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DISCOVERY OF NEW DRUGS AND COMPUTATIONAL STUDIES OF COUMARIN-CARPROFEN SCAFFOLDS AS A NOVEL CLASS OF ANTI-TUBERCULAR, ANTI-INFLAMMATORY AND ANTI-BACTERIAL AGENTS

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ABSTRACT

Carprofen and coumarins are biologically promising potent heterocyclic compounds as they posses very good anti-tubercular, antimicrobial, anti-inflammatory and anticancer activities. In the present study, the condensation of substituted 4-bromomethyl coumarin and carprofen in the presence of anhydrous potassium carbonate gives exclusively coumarim-carprofen hybrids (3a-3i). Anti-tubercular assays against *M. tuberculosis* (H37Rv) coupled with in silico molecular docking studies indicated that dimethyl substituents **(3a)**, **(3f)** and **(3g)** showed promising activity with higher C-score values. The synthesized compounds were also screened for *in-vitro* antimicrobial and anti-inflammatory. However, the compounds of **(3c)** and **(3f)** exhibit excellent *in-vitro* activity against *M.tuberculosis* ($H_{37}Rv$). These results suggested that the as synthesized compounds of **(3c)** and (**3f)** own promising lead for subsequent investigation in search of new anti-tubercular agents. Accordingly, the *in-vitro* anti-inflammatory activity revealed that compounds of **(3a), (3b), (3c)** and **(3i)** exhibited highest prominent anti-inflammatory activity. Compounds **(3a), (3b), (3f)** and **(3i)** have shown excellent antibacterial activity.

KEYWORDS: Coumarin-carprofen, Anti-tubercular, Molecular docking, Anti-inflammatory and Antibacterial.

INTRODUCTION

Despite tremendous progress in understanding tuberculosis disease, there remains the challenge to develop agents for its therapy with fewer side effects. Most of the approved drugs have maximum amount of side effect. Tuberculosis (TB) is an infectious disease mainly caused by *Mycobacterium tuberculosis* and characterized by tubercle lesions in the lungs.[1] It is a leading cause of death worldwide, TB is one of the global health emergency because of the increase in secondary infections and/or co-infection in immune-compromised patients [such as those infected with human immunodeficiency virus (HIV)] and most worrying is the emergence of resistant strains of *M. tuberculosis* [multidrug-resistant (MDR) and extensively drug resistant (XDR) TB strains].[2] The WHO has estimated that every year about eight million new cases of tuberculosis occur and up to three million individuals die due to this disease (one person dies every 10 s).[3] It is also estimated that between 2002 and 2020, approximately a billion people will be newly infected, more than 150 million people will get sick and 36 million will die of TB.[4] Therefore, the development of new and more efficacious drugs against *Mycobacterium tuberculosis* is urgently needed. This made the researchers to design the drug which can exhibit less amount or zero amount of side effect.

Coumarin has become an essential biomolecule in the area of drug discovery, since incorporation of coumarin group into potent heterocyclic moieties, results in significant enhancement in efficacy of a drug. Further, coumarin has acquired the special significance in the medicinal chemistry arena, which is a class of compound widely available in the natural products, exhibits remarkable arrays of biological properties, like anti-microbial, anti-inflammatory, anti-oxidant effects, anti-coagulant, anti-tumor, anti-viral, as well as enzyme inhibitory actions. Most of the anti-tuberculosis drug available in the market has the coumarin nucleas, namely novobiocin and coumarin-4-acetic acid benzylidene hydrazide. In view of their extensive application of plant

origin and diverse biological properties, a large range of coumarin containing nucleus have been synthesized.

Coumarins with numerous structural functions and versatile biological properties such as antimicrobial, anticancer, anti inflammatory and anti-HIV activities have been currently reviewed.[5] They form a large class of important lactones with a fused structure, and virtually contain π -π conjugated system with rich electron and good charge-transport properties. This kind of fused ring endues coumarin-based derivatives with various applications in the fields of bromatology, material and supramolecular chemistry as well as medicinal chemistry. Moreover, the unique shape of coumarin has a unique potential which permits its derivatives readily have interaction with diversity of enzymes and receptors in organisms through weak bond interactions and thereby exhibit wide potentiality as medicinal drugs.[6] As a result, coumarin as medicinal drugs have been an increasing number of attracting special interest due to their capability terrific contributions in prevention and treatment of diseases and their related researches and trends have turn out to be incredibly attractive highlights.[7,9] Whereas several coumarin scaffolds such as Coumarin-triazole coupled hybrids[1], Chalconated coumarin[2], Iodinated-4-aryloxymethyl-coumarin[3] and 4aryl/alkyl sulfonyl methyl coumarin[4] has shown potent anti-TB activities with MICs of 6.25, 3.5, 1.56 and 0.78μg/mL respectively.[10,14] Whereas coumarin–thiazoline hybrids[5] and Benzocoumarin-pyrimidine hybrids[6] have been reported from our laboratory as potential anti-tubercular agent.[15,16] Compounds mentioned above are represented in **Figure 1.**

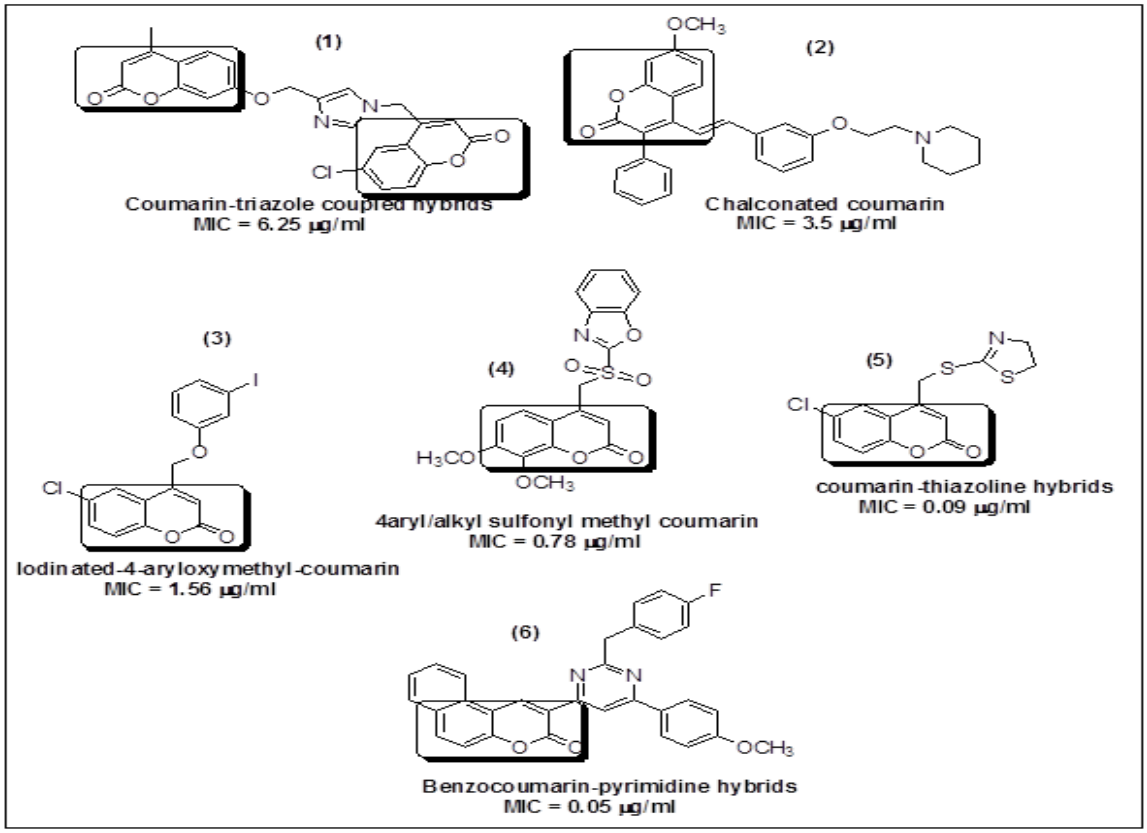


Figure 1. Structures of some potent coumarin derivatives exhibiting anti-tubercular activity.

On other hand, Carprofen is an aryl-propionic acid class of a non-steroidal anti-inflammatory drug (NSAID), containing carbazole nucleus. Whereas carbazole is an aromatic heterocyclic organic compound. It has a tricyclic structure, consisting of two six-membered benzene rings fused on either side of a five-membered nitrogen-containing ring. Although carbazole resemble the indole structure, but in which a second benzene ring is fused onto the five-membered ring at the 2–3 position of indole. Because of, indole nucleus in the carbazole exhibits better pharmacological activities compared to other nitrogen containing alkaloids.

Indole nucleus is also involved in the proteins as an amino acid such as tryptophan, therefore indole nucleus, a biologically accepted pharmacophore in medicinal chemistry, has made it versatile heterocyclic possessing a large spectrum of biological activities.

Carbazole scaffolds are biologically potent and possess a wide spectrum of pharmacological activities, such as antibacterial,[17,19] anti-inflammatory, antifungal,[20,21] antitumour, antineoplastic,[22,26] anticonvulsant,[27] antioxidant,[28] antidiabetic.[29] The recent studies has shown the importance of carbazole based compound as a promising anti-tuberculosis agent such as Clausenine [1],

Clausine-A [2], 7-Methoxymukonal [3], Murrayaline-C [4] and Carbalexin-C [5] has shown anti-TB activities with MICs of 45.1µM, 29.3µM, 24.3µM, 2.8 µM and 1.5µM respectively.[30] Compounds are represented in **Figure 2.**

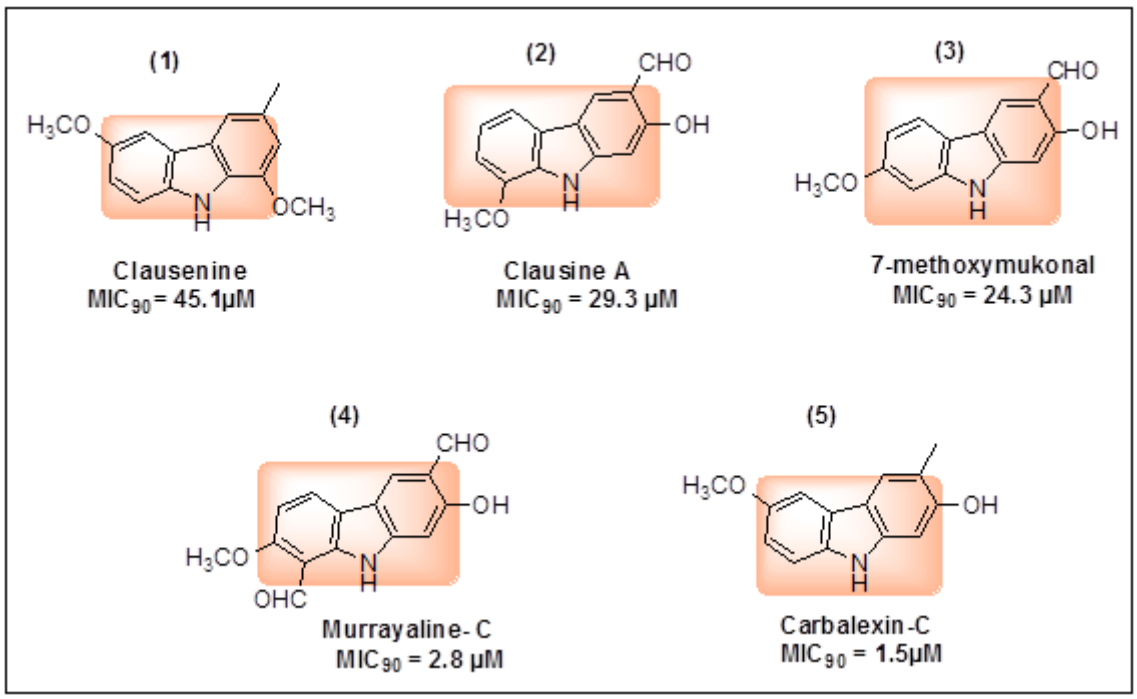


Figure 2. Anti-tubercular carbazole analogues.

An integrated venture towards medicinal chemistry, in this context it was achieved by the introduction of pharmacophoric moieties into coumarin to obtained Coumarin- Carprofen scaffold hybrids. Thus, the title compounds **(3a-3i)** are obtained by activating the condensation of 4- bromomethyl coumarin with carprofen in **Figure 3**. Therefore, an exhaustive survey of the literature reveals that no work has been reported on the synthesis of Coumarin- Carprofen hybrids. Hence, to the best of our knowledge, the unique synthesis of title compounds is the first report of heterocyclic scaffolds **(Scheme 1)**. The preliminary screening of title compounds have been carried out for their pharmacological activities such as anti-TB, anti-inflammatory and antibacterial and also molecular docking study.

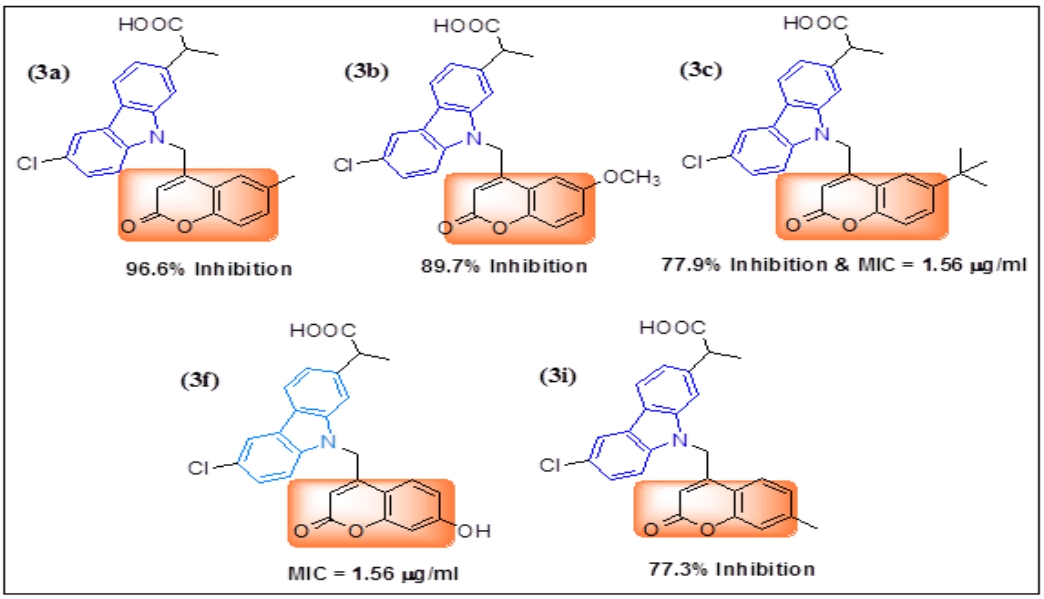
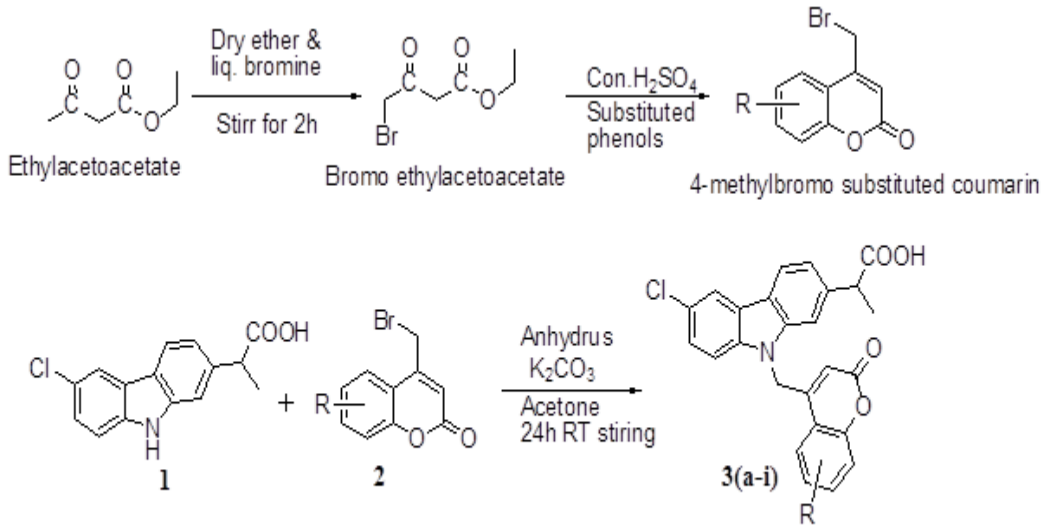


Figure 3. Some of the synthesized compounds shown excellent anti-inflammatory and anti-tubercular activity.

CHEMISTRY

Carprofen (CPF) **1** is commercially available and can be synthesized according to literature method.[31] The derivatives of 4-(bromomethyl)-2*H*-chromen-2-ones **(2a-2i)** were synthesized via Pechmann cyclization of phenols with 4-bromoethylacetoacetate [32] presented in **Scheme-1**.

Further, carprofen **(1)** (0.01mole) in presence of anhydrous K_2CO_3 (0.03mole) was treated with the substituted 4-(bromomethyl)-2*H*-chromen-2-ones **(2a-2i)** (0.011mole) using acetone as the solvent afforded derivatives of 2-(6-chloro-9-((2-oxo-2*H*-chromen-4-yl)methyl)-9*H*-carbazol-2-yl)propanoic acid **3(a-i)** (**Scheme-1**).



Scheme 1. Schematic represent of Coumarin - Carprofen hybrids.

RESULT AND DISCUSSION

The newly synthesized compounds **(3a-3i)** structure was confirmed by spectroscopic techniques. Where in IR spectrum of compound **3a** (R=6-CH_3) exhibited two stretching band at 1716.14 and 1738.79cm^{-1} due to lactone and acid carbonyl respectively. Single band was observed at 3319.53cm^{-1} due to -OH stretching of carboxylic acid. In case of GC-mass spectrum peak of compound **3a** was observed at *m/z* 445. Further 3a was confirmed by ^{1}H-NMR wherein -OH of propionic carboxylic acid is resonated at 11.387ppm as singlet, C_3-H of coumarin resonated as a singlet at 6.205 ppm, methylene linker C_{11}-H resonated at 5.355ppm as a singlet and coumarin C_6-CH_3 resonated at 2.184 ppm. C_{16}-H of carprofen resonated as quartet at 4.179 ppm whereas C_{17}-H of CPF appeared as doublet at 1.548 ppm. Aromatic protons resonated as multiplets between 7.13-8.18 ppm. Numbering of the structure is shown in **Figure 4**.

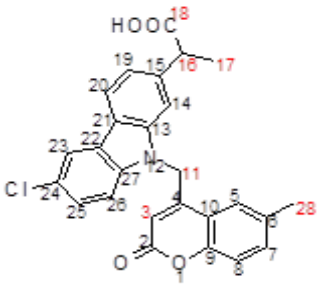


Figure 4. Numbering of the compound 3a.

BIOEVALUATION

All the Coumarin-carprofen hybrids were evaluated for their *in-vitro* anti-tubercular, anti-bacterial, anti-inflammatory and anti-cancer activity. From these evaluations it is cleared that, coumarin-carprofen hybrids are potent anti-tuberculosis agent as well as anti-inflammatory.

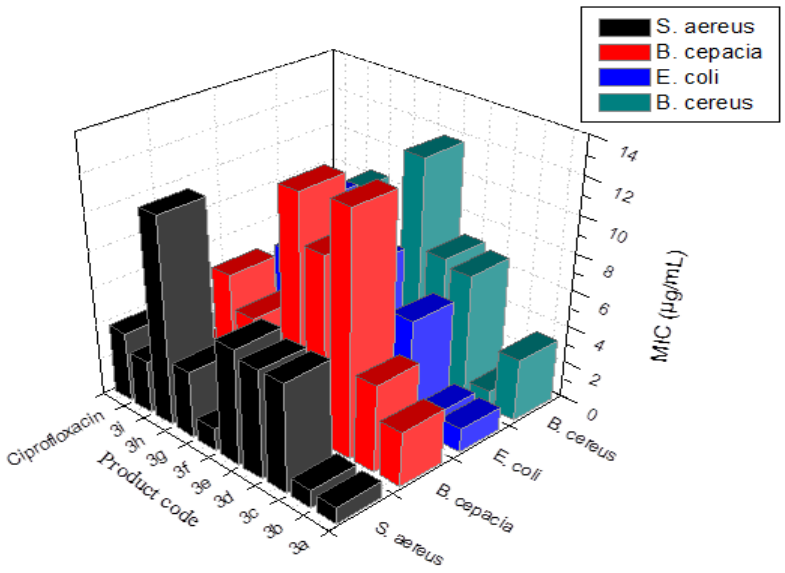
***In-vitro* anti-bacterial activity.**

In-vitro antibacterial activity of synthesized compounds was performed using disk diffusion method.[33,34] The bacterial strains namely *Styphylococcus aereus* (MTCC 12598), *Burkholderia cepacia* (MTCC 438), *Escherichia coli* (ATCC 25992) and *Bacillus cereus* (ATCC 11778). The microbial strains were cultured overnight at 37°C in nutrient broth agar medium. The broth cultures were compared to the turbidity with that of the standard 0.5 McFarland solution. All the bacterial organisms were maintained at 4°C for further use. The newly synthesized compounds were tested for antibacterial activity under disc diffusion method by taking ciprofloxacin as standard. Sterile disks (6 mm, Himedia) impregnated with 20 µl of diluted synthetic compounds with DMSO (2 mg/ml), disks saturated with only DMSO treated as negative control and disks with ciprofloxacin served as positive controls, and were placed on the surface of the agar plates. Further, all the plates were incubated for 24 hrs at 37^{0}C. The zone of inhibition around the well in each plate was measured in mm.

Minimal inhibitory concentration (MIC) determination

The minimum inhibitory concentration of the **3(a-i)** was determined by dilution method[35] as follows. The lowest concentration of compounds which were able to produce bacterial inhibition zone around the disk were considered as MIC values. The as synthesized were dissolved and diluted to give two-fold serial concentrations of the compounds was employed to determine the MIC. The MIC value was determined as the lowest concentration of the coumarin-carprofen hybrids inhibiting the visual growth of the microorganism on the agar plate.

In vitro antibacterial activities of synthetic compounds were evaluated by disk diffusion method. As a result, compound **3a** and **3b** found to be more effective against the tested bacterial strains and observed high zone of inhibition around 30 mm for higher concentration (40µg/mL). Further MIC value of **3a** and **3b** synthesized compounds was found to be 0.90 and 1.0 µg/ml respectvely for *S. aereus* and it has shown 12 mm zone of inhibition for *E. coli* with the MIC value of 1.3 and 1.35 µg/ml respectively. The compounds **3i** exhibits 11 mm of zone of inhibition and MIC was found to be 2.85 and 0.95 µg/ml for *S. aereus and B. cepacia* respectively. Compounds **3d** and **3f** showed 12 mm of zone of inhibition with MIC value of 1.9 and 1.7 µg/ml for *E. coli*. Additionally, compounds **3c** and **3e** are reactive to bacteria used, both the compounds got their MIC at the concentration 5.85 and 7.55 µg/ml for *E. coli*. However, **3g** was found to be less reactive in the study, 4.8 µg/ml was found to be MIC value with treatment of *B. cereus*. **3h** observed to be less reactive to bacterial strains and showed comparatively lesser biological activity than the other compounds with highest MIC value of 11.25 µg/ml for *S. aereus*. Ciprofloxacin served as control and yielded the MIC of 3.8, 0.9, 1.0 and 4.55 µg/ml for S. *aereus, B. cepacia, E. coli* and *B. cereus* respectively. the detailed account of antibacterial activity of all the compounds is tabulated in the **Graph 1**.



Graph 1. Graphical representation of *In-vitro* anti-bacterial activity of compound (3a-3i).

***In-vitro* anti-inflammatory activity**

In vitro anti-inflammatory activity of the synthesized compounds (3a-3i) were evaluated by protein denaturation method[36] as follows. Diclofenac sodium (non steroidal anti- inflammatory drug) was used as a standard drug. The reaction mixture consisting of 2.0 mL of known concentration of compounds **3a-3i** (100 µg/mL) or standard diclofenac sodium (100 µg/mL) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 2.0 mL of egg albumin (from fresh hen's egg) and incubated at 27°C for about 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for about 10 min. After cooling, the absorbance was measured by recording at 660 nm using double distilled water as blank. Each experiment was done in triplicate and the average values were taken. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$[\%\ inhibition\ = \frac{(Ac - At)}{Ac} \times 100]$$

Where, A_c=absorbance of control; A_t =absorbance of test sample.

Statistical analysis

All experiments were performed in triplicates (n=3) and the data are presented as the mean ± standard deviation. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software 20 Version (IBM). The significance of differences was defined at the $p < 0.05$ and $p < 0.01$ level.

In the present study known concentration of compound **(3a-3i)** were subjected for anti-inflammatory activity on protein denaturation. *In-vitro* anti-inflammatory activity of the tested compounds was comparable to the Diclofenac sodium, a reference drug, along with carprofen as a standard drug. A significant difference was observed in the inhibition of thermally induced protein denaturation. It was observed that all tested compounds were compared with standard drug at concentration of 100µg/ml. In all tested compounds inhibition activity of compound **(3a)** was more than reference drug diclofenac sodium but less than the carprofen, whereas remaining tested compounds showed less inhibitory activity which is as shown in **Table 1.** The results revealed that compound (**3a)** exhibited prominent anti-inflammatory activity with **96.59%** of inhibition, whereas compound **(3b), (3c), (3i)** & **(3f)** has shown **89.70%, 77.94%, 77.25%** and **70.60%** of inhibition respectively, whereas remaining derivatives has shown moderate activity.

Table 1. Anti-inflammatory Assay.

Test Compounds	Used concentration	% Inhibition
Diclofenac Sodium	100 µg	**95.4833±1.58863****
Carprofen	100 µg	**98.0833±0.30022****
3a	100 µg	**96.5967±1.30021****
3b	100 µg	**89.7000±0.99947****
3c	100 µg	**77.9467±1.82618***
3d	100 µg	65.2600±1.38005*
3e	100 µg	49.8233±1.67727
3f	100 µg	**70.6067±2.17054***
3h	100 µg	53.1200±1.20089
3g	100 µg	47.5633±0.91719
3i	100 µg	**77.2533±0.75554***

Bold values signifies compounds having highest activity.

Results are expressed as Mean±SE (n=3); ****significant at the p < 0.01.

*** Correlation is significant at the 0.01 level (2-tailed)***

** Correlation is significant at the 0.05 level (2-tailed)**

***In-vitro* anti-tubercular activity**

All the coumarin-carprofen hybrids are evaluated for their potent *In-vitro* anti-tubercular activity against *M. tuberculosis* ($H_{37}Rv$) strain (ATCC No- 27294) in BACTEC 12 B medium, using Microplate Alamar Blue Assay (MABA). The results are summarized in **Table. 2.** All the synthesized compounds showed excellent results with MIC ranging from 50-1.56 µg/mL. Compounds (**3c)** and (**3f)** has shown more significant anti-tubercular activity with MIC of 1.56 µg/mL, which is more significant than the standard drug, pyrazinamide and ciprofloxacin having MICs of 3.125 µg/mL. Compound (**3a)** has shown MIC values of 3.12 µg/mL. (**3i)** exhibited MIC values of 6.25 µg/mL, which is equal to streptomycin with MIC values of 6.25 µg/mL. Whereas compounds (**3d)** exhibited MIC values of 12.5 µg/mL, remaining (**3b), (3e), (3g)** and (**3h)** showed moderate activity with MIC values of 25, 25, 50 and 50 µg/mL respectively.

Table 2. In vitro anti-tubercular activity of compounds (3a-3i) against *M.tuberculosis* (H37 RV strain).

Compound	R	MIC (µg/mL)
3a	6-CH_3	3.12
3b	6-OCH_3	25
3c	**6-$C(CH_3)_3$**	**1.56**
3d	6-Cl	12.5
3e	5,7- DiCH_3	25
3f	**7-OH**	**1.56**
3g	5,6- Benzo	50
3h	7,8- Benzo	50
3i	7- CH_3	6.25
Pyrazinamide	3.125	
Streptomycin	6.25	
Ciprofloxacin	3.125	

Bold values signifies compounds having highest activity.

Computational studies

Molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0.[39] Crystal structure of *Mycobacterium tuberculosis* DprE1 in complex with CT319 under code 4FDO[40] was extracted from the Brookhaven Protein Database (PDB:

http://www.rcsb.org/pdb). All the essential hydrogen atoms were added to define the correct configuration and tautomeric states. Then the modelled structure was energy-minimized using Tripos force field with distance dependent dielectric function and partial atomic charges were calculated by AMBER7 FF02 method and finally water molecules were removed from the model. The geometry of the compounds **(3a-3i)** was subsequently optimized to minimal energy using the Powell energy minimization algorithm, Tripos force field with MMFF94 charges. The compounds **(3a-3i)** was then docked onto the binding pocket of the enzyme for docking-scoring analysis. To identify the ligand-protein interactions, the top pose and protein were loaded into work area and the MOLCAD (Molecular Computer Aided Design) program was employed to visualize the binding mode between the protein and ligand. **Table.3** represents the docking score of the coumarin - carprofen scaffolds.

Surflex-docking was employed to understand the interaction between enzyme and compounds **(3a-3i)** and to ultimately elucidate the interaction mechanism. Results obtained by Surflex-docking tools presented 20 conformations of compounds **(3a-3i)**. We selected the best.

Table 3. Surflex Docking score (kcal/mol) of the coumarin derived carprofen.

Compds	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
3a	**7.55**	**-2.39**	**0.95**	**-130.429**	**26.678**	**-280.924**	**33.022**
3b	6.65	-1.52	1.91	-141.098	-42.839	-251.357	-34.628
3c	6.62	-1.41	0.01	-141.962	-44.405	-279.358	-32.762
3d	6.65	-1.17	0.01	116.839	36.295	-244.651	-31.209
3e	6.65	-1.17	0.01	-116.839	-36.295	-244.651	-31.209
3f	**6.83**	**-1.27**	**2.40**	**-122.216**	**-59.659**	**-285.740**	**-33.961**
3g	**7.42**	**-3.72**	**1.08**	**-159.210**	**-48.823**	**-366.426**	**-38.365**
3h	6.27	-2.61	1.10	-147.474	-32.749	-290.794	-36.614
3i	6.55	-2.77	0.88	-150.468	-38.615	-299.134	-35.809

Bold values signifies compounds having highest activity

**Asterisk indicates compound with highest C Score.*

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

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

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

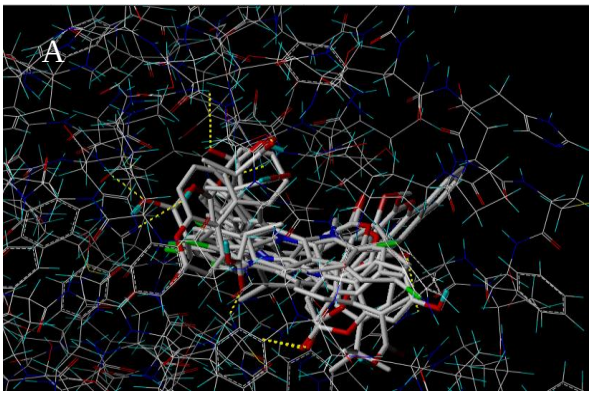
[d] *D-score for charge and van der Waals interactions between the protein and the ligand.*[41]

[e] *PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF).*[42]

[f] *G-score showing hydrogen bonding, complex (ligand-protein) and internal (ligand-ligand) energies.*[43]

[g] *Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.*[44]

Conformation for further analysis, owing to its higher binding affinity and lowest molecular energy. **Figure 5. (A & B)** shows the docked view of all the compounds at the active site of the enzyme (PDB ID: *4FDO*), among all the studied compounds (**3a** and **3f**) have showed better scores.



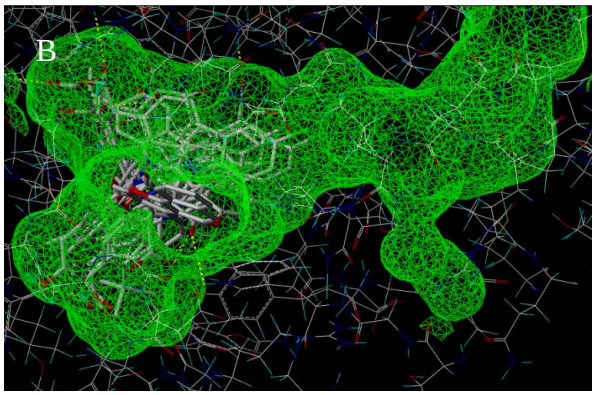


Figure 5. Docked view of all 9 compounds (3a-3i) at the active site of the enzyme PDB ID:4FDO.

Compound **(3a),** has showed two hydrogen bonding interaction as depicted in **Figure 6. (A-C)** and **Figure 7.** oxygen atom of carbonyl group present at 2nd position of coumarin ring makes an hydrogen bonding interaction with hydrogen atom of amino acid residue GLY117 (C=O ----- H-GLY117, 2.36 Å) and hydrogen atom of

hydroxyl group makes an hydrogen bonding interaction with oxygen atom of amino acid residue LEU363 (O-H ---- H-LEU363, 2.20 Å). As depicted in **Figure 8. (A-C)** and **Figure 9.** compound **(3f)**, has showed two hydrogen bonding interaction, oxygen atom of carbonyl group present at 2nd position of coumarin ring makes an hydrogen bonding interaction with hydrogen atom of amino acid residue LYS418 (C=O ----- H-LYS418, 1.82 Å) and oxygen atom of carbonyl group makes an hydrogen bonding interaction with hydrogen atom of amino acid residue SER228 (C=O ----- H-SER228, 2.04 Å).

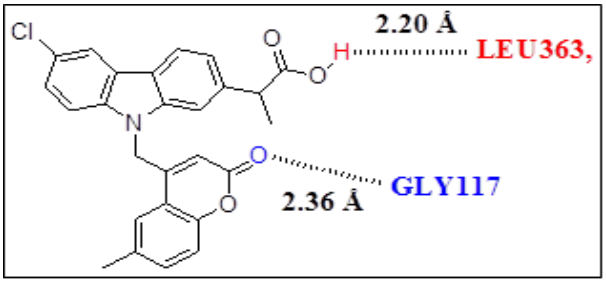
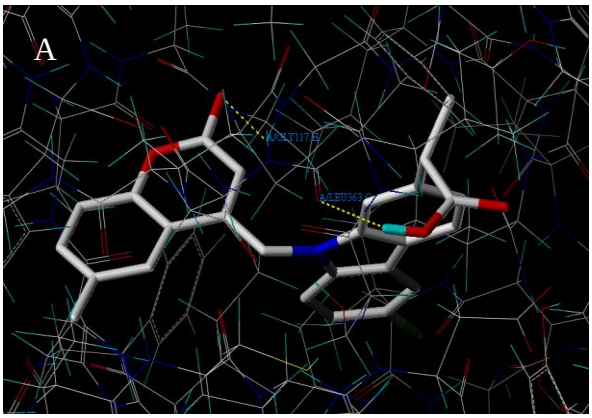
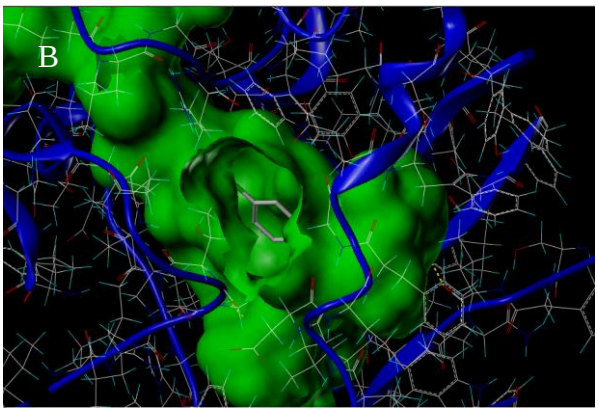


Figure 7. Binding interactions of (3a) at the active site of the enzyme 4FDO.





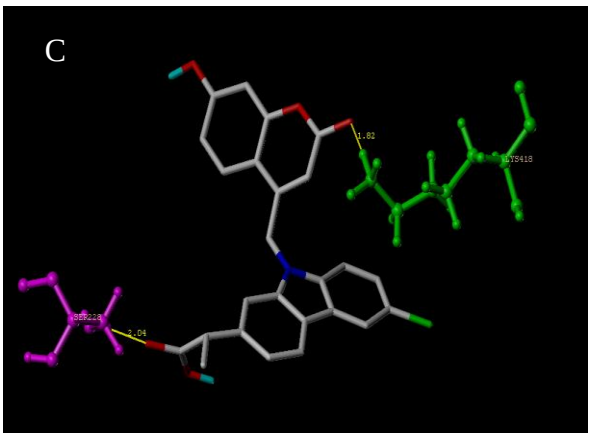
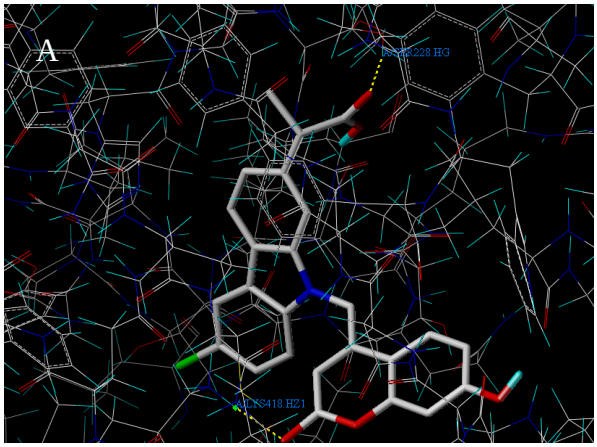
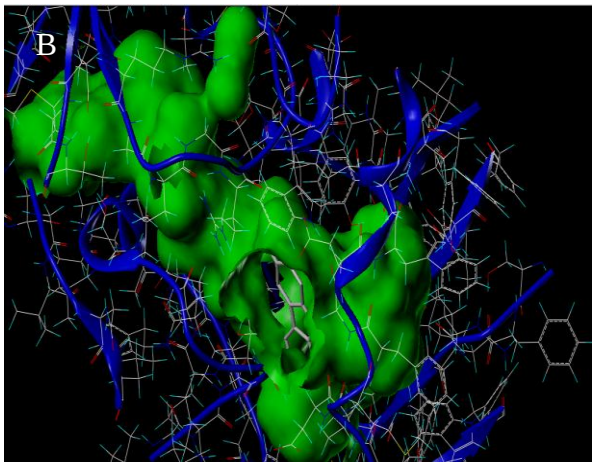


Figure 6. Details of the interactions between the enzyme and compound (3a) in the docked model.





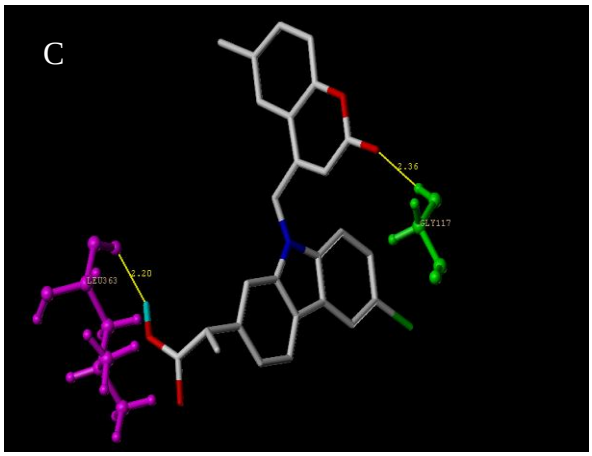


Figure 8. Details of the interactions between the enzyme and compound (3f) in the docked model.

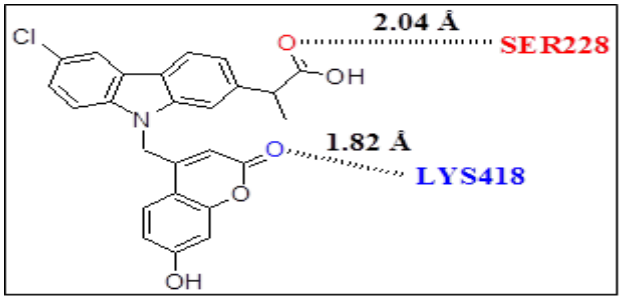
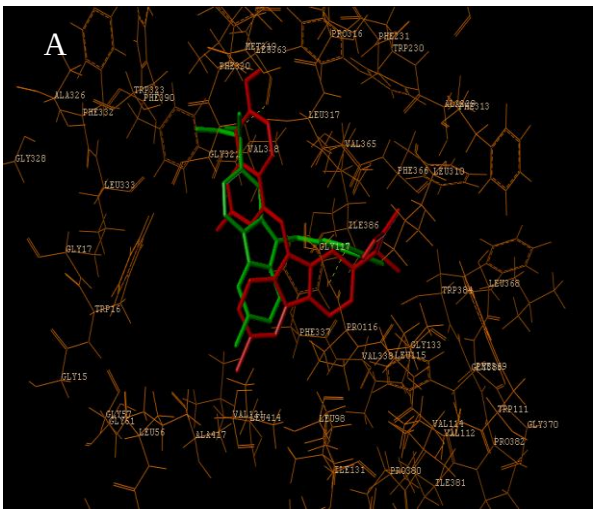


Figure 9. Binding interactions of (3f) at the active site of the enzyme 4FDO.

Both the compounds were surrounded by hydrophobic amino acids (ALA326, PHE332, LEU333, GLY17, GLY328, TRP323, PHE390, PHE320, LEU317, VAL365, ILE386, PRO316, GLY117, LEU98, GLY370) and hydrophilic amino acids (CYS387, ASN385, LYS367, SER228, SER227, SER246, GLN336, GLN334, TYR335, ASN324, LYS418THR416, ARG325, ASP318, HIS136, ASN135, TYR314, LYS134) (**Figure 10. A** and **B**).



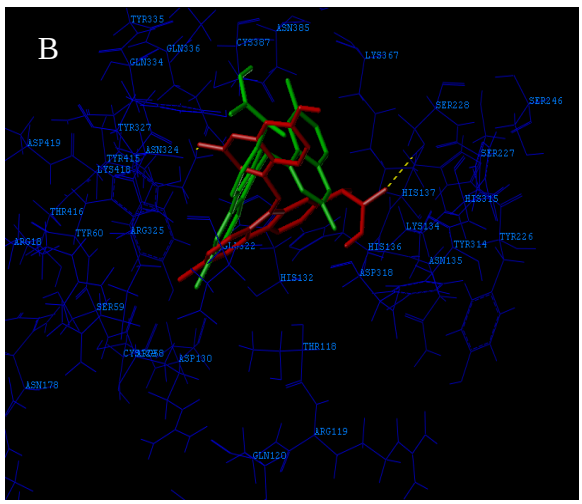


Figure 9. A) Hydrophobic amino acids surrounded to compounds (3a) (green colour) and (3f) (redcolour) B) Hydrophilic amino acids surrounded to compounds (3a) and (3f).

CONCLUSION

In summary, the newly synthesized coumarin - carprofen hybrids, **3(a-i)** have been designed and synthesized by reacting 2-(6-chloro-9H-carbazol-2-yl)propanoic acid with different substituted 4-(bromomethyl)-2H-chromen-2-one in presence of anhydrous potassium carbonate. The ease of separation of pure product and high yields of compounds are having unique features of this reaction. These compounds were purified by preparative TLC and also using column chromatography. The compounds were characterized by IR, mass spectrometry, ^{1}H NMR ^{13}C NMR and Elemental analyses. All these compounds are screened for *in-vitro* antibacterial, anti-inflammatory and exhibited potent *in-vitro* anti-mycobacterial activity against *M. tuberculosis* H37Rv (ATCC 27294 strain). Some of the derivatives displayed MIC values below 6.25 µg/ mL. Whereas, molecular docking study reveals, the compounds **(3a)**, **(3f)** and **(3g)** have shown highest CScore value. From all tested compounds, two compounds, **(3c)** and **(3f)**, possess the maximum M. tuberculosis inhibitory activity with MIC = 1.56 µg/mL and is 13 times more active than pyrazinamide and ciprofloxacin. However anti-inflammatory activity of compound **(3a)** is more than reference drug diclofenac sodium but less than the carprofen. Based on the pharmacological aspects, we believe that the observed results should be useful in guiding future global efforts to discover new drugs for exploration in medicinal chemistry.

EXPERIMENTAL SECTION

General Chemistry Methods

Melting points were determined with open capillary method on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument (Nicolet, Madison, WI, USA) as using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400MHz Spectrometer using DMSO-d_6 as solvent and TMS as an internal standard and chemical shifts were reported as δ values (ppm). Mass spectra were recorded using Shimadzu GCMSQP2010S. The elemental analysis was carried out using Hereaus CHN rapid analyzer. Purity of the compound was checked by TLC. All the chemicals purchased were of analytical grade and were used without further purification unless otherwise stated.

General procedure for the synthesis of 2-(6-chloro-9-((6-methyl-2-oxo-2H-chromen-4-yl)methyl)-9H-carbazol-2-yl)propanoic acid (3a). To a solution of compound **1** (1.0 molar equiv) in acetone (3 molar equiv) anhydrous K_2CO_3 were added, and stirred the mixture at room temperature for 2 to 3h to this mixture, **2** (1.1 molar equiv) were added. The mixture was stirred at room temperature for 12h, and completion of the reaction was confirmed by TLC. Then, the reaction mixture was cooled, quenched in ice water, separated solid was filtered and washed with water and recrystallized from acetone. Yield 84%; m.p: 210-212°C; IR (KBr) cm^{-1} 1716.14 (C=O lactone) and 1738.79 (C=O acid); ^{1}H-

NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 11.38 (s, 1H, -COOH), 7.13-8.18 (m, 9H, Ar-H), 6.20 (s, 1H, C_3-H), 5.35 (s, 2H, -NCH_2), 4.17 (q, J = 5.6 Hz, 1H, C_{16}-H), 2.18 (s, 3H, C_6-CH_3), 1.54 (d, J=4.8 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.5, 20.1, 44.7, 61.514, 109.9, 112.2, 112.3, 116.2, 116.3, 118.4, 119.6, 120.7, 120.8, 122.8, 123.5, 124.2, 125.1, 132.9, 133.6, 138.3, 138.5, 140.5, 149.9, 151.0, 159.4, 173.4; EI-MS: $M^{+\cdot}$ *m/z* 445; Anal Calcd. for $C_{26}H_{20}ClNO_4$ (%), Calcd: C, 70.03; H, 4.52; N, 3.14; found: C, 70.01; H, 4.50; N, 3.13.

2-(6-chloro-9-((6-methoxy-2-oxo-2H-chromen-4-yl)methyl)-9H-carbazol-2-yl)propanoic acid ***(3b).*** Yield 87%; m.p: 221-225°C; IR (KBr) cm^{-1} 1714.53 (C=O lactone) and 1736.53 (C=O acid); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 11.39 (s, 1H, -COOH), 7.03-8.18 (m, 9H, Ar-H), 6.22 (s, 1H, C_3-H), 5.40 (s, 2H, -NCH_2), 4.18 (q, J=6 Hz, 1H, C_{16}-H), 3.687 (s, 3H, C_6-OCH_3), 1.54 (d, J= 6 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.6, 44.7, 55.5, 61.5, 107.2 109.9, 112.3, 112.4, 117.6, 118.4, 119.5, 119.6, 120.8, 122.8, 123.5, 125.1, 138.3, 138.5, 140.5, 147.2 149.9, 155.4, 159.5, 173.4; EI-MS: $M^{+\cdot}$ *m/z* 461; Anal Calcd. for $C_{26}H_{20}ClNO_5$ (%), Calcd: C, 67.61; H, 4.36; N, 3.03, found: C, 67.58; H, 4.35; N, 3.02.

2-(9-((6-tert-butyl-2-oxo-2H-chromen-4-yl)methyl)-6-chloro-9H-carbazol-2-yl)propanoic acid ***(3c).*** Yield 77%; m.p: 230-232°C; IR (KBr) cm^{-1} 1713.03 (C=O lactone) and 1738.13 (C=O acid); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 11.35 (s, 1H, -COOH), 7.24-8.34 (m, 9H, Ar-H), 6.42 (s, 1H, C_3-H), 5.34 (s, 2H, -NCH_2), 4.06 (q, J=8 Hz, 1H, C_{16}-H), 1.31 (s, 9H, C_6-$(CH_3)_3$), 1.70 (d, J=4 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.66, 31.2, 34.5, 45.86, 61.4, 109.6, 111.6, 112.9, 116.8, 119.31, 119.37, 119.9, 120.8, 121.9, 124.1, 124.9, 125.9, 129.7, 138.1, 138.2, 140.3, 147.5, 149.2, 160.7, 173.86; EI-MS: $M^{+\cdot}$ *m/z* 487, $[M+1]^{+\cdot}$ 488; Anal Calcd. for $C_{26}H_{20}ClNO_4$ (%), Calcd: C, 71.38; H, 4.37; N, 2.87, found: C, 71.36; H, 4.34; N, 2.86.

2-(6-chloro-9-((6-chloro-2-oxo-2H-chromen-4-yl)methyl)-9H-carbazol-2-yl)propanoic acid ***(3d)***. Yield 87%; m.p: 215-217°C; IR (KBr) cm^{-1} 1716.28 (C=O lactone) and 1734.57 (C=O acid); 11.39 (s, 1H, -COOH), 7.03-8.18 (m, 9H, Ar-H), 6.22 (s, 1H, C_3-H), 5.40 (s, 2H, -NCH_2), 4.18 (q, J=6 Hz, 1H, C_{16}-H), 1.54 (d, J= 6 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.6, 44.7, 55.5, 107.2 109.9, 112.3, 112.4, 117.6, 118.4, 119.5, 119.6, 120.8, 122.8, 123.5, 125.1, 138.3, 138.5, 140.5, 147.2 149.9, 155.4, 159.5, 173.4; EI-MS: $M^{+\cdot}$ *m/z* 465; Anal Calcd. for $C_{25}H_{17}Cl_2NO_4$ (%), Calcd: C, 64.39; H, 3.67; N, 3.00, found: C, 64.36; H, 3.35; N, 2.97.

2-(6-chloro-9-((5,7-dimethyl-2-oxo-2H-chromen-4-yl)methyl)-9H-carbazol-2-yl)propanoic acid ***(3e)***. Yield 71%; m.p: 198-200°C; 1720.31 (C=O lactone) and 1735.51 (C=O acid); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 11.40 (s, 1H, -COOH), 6.87-8.17 (m, 9H, Ar-H), 6.14 (s, 1H, C_3-H), 5.44 (s, 2H, -NCH_2), 4.17 (q, J=4 & 8 Hz, 1H, C_{16}-H), 2.47 (s, 3H, C_7-CH_3), 2.25 (s, 3H, C_5-CH_3), 1.54 (d, J=8 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 19.05, 20.98, 23.14, 45.23, 64.16, 110.45, 111.54, 111.79, 112.86, 114.58, 115.81, 119.00, 120.20, 121.23, 121.37, 123.38, 125.68, 129.88, 136.55, 138.86, 138.93, 141.02, 142.55, 152.33, 154.96, 159.63, 173.75; EI-MS: $M^{+\cdot}$ *m/z* 459; Anal Calcd. for $C_{27}H_{22}ClNO_4$ (%),Calcd: C, 70.51; H, 4.82; N, 3.05, found: C, 70.50; H, 4.80; N, 3.03.

2-(6-chloro-9-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-9H-carbazol-2-yl)propanoic acid ***(3f)***. Yield 80%; m.p: 220-222°C; IR (KBr) cm^{-1} 1700.76 (C=O lactone) and 1732.33 (C=O acid); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 11.37 (s, 1H, -COOH), 7.09-8.17 (m, 9H, Ar-H), 6.69 (s, 1H, C_3-H), 5.32 (s, 2H, -NCH_2), 3.83 (q, J=8 Hz, 1H, C_{16}-H), 10.62 (s, 3H, C_7-OH), 1.43 (d, J=8 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 19.41, 45.56, 61.99, 100.64, 109.78, 110.25, 112.82, 119.20, 120.11, 120.87, 121.10, 123.32, 125.57, 131.51, 133.42, 136.82, 138.78, 140.21, 141.00, 145.93, 159.62, 161.98, 176.04; EI-MS: $M^{+\cdot}$ *m/z* 447; Anal Calcd. for $C_{25}H_{18}ClNO_5$ (%), Calcd: C, 67.04; H, 4.05; N, 3.13; found: C, 67.02; H, 4.04; N, 3.11.

2-(6-chloro-9-((3-oxo-3H-benzo[f]chromen-1-yl)methyl)-9H-carbazol-2-yl)propanoic acid ***(3g)***. Yield 69%; m.p: 247-249°C; IR (KBr) cm^{-1} 1714.31(C=O lactone) and 1736.50 (C=O acid); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 11.37 (s, 1H, -COOH), 8.03-8.31 (m, 3H, Ar-H), 7.512-7.70 (m, 9H, Ar-H), 6.21 (s, 1H, C_3-H), 5.32 (s, 2H, -NCH_2), 4.11 (q, J=4.8 & 9.6 Hz, 1H, C_{16}-H), 1.47 (d, J=2.4 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.79, 44.08, 48.01, 101.84, 107.05, 107.18, 108.66, 111.18, 112.91, 113.14, 113.84, 115.51, 118.06, 119.83, 122.46, 123.47, 126.05, 126.23, 126.36, 129.08, 129.28, 130.10, 131.44, 134.72, 142.48, 148.60, 155.03, 160.0, 174.717; EI-MS: $M^{+\cdot}$ *m/z* 481; Anal Calcd. for $C_{29}H_{20}ClNO_4$ (%), Calcd: C, 72.27; H, 4.18; N, 2.91; found: C, 72.25; H, 4.17; N, 2.90.

2-(6-chloro-9-((2-oxo-2H-benzo[h]chromen-4-yl)methyl)-9H-carbazol-2-yl)propanoic acid ***(3h)***. Yield 70%; m.p: 254-256°C; IR (KBr) cm^{-1} 1718.23 (C=O lactone) and 1734.31 (C=O acid); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 11.46 (s, 1H, -COOH), 8.09-8.40 (m, 3H, Ar-H), 7.60-7.81 (m, 9H, Ar-H), 6.30 (s, 1H, C_3-H), 5.41 (s, 2H, -NCH_2), 4.20 (q, J=4.8 & 9.6 Hz, 1H, C_{16}-H), 1.46 (d, J=4.4 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.24, 45.09, 47.06, 102.08, 107.51, 108.99, 111.03, 113.34, 114.17, 116.55, 118.39, 120.58, 120.80, 122.99, 125.41, 126.56, 129.41, 129.61, 130.44, 131.77, 132.98, 135.05, 137.02, 142.81, 148.93, 155.24, 160.24, 174.99; EI-MS: $M^{+\cdot}$ *m/z* 481; Anal Calcd. for $C_{26}H_{20}ClNO_4$ (%), Calcd: C, 72.27; H, 4.18; N, 2.91; found: C, 72.26; H, 4.15; N, 2.89.

2-(6-chloro-9-((7-methyl-2-oxo-2H-chromen-4-yl)methyl)-9H-carbazol-2-yl)propanoic acid ***(3i)***. Yield

83%; m.p: 195-197°C; IR (KBr) cm^{-1} 1718.09 (C=O lactone) and 1738.26 (C=O acid); ^{1}H-NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 11.38 (s, 1H, -COOH), 6.87-8.18 (m, 9H, Ar-H), 6.14(s, 1H, C_3-H), 5.31 (s, 2H, -NCH_2), 4.14 (q, J=8 & 4 Hz, 1H, C_{16}-H), 2.24 (s, 3H, C_7-CH_3), 1.54 (d, J=4 Hz, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 17.98, 20.42, 44.31, 61.11, 109.58, 110.82, 111.93, 113.74, 116.04, 118.05, 119.27, 120.3, 120.43, 122.48, 123.09, 123.75, 124.76, 124.81, 137.94, 138.03, 140.11, 142.50, 149.56, 152.51, 159.13, 172.97; EI-MS: $M^{+\cdot}$ *m/z* 445; Anal Calcd. for $C_{26}H_{20}ClNO_4$ (%), Calcd: C, 70.03; H, 4.52; N, 3.14; found: C, 70.00; H, 4.51; N, 3.13.

GENERAL PROCEDURE FOR ANTI-TUBERCULAR ASSAY

The anti-mycobacterial activity of compounds were assessed against *M. tuberculosis* H37 RV strain (ATCC No- 27294) using micro plate alamar blue assay (MABA). Briefly, 200 µl of sterile water was added to outer perimeter wells of sterile 96 wells plate to avoid the evaporation rate of medium in the wells during the incubation. 100 µl of the Middlebrooks 7H9 broth was added to the wells and serial dilution of compounds (100 to 0.8 µg/ml) was carried out, plates were covered parafilm and incubates for five days at 37°C. After the desired incubation period freshly prepared 25µl of 1:1 mixture of Alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. Further, based on coloration, the growth was monitored i.e. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC value of each compounds were assessed by lowest concentration which prevented the color change from blue to pink.

COMPUTATIONAL STUDIES

Molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0. [1]. Crystal structure of *Mycobacterium tuberculosis* DprE1 in complex with CT319 under code 4FDO [2] was extracted from the Brookhaven Protein Database (PDB: http://www.rcsb.org/pdb). All the essential hydrogen atoms were added to define the correct configuration and tautomeric states. Then the modelled structure was energy-minimized using Tripos force field with distance dependent dielectric function and partial atomic charges were calculated by AMBER7 FF02 method and finally water molecules were removed from the model. The geometry of the compounds **(3a-3i)** was subsequently optimized to minimal energy using the Powell energy minimization algorithm, Tripos force field with MMFF94 charges. The compounds **(3a-3i)** were then docked onto the binding pocket of the enzyme for docking-scoring analysis. To identify the ligand-protein interactions, the top pose and protein were loaded into work area and the MOLCAD (Molecular Computer Aided Design) program was employed to visualize the binding mode between the protein and ligand.

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