6.998 (s, 1H, C2-phenyl), 6.751, 6.735 (d, J=8 Hz, 1H, C4-phenyl), 6.592-6.564 (t, J=7 Hz, 1H, C5-phenyl), 6.398, 6.382 (d, J= 8 Hz, 1H, C6-phenyl), 4.201 (s, 2H, methylene-CH_2), 3.762 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.194 (C=O), 146.092, 140.125, 134.916, 135.851, 130.473, 128.291, 127.132, 126.253, 123.277, 119.854, 114.973, 112.325 (aromatic carbons), 53.526 (methylene-CH_2); MS (ESI) *m/z*: 337.4 (24%), 335.2 (74%); Anal. Calcd for $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 8.37; Found: C, 71.83; H, 4.56; N, 8.36 %.

*5.3.3.6. 1-(9H-carbazol-9-yl)-2-((4-chlorophenyl)amino)ethan-1-one (**5c**)*

Yield: 67%; MP: 154-155ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.118, 8.102 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.957, 7.942 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7.413-7.380 (td, J=15, 1.5 Hz, 2H, C2, C7- carbazole), 7.137-7.104 (td, J=15, 1.5 Hz, 2H, C3, C6-carbazole), 7.115, 7.102 (d, J=6.5 Hz, 2H, C3, C5-phenyl), 6.625, 6.607 (d, J=8.5 Hz, 2H, C2, C6-phenyl), 4.189 (s, 2H, methylene-CH_2) 3.661 (1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.932 (C=O), 145.736, 139.235, 133.873, 130.579, 128.461, 127.992, 126.931, 125.134, 122.119, 115.174 (aromatic carbons), 54.981 (methylene carbon); MS (ESI) *m/z*: 337.3 (24%), 335.3 (75%); Anal. Calcd for $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 8.37; Found: C, 71.82; H, 4.51; N, 8.39 %.

*5.3.3.7. 1-(9H-carbazol-9-yl)-2-((3-fluorophenyl)amino)ethan-1-one (**6c**)*

Yield: 61%; MP: 155-156ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.029, 8.013 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.943-7.924 (dd, J=9.5, 1.5 Hz, 2H, C4, C5- carbazole), 7.383-7.349 (td, J=7.5, 1.5 Hz, 2H, C2, C7- carbazole), 7.153-7.122 (td, J=6, 1.5 Hz, 2H, C3,

C6-carbazole), 7.012 (s, 1H, C2-phenyl), 6.732, 6.716 (d, J=8 Hz, 1H, C4-phenyl), 6.621-6.593 (t, J=7 Hz, 1H, C5-phenyl), 6.412, 6.396 (d, J= 8 Hz, 1H, C6-phenyl), 4.201 (s, 2H, methylene CH_2), 3.823 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.371 (C=O), 146.097, 140.983, 135.692, 134.321, 130.657, 128.239, 126.984, 126.186, 124.903, 120.004, 115.735, 112.364 (aromatic carbons), 54.299 (methylene carbon); MS (ESI) *m/z*: 319.2 (78%); Anal. Calcd for $C_{20}H_{15}FN_2O$: C, 75.46; H, 4.75; N, 8.80; Found: C, 75.55; H, 4.77; N, 8.83 %.

*5.3.3.8. 1-(9H-carbazol-9-yl)-2-((4-fluorophenyl)amino)ethan-1-one (**7c**)*

Yield: 61%; MP: 157-159ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.176, 8.160 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.983, 7.968 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7.351-7.316 (td, J=15, 1.5 Hz, 2H, C2, C7- carbazole), 7.146-7.113 (td, J=15, 1.5 Hz, 2H, C3, C6-carbazole), 7.093, 7.079 (d, J=7 Hz, 2H, C3, C5-phenyl), 6.691, 6.673 (d, J=8.5 Hz, 2H, C2, C6-phenyl), 4. 105 (s, 2H, methylene-CH_2) 3.701 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.943 (C=O), 145.532, 138.443, 133.519, 130.563, 128.904, 127.527, 126.781, 125.212, 122.654, 115.181 (aromatic carbons), 54.397 (methylene carbon); MS (ESI) *m/z*: 319.4 (75%); Anal. Calcd for $C_{20}H_{15}FN_2O$: C, 75.46; H, 4.75; N, 8.80; Found: C, 75.52; H, 4.74; N, 8.81 %.

*5.3.3.9. 2-((3-bromophenyl)amino)-1-(9H-carbazol-9-yl)ethan-1-one (**8c**)*

Yield: 52%; MP: 163-165ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.132, 8.117 (d, J=7.5 Hz, 2H, C1, C8-carbazole), 7.948-7.920 (dd, J=6, 1.5 Hz, 2H, C4, C5-carbazole), 7.351-7.218 (m, 4H, C2, C3, C6, C7-carbazole), 6.939 (s, 1H, C2-phenyl), 6.824-6.807 (d, J=8.5 Hz, 1H, C4-phenyl), 6.703-6.673 (t, J=7.5 Hz, 1H, C5-phenyl), 6.536-6.520 (dd, J=8, 1.5 Hz, 1H, C6-phenyl), 4.192 (s, 2H, methylene-CH_2), 3.761 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.393 (C=O), 148.327, 142.394, 136.725, 134.903, 131.695, 128.328, 127.101, 126.139, 124.245, 120.863, 115.993, 112.715 (aromatic

carbons), 54.023 (methylene carbon); MS (ESI) *m/z*: 382.3 (49%), 380.2 (50%); Anal. Calcd for $C_{20}H_{15}BrN_2O$: C, 63.34; H, 3.99; N, 7.39; Found: C, 63.42; H, 4.04; N, 7.42 %.

*5.3.3.10. 2-((4-bromophenyl)amino)-1-(9H-carbazol-9-yl)ethan-1-one (**9c**)*

Yield: 49%; MP: 169-171ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.196, 8.181 (d, J=7.5 Hz, 2H, C1, C8- carbazole), 7.954-7.934 (dd, J=9,1.5 Hz, 2H, C4, C5- carbazole), 7.391-7.376 (dd, J=7.5, 1.5 Hz, 2H, C3, C5-phenyl), 7.344-7.219 (m, 4H, C2, C3, C6, C7-carbazole), 6.663-6.641 (dd, J=11, 1.5 Hz, 2H, C2,C6-phenyl), 4.215 (s, 2H, methylene-CH_2), 3.766 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.312 (C=O), 148.652, 142.744, 136.285, 134.914, 131.004, 128.061, 127.429, 126.103, 124.189, 120.631, 116.534, 112.875 (aromatic carbons), 54.328 (methylene carbon); MS (ESI) *m/z*: 382.3 (50%), 380.2 (48%); Anal. Calcd for $C_{20}H_{15}BrN_2O$: C, 63.34; H, 3.99; N, 7.39; Found: C, 63.40; H, 4.02; N, 7.41 %.

*5.3.3.11.1-(9H-carbazol-9-yl)-2-((3,5-dichlorophenyl)amino)ethan-1-one (**10c**)*

Yield: 55%; MP: 170-173ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.109, 8.094 (d, J=7.5 Hz, 2H, C1, C8- carbazole), 7.914-7.896 (dd, J=9, 1.5 Hz, 2H, C4, C5- carbazole), 7. 362-7.347 (td, J=7.5, 1.5 Hz, 2H, C2, C7- carbazole), 7.136-7.106 (td, J=7.5, 1.5 Hz, 2H, C3, C6- carbazole), 6.811 (s, 2H, C2, C6-phenyl), 6.563 (s, 1H, C4-phenyl), 4.241, (s, 2H, methylene-CH_2), 3.819, (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.220 (C=O), 148.832, 142.334, 136.540, 132.312, 130.422, 128.438, 126.392, 123.659, 121.966, 115.054 (aromatic carbons), 54.612 (methylene carbon); MS (ESI) *m/z*: 374.2 (5%), 372.4 (32%), 370.3 (74%); Anal. Calcd for $C_{20}H_{14}Cl_2N_2O$: C, 65.06; H, 3.82; N, 7.59; Found: C, 65.15; H, 3.86; N, 7.57 %.

*5.3.3.12.1-(9H-carbazol-9-yl)-2-((3,5-dibromophenyl)amino)ethan-1-one (**11c**)*

Yield: 54%; MP: 182-184ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.062, 8.017 (dd, J=22.5, 8 Hz, 2H, C1, C8- carbazole), 7.972-7.930 (dd, J=21, 7.5 Hz, 2H, C4, C5- carbazole), 7.371-7.313 (td, J=14, 7.5 Hz, 2H, C2, C7- carbazole), 7.211-7.155 (m, 3H, C3, C6-carbazole, C4-phenyl), 6.524 (s, 2H, C2, C6-phenyl), 4.235, (s, 2H, methylene-CH_2), 3.629, (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.625 (C=O), 147.274, 142.526, 136.311, 133.401, 130.828, 127.421, 126.264, 123.616, 120.108, 115.324 (aromatic carbons), 54.771 (methylene carbon); MS (ESI) *m/z*: 463.2 (46%), 461.1 (97%), 459.3 (49%); Anal. Calcd for $C_{20}H_{14}Br_2N_2O$: C, 52.43; H, 3.08; N, 6.11; Found: C, 52.51; H, 3.09; N, 6.14 %.

*5.3.3.13.1-(9H-carbazol-9-yl)-2-((3-chloro-4-fluorophenyl)amino)ethan-1-one (**12c**)*

Yield: 52%; MP: 171-172°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm); 8.069, 8.024 (d, J=7.5 Hz, 2H, C1, C8- carbazole), 7.921-7.906 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7.372-7.309 (td, J=14, 7.5 Hz, 2H, C2, C7- carbazole), 7.195-7.131 (td, J=14, 7.5 Hz, 2H, C3, C6-carbazole), 7.108-6.892 (m, 3H, C2, C5, C6-phenyl), 4.178 (s, 2H, methylene-CH_2), 3.862 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.241 (C=O), 146.424, 142.389, 140.174, 134.668, 130.914, 128.553, 127.166, 126.321, 123.632, 121.326, 120.339, 115.228 (aromatic carbons), 54.417 (methylene carbon); MS (ESI) *m/z*: 355.2 (24%), 353.4 (75%); Anal. Calcd for $C_{20}H_{14}ClFN_2O$: C, 68.09; H, 4.00; N, 7.94; Found: C, 68.16; H, 3.98; N, 7.97 %.

*5.3.3.14.2-((2-bromo-4-fluorophenyl)amino)-1-(9H-carbazol-9-yl)ethan-1-one (**13c**)*

Yield: 57%; MP: 179-182ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.138, 8.121 (d, J=8.5 Hz, 2H, C1, C8- carbazole), 7.926-7.908 (dd, J=9, 1.5 Hz, 2H, C4, C5- carbazole), 7.341-7.307 (td, J=17, 7.5 Hz, 2H, C2, C7- carbazole) 7.197-7.166 (td, J=15.5, 7.5 Hz, 2H, C3,C6- carbazole), 7.021 (s, C3-phenyl), 6.852, 6.836 (d, , J=8 Hz, 1H, C5-phenyl),

6.439, 6.424, (d, J=7.5 Hz, 1H, C6-phenyl), 4.185 (s, 2H, methylene-CH_2), 3.793 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.392 (C=O), 146.501, 142.263, 134.354, 132.632, 130.126, 128.941, 127.138, 126.235, 124.432, 122.435, 120.527, 115.008 (aromatic carbons), 53.522 (methylene carbon); MS (ESI) *m/z*: 400.4 (49%), 398.3 (49%); Anal. Calcd for $C_{20}H_{14}BrFN_2O$: C, 60.47; H, 3.55; N, 7.05; Found: C, 60.53; H, 3.57; N, 7.04 %.

*5.3.3.15. 1-(9H-carbazol-9-yl)-2-((3-nitrophenyl)amino)ethan-1-one (**14c**)*

Yield: 48%; MP: 189-191ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.261, 8.245 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.913-7.894 (dd, J=9.5, 1.5 Hz, 2H, C4, C5- carbazole), 7.362-7.328 (td, J=7.5, 1.5 Hz, 2H, C2, C7-carbazole), 7.274-7.243 (td, J=6, 1.5 Hz, 2H, C3,C6-carbazole), 6.932 (s, 1H, C2-phenyl), 6.813, 6.797 (d, J=8 Hz, 1H, C4-phenyl), 6.661-6.633 (t, J=7 Hz, 1H, C5-phenyl), 6.592, 6.575 (d, J= 8.5 Hz, 1H, C6-phenyl), 4.220 (s, 2H, methylene-CH_2), 3.815 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.931 (C=O), 148.136, 141.520, 135.391, 134.145, 132.515, 129.219, 127.835, 126.301, 123.664, 120.392, 116.523, 112.839 (aromatic carbons), 54.229 (methylene carbon); MS (ESI) *m/z*: 346.4 (37%); Anal. Calcd for $C_{20}H_{15}N_3O_3$: C, 69.56; H, 4.38; N, 12.17; Found: C, 69.61; H, 4.38; N, 12.16 %.

*5.3.3.16.1-(9H-carbazol-9-yl)-2-((4-nitrophenyl)amino)ethan-1-one (**15c**)*

Yield: 50%; MP: 191-192ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.259, 8.243 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.931, 7.916 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7.314-7.294 (td, J=15, 1.5 Hz, 2H, C2, C7- carbazole), 7.251-7.221 (td, J=15, 1.5 Hz, 2H, C3, C6-carbazole), 6.992, 6.978 (d, J=7 Hz, 2H, C3, C5-phenyl), 6.621, 6.602 (d, J=9.5 Hz, 2H, C2, C6-phenyl), 4. 162 (s, 2H, methylene-CH_2) 3.839 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 170.285 (C=O), 146.517, 140.913, 134.381, 132.155, 130.516, 128.835, 126.253, 124.793, 122.141, 116.723 (aromatic carbons), 54.233 (methylene

carbon); MS (ESI) *m/z*: 346.5 (35%); Anal. Calcd for $C_{20}H_{15}N_3O_3$: C, 69.56; H, 4.38; N, 12.17; Found: C, 69.63; H, 4.39; N, 12.19 %.

*5.3.3.17.1-(9H-carbazol-9-yl)-2-((2-chloro-4-nitrophenyl)amino)ethan-1-one (**16c**)*

Yield: 51%; MP: 195-198ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.312 (s, 1H, C3-phenyl), 8.279-8.256 (dd, J=11.5, 2.5 Hz, 1H, C5-phenyl), 8.091, 8.076 (d, J=7.5 Hz, 2H, C1, C8- carbazole), 7.469-7.451 (dd, J=7.5, 1.5 Hz, 2H, C4, C5- carbazole), 7.378-7.344 (td, J=8, 1.5 Hz, 2H, C2, C7- carbazole), 7.191-7.158 (td, J=9, 1.5 Hz, 2H, C3, C6- carbazole), 6.713, 6.695 (d, J=9 Hz, 1H, C6-phenyl), 6.219 (s, 1H, NH), 4.232 (s, 2H, methylene-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 171.832 (C=O), 147.413, 141.122, 136.559, 132.642, 130.129, 128.231, 127.243, 126.321, 124.424, 122.111, 120.132, 116.198 (aromatic carbons), 54.332 (methylene carbon); MS (ESI) *m/z*: 382.6 (25%), 380.4 (74%); Anal. Calcd for $C_{20}H_{14}ClN_3O_3$: C, 63.25; H, 3.72; N, 11.06; Found: C, 63.31; H, 3.74; N, 11.04 %.

*5.3.3.18.1-(9H-carbazol-9-yl)-2-((4-chloro-2-nitrophenyl)amino)ethan-1-one (**17c**)*

Yield: 45%; MP: 197-198ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.293 (s, 1H, C3-phenyl), 8.131, 8.115 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.919, 7.905 (d, J=7 Hz, 2H, C4, C5- carbazole), 7.371-7.346, (t, J=6 Hz, 2H, C2, C7- carbazole), 7.289-7.202 (m, 3H, C3, C6- carbazole, C5-phenyl), 6.862, 6.845 (d, J=8.5 Hz, 1H, C6-phenyl), 6.193 (s, 1H, NH), 4.235 (s, 2H, methylene-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 171.635 (C=O), 147.715, 141.631, 137.323, 132.893, 130.889, 128.713, 127.617, 126.114, 124.311, 122.510, 120.113, 116.232 (aromatic carbons), 54.126 (methylene carbon); MS (ESI) *m/z*: 382.2 (24%), 380.4 (74%); Anal. Calcd for $C_{20}H_{14}ClN_3O_3$: C, 63.25; H, 3.72; N, 11.06; Found: C, 63.33; H, 3.71; N, 11.07 %.

*5.3.3.19.2-((2-(9H-carbazol-9-yl)-2-oxoethyl)amino)benzoic acid (**18c**)*

Yield: 48%; MP: 170-171ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 12. 197 (s, 1H, OH), 8.191, 8.175 (d, J=8 Hz, 1H, C3-phenyl), 8.039, 8.023 (d, J=8 Hz, 2H, C1, C8-carbazole), 7.842-7.827 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7.353-7.338 (td, J=7.5, 1.5 Hz, 2H, C2, C7- carbazole), 7.361-7.346 (td, J=7.5, 1.5 Hz, 2H, C3, C6- carbazole), 7.256-7.230 (t, J=6.5 Hz, 1H, C5-phenyl), 6.995, 6.918 (m, 2H, C4, C6-phenyl, 6.718-6.692 (t, J=6.5 Hz, 1H, C5-phenyl), 4.251 (s, 2H, methylene-CH_2) 3.813 (1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 183.835 (COOH), 171.117 (C=O), 146.318, 141.216, 135.314, 132.553, 130.616, 128.219, 127.438, 126.431, 123.192, 121.896, 118.935, 115.361 (aromatic carbons), 53.228 (methylene carbon); MS (ESI) *m/z*: 345.5 (36%); Anal. Calcd for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13; Found: C, 73.30; H, 4.69; N, 8.14 %.

*5.3.3.20.1-(9H-carbazol-9-yl)-2-(4-(4-chlorophenyl)piperazin-1-yl)ethan-1-one (**19c**)*

Yield: 79%; MP: 172-175ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.192, 8.176 (d, J=8 Hz, 2H,C1, C8- carbazole), 7.941, 7.926 (d, J=7.5 Hz, 2H, C4, C5- carbazole), 7.381-7.350 (td, J=15.5, 1.5 Hz, 2H, C2, C7- carbazole), 7.259-7.226 (td, J=15, 1.5 Hz, 2H, C3, C6-carbazole), 7.103, 7.090 (d, J=6.5 Hz, 2H, C3, C5-phenyl), 6.664, 6.646 (d, J=9 Hz, 2H, C2, C6-phenyl), 4.294 (s, 2H, methylene-CH_2) 3.223-3.209 (m, 4H, piperazine); 2.739-2.716 (m, 4H, piperazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.928 (C=O), 146.135, 139.402, 134.815, 131.823, 129.119, 127.325, 126.411, 125.428, 122.385, 115.459 (aromatic carbons), 53.948 (methylene carbon); 53.334, 50.183 (piperazine carbons); MS (ESI) *m/z*: 406.4 (24%), 404.1 (74%); Anal. Calcd for $C_{24}H_{22}ClN_3O$: C, 71.37; H, 5.49; N, 10.40; Found: C, 71.44; H, 5.47; N, 10.43 %.

*5.3.3.21.1-(9H-carbazol-9-yl)-2-(4-(4-nitrophenyl)piperazin-1-yl)ethan-1-one (**20c**)*

Yield: 71%; MP: 185-187ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 8.168, 8.152 (d, J=8 Hz, 2H, C1, C8- carbazole), 7.953, 7.936 (d, J=8.5 Hz, 2H, C4, C5- carbazole), 7.485-7.453 (td, J=16, 1.5 Hz, 2H, C2, C7- carbazole), 7.281-7.251 (td, J=15, 1.5 Hz, 2H, C3, C6-carbazole), 7.193, 7.179 (d, J=7 Hz, 2H, C3, C5-phenyl), 6.651, 6.633 (d, J=9 Hz, 2H, C2, C6-phenyl), 4. 297 (s, 2H, methylene-CH_2) 3.251-3.227 (m, 4H, piperazine); 2.738-2.715 (m, 4H, piperazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 169.893 (C=O), 146.201, 139.919, 134.631, 131.725, 129.334, 127.452, 126.591, 125.762, 122.492, 115.629 (aromatic carbons), 54.339 (methylene carbon); 52.034, 50.214 (piperazine carbons); MS (ESI) *m/z*: 415.1 (42%); Anal. Calcd for $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52; Found: C, 69.64; H, 5.36; N, 13.56 %.

*5.3.3.22. 1-(9H-carbazol-9-yl)-2-(cyclopropylamino)ethan-1-one (**S13**)*

Yield: 72%; MP: 105-106ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 8.112,8.096 (d, J=13 Hz, 2H, Ar-CH), 7.483-7.466 (d, J=12.5 Hz, 2H, Ar-CH), 7.390-7.358 (t, J=4.5 Hz, 2H, Ar-CH), 7.163-7.134 (t, J=6 Hz, 2H, Ar-CH), 5.754 (s, 2H, methylene-CH_2), 3.909 (s, 1H, NH), 1.569-0.593 (m, 5H, cyclopropyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 169.905 (C=O), 139.808, 125.901, 123.490, 120.459, 119.426, 110.745 (aromatic carbons), 53.435 (methylene carbon), 24.067, 7.756 (cyclopropyl carbons); ESI-MS (m/z) = 265.6 (35%). Anal. Calcd for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60; Found: C, 77.48; H, 6.12; N, 10.58 %.

*5.3.3.23. 1-(9H-carbazol-9-yl)-2-(cyclobutylamino)ethan-1-one (**S14**)*

Yield: 67%; MP: 109-110ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.025,8.010 (d, J=7.5 Hz, 2H, Ar-CH), 7.376-7.334 (dd, J=13, 6.5 Hz, 2H, Ar-CH), 7.196-7.183 (t, J=6.5 Hz, 2H, Ar-CH), 7.171-7.155 (t, J=8 Hz, 2H, Ar-CH), 3.578 (s, 2H, methylene-CH_2), 3.158 (s, 1H, NH), 2.113-0.950 (m, 7H, cyclobutyl); ^{13}C NMR (125 MHz,

$CDCl_3$) δ (ppm): 169.791 (C=O), 139.642, 125.708, 123.260, 120.292, 119.193, 110.529 (aromatic carbons), 52.919 (methylene carbon), 38.451, 24.067, 15.123 (cyclobutyl carbons); ESI-MS (m/z) = 279.3 (25%). Anal. Calcd for $C_{18}H_{18}N_2O$: C, 77.67; H, 6.52; N, 10.06; Found: C, 77.82; H, 6.54; N, 09.99 %.

*5.3.3.24.1-(9H-carbazol-9-yl)-2-(cyclopentylamino) ethan-1-one (**S15**)*

Yield: 71%; MP: 112-113ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.016,8.001 (d, J=7.5 Hz, 2H, Ar-CH), 7.367-7.325 (dd, J=13, 6.5 Hz, 2H, Ar-CH), 7.187-7.174 (t, J=6.5 Hz, 2H, Ar-CH), 7.162-7.146 (t, J=8 Hz, 2H, Ar-CH), 3.569 (s, 2H, methylene-CH_2), 3.149 (s, 1H, NH), 2.369-1.250 (m, 9H, cyclopentyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 168.992 (C=O), 139.352, 125.428, 122.995, 120.089, 119.057, 110.243 (aromatic carbons), 54.028 (methylene carbon), 47.571, 33.159, 24.067 (cyclopentyl carbons); ESI-MS (m/z) = 293.2 (25%). Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.90; N, 9.58; Found: C, 78.29; H, 6.93; N, 9.54 %.

*5.3.3.25. 1-(9H-carbazol-9-yl)-2-(cyclohexylamino)ethan-1-one (**S16**)*

Yield: 65%; MP: 115-116ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 8.112,8.096 (d, J=8 Hz, 2H, Ar-CH), 7.483-7.466 (d, J=8.5 Hz, 2H, Ar-CH), 7.390-7.358 (td, J=8, 1 Hz, 2H, Ar-CH), 7.163-7.134 (td, J=7.5, 1 Hz, 2H, Ar-CH), 3.551 (s, 2H, methylene-CH_2), 3.165 (s, 1H, NH), 2.113-1.018 (m, 11H, cyclohexyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 169.325 (C=O), 139.819, 125.883, 123.480, 120.454, 119.400, 110.745 (aromatic carbons), 52.921 (methylene carbon), 38.582, 28.256, 24.280, 20.122 (cyclohexyl carbons); ESI-MS (m/z) = 307.2 (25%). Anal. Calcd for $C_{20}H_{22}N_2O$: C, 78.40; H, 7.24; N, 9.14; Found: C, 78.62; H, 7.25; N, 9.16 %.

*5.3.3.26. 1-(9H-carbazol-9-yl)-2-(cycloheptylamino)ethan-1-one (**S17**)*

Yield: 63%; MP: 118-119ºC; ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 8.121,8.105 (d, J=8 Hz, 2H, Ar-CH), 7.492-7.475 (d, J=8.5 Hz, 2H, Ar-CH), 7.399-7.367 (td, J=8, 1

Hz, 2H, Ar-CH), 7.172-7.143 (td, J=7.5, 1 Hz, 2H, Ar-CH), 3.572 (s, 2H, methylene-CH_2), 3.128 (s, 1H, NH), 2.109-0.996 (m, 13H, cycloheptyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 169.725 (C=O), 139.552, 125.521, 122.294, 120.102, 119.268, 110.313 (aromatic carbons), 53.169 (methylene carbon), 37.975, 30.091, 25.274, 19.879 (cycloheptyl carbons); ESI-MS (m/z) = 321.5 (25%). Anal. Calcd for $C_{21}H_{24}N_2O$: C, 78.71; H, 7.55; N, 8.74; Found: C, 78.90; H, 7.57; N, 8.68 %.

*5.3.3.27. 1-(9H-carbazol-9-yl)-2-(cyclooctylamino)ethan-1-one (**S18**)*

Yield: 61%; MP: 121-123ºC; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.006,7.981 (d, J=12.5 Hz, 2H, Ar-CH), 7.459-7.434 (dd, J=12.5, 6.5 Hz, 2H, Ar-CH), 7.180-7.167 (t, J=4.5 Hz, 2H, Ar-CH), 7.155-7.139 (t, J=6 Hz, 2H, Ar-CH), 3.312 (s, 2H, methylene-CH_2), 2.891 (s, 1H, NH), 2.467-0.954 (m, 15H, cyclooctyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 168.698 (C=O), 139.691, 125.702, 122.578, 119.971, 118.997, 110.025 (aromatic carbons), 56.005 (methylene carbon), 49.794, 36.215, 27.193, 26.532, 20.384 (cyclooctyl carbons); ESI-MS (m/z) = 335.4 (25%). Anal. Calcd for $C_{22}H_{26}N_2O$: C, 79.00; H, 7.84; N, 8.38; Found: C, 79.21; H, 7.85; N, 8.35 %.

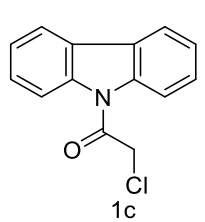
5.3.4. Antitubercular screening

The synthesized compounds were screened for antitubercular activity against *Mtb* H37Rv by MABA method. The potency of the compounds was recorded as MIC (**Table 5-13)**. The relationship between the structure and activity was established and was found that the nitro compounds **(14c, 15c, 16c and 17c)** were most active among all the screened compounds at MIC of 1.56µg/mL. The strong electron withdrawing nature of nitro group might have influenced the biological activity of the compounds. The di-substituted halogen compounds (**10c, 11c, 12c**) were better over the mono substituted halogen compounds (**3c, 4c, 5c, 6c, 7c, 8c, 9c**) at MIC 3.13µg/mL and this

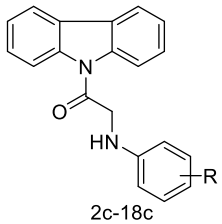
could be due to effective binding into the active site of the target. Further, the nitro compounds were more active in comparison to the di-halogen compounds. The linker was also found to have an essential role in the activity. A drastic reduction in activity was observed in compounds **19c** and **20c,** where the acyl linker was replaced with piperazinyl type linker and this could be due restricted rotation of single bond in piperazinyl linker. Different substitutions on the phenyl ring altered the activity, while the carbazole core maintained the activity at micro molar range. Therefore, it can be inferred that the carbazole core and nitro substitution on the phenyl ring are important for producing antitubercular activity.

5.3.5. Antibacterial screening

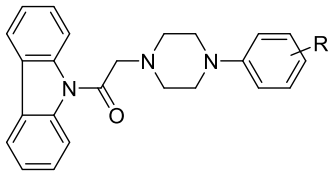
The chlorpromazine and several phenothiazines were also reported to be effective against other bacterial strains. Therefore, we extended our study to screen our compounds against *S. aureus* and *E. coli* by disc diffusion method. Compounds **14c, 15c, 16c and 17c** were most active among all the compounds screened against *S. aureus*, having MIC of 0.98µg/mL. The activity was reduced against *E. coli* and the most active compounds produced MIC of 7.81µg/mL (**Table 5-13**). It was observed that the nitro compounds were more active against *S. aureus* than the di-halogen substituted compounds, but it remained same in *E. coli*. The difference in activity between *S. aureus* and *E. coli* could be due to their difference in permeability of the compounds across the cell wall.



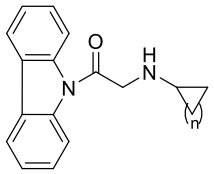
1c



2c-18c



19c, 20c



n =1,2,3,4,5,6
S13-S18

Code	R/n	MIC in µg/mL (µM) [a]	MIC in µg/mL (µM) [b]	MIC in µg/mL (µM) [c]
1c	-	62.50 (256.47)	62.50 (256.47)	50 (205.17)
2c	H	15.63 (52.03)	31.25 (104.04)	25 (83.23)
3c	2-Cl	15.63 (46.68)	31.25 (93.33)	25 (74.67)
4c	3-Cl	7.81 (23.32)	15.63 (46.68)	12.5 (37.33)
5c	4-Cl	7.81 (23.32)	15.63 (46.68)	12.5 (37.33)
6c	3-F	7.81 (24.53)	15.63 (49.09)	12.5 (39.26)
7c	4-F	7.81 (24.53)	15.63 (49.09)	12.5 (39.26)
8c	3-Br	7.81 (20.59)	15.63 (41.21)	12.5 (32.95)
9c	4-Br	7.81 (20.59)	15.63 (41.21)	12.5 (32.95)
10c	3,5-diCl	1.95 (5.28)	7.81 (21.15)	3.13 (8.47)
11c	3,5-diBr	1.95 (4.25)	7.81 (17.04)	3.13 (6.83)
12c	3-Cl,4-F	1.95 (5.52)	7.81 (22.13)	3.13 (8.87)
13c	2-Br,4-F	3.91 (9.84)	7.81 (19.66)	6.25 (15.73)
14c	3-NO_2	0.98 (2.83)	7.81 (22.61)	1.56 (4.51)
15c	4-NO_2	0.98 (2.83)	7.81 (22.61)	1.56 (4.51)
16c	2-Cl,4-NO_2	0.98 (2.58)	7.81 (20.56)	1.56 (4.10)
17c	2-NO_2,4-Cl	0.98 (2.58)	7.81 (20.56)	1.56 (4.10)
18c	2-COOH	7.81 (22.67)	15.63 (45.38)	12.5 (36.29)
19c	4-Cl	7.81 (19.33)	15.63 (38.69)	12.5 (30.94)
20c	4-NO_2	3.91 (9.43)	7.81 (18.84)	3.13 (7.55)
S13	1	15.63 (59.13)	125 (472.89)	25 (94.57)
S14	2	15.63 (56.15)	125 (449.05)	25 (89.81)
S15	3	7.81 (26.71)	62.50 (213.76)	25 (85.50)
S16	4	3.90 (12.72)	31.25 (101.98)	12.5 (40.79)
S17	5	15.63 (48.77)	62.50 (195.04)	25 (78.01)
S18	6	15.63 (46.73)	125 (373.73)	50 (149.49)
Chlorpromazine	-	7.81 (24.49)	15.63 (49.01)	12.5 (39.20)
Ciprofloxacin	-	1.95 (5.88)	3.91 (11.80)	3.13 (9.44)
Pyrazinamide	-	-	-	3.13 (25.42)

Table 5-13: Antitubercular and antibacterial activities of carbazole derivatives

[a] Minimum Inhibitory Concentration (MIC) against *S. aureus* (ATCC 25323)

[b] Minimum Inhibitory Concentration (MIC) against *E. coli* (ATCC35218)

[c] Minimum Inhibitory Concentration (MIC) against *M. tuberculosis* H37Rv (ATCC 27294)

5.3.6. BBB permeability screening

BBB permeability is an important determinant of drugs to produce CNS effects and therefore, it is imperative to check the BBB permeability of compounds required to act peripherally. It is particularly to avoid inadvertent side effects and possible toxicity. Therefore, all the developed carbazole derivatives were screened for BBB permeability by Parallel artificial membrane permeability assay (PAMPA) to ascertain their effect on CNS. The permeability of the compounds was compared with commercial drugs and classified as high permeable (CNS+), low permeable (CNS-) and permeable uncertain (CNS+/-). The effective permeability (Pe) of chlorpromazine and diazepam was found as 6.1 $x10^{-6}$ and 12.4 $x10^{-6}$ cm/s, respectively and were classified as high permeable drugs, whereas, the effective permeability of atenolol, verapamil and levofloxacin was found at 1.1 $x10^{-6}$, 0.0 and 0.0 cm/s, respectively and were classified as low permeable drugs. The test compounds (**1c to 20c**) have produced effective permeability in the range from 2.5 $x10^{-6}$ to 4 $x10^{-6}$ cm/s and were classified as permeability uncertain (**Table 5-14**). The reduced BBB permeability of the test compounds is expected to diminish the CNS effects in comparison to chlorpromazine.

5.3.7. In-vitro *cytotoxicity screening*

A drug entering the human system should be free from toxicity towards normal cells. Therefore, all the compounds were tested against kidney epithelial (VERO) cells to check their toxicity. The 50% cytotoxicity concentration (CC_{50}) determined was in the range of 90.7 to 207.3µg/mL **(Table 5-15)**, indicating that the compounds were free from toxicity towards mammalian cells. The drug molecules should be selective towards a desired activity to avoid unwanted side effects. Here, the compounds should kill the microbial organisms but not the normal human cells. The selectivity index (SI) was calculated by dividing the obtained cytotoxicity (CC_{50}) with the antitubercular

MIC. The potent compounds (**14c to 17c**) showed SI more than 63, therefore, they were selective towards *Mtb* with less or no mammalian cell toxicity.

Compound Code	P_e x10^{-6} cm/s	Classification
Chlorpromazine	6.1±0.059	CNS+
Atenolol	1.1±0.021	CNS-
Verapamil	0.0	CNS-
Diazepam	12.4±0.263	CNS+
Levofloxacin	0.0	CNS-
1c	2.5±0.020	CNS+/-
2c	2.6±0.128	CNS+/-
3c	2.8±0.047	CNS+/-
4c	2.8±0.025	CNS+/-
5c	2.7±0.061	CNS+/-
6c	2.6±0.103	CNS+/-
7c	2.6±0.058	CNS+/-
8c	3.0±0.033	CNS+/-
9c	3.0±0.084	CNS+/-
10c	3.2±0.391	CNS+/-
11c	3.3±0.075	CNS+/-
12c	3.3±0.419	CNS+/-
13c	3.4±0.088	CNS+/-
14c	2.9±0.026	CNS+/-
15c	2.9±0.035	CNS+/-
16c	3.3±0.071	CNS+/-
17c	3.3±0.233	CNS+/-
18c	2.8±0.086	CNS+/-
19c	4.0±0.152	CNS+/-
20c	3.9±0.069	CNS+/-
S16	5.1±0.271	CNS+

Table 5-14: BBB permeability of commercial drugs and carbazole derivatives

Data are expressed as mean ± SEM (n =3)

CNS+ = high BBB permeation compounds, *i.e.* Pe= > 4.0 * 10^{-6} cm s^{-1}

CNS- =low BBB permeation compounds, *i.e.* Pe= < 2.0 * 10^{-6} cm s^{-1}

CNS+/- =BBB permeation uncertain compounds, *i.e.* Pe =4.0 - 2.0 *10^{-6} cm s^{-1}

Compound Code	CC_{50} (µg/mL) [a]	Selectivity Index [b]
1c	207.31±9.825	4.1
2c	171.24±0.451	6.8
3c	167.68±0.637	6.7
4c	161.35±0.781	12.9
5c	162.13±0.732	13.0
6c	168.20±0.747	13.4
7c	165.29±10.542	13.2
8c	168.71±0.768	13.5
9c	133.45±0.393	10.7
10c	138.18±10.189	44.1
11c	125.33±0.806	40.0
12c	129.17±0.781	41.2
13c	122.72±0.625	19.6
14c	104.56±0.753	67.0
15c	98.44±0.788	63.1
16c	105.10±10.190	67.4
17c	99.42±10.167	63.7
18c	115.28±0.691	9.2
19c	95.89±0.554	7.7
20c	90.77±8.332	29.0
S16	135.04±2.597	10.8

Table 5-15: Cytotoxicity study of carbazole derivatives against VERO cells

[a] Data are expressed as mean ± SEM (n =3)

[b] Selectivity index is ratio of cytotoxicity (CC_{50}) to *Mtb* MIC

5.3.8. NDH-2 inhibitory screening

Compounds that produced strongest growth inhibition against whole *Mtb* were screened for NDH-2 inhibition to understand their mechanism of action. Percentage inhibition of residual NADH oxidation activity was recorded through NADH:menadione oxidoreduction assay. Among the screened compounds, **15c** produced the highest percentage inhibition (25.80%) at 50µM concentrations, followed by **14c**, **16c** and **17c** with 17.84, 16.23 and 15.51 percent inhibitions respectively. Rest of the compounds produced around 10 percent inhibitions at 50µM concentration (**Table 5-16**). None of

the compounds in the study produced greater than 50% inhibition at 50µM concentration. The IC_{50} was recorded as greater than 50 micromolar (>50µM).

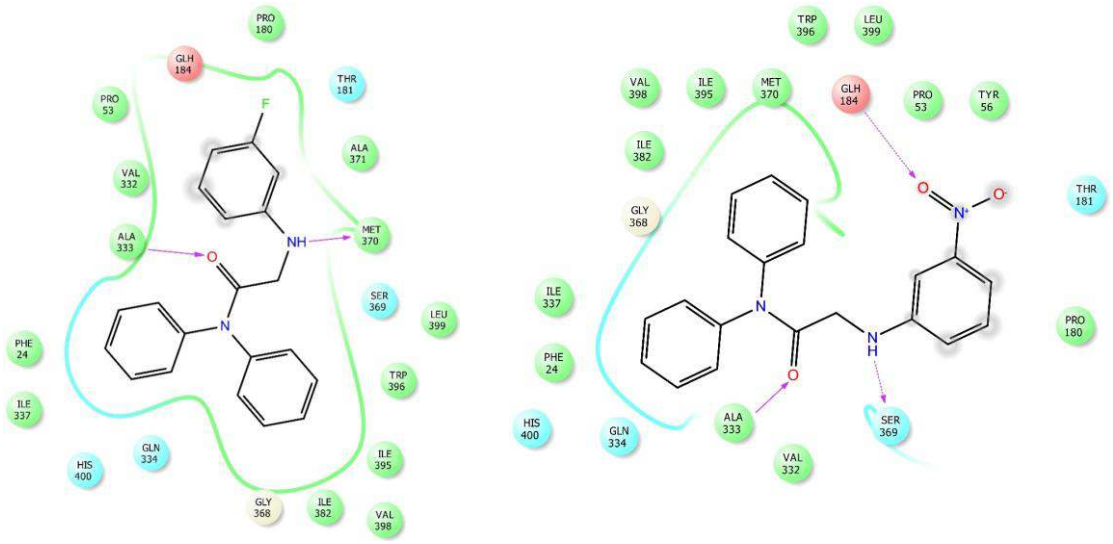
Compound Code	% inhibition of residual NADH oxidation activity at 50µM concentration
10c	13.09±2.134
11c	10.48±1.082
12c	7.92±1.590
13c	10.86±1.061
14c	17.84±8.735
15c	25.80±1.771
16c	16.23±4.931
17c	15.51±2.082
HQNO	$82.81\pm0.524^{x,y,z,@,\#,\$,\%,\&}$

Table 5-16: NDH-2 inhibitory study of carbazole derivatives. Data expressed as a Mean ±SEM (n=3). $^{x}P< 0.05$ compared to 10c, $^{y}P< 0.05$ compared to 11c, $^{z}P< 0.05$ compared to 12c, $^{@}P< 0.05$ compared to 13c, $^{\#}P< 0.05$ compared to 14c, $^{\$}P< 0.05$ compared to 15c, $^{\%}P< 0.05$ compared to 16c, $^{\&}P< 0.05$ compared to 17c [One- way ANOVA followed by Newmann-Keuls test].

5.4. Section 4: Molecular Docking, Synthesis, Characterization and Biological Profiling of Biphenyl Derivatives

5.4.1. Extra precision molecular docking

The biphenyl derivatives were docked against the protein structure of *Mtb* NDH-2 that was obtained through homology modeling. All the molecules in the study reached quinone binding motif ("333-AQxAxQ-338") and produced the essential interactions. The docking scores were observed in the range of -5.2194 to -1.5277 Kcal/mol. Molecules **15b**, **16b** and **17b** bind to the active site with binding energy of -5.2023, -5.2194 and -5.1905 Kcal/mol, respectively. The essential hydrogen bond interaction with Ala-333, a critical residue in quinone binding, was conserved (**Figure 5-8 & Table 5-17**). The molecules can arrest the synthesis of ATP by inhibiting NDH-2.



6b 14b

Figure 5-8: Docking poses of molecules 6b and 14b against NDH-2

Ligand code	Lowest binding energy (Kcal/mol)	Residues forming the quinone binding motif (residues forming H-bonding with ligand were indicated in bold)
1b	-3.1018	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
2b	-3.5661	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
3b	-3.5824	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
4b	-3.5710	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
5b	-3.4999	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
6b	-3.6014	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
7b	-3.6028	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
8b	-3.6132	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
9b	-3.5907	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
10b	-3.8011	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
11b	-3.8146	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
12b	-3.9038	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
13b	-4.1121	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
14b	-4.5008	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
15b	-5.2023	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
16b	-5.2194	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
17b	-5.1905	**Ala-333**,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338
18b	-3.5851	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
19b	-2.9814	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
20b	-2.9055	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S7	-1.5277	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S8	-1.6082	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S9	-1.9906	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S10	-1.9912	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S11	-1.6582	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)
S12	-1.6241	Ala-333,Gln-334,Gly-335, Ala-336,Ile-337,Gln-338 (no hydrogen bond interaction)

Table 5-17: Docking of designed biphenyl molecules against NDH-2 of *Mtb*

5.4.2. Molecular property and toxicity prediction

Computational study was performed using OSIRIS DataWarrior to predict the molecular property of the designed compounds. Different molecular properties could alter the biological activity. The designed molecules should follow 'Lipinski's rule' to be drug-like. All the molecules in biphenyl series followed all the rules *viz.* molecular weight, partition coefficient (LogP), hydrogen bond donor and hydrogen bond acceptor. cLogP of the molecules was found in the range between 1.2907 and 4.4578. Molecules **14b, 15b, 16,** and **17b** showed cLogP of 2.0858, 2.0858, 2.6918, 2.6918 respectively. Topological polar surface area (TPSA) was also calculated for all the molecules in biphenyl series. It was found in the range between 20.31 and 78.16. Molecules **14b, 15b, 16,** and **17b** showed the highest TPSA at 78.16, while molecules **2b** to **13b** at 32.34. The cycloalkane derivatives (**S7-S12**) also showed TPSA at 32.34. Druglikeness was also found in the desired range (**Table 5-18**). Toxicities *viz*. mutagenicity, tumorigenicity, and irritancy were predicted through OSIRIS DataWarrior. None of the molecules in the study were found mutagenic and tumorigenic, except **1b**. Molecules **1b, 19b, 20b,** and **S10** were predicted for some kind of associated irritancy (**Table 5-18**). Druglikeness and reduced toxicity of biphenyl molecules could be rewarded in further development as drug.

Code	cLogP	cLogS	HBA	HBD	TSA	TPSA	DL	MU	TU	IR	SI	MF	MC	EA	RB	NR	AR	SA
1b	2.501	-4.355	2	0	216	20.31	1.25	H	H	H	0.52	0.58	0.55	3	3	2	2	8
2b	3.0074	-4.946	3	1	281	32.34	2.28	N	N	N	0.52	0.58	0.61	3	5	3	3	10
3b	3.6134	-5.682	3	1	277	32.34	2.33	N	N	N	0.5	0.57	0.66	4	5	3	3	8
4b	3.6134	-5.682	3	1	291	32.34	2.33	N	N	N	0.5	0.59	0.66	4	5	3	3	8
5b	3.6134	-5.682	3	1	290	32.34	2.33	N	N	N	0.54	0.59	0.64	4	5	3	3	10
6b	3.1082	-5.26	3	1	286	32.34	0.94	N	N	N	0.5	0.59	0.66	4	5	3	3	8
7b	3.1082	-5.26	3	1	271	32.34	0.94	N	N	N	0.54	0.59	0.64	4	5	3	3	10
8b	3.7326	-5.78	3	1	299	32.34	0.49	N	N	N	0.54	0.59	0.66	4	5	3	3	8
9b	3.7326	-5.78	3	1	276	32.34	0.49	N	N	N	0.54	0.59	0.64	4	5	3	3	10
10b	4.2194	-6.418	3	1	311	32.34	2.33	N	N	N	0.48	0.58	0.68	5	5	3	3	11
11b	4.4578	-6.614	3	1	324	32.34	0.49	N	N	N	0.48	0.58	0.68	5	5	3	3	11
12b	3.7142	-5.996	3	1	299	32.34	0.99	N	N	N	0.52	0.58	0.68	5	5	3	3	8
13b	3.8334	-6.094	3	1	309	32.34	-0.84	N	N	N	0.52	0.56	0.69	5	5	3	3	8
14b	2.0858	-5.406	6	1	310	78.16	-2.87	N	N	N	0.50	0.60	0.69	6	6	3	3	8
15b	2.0858	-5.406	6	1	302	78.16	-2.87	N	N	N	0.53	0.60	0.66	6	6	3	3	10
16b	2.6918	-6.142	6	1	318	78.16	-2.81	N	N	N	0.51	0.56	0.73	7	6	3	3	8
17b	2.6918	-6.142	6	1	316	78.16	-2.81	N	N	N	0.48	0.57	0.74	7	6	3	3	8
18b	2.4925	-4.959	5	2	299	69.64	2.12	N	N	N	0.46	0.57	0.70	5	6	3	3	8
19b	3.7448	-5.227	4	0	336	26.79	6.43	N	N	H	0.55	0.56	0.70	5	5	4	3	12
20b	1.2907	-4.951	7	0	349	72.61	1.23	N	N	H	0.54	0.57	0.72	7	6	4	3	12
S7	1.82	-4.20	3	1	251	32.34	2.80	N	N	N	0.5	0.58	0.59	3	5	3	2	9
S8	2.17	-4.47	3	1	261	32.34	1.76	N	N	N	0.52	0.58	0.60	3	5	3	2	9
S9	2.51	-4.74	3	1	277	32.34	0.09	N	N	N	0.5	0.56	0.60	3	5	3	2	10
S10	2.85	-5.01	3	1	272	32.34	-2.74	N	N	L	0.52	0.58	0.61	3	5	3	2	10
S11	3.19	-5.28	3	1	285	32.34	-2.27	N	N	N	0.5	0.58	0.62	3	5	3	2	11
S12	4.26	-5.55	3	1	302	32.34	-2.44	N	N	N	0.52	0.59	0.62	3	5	2	2	11

Table 5-18: Molecular properties and predicted toxicity of biphenyl molecules. HBA= H-Acceptors; HBD= H-Donor; TSA=Total surface area; TPSA= Total Polar surface area; DL= Druglikeness; MU=Mutagenic; TU=Tumorigenic; IR=Irritant; SI=Shape Index; MF=Molecular Flexibility; MC=Molecular Complexity; EA=Electronegative Atoms; RB=Rotatable Bonds; AR=Aromatic Rings; SA=Symmetric atoms; NR=No. of Rings; PA= Percentage of absorbance; N=none; H=High; L=low.

5.4.3. Synthesis and characterization

The designed biphenyl molecules were synthesized as shown in **Scheme 3**, involving the synthesis of intermediate, 2-chloro-N,N-diphenylacetamide **(1b)**. The intermediate (**1b**) was then reacted with different phenyl amines/ phenyl piperazines/ cyclic amines to afford the final derivatives of biphenyl scaffold. The progress and completion of reaction was closely monitored through thin layer chromatography. Column chromatography was performed to get pure biphenyl derivatives. The yield of the compounds was found in the range of 43 to 97 percent. Melting point, elemental composition, ^{1}H NMR, ^{13}C NMR and Mass data of all the compounds in biphenyl series are as follows,

*5.4.3.1. 2-chloro-N,N-diphenylacetamide (**1b**)*

Yield: 97%; MP: 105-107°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.392-7.257 (m, 10H, biphenyl-CH), 4.027 (s, 2H, Methylene-CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 166.289 (C=O), 141.889, 141.678, 130.620, 130.102, 129.334, 128.662, 127.212, 126.838, 126.185 (aromatic carbons), 42.744 (methylene carbon); MS (ESI) m/z: 248.3 (25%), 246.3 (75%). Anal. Calcd for $C_{14}H_{12}ClNO$: C, 68.44; H, 4.92; N, 5.70; Found: C, 68.53; H, 4.91; N, 5.72 %.

*5.4.3.2. N,N-diphenyl-2-(phenylamino)acetamide (**2b**)*

Yield: 78%; MP: 132-135°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm); 7.395-7.259 (m, 10H, biphenyl-CH), 7.224-7.208 (t, 2H, J=8, C3, C5-phenyl), 7.125,7.110 (d, 2H, J=7.5, C2, C6-phenyl), 7.096-7.080 (t, 1H, J=8, C4-phenyl), 4.031 (s, 2H, methylene-CH_2); 3.328 (s, 1H, NH); ^{13}C-NMR (125 MHz $CDCl_3$) δ: 167.323 (C=O), 142.012, 141.884, 130.624, 130.108, 129.337, 128.670, 127.215, 126.843, 126.197 (aromatic carbons), 43.297 (methylene carbon); MS (ESI) *m/z*: 303.5 (45.2%);

Anal. Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.26; Found: C, 79.49; H, 6.02; N, 9.27 %.

*5.4.3.3. 2-((2-chlorophenyl)amino)-N,N-diphenylacetamide (**3b**)*

Yield: 79%; MP: 143-145ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.377-7.290 (m, 10H, biphenyl-CH), 7.253-7.220 (m, 3H, C3, C5, C6-phenyl), 7.153-7.140 (t, 1H, J=6.5, C4-phenyl), 4.018 (s, 2H, methylene-CH_2) 3.791 (s, 1H, NH) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.308 (C=O), 143.079, 141.946, 129.986, 129.679, 129.468, 129.228, 128.854, 128.633, 128.268, 127.711, 126.761, 126.262, 125.772, 119.332, 119.159, 119.025, 115.991 (aromatic carbons), 42.801 (methylene carbon); MS (ESI) *m/z*: 339.8 (25%), 337.7 (74%); Anal. Calcd for $C_{20}H_{17}ClN_2O$: C, 71.32; H, 5.09; N, 8.32; Found: C, 71.41; H, 5.10; N, 8.36 %.

*5.4.3.4. 2-((3-chlorophenyl)amino)-N,N-diphenylacetamide (**4b**)*

Yield: 77%; MP: 144-146ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.381-7.293 (m, 10H, biphenyl-CH), 7.058 (s, 1H, C2-phenyl), 6.714, 6.697 (d, 1H, J=8.5, C4-phenyl), 6.563-6.512 (m, 2H, C5, C6-phenyl), 4. 025 (s, 2H, methylene-CH_2) 3.804 (s, 1H, NH) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.312 (C=O), 143.084, 141.952, 129.991, 129.683, 129.472, 129.235, 128.857, 128.639, 128.274, 127.715, 126.763, 126.266, 125.778, 119.333, 119.165, 119.028, 116.002 (aromatic carbons), 42.810 (methylene carbon); MS (ESI) *m/z*: 339.7 (25%), 337.7 (74%); Anal. Calcd for $C_{20}H_{17}ClN_2O$: C, 71.32; H, 5.09; N, 8.32; Found: C, 71.43; H, 5.11; N, 8.35 %.

*5.4.3.5. 2-((4-chlorophenyl)amino)-N,N-diphenylacetamide (**5b**)*

Yield: 75%; MP: 147-148ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.383-7.248 (m, 10H, biphenyl-CH), 7.091-7.068 (dd, J=9, 1.5 Hz, 2H, C3, C5-phenyl), 6.605-6.576 (dd, J=8.5, 1.5 Hz, 2H, C2, C6-phenyl), 4. 018 (s, 2H, methylene-CH_2) 3.397 (1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.289 (C=O), 145.018, 141.889, 141.668, 130.999,

130.236, 130.025, 129.727, 129.410, 129.190, 128.575, 128.364, 128.115, 126.819, 126.579, 126.291, 126.003, 123.248, 116.337 (aromatic carbons), 42.744 (methylene carbon); MS (ESI) *m/z*: 339.7 (24%), 337.5 (75%); Anal. Calcd for $C_{20}H_{17}ClN_2O$: C, 71.32; H, 5.09; N, 8.32; Found: C, 71.45; H, 5.10; N, 8.33 %.

*5.4.3.6. 2-((3-fluorophenyl)amino)-N,N-diphenylacetamide (**6b**)*

Yield: 68%; MP: 146-148ºC, ^{1}H-NMR (500 MHz, $CDCl_3$)δ; 7.384-7.296 (m, 10H, biphenyl-CH), 7.052 (s, 1H, C2-phenyl), 6.710, 6.693 (d, 1H, J=8.5, C4-phenyl), 6.559-6.508 (m, 2H, C5, C6-phenyl), 4.021 (s, 2H, methylene CH_2), 3.293 (s, 1H, NH); ^{13}C-NMR (125 MHz $CDCl_3$) δ: 166.298 (C=O), 147.725, 141.937, 134.910, 130.370, 130.063, 129.689, 129.410, 129.199, 128.700, 128.124, 126.809, 126.300, 126.070, 125.859, 125.542, 118.506, 115.051, 113.323 (aromatic carbons), 42.763 (methylene carbon); MS (ESI) *m/z*: 321.2 (72%); Anal. Calcd for $C_{20}H_{17}FN_2O$: C, 74.98; H, 5.35; N, 8.74; Found: C, 75.09; H, 5.34; N, 8.75 %.

*5.4.3.7. 2-((4-fluorophenyl)amino)-N,N-diphenylacetamide (7**b**)*

Yield: 65%; MP: 151-152ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.384-7.308 (m, 10H, biphenyl-CH), 7.250, 7.235 (d, 2H, J=7.5, C3, C5-phenyl), 6.855, 6.838 (d, 2H, J=8.5, C2, C6-phenyl), 4.021 (s, 2H, methylene-CH_2) 3.173 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.289 (C=O), 143.770, 142.455, 141.908, 130.102, 129.785, 129.401, 129.055, 128.700, 126.560, 126.195, 125.792, 116.164, 115.799, 115.627, 113.985, 113.928 (aromatic carbons), 42.763 (methylene carbon); MS (ESI) *m/z*: 321.3 (74%); Anal. Calcd for $C_{20}H_{17}FN_2O$: C, 74.98; H, 5.35; N, 8.74; Found: C, 75.05; H, 5.33; N, 8.78 %.

*5.4.3.8. 2-((3-bromophenyl)amino)-N,N-diphenylacetamide (**8b**)*

Yield: 55%; MP: 164-166ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.384-7.296 (m, 10H, biphenyl-CH), 7.034 (s, 1H, C2-phenyl), 6.811, 6.794 (d, 1H, J=8.5, C4-

phenyl), 6.534-6.482 (m, 2H, C5, C6-phenyl), 4.022 (s, 2H, methylene-CH_2), 3.299 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.298 (C=O), 147.926, 141.937, 130.658, 130.543, 130.044, 129.766, 129.410, 128.959, 128.585, 128.115, 127.040, 126.665, 126.195, 125.734, 123.104, 121.386, 117.921, 113.736 (aromatic carbons), 42.753 (methylene carbon); MS (ESI) *m/z*: 384.2 (49%), 382.4 (50%); Anal. Calcd for $C_{20}H_{17}BrN_2O$: C, 63.00; H, 4.49; N, 7.35; Found: C, 63.12; H, 4.52; N, 7.32 %.

*5.4.3.9. 2-((4-bromophenyl)amino)-N,N-diphenylacetamide (**9b**)*

Yield: 52%; MP: 169-171ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.389-7.308 (m, 10H, biphenyl-CH), 7.253-7.226 (dd, J=11.5, 3 Hz, 2H, C3, C5-phenyl), 6.564-6.549 (dd, J=11, 1.5 Hz, 2H, C2, C6-phenyl), 4.183 (s, 2H, methylene CH_2), 3.551 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.289 (C=O), 145.546, 141.937, 132.079, 130.159, 129.977, 129.785, 129.410, 129.209, 128.662, 128.537, 128.297, 127.942, 127.385, 126.636, 126.291, 126.108, 125.888, 116.798, 110.242 (aromatic carbons), 42.753 (methylene carbon); MS (ESI) *m/z*: 384.3 (49%), 382.3 (50%); Anal. Calcd for $C_{20}H_{17}BrN_2O$:C, 63.00; H, 4.49; N, 7.35; Found:C, 63.14;H, 4.52; N, 7.33 %.

*5.4.3.10.2-((3,5-dichlorophenyl)amino)-N,N-diphenylacetamide (**10b**)*

Yield: 50%; MP: 167-168ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.392-7.258 (m, 10H, biphenyl-CH), 7.194 (s, 1H, C4-phenyl), 6.535 (s, 2H, C2, C6-phenyl), 4.027 (s, 2H, methylene CH_2), 3.824 (s, 1H, NH); ^{13}C-NMR (125 MHz $CDCl_3$) δ: 166.289 (C=O), 148.329, 142.157, 141.927, 135.477, 130.236, 129.977, 129.410, 128.969, 128.220, 128.009, 127.855, 127.510, 126.876, 126.233, 125.830, 118.372, 113.294 (aromatic carbons), 42.725 (methylene carbon); MS (ESI) *m/z*: 376.5 (5%), 374.3 (32%), 372.4 (74%); Anal. Calcd for $C_{20}H_{16}Cl_2N_2O$: C, 64.70; H, 4.34; N, 7.55; Found: C, 64.82; H, 4.30; N, 7.58 %.

*5.4.3.11.2-((3,5-dibromophenyl)amino)-N,N-diphenylacetamide (**11b**)*

Yield: 45%; MP: 174-175ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.392-7.301 (m, 10H, biphenyl-CH), 7.256 (s, 1H, C4-phenyl), 6.732 (s, 2H, C2, C6-phenyl), 4.026 (s, 2H, methylene CH_2), 3.891 (s, 1H, NH); ^{13}C-NMR (125 MHz $CDCl_3$) δ: 166.289 (C=O), 148.771, 141.927, 141.860, 130.015, 129.823, 129.458, 129.055, 128.710, 128.355, 128.057, 126.963, 126.694, 126.243, 126.003, 125.619, 123.709, 123.402, 116.587 (aromatic carbons), 42.725 (methylene carbon); MS (ESI) *m/z*: 465.2 (46%), 463.2 (97%), 461.4 (49%); Anal. Calcd for $C_{20}H_{16}Br_2N_2O$: C, 52.20; H, 3.50; N, 6.09; Found: C, 52.32; H, 3.51; N, 6.11 %.

*5.4.3.12.2-((3-chloro-4-fluorophenyl)amino)-N,N-diphenylacetamide (**12b**)*

Yield: 55%; MP: 168-169°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm); 7.394-7.258 (m, 10H, biphenyl-CH), 6.927-6.892 (m, 2H, C2, C6-phenyl), 6.696-6.680 (dd, J=8, 2 Hz, 1H, C5-phenyl) 4.027 (s, 2H, methylene-CH_2), 3.328 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.289 (C=O), 152.649, 150.748, 143.204, 141.908, 130.169, 129.257, 128.595, 128.259, 126.761, 126.204, 125.926, 121.117, 120.973, 116.999, 116.827, 116.491, 114.312 (aromatic carbons), 42.734 (methylene carbon); MS (ESI) *m/z*: 357.4 (24%), 355.4 (75%); Anal. Calcd for $C_{20}H_{16}ClFN_2O$: C, 67.70; H, 4.55; N, 7.90; Found: C, 67.78; H, 4.52; N, 7.95 %.

*5.4.3.13.2-((2-bromo-4-fluorophenyl)amino)-N,N-diphenylacetamide (**13b**)*

Yield: 48%; MP: 175-176ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.391-7.256 (m, 10H, biphenyl-CH), 7.036 (s, 1H, C3-phenyl), 6.943, 6.927 (d, 1H, J=8, C5-phenyl), 6.602, 6.588, (d, 1H, J=7, C6-phenyl), 4.026 (s, 2H, methylene CH_2), 3.871 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.289 (C=O), 141.917, 141.754, 130.505, 130.082, 129.506, 129.410, 128.930, 128.575, 128.335, 127.155, 126.876, 126.550, 126.070, 125.696, 119.389, 119.188, 116.107, 115.416 (aromatic carbons), 42.744

(methylene carbon); MS (ESI) *m/z*: 402.2 (49%), 400.2 (49%); Anal. Calcd for $C_{20}H_{16}BrFN_2O$: C, 60.17; H, 4.04; N, 7.02; Found: C, 60.26; H, 4.07; N, 7.06 %.

*5.4.3.14.2-((3-nitrophenyl)amino)-N,N-diphenylacetamide (**14b**)*

Yield: 43%; MP: 181-182ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.375-7.258 (m, 10H, biphenyl-CH), 7.030 (s, 1H, C2-phenyl), 6.891, 6.875 (d, 1H, J=8, C4-phenyl), 6.673-6.632 (m, 2H, C5, C6-phenyl), 4.063 (s, 2H, methylene CH_2), 3.419 (s, 1H, NH); ^{13}C-NMR (125 MHz $CDCl_3$) δ: 166.356 (C=O), 152.754, 142.071, 141.879, 141.754, 139.086, 130.284, 130.140, 129.689, 129.410, 128.921, 128.633, 128.326, 127.155, 126.742, 126.425, 126.137, 113.429 (aromatic carbons), 42.763 (methylene carbon); MS (ESI) *m/z*: 348.2 (68%); Anal. Calcd for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10; Found: C, 69.29; H, 4.94; N, 12.14 %.

*5.4.3.15.2-((4-nitrophenyl)amino)-N,N-diphenylacetamide (**15b**)*

Yield: 45%; MP: 182-184ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.371-7.253 (m, 10H, biphenyl-CH), 7.251, 7.237 (d, 2H, J=7, C3, C5-phenyl), 6.852, 6.836 (d, 2H, J=8, C2, C6-phenyl), 4.104 (s, 2H, methylene-CH_2) 3.569 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.356 (C=O), 152.753, 142.102, 141.881, 141.754, 139.095, 130.285, 130.141, 129.691, 129.464, 128.936, 128.630, 128.321, 127.251, 126.447, 126.421, 126.034, 113.668 (aromatic carbons), 42.763 (methylene carbon); MS (ESI) *m/z*: 348.3 (70%); Anal. Calcd for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10; Found: C, 69.29; H, 4.94; N, 12.14 %.

*5.4.3.16.2-((2-chloro-4-nitrophenyl)amino)-N,N-diphenylacetamide (**16b**)*

Yield: 49%; MP: 186-188ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.115 (s, 1H, C3-phenyl), 8.003-7.987 (d, J=8 Hz, 1H, C5-phenyl), 7.389-7.252 (m, 10H, biphenyl-CH), 6.895, 6.881 (d, J=8 Hz, 1H, C6-phenyl), 4.845 (s, 1H, NH), 4.027 (s, 2H, methylene-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.317 (C=O), 149.059, 141.937, 141.678, 138.798,

129.353, 128.710, 128.489, 128.143, 127.932, 126.867, 126.329, 126.032, 125.868, 125.475, 124.390, 117.690, 113.803 (aromatic carbons), 42.744 (methylene carbon); MS (ESI) *m/z*: 384.4 (26%), 382.4 (75%); Anal. Calcd for $C_{20}H_{16}ClN_3O_3$: C, 62.92; H, 4.22; N, 11.01; Found: C, 63.05; H, 4.25; N, 11.06 %.

*5.4.3.17.2-((4-chloro-2-nitrophenyl)amino)-N,N-diphenylacetamide (**17b**)*

Yield: 51%; MP: 186-187ºC, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.120 (s, 1H, C3-phenyl), 7.394-7.259 (m, 11H, biphenyl-CH, C5-phenyl); 6.780, 6.762 (d, J=9 Hz, 1H, C6-phenyl), 6.106 (s, 1H, NH), 4.029 (s, 2H, methylene-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.317 (C=O), 143.338, 141.908, 135.909, 132.127, 130.322, 130.034, 129.814, 129.487, 129.151, 128.700, 128.076, 126.867, 126.646, 126.233, 125.916, 125.398, 121.559, 120.167 (aromatic carbons), 42.715 (methylene carbon); MS (ESI) *m/z*: 384.5 (26%), 382.6 (75%); Anal. Calcd for $C_{20}H_{16}ClN_3O_3$: C, 62.92; H, 4.22; N, 11.01; Found: C, 63.02; H, 4.23; N, 11.04 %.

*5.4.3.18.2-((2-(diphenylamino)-2-oxoethyl)amino)benzoic acid (**18b**)*

Yield: 43%; MP: 170-171ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 12.203 (s, 1H, OH), 7.814, 7.798 (d, J=8 Hz, 1H, C3-phenyl), 7.587-7.550 (m, 10H, biphenyl-CH), 7.013, 6.988 (m, 2H, C4, C6-phenyl, 6.794-6.768 (t, J=6.5 Hz, 1H, C5-phenyl), 4.138 (s, 2H, methylene-CH_2) 3.743 (1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 182.373 (COOH), 170.948 (C=O), 151.364, 143.205, 141.879, 141.748, 139.095, 130.291, 130.143, 129.695, 129.329, 128.718, 128.631, 128.323, 127.254, 126.447, 126.428, 126.034, 1143.281 (aromatic carbons), 42.369 (methylene carbon);MS (ESI) *m/z*: 347.4 (38%); Anal. Calcd for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09; Found: C, 72.90; H, 5.26; N, 8.13 %.

*5.4.3.19. 2-(4-(4-chlorophenyl)piperazin-1-yl)-N,N-diphenylacetamide (**19b**)*

Yield: 71%; MP: 162-165ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.391-7.254 (m, 10H, biphenyl-CH), 7.116, 7.102 (d, J=7 Hz, 2H, C3, C5-phenyl), 6.692, 6.678 (d, J=7 Hz, 2H, C2, C6-phenyl), 4.303 (s, 2H, methylene-CH_2) 3.219-3.205 (m, 4H, piperazine); 2.728-2.705 (m, 4H, piperazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.293 (C=O), 145.018, 141.889, 141.668, 130.999, 130.236, 130.025, 129.727, 129.410, 129.190, 128.575, 128.364, 128.115, 126.819, 126.579, 126.291, 126.003, 123.248, 116.337 (aromatic carbons), 48.739, 48.627 (piperazine carbons); 43.932 (methylene carbon); MS (ESI) *m/z*: 408.4 (24%), 406.4 (74%); Anal. Calcd for $C_{24}H_{24}ClN_3O$: C, 71.01; H, 5.96; N, 10.35; Found: C, 71.14; H, 5.97; N, 10.32 %.

*5.4.3.20.2-(4-(4-nitrophenyl)piperazin-1-yl)-N,N-diphenylacetamide (**20b**)*

Yield: 71%; MP: 169-171ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ; 7.380-7.243 (m, 10H, biphenyl-CH), 7.121, 7.107 (d, J=7 Hz, 2H, C3, C5-phenyl), 6.701, 6.687 (d, J=7 Hz, 2H, C2, C6-phenyl), 4.311 (s, 2H, methylene-CH_2) 3.225-3.210 (m, 4H, piperazine); 2.731-2.711 (m, 4H, piperazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.319 (C=O), 145.109, 141.901, 141.521, 131.104, 130.452, 130.148, 129.865, 129.410, 129.213, 128.599, 128.367, 128.119, 126.822, 126.584, 126.298, 125.999, 123.245, 116.332 (aromatic carbons), 49.432, 49.339 (piperazine carbons); 44.562 (methylene carbon); MS (ESI) *m/z*: 417.2 (31%); Anal. Calcd for $C_{24}H_{24}N_4O_3$: C, 69.21; H, 5.81; N, 13.45; Found: C, 69.32; H, 5.82; N, 13.43 %.

*5.4.3.21. 2-Cyclopropylamino-N,N-diphenyl-acetamide (**S7**)*

Yield: 82%; MP: 121-123ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.336-7.253 (m, 10H, Ar-CH), 3.646 (s, 2H, methylene CH_2), 2.888 (s, 1H, NH), 1.888-1.872 (m, 1H, cyclopropane-CH), 0.882-0.869 (m, 2H, cyclopropane-CH_2), 0.763-0.750 (m, 2H, cyclopropane-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 171.25 (C=O), 142.60,

129.36, 126.78 (aromatic carbons), 55.87 (methylene carbon), 36.29, 7.63 (cyclopropyl carbons); MS (ESI) m/z: 267.5 (27%). Anal. Calcd for $C_{17}H_{18}N_2O$: C, 76.66; H, 6.81; N, 10.52; Found: C, 76.85; H, 6.83; N, 10.55 %.

*5.4.3.22.2-Cyclobutylamino-N,N-diphenyl-acetamide (**S8**)*

Yield: 75%; MP: 126-128ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.324-7.189 (m, 10H, Ar-CH), 3.954 (s, 2H, methylene-CH_2), 2.914 (s, 1H, NH) 2.530-2.446 (m, 1H, cyclobutane-CH), 1.333-1.230 (m, 6H, cyclobutane-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 169. 23 (C=O), 140.62, 129.73, 126.61 (aromatic carbons), 54.16 (methylene carbons), 47.69, 24.13, 10.78 (cyclobutane carbons) MS (ESI) m/z: 281.6 (22%). Anal. Calcd for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99; Found: C, 77.38; H, 7.21; N, 10.03 %.

*5.4.3.23.2-Cyclopentylamino-N,N-diphenyl-acetamide (**S9**)*

Yield: 79%; MP: 131-132ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.332-7.250 (m, 10H, Ar-CH), 3.584 (s, 2H, methylene CH_2), 3.259 (s, 1H, NH), 1.637-1.143 (m, 9H, cyclopentane); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 171.25 (C=O), 142.60, 129.36, 126.78 (aromatic carbons), 55.87 (methylene carbon), 36.29, 29.80, 7.63 (cyclopentyl carbons); MS (ESI) m/z: 295.5 (25%). Anal. Calcd for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52; Found: C, 77.71; H, 7.55; N, 9.49 %.

*5.4.3.24. 2-Cyclohexylamino-N,N-diphenyl-acetamide (**S10**)*

Yield: 87%; MP: 137-139ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.461-7.379 (m, 10H, Ar-CH), 4.143 (s, 2H, methylene CH_2), 3.698 (s, 1H, NH), 3.221-3.195 (m, 1H, cyclohexane-CH), 1.632-1.098 (m, 10H, cyclohexane-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 171.25 (C=O), 141.96, 129.43, 126.72 (aromatic carbons), 62.83 (methylene carbon), 54.36, 35.27, 24.99, 24.12 (cyclohexyl carbons); MS (ESI) m/z:

309.2 (27%). Anal. Calcd for $C_{20}H_{24}N_2O$: C, 77.89; H, 7.84; N, 9.08; Found: C, 78.12; H, 7.85; N, 9.05 %.

*5.4.3.25.2-Cycloheptylamino-N,N-diphenyl-acetamide (**S11**)*

Yield: 81%; MP: 143-145ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.306-7.240 (m, 10H, Ar-CH), 3.940 (s, 2H, methylene CH_2), 3.600 (s, 1H, NH), 2.913-2.876 (m, 1H, cycloheptyl-CH), 1.736-1.236 (m, 12H, cycloheptyl-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 168.26 (C=O), 140.16, 129.26, 126.22 (aromatic carbons), 62.17 (cycloheptyl carbon), 55.92 (methylene carbon), 36.81, 31.89, 26.67 (cycloheptyl carbons); MS (ESI) m/z: 323.6 (27%). Anal. Calcd for $C_{21}H_{26}N_2O$: C, 78.22; H, 8.13; N, 8.69; Found: C, 78.45; H, 8.15; N, 8.65 %.

*5.4.3.26.2-Cyclooctylamino-N,N-diphenyl-acetamide (**S12**)*

Yield: 72%; MP: 147-149ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.332-7.186 (m, 10H, Ar-CH), 3.951 (s, 2H, methylene CH_2), 3.319 (s, 1H, NH), 2.733-2.716 (m, 1H, cyclooctyl-CH) 1.689-1.243 (m, 14H, cyclooctyl-CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 169.19 (C=O), 141.28, 129.14, 126.51 (aromatic carbons), 61.98 (methylene carbon), 55.23, 34.95, 26.19, 23.84 (cyclohexyl carbons); MS (ESI) m/z: 337.3 (27%). Anal. Calcd for $C_{22}H_{28}N_2O$: C, 78.53; H, 8.39; N, 8.33; Found: C, 78.77; H, 8.41; N, 8.36 %.

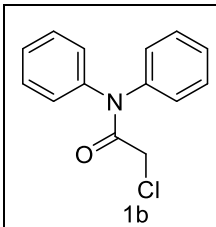
5.4.4. Antitubercular Screening

The antitubercular activity of biphenyl derivatives was assessed against *Mtb* H37Rv by Microplate Alamar Blue Assay (MABA) and the results are depicted in **Table 5-19**. Compounds **15b** and **16b** were the most active with MIC of 0.78µg/mL. They were followed by the compounds **14b** and **17b** with MIC of 1.56µg/mL. The activity was stronger than the template molecule chlorpromazine (12.5µg/mL) and standard drugs pyrazinamide and ciprofloxacin (3.13µg/mL). Substitution of a nitro group on the

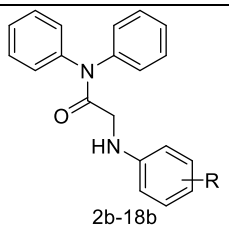
phenyl ring at *para* position (**15b** and **16b**) was found essential for the activity. Di-substituted halogen compounds (**10b-13b**) were found active with MIC of 3.13µg/mL, followed by mono-substituted halogen compounds (**3b-9b**) with MIC ≥6.25µg/mL. Among the cycloalkane compounds, the cyclopentane (**S9**) and cyclohexane (**S10**) derivatives produced better inhibition with MIC of 6.25µg/mL. Thus, the modification of phenothiazine ring to biphenyl ring produced interesting results, where the activity has significantly improved.

5.4.5. Antibacterial Screening

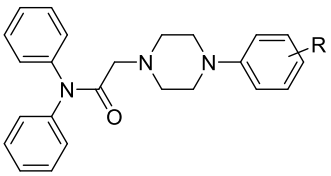
The biphenyl derivatives were further screened against a *gram* positive (*S. aureus)* and a *gram* negative (*E. coli)* strain to understand the spectrum of activity. Compound **16b** was the most effective against *S. aureus* with MIC of 0.49µg/mL, followed by **14b, 15b** and **17b** with MIC of 0.98µg/mL. Compounds **14b-17b** were the most effective against *E. coli* with MIC of 7.81µg/mL. It was observed that the activity was substantially higher against *S. aureus* than against *E. coli*. This may be due to increased permeability of the compounds through gram positive organism (*S. aureus*) over the gram negative one. The standard drug ciprofloxacin produced marginally higher inhibition against *E. coli* than the tested compounds, while the case is reverse against *S. aureus* (**Table 5-19**). Among cycloalkane derivatives, compound **S10** was found to be the most effective with MIC of 3.90 and 31.25µg/mL against *S. aureus* and *E. coli* respectively. A drastic reduction in activity was observed on varying the cyclohexane ring to cyclopropyl or cyclooctyl (**Table 5-19**).



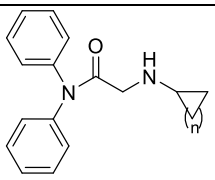
1b



2b-18b



19b, 20b



n =1,2,3,4,5,6
S7-S12

Code	R/n	MIC in µg/mL (µM) [a]	MIC in µg/mL (µM) [b]	MIC in µg/mL (µM) [c]
1b	-	62.50 (254.36)	125 (508.72)	50 (203.49)
2b	H	15.63 (51.68)	31.25 (103.34)	25 (82.67)
3b	2-Cl	7.81 (23.18)	31.25 (92.77)	12.5 (37.11)
4b	3-Cl	7.81 (23.18)	31.25 (92.77)	12.5 (37.11)
5b	4-Cl	7.81 (23.18)	31.25 (92.77)	6.25 (18.55)
6b	3-F	7.81 (24.37)	31.25 (97.54)	6.25 (19.50)
7b	4-F	7.81 (24.37)	31.25 (97.54)	6.25 (19.50)
8b	3-Br	3.91 (10.25)	15.63 (40.99)	6.25 (16.39)
9b	4-Br	3.91 (10.25)	15.63 (40.99)	6.25 (16.39)
10b	3,5-diCl	1.95 (5.25)	15.63 (42.09)	3.13 (8.43)
11b	3,5-diBr	1.95 (4.23)	7.81 (16.91)	3.13 (6.80)
12b	3-Cl,4-F	1.95 (5.49)	15.63 (44.05)	3.13 (8.82)
13b	2-Br,4-F	3.91 (9.79)	15.63 (39.14)	3.13 (7.83)
14b	3-NO_2	0.98 (2.82)	7.81 (22.48)	1.56 (4.49)
15b	4-NO_2	0.98 (2.82)	7.81 (22.48)	0.78 (2.24)
16b	2-Cl,4-NO_2	0.49 (1.28)	7.81 (20.45)	0.78 (2.04)
17b	2-NO_2,4-Cl	0.98 (2.56)	7.81 (20.45)	1.56 (4.08)
18b	2-COOH	7.81 (22.54)	15.63 (45.12)	6.25 (18.04)
19b	4-Cl	3.91 (9.63)	15.63 (38.50)	6.25 (15.39)
20b	4-NO_2	1.95 (4.68)	15.63 (37.52)	3.13 (7.51)
S7	1	15.63 (58.68)	125 (469.32)	25 (93.86)
S8	2	15.63 (55.74)	125 (445.83)	25 (89.16)
S9	3	7.81 (26.52)	31.25 (106.14)	6.25 (21.22)
S10	4	3.90 (12.64)	31.25 (101.31)	6.25 (20.26)
S11	5	31.25 (96.91)	125 (387.65)	25 (77.53)
S12	6	31.25 (92.87)	125 (371.49)	50 (148.59)
Chlorpromazine	-	7.81 (24.49)	15.63 (49.01)	12.5 (39.20)
Ciprofloxacin	-	1.95 (5.88)	3.91 (11.80)	3.13 (9.44)
Pyrazinamide	-	-	-	3.13 (25.42)

Table 5-19: Antitubercular and antibacterial activities of biphenyl derivatives

[a] Minimum Inhibitory Concentration (MIC) against *S. aureus* (ATCC 25323)

[b] Minimum Inhibitory Concentration (MIC) against *E. coli* (ATCC35218)

[c] Minimum Inhibitory Concentration (MIC) against *M. tuberculosis* H37Rv (ATCC 27294)

5.4.6. BBB Permeability Screening

BBB permeability is an indicator for possible CNS effects of the drugs. Therefore, biphenyl derivatives were screened for BBB permeability to understand the effect(s) of structural modification of the template molecule, chlorpromazine on CNS activity. The effective permeability (Pe) was determined for some commercial drugs and biphenyl derivatives. Pe of commercial drugs was compared and classified as high permeable (CNS+), low permeable (CNS-) and permeable uncertain (CNS+/-). Chlorpromazine and diazepam were classified as high permeable drugs with Pe of 6.1 x 10-6 and 12.4 x 10-6 cm/s, respectively. Atenolol, verapamil and levofloxacin were classified as low permeable drugs with Pe of 1.1 x 10^{-6}, 0.0 and 0.0 cm/s, respectively. The screened biphenyl derivatives were classified as permeability uncertain with Pe in the range between 2.1 x 10^{-6} and 3.0 x 10^{-6} cm/s. The most potent compounds in whole cell antitubercular screening (**15b** and **16b**) produced Pe of 2.5 and 2.7 x 10-6 cm/s, respectively (**Table 5-20**). A drastic reduction in BBB permeability of biphenyl derivatives was observed in comparison to chlorpromazine. CNS effect(s) of all the biphenyl derivatives is expected to be reduced.

5.4.7. In-vitro *cytotoxicity screening*

The antimicrobial drugs should be devoid of toxicity to normal mammalian cells. Therefore, biphenyl derivatives were screened against VERO (monkey kidney epithelial) cells for their toxicity. The concentration that reduced the cell viability by 50% (CC_{50}) was determined and was found to be > 100µg/mL for all the compounds in the study (**Table 5-21**). The most potent compounds in whole cell antitubercular screening (**15b** and **16b**) produced CC_{50} of 114 and 121µg/mL respectively. The selectivity of a drug molecule towards desired activity is a major challenge in antitubercular drug discovery, where the compound should be toxic only towards *Mtb*

rather than normal human cell. Selectivity index (SI) was calculated by dividing VERO cell CC_{50} to *Mtb* MIC and was found to be > 70 with compounds **14b** to **17b**, indicating their higher selectivity towards *Mtb* rather than normal mammalian cells.

Compound Code	P_e x10^{-6} cm/s	Classification
Chlorpromazine	6.1±0.059	CNS+
Atenolol	1.1±0.021	CNS-
Verapamil	0.0	CNS-
Diazepam	12.4±0.263	CNS+
Levofloxacin	0.0	CNS-
1b	2.2±0.029	CNS+/-
2b	2.3±0.107	CNS+/-
3b	2.4±0.083	CNS+/-
4b	2.5±0.214	CNS+/-
5b	2.2±0.068	CNS+/-
6b	2.1±0.051	CNS+/-
7b	2.1±0.070	CNS+/-
8b	2.6±0.065	CNS+/-
9b	2.6±0.033	CNS+/-
10b	2.8±0.320	CNS+/-
11b	2.8±0.057	CNS+/-
12b	2.8±0.083	CNS+/-
13b	3.0±0.311	CNS+/-
14b	2.5±0.072	CNS+/-
15b	2.5±0.089	CNS+/-
16b	2.7±0.091	CNS+/-
17b	2.8±0.062	CNS+/-
18b	2.4±0.074	CNS+/-
19b	3.5±0.138	CNS+/-
20b	3.3±0.202	CNS+/-
S9	2.7±0.071	CNS+/-
S10	3.0±0.093	CNS+/-

Table 5-20: BBB permeability of commercial drugs and biphenyl derivatives

Data are expressed as mean ± SEM (n =3)

CNS+ = high BBB permeation compounds, *i.e.* Pe= > 4.0 * 10^{-6} cm s^{-1}

CNS- =low BBB permeation compounds, *i.e.* Pe= < 2.0 * 10^{-6} cm s^{-1}

CNS+/- =BBB permeation uncertain compounds, *i.e.* Pe =4.0 - 2.0 *10^{-6} cm s^{-1}

Compound Code	CC_{50} (µg/ml) [a]	Selectivity Index [b]
1b	222.53±10.642	4.5
2b	187.15±3.825	7.5
3b	182.39±9.361	7.3
4b	177.81±14.538	14.2
5b	176.46±2.831	14.1
6b	184.74±2.429	14.8
7b	181.18±2.662	14.5
8b	183.84±1.264	14.7
9b	148.29±4.831	11.9
10b	152.81±1.216	48.8
11b	141.20±2.557	45.1
12b	144.54±10.491	46.2
13b	137.86±2.818	44.0
14b	120.22±2.439	77.0
15b	114.28±5.452	73.2
16b	121.31±1.294	77.8
17b	115.15±6.716	73.8
18b	130.91±1.384	10.5
19b	111.37±1.822	17.8
20b	108.45±1.715	34.6
S9	131.90±3.261	21.1
S10	150.43±7.055	24.0

Table 5-21: Cytotoxicity of biphenyl derivatives against VERO cells

[a] Data are expressed as mean ± SEM (n =3)

[b] Selectivity index is ratio of cytotoxicity (CC_{50}) to *Mtb* MIC

5.4.8. NDH-2 inhibitory screening

Compounds with strongest growth inhibition against whole *Mtb* were selected for NDH-2 inhibition screening. Percentage inhibition of residual NADH oxidation activity was recorded through NADH:menadione oxidoreduction assay. Among the screened compounds (**14b** to **17b**), **16b** produced highest percentage inhibition (22.55%) at 50µM concentrations, followed by **15b**, **14b** and **17b** with 13.41, 10.64 and 8.63 percent inhibitions respectively (**Table 5-22**). All the compounds produced < 50 percent inhibitions at 50µM concentration. Therefore, it was concluded that the IC_{50} of these biphenyl compounds could be >50µM.

Code	% inhibition of residual NADH oxidation activity at 50µM concentration
14b	10.64±1.003
15b	13.41±1.481
16b	22.55±2.714
17b	8.63±2.112
HQNQ	$82.81\pm0.524^{\#,\$,\%,\&}$

Table 5-22: NDH-2 inhibitory study of biphenyl derivatives.

Data expressed as a Mean ± SEM (n=3). $^{\#}P< 0.05$ compared to 14b, $^{\$}P< 0.05$ compared to 15b, $^{\%}P< 0.05$ compared to 16b, $^{\&}P< 0.05$ compared to 17b [One- way ANOVA followed by Newmann-Keuls test].

5.5. ATP synthase inhibition study

5.5.1. Extra-precision molecular docking

In a pilot study in **Section 5.1.3.1**, the purchasable subset of ZINC database was docked against ATP synthase. ZINC38959526 produced docking score of -6.10790 Kcal/mol, which was comparable to bedaquiline (-6.067435 Kcal/mol). There we observed structural similarity of ZINC38959526 with our designed molecules. We hypothesized that our compounds could also inhibit ATP synthase. Therefore, we did extra-precision docking of all the designed molecules against ATP synthase (PBD code: 4V1F) to understand the binding energy of the molecules against the target. Molecules **S3, S4, S9, S10** and **S16** reached the active site and produced all the essential interactions. Rest of the molecule find no pose to interact with the active site residues and died during docking. The cycloalkane class of molecules **S3, S4, S9, S10** and **S16** produced docking score of -4.9711, -5.1939, -5.64231, -5.80292 and -4.9137 Kcal/mol respectively (**Table 5-23**). These were comparable to the standard drug bedaquiline (-6.06743 Kcal/mol) and all the essential interactions with the active site residues (GLU 65 and PHE 69) were retained. The two phenyl rings of the molecule **S10** have produced π-π interaction with PHE 69 but only a single phenyl ring of **S9** was involved (**Figure 5-9**). The increased *in-vitro* inhibitory activity of **S10** in comparison to **S9** can be attributed to the involvement of two π-π interactions. A better correlation between the *in-vitro* and *in-silico* studies indicated that compounds **S9** and **S10** are potent mycobacterial ATP synthase inhibitors.

Ligand code	Lowest binding energy (Kcal/mol)	Residues forming interactions
1p-20p	-	no pose/ molecule died
1c-20c	-	no pose/ molecule died
1b-20b	-	no pose/ molecule died
S3	-4.9711	Glu 65 and Phe 69
S4	-5.1939	Glu 65 and Phe 69
S9	-5.6423	Glu 65 and Phe 69
S10	-5.8029	Glu 65 and Phe 69
S16	-4.9137	Glu 65 and Phe 69
Bedaquiline	-6.0674	Glu 65 and Phe 69

Table 5-23: Docking result of phenothiazine/carbazole/biphenyl molecules against ATP synthase

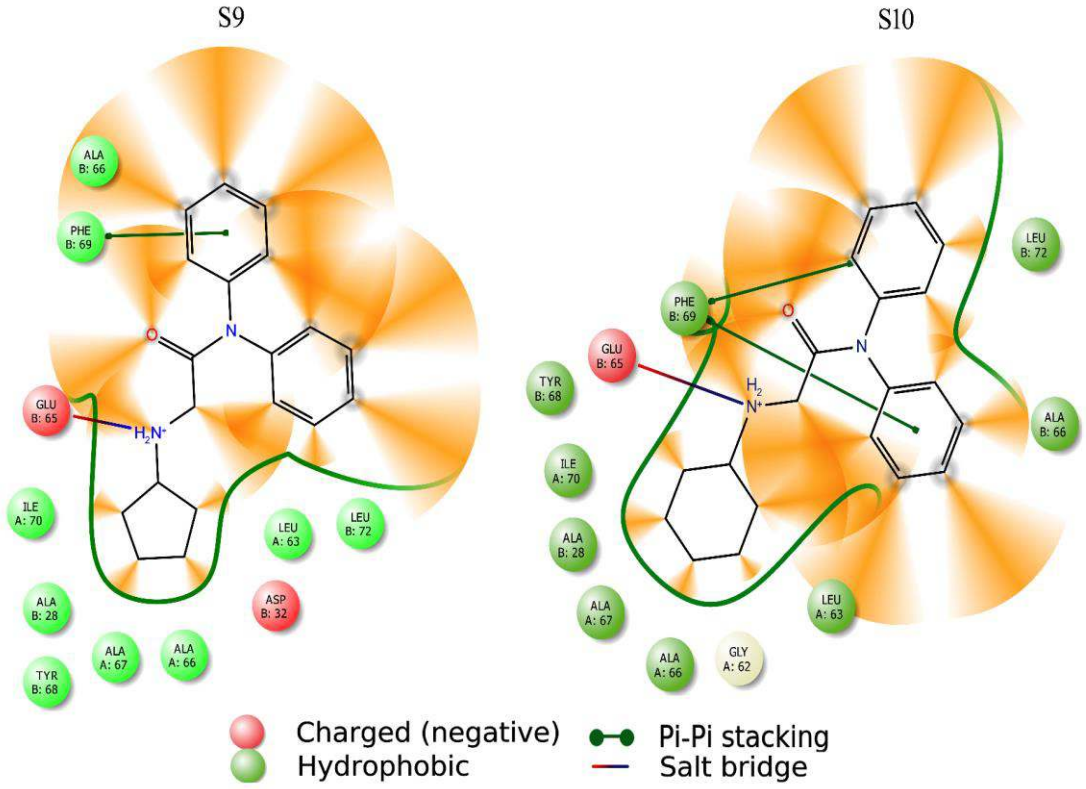


Figure 5-9: Docking pose of molecules S9 and S10 showing interactions with the active site residues of mycobacterial ATP synthase (PDB code: 4V1F)

5.5.2. In-vitro *ATP synthase inhibition assay*

The compounds **S3, S4, S9, S10** and **S16** with strongest binding with ATP synthase (PDB code: 4V1F) were screened for *in-vitro* enzyme inhibition activity. The ATP produced from the inverted membrane vesicles (IMVs) was quantified to determine the potency of the compounds. Among all the screened compounds, **S9** and **S10** showed IC_{50} of 14 and 10.4 µM, respectively. Rest of the compounds were less active at IC_{50} ≥100 µM (**Table 5-24**). At 100 µM concentration, compounds **S9** and **S10** showed 69 and 100 percent inhibition, respectively (**Figure 5-10**).

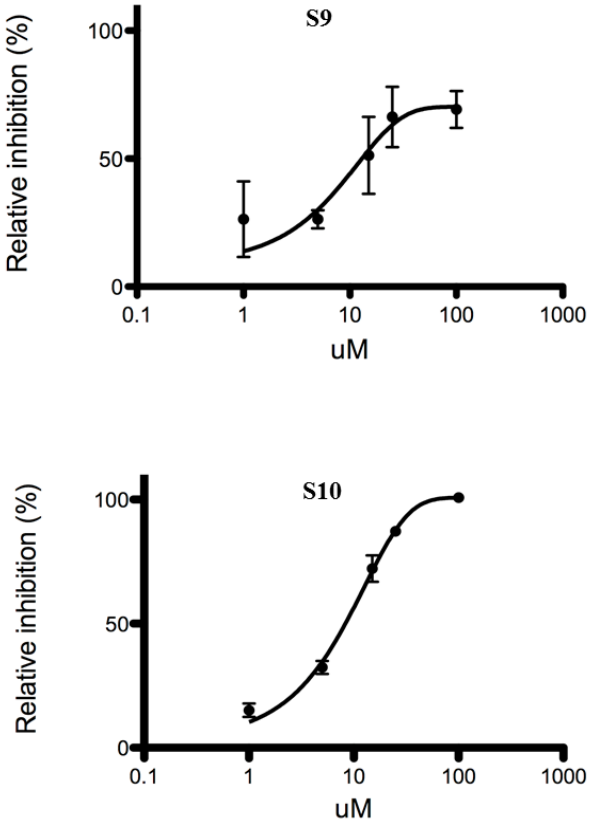


Figure 5-10: Dose response curve of compounds S9 and S10 against ATP synthesis inhibition

Compound Code	IC_{50} (µM)	Max inhibition (%) at 100 µM concentration (Mean±SD)
S3	>100	45±14.5
S4	>100	62±11.9
S9	14	69±10.18
S10	10.4	100±0.009
S16	>100	41±15.61

Table 5-24: *In-vitro* ATP synthase inhibition study; Molecules with strong binding against ATP synthase (PDB code: 4V1F) were selected for this biochemical assay