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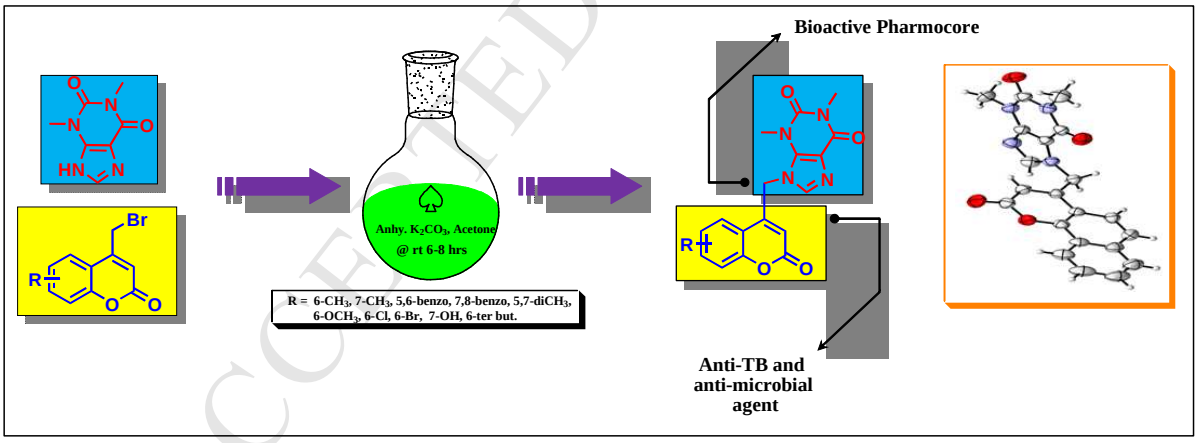
Graphical abstract



Synthesis of coumarin-theophylline hybrids as a new class of anti-tubercular and anti-microbial agents

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Abstract

A series of novel coumarin-theophylline hybrids were synthesized and examined for their anti-tubercular activity *in vitro* against *Mycobacterium tuberculosis* $H_{37}Rv$, anti-microbial activity *in vitro* against gram-positive bacteria (Staphylococcus aureus) and gram-negative bacterias (Escherichia coli, Salmonella typhi) as well as fungi (Candida albicans). The compound (**3a**) has shown excellent anti-tubercular activity with MIC of 0.12 µg/mL. Electron donating compounds (**3a, 3f**) have displayed significant anti-microbial activity. The compounds have also been precisely elucidated using single crystal X-ray diffraction techniques. Molecular docking study has been performed against 4DQU enzyme of *Mycobacterium tuberculosis* showed good binding interactions and is in agreement with the *in vitro* results.

Key words: Coumarin-theophylline hybrids; Molecular docking; Anti-tuberculosis; Anti-microbial; X-ray structure.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by the pathogenic bacterium *Mycobacterium tuberculosis* and remains a major global health problem till date. In the year 2015, the World Health Organization (WHO), has estimated TB prevails one of the top 10 causes of death worldwide and about 6.1 million new TB cases with 1.4 million TB deaths [1, 2]. At times, the drug resistant being one of the reasons as the patient doesn't respond to treatment with anti-TB drug [3]. Despite the availability of effective anti-TB drugs, such as Isoniazid and rifampicin, mortality rates due to TB is still increasing [4]. Thus, there is an emergent need of novel drug entities to counter Multi-drug resistant (MDR) and extensively drug resistant (XDR) mycobacterium tuberculosis strains which compromises longer resistance and recovery of the immune compromised patients [5]. Anti-TB drugs consist of two groups: first-line drugs which are usually used for the treatment of TB patients and second-line drugs are used for the treatment of MDR-TB. Second-line drugs have many more adverse effects than the first-line anti-TB drugs [6]. Development of new anti-TB agents that reduce toxicity, side effects with ability to penetrate host cells as well as effectively threat MDR and XDR are in great interest and would have major impact on public health [4, 7].

Coumarins are the elite class of oxygen-containing fused heterocycles, which are widely distributed in plants [8]. Many of them have been extensively recognized as the key subunits to design synthetic drug candidates in terms of their dynamic pharmacological activities such as anti-inflammatory [9], anti-cancer [10], anti-HIV [11] and anti-malarial [12]. In recent studies naturally occurring coumarins have been reported to possess potent antimycobacterial activity. Noteworthy, (+) Calanolide A was found to be extremely active against *M. tuberculosis* $H_{37}Rv$ and number of MDR-TB stains (A, Figure 1) [13]. Some coumarin derivatives conjugated with nitrogen containing heterocyclic moieties such as pyridine, thiazolyl, Pyrazolo and pyrazole pyrimidine exhibited antimicrobial and anti-tubercular activity profiles. Thus, the hybrids of coumarin nucleus with other moieties have afforded new molecules with improved activity [14]. Recently, 2-mercaptobenzimidazole linked coumarinyl triazoles and coumarin containing thiazole derivatives have been reported as potential anti-tubercular agents (B, C Figure 1) [15, 16]. Purines are nitrogen containing heterocycles which are found abundantly in nature. Purines and its derivatives play an important role in biological activity [17]. In recent years purine derivatives are found to be an important class of drug candidate against *Mycobacterium tuberculosis* (MTB). Yulian Voynikov and co-workers synthesized amino acid derived from theophylline 7-acetamides

which substituent's (R= H, Me, *i*Pr, *i*Bu, 2-CH_2-indole), varying at α-position are found to be highly active against MTB bacilli ($H_{37}Rv$) (D, Figure 1) [18]. Some purine derivatives like 6-(2-furyl) purines are found to be potent MTB. It is demonstrated that nature of the N-9 substituent is vital for activity against MTB. The compounds with the N-9 substituent such as N-benzylated purine are highly potent. Alkylation at N-9 position are essentially inactive, when the length of alkyl side chain was increased the activity was decreased (E, F and G, Figure 1) [19, 20].

The design concept of the presently synthesised compounds inherently comes by taking into account of the bioactivity related to nitrogen heterocycles linked to various other pharmacophoric moieties as reported in literature, hence clubbing of theophylline with coumarin will serve an interesting bioactive template for anti-microbial and tuberculosis screening.

Insert Figure 1

2. Results and Discussion

2.1. Chemistry

The substituted 4-bromomethyl coumarins **1**(**a-j**) were synthesized using Pechman cyclisation of phenols with 4-bromoethylacetoacetate using sulphuric acid as the condensing agent [21]. Condensation of 4-bromomethyl coumarin **1**(**a-j**) with theophylline (**2**) in anhydrous K_2CO_3 using acetone as solvent afforded 1,3-dimethyl-9-[(substituted-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H]-dione derivatives **3**(**a-j**). Synthesis of target compounds was carried out as outlined in Scheme 1. The newly synthesized compounds were characterized ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. The spectral data of newly synthesized compounds **3**(**a-j**) are provided in the experimental section, and in accordance with the assigned structures of the compounds.

Insert Scheme 1

Formation of compounds **3**(**a-j**) was supported by spectroscopic data. In case of compound (**3j**), (R = ter but). The IR Spectrum exhibited band at 1725 cm^{-1} assignable to lactone carbonyl stretching, and 1706cm^{-1} and 1664cm^{-1} are assignable to theophylline (C=O) carbonyls and 1550 cm^{-1} assignable to diazine (–C=N) of theophylline. Formation of product was further established by ^{1}H NMR spectrum, wherein (N_1–CH_3) of theophylline resonated as singlet at 3.42 ppm and (N_3–CH_3) of theophylline resonated as singlet at 3.32

ppm. C_3–H of coumarin and C_4–CH_2 resonated at 5.68 and 5.86 ppm as a singlet respectively. Methyl protons of coumarin resonated at 1.30 ppm as a singlet, H–C=N of the azomethine ring appeared at 8.17 ppm as a singlet and C_5–H of coumarin ring appeared at 7.70 ppm as a doublet (J_{meta}=2.8Hz). C_7–H of coumarin resonated at 7.73 ppm as a doublet of doublet (J_{ortho}=8.8Hz, J_{meta}=2.8Hz), C_8–H of coumarin resonated at 7.36 as a doublet (J_{ortho}= 8.8Hz). Further, molecular ion peak in EI-MS at m/z 394 (100%), lent an additional support to the architecture of compound (**3j**). Rest all the compounds are in good agreement with the expected molecular weight and are an indication of the stability of ion.

3. X-Ray Diffraction Studies

Single crystals for compounds (**3c**), (**3d**) and (**3e**) were developed by slow evaporation of chloroform at room temperature. Compounds (**3c**) and (**3d**) crystallized under a monoclinic system with the space group C2/c and P21/n respectively and the compound (**3e**) crystallized under a triclinic system with the space group P-1. The unit cell dimensions of compound (**3c**) are as follows: a = 19.8675(9) Å, b = 8.3781(3) Å, c = 22.2513(8) Å, α = 90°, β = 108.902(3)°, γ =90°, Z = 8. For compound (**3d**) the unit cell dimensions are: a = 10.8667(16) Å, b = 13.567(2) Å, c = 12.4117(18) Å, α = 90°, β = 104.347(4)°, γ = 90(6)°, Z = 4. And the unit cell dimensions of compound (**3e**) are a = 7.9181(6) Å, b = 8.4627(8) Å, c = 14.2273(13) Å, α = 97.649(5)°, β = 90.998(5)°, γ = 117.355(4)°, Z = 4. Data collection and reduction were performed using CrysAlisPro (version 1.171.36.32) [22], OLEX2 (version 1.2) [23] and SHELXS-97 [24] was used to solve and refine the crystal structures.

The crystal structure for compound (**3c)**. The compound 1,3-dimethyl-7-[(3-oxo-3*H*-benzo[*f*]chromen-1-yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione was crystallized using chloroform by slow evaporation at room temperature and crystalline state is characterized by a long range, well defined three dimensional orders. The asymmetric unit contains only one independent molecule is depicted in **Figure 2**. In the crystal structure, intermolecular C---H…O and weak intramolecular C—H...N & C---H…O hydrogen bonds are observed. In the crystal, π-π interaction with C---H…π are stabilized by crystal packing. Bond lengths and angles are within the normal ranges [R]. The X-ray structure parameters and refinement for compound (**3c**) are presented in **Table 1**. The ORTEP and packing diagrams of compound (**3c**) are portrayed in **Figure 2.**

Insert Table 1

Insert Figure 2

The crystal structure for compound (**3d)**. From the crystal data it was observed that the asymmetric unit of compound 1,3-dimethyl-9-[(2-oxo-2H-benzo[h]chromen-4-yl)methyl]-3,9-dihydro-1H-purine-2,6-dione was crystallized using chloroform by slow evaporation at room temperature and crystalline state is characterized by a long range, well defined three dimensional orders. The asymmetric unit contains only one independent molecule is depicted in **Figure 3.** In the crystal structure, intermolecular C--H...N & C---H…O and intramolecular C--H...N & C---H…O hydrogen bonds are observed. In the crystal, inversion related C---H...O interactions generate an $R_2^2(8)$ ring pattern and link pair of independent molecules into dimers. In addition, π–π interactions between inversions related molecules and C---H…π is stabilized by crystal packing. Bond lengths and angles are within the normal ranges [R]. The crystal structure contains weak intramolecular C---H...N and C---H...O hydrogen bonds. The X-ray structure parameters and refinement for compound (**3d**) are presented in **Table 2.** The ORTEP and packing diagram of compound (**3d**) are portrayed in **Figure 3**.

Insert Table 2

Insert Figure 3

The crystal structure for compound (**3e**). The compound 9-[(5,7-dimethyl-2-oxo-2*H*-chromen-4-yl)methyl]-1,3-dimethyl-3,9-dihydro-1*H*-purine-2,6-dione was crystallized using chloroform by slow evaporation at room temperature and crystalline state is characterized by a long range, well defined three dimensional orders. The asymmetric unit contains only one independent molecule is depicted in **Figure 4.** In the crystal structure, intermolecular C---H…O and weak intramolecular C—H...N & C---H…O hydrogen bonds are observed. In the crystal, π-π interaction with C---H…π are stabilized by crystal packing. Bond lengths and angles are within the normal ranges [R]. The X-ray structure parameters and refinement for compound (**3e**) are presented in **Table 3.** The ORTEP and packing diagram of compound (**3e**) are portrayed in **Figure 4.**

Insert Table 3

Insert Figure 4

4. Biological activity

All the compounds prepared herein were screened for their potential *in-vitro* anti-tubercular activity against MtbH37Rv strain by MicroplateAlamar Blue Assay (MABA) [25]. Anti-bacterial activity against Gram positive and Gram negative bacteria and also fungal activity against *Candida albicans* by MIC method [26].

4.1. Anti-tubercular activity

All the compounds **3(a-j)** were primarily screened at a single concentration of 7.8 µg/ mL against MtbH_{37}Rv (ATCC-27294) in BACTEC 12 B medium, using a Microplate Alamar Blue Assay (MABA). The results are summarized in **Table 4**. Compounds exhibiting >90% inhibition in the primary test were further screened using 2-fold dilution to determine the exact MIC values of the test compounds. In the primary screening, seven compounds (**3a, 3b, 3c, 3e, 3g, 3h** and **3j**) demonstrated 92–96% inhibition of Mtb. In the secondary level of screening, these seven compounds (**3a**, **3b**, **3c**, **3e**, **3g**, **3h** and **3j**) inhibited Mtb with a MIC 0.12, 1.95, 1.95, 0.97, 3.9, 3.9 and 0.48 µg/mL respectively. Compared to that of control Isoniazid with 100 % inhibitions of Mtb at 0.06 µg/mL. We were found a good number of active anti-TB compounds (**3a**, **3b**, **3c**, **3e**, **3g**, **3h** and **3j**).

4.1.1. Structure Activity Relationship (SAR) Studies for Anti-tuberculosis

It was observed from structural activity relationship studies, the methyl substitution at C-6 position of the coumarin moiety showed excellent activity with MIC of 0.12 µg/mL. Second line of activity was observed by tertiary butyl at C-6 position of the coumarin with MIC of 0.48 µg/mL. Whereas, the dimethyl substitution at C-5,7 position of coumarin showed good activity with MIC of 0.97µg/mL. Methyl substitution at C-7 and benzo substitution at C-5,6 position of coumarin moiety have shown similar activity with MIC of 1.95µg/mL. It was also observed that the halogen substituted compounds at C-6 of coumarin moiety (Cl and Br) were also found to exhibit similar MIC value of 3.9 µg/mL. Whereas, the other substitutions i.e., 6-OCH_3, 7,8-benzo and 7-OH showed no activity against MtbH_{37}Rv.

From the overall structural activity relationship study it was observed that the mono substituted methyl group at C-6 position of coumarin enhances the biological activity of the compound [27]. It was also observed that tertiary butyl substitution at C-6 position exhibit significant anti-TB activity. Dimethyl substituted compound (5,7-diCH_3) exhibited good activity. Further the methyl at C-7 position was found to exhibit less activity compared to methyl substitution at C-6 of coumarin. The second line of anti-Tb activity were flaunted by

7-CH_3 and 5.6-benzo substituted coumarin derivative exhibiting good activity. Whereas, the halogen substituted compounds (Cl and Br) at C-6 of coumarin was found to exhibit moderate activity with MIC of 3.9µg/mL. Whereas, the other substituted compounds -OCH_3, 7,8-benzo and 7-OH was found to be less active.

Insert Table 4

4.2. In-vitro antimicrobial studies

The investigation of antibacterial screening of all the tested compounds **3**(**a-j**) against both gram positive and gram negative human pathogenic bacterial strains showed moderate to excellent activity and was compared with the standard antibiotics like tetracyclin. Screening results are summarized in **Table 5** The best antibacterial effect has compound 6-CH_3 (**3a**) with MIC–3.9µg/mL against *S. aureus, E. coli* and 7.8 µg/mL against *S. typhi*. Moreover, compound 5,6-Benzo (**3c**), 6-OCH_3 (**3f**), and 6-ter but (**3j**) also showed good antibacterial activity with MIC–7.8µg/mL against *S. aureus* and for *E. Coli*, these compounds (**3c**, **3f** and **3j**) showed the activity with MIC 7.8, 7.8 and 3.9µg/mL respectively, whereas the antibacterial activity of these compounds against *S. typhi* was shown to be 15.6, 7.8 and 7.8 µg/mL respectively. Compounds (**3b**, **3d**, **3e**, **3g**, **3h** and **3i**) showed moderate antibacterial activity against both gram positive and gram negative bacteria.

The antifungal activity was evaluated against fungal strain such as *Candida albicans.* Ampoterecin B was used as a standard for the comparison of antifungal activity. It is evident from the result that, compounds 6-CH_3 (**3a**), 7-CH_3 (**3b**), and 6-OCH_3 (**3f**) were observed as most active against *Candida albicans* with MIC value 31.3 µg/mL. Especially, compounds (**3c, 3d, 3i,** and **3j**) exhibited sensible activity against *Candida albicans* (MIC 62.5 µg/mL). While compounds (**3g**) and (**3h**) showed moderate activity against the tested organism. The test compound 5,7-diCH_3 (**3e**) was fairly active against *Candida albicans.*

4.2.1. Structure Activity Relationship (SAR) Studies for Anti-bacterial

The coumarin-theophylline hybridsweretested forbroad diversity regarding growth inhibitory activity with minimum inhibitory concentrations ranging from 3.9 to 250µg/mL.The presence of electron donating groups in the coumarin skeleton is found to contribute positive effect to the antibacterial activity. Methylsubstitution, at C-6 of coumarin has shown maximum activity with MIC of 3.9 µg/mL against Gram positive bacterial stain *S. aureus*, Gram negative bacteria *E. coli*, and MIC of 7.8µg/mL for Gram negative bacteria *S.*

typhi. Further substitution -OCH_3 at C-6 f coumarin has shown MIC of 7.8µg/mL for all bacterial stains*S. aureus*, *E.coli*and *S. typhi*. The -ter but group at C-6 of coumarin has shown MIC of 7.8µg/mL for *S. aureus* and *S. typhi* and MIC of 3.9µg/mL for *E. coli*. For halogens (-Cl and -Br), -Br has shown MIC of 15.6µg/mL for *S. aureus*and MIC of 31.3µg/mL for *E. coli* and *S. typhi.* And -Cl has shown MIC of 31.3µg/mL for *S. areus* and *E. coli*and MIC of 62.5 µg/mL for *S. typhi.* Among the halogens -Br has shown maximum activity for *S. aureus* and *S. typhi.* The efficacy of substituent at C-6 position decreased in the orderof -CH_3>ter but > OCH_3. 5,6-benzo have shown MIC of 7.8 µg/mL for *S. aureus* and Gram negative *E. coli* and MIC of 15.6µg/mL for *S. typhi.* Also the remaining substitutions like -CH_3at C-7, 5,7-diCH_3, 7,8-benzo, -OH at C-7 position of coumarin moiety have shown moderate activity.

4.2.2. Structure Activity Relationship (SAR) Studies for Anti-fungal

SAR studies on coumarin-theophylline hybrids have carried out using fungal strain (Candida albicans) with reference to standard Amphotericin B. It is observed the methyl, methoxy at C-6 position and methyl at C-7 position of coumarin have shown maximum activity with MIC of 31.3µg/mL. Followed by ter but at C-6 position, 5,6-benzo, 7,8-benzo and hydroxyl group at C-7 position with MIC of 62.5µg/mL. Halogens (-Cl and -Br) at C-6 of coumarin with MIC of 125µg/mL. Finally 5,7-dimethyl of coumarin has shown least activity with MIC of 250µg/mL.

Insert Table 5

5. Molecular Docking studies

Molecular docking study was performed to support the interaction and preferred binding mode of coumarin-theophylline derivatives with enzyme. All the 10 inhibitors were docked into the active site of enzyme as shown in **Figure 5 (A & B)**. The predicted binding energies of all the compounds are listed in **Table 6** which shows that the compound (**3a**) exhibits the highest C-score value of 6.27 which is in agreement with our observed *in vitro* anti-TB results. As depicted in **Figure 6 (A-D)**, compound (**3a**) makes four hydrogen bonding interactions with amino acid residues, among them one bonding interaction raised from the oxygen atom of coumarin moiety with the hydrogen of ILE21 (O ---- H-ILE21, 1.98 4Å), three interactions were of oxygen atom of keto group of coumarin ring with hydrogen of ILE21, SER94 and ALA22 (-C=O------ H-ILE21, 2.42 Å; SER94, 2.72Å and H-ALA22, 1.81 Å).

As depicted in **Figure 7 (A-D)**, compound (**3e**), makes five hydrogen bonding interactions at the active site of the enzyme (PDB ID: 4DQU), -C=O group of coumarin ring makes three binding interactions (-C=O------H-LEU197, 2.73 Å; THR196, 2.39 Å and H-ALA198, 2.01 Å), one bonding interaction raised from the oxygen atom of coumarin moiety with the hydrogen of THR196 (O ---THR196, 1.85 Å) and remaining another hydrogen bonding interaction raised from the carbonyl group of theophylline (purine) ring with hydrogen of LYS165 (-C=O----- H-LYS165, 1.75 Å). Anti-tubercular assays against M. tuberculosis $H_{37}Rv$ strain shows excellent results with compounds (**3a**) and (**3j**) with $-CH_3$ and $-(CH_3)_3$ groups at C-6 position. In addition, coumarin C4-bridged theophylline moiety has increased the binding ability of the new molecular entities at the active site as revealed by *in vitro* studies.

Insert Figure 5

Insert Figure 6

Insert Figure 7

Insert Table 6

6. Experimental section

6.1. Instrumentation

Melting points were determined using an open capillary method on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument (Nicolet, Madison. WL, USA) using KBr discs. ^{1}H NMR spectra were recorded on a Bruker 400 MHz and Geol 400 MHz spectrometers using $CDCl_3$ and DMSO as solvents and TMS as an internal standard. All chemical shifts are reported as δ values (ppm). EI-MS were recorded using a shimadzu GCMSQP2010S. The elemental analyses were carried out using a Hereaus CHN rapid analyzer. The microwave irradiation syntheses were carried out using a CEM-Discover Focused Microwave system. All the synthesized derivatives were subjected to TLC analysis to ensure the completion of the reaction. The X-ray single crystal structures of compounds (**3c, 3d** and **3e**) were recorded using CrysAlisPro (version 1.171.36.32), OLEX2 (version 1.2) and SHELXS-97.

*6.2. General procedure for the preparation of compounds **3(a–j)***

A mixture of Theophylline (0.01 mol) and powdered anhydrous K_2CO_3 (0.02 mol) with the substituted 4-bromomethyl coumarin (0.01 mol) in 10 mL acetone was stirred at room temperature for 6-8 h. The progress of the reaction was monitored by TLC. After completion of the reaction the reaction mixture was quenched in crushed-ice. Separated solid was filtered and recrystallized from suitable solvent.

*6.2.1. 1,3-dimethyl-9-((6-methyl-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3a**).* Colourless crystals; Yield 85%; m.p: 272-275 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1726 (C=O of lactone), 1700 (C=O of theophylline), 1666 (C=O of theophylline), 1545 (C=N diazine of theophylline); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): δ 2.44 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 3.65 (s, 3H, CH_3), 5.67 (s, 2H, CH_2), 5.72 (s, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.41 (d, 1H, Ar-H, *J*=8.6Hz), 7.29 (d, 1H, Ar-H, *J*=8.6 Hz), 7.66 (s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): δ 21.18, 28.12, 29.83, 46.21, 106.94, 112.72, 116.69, 117.54, 122.99, 133.86, 134.76, 141.68, 149.17, 149.74, 151.67, 151.84, 155.29, 160.40; MS m/z (%): 352 $[M]^+$ (10.2 %); Anal. Calcd for $C_{18}H_{16}N_4O_4$: C, 61.36; H, 4.58; N, 15.90; O, 18.16 Found: C, 61.84; H, 4.14; N, 16.01; O, 18.02%.

*6.2.2. 1,3-dimethyl-9-((7-methyl-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3b**).* Colourless crystals; Yield 84%; Mp: 220-222 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1722 (C=O of lactone), 1700 (C=O of theophylline) 1621 (C=O of theophylline), 1569 (C=N diazine of theophylline); ^1HNMR (400 MHz, $CDCl_3$, δ ppm) δ 2.45 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 3.62 (s, 3H, CH_3), 5.63(s, 1H, Ar-H), 5.69 (s, 1H, CH_2), 7.14 (s, 1H, Ar-H), 7.17 (d, 1H, Ar-H, *J*=4.8Hz), 7.47 (d, 1H, Ar-H, *J*=8Hz), 7.65 (s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) 21.64, 27.92, 29.84, 46.00, 106.77, 111.85, 114.44, 117.74, 122.71, 125.93, 141.50, 144.11, 148.98, 149.56, 151.49, 153.69, 155.13, 160.23; MS m/z (%): 352 $[M]^+$ (10%); Anal. Calcd for $C_{18}H_{16}N_4O_5$: C, 61.36; H, 4.58; N, 15.90; O, 18.16; Found: C, 61.12; H, 4.62; N, 15.19; O, 18.06%.

*6.2.3. 1,3-dimethyl-9-((3-oxo-3H-benzo[f]chromen-1-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3c**).* Colourless crystals; Yield 80%; m.p: 288-292 ^oC; IR (KBr) (υ_{max}/ cm^{-1}): 1740 (C=O of lactone), 1703 (C=O of theophylline), 1657 (C=O of theophylline) 1549 (C=N diazine of theophylline); ^1H NMR (400 MHz, DMSO-d_6, δ ppm), δ 3.14 (s, 3H, CH_3), 3.43 (s, 3H, CH_3), 5.73 (s, 1H, Ar-H), 5.90 (s, 2H, CH_2), 8.34 (s, 1H, Ar-H, *J*=2.4Hz), 8.21 (s, 1H,

CH), 8.06 (s, 1H, Ar-H, *J*=2.4Hz), 7.89-7.91 (m, 2H, Ar-H), 7.70-7.52 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 28.06, 31.24, 46.84, 106.68, 111.51, 113.10, 120.58, 122.26, 122.70, 125.04, 128.29, 128.62, 129.65, 135.00, 143.73, 149.21, 150.41, 151.68, 153.06, 154.97, 159.96; MS m/z (%): 388 $[M]^+$ (20%); Anal. Calcd for $C_{21}H_{16}N_4O_4$: C, 64.94; H, 4.15; N, 14.43; O, 16.48; Found: C, 64.68; H, 4.18; N, 14.234; O, 16.02%.

*6.2.4. 1,3-dimethyl-9-((2-oxo-2H-benzo[h]chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3d**).* Colourless crystals; Yield 83%; m.p: 290-293 ^oC; IR (KBr) (υ_{max}/ cm^{-1}): 1722 (C=O of lactone), 1699 (C=O of theophylline), 1680 (C=O of theophylline), 1548 (C=N diazine of theophylline); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ 3.36 (s, 3H, CH_3), 3.65 (s, 3H, CH_3), 5.69 (s, 2H, CH_2), 6.21 (s, 1H, Ar-H), 7.51 (d, 1H, Ar-H, *J*= 8Hz), 7.64 (dd, 1H, Ar-H, *J*=8Hz, 4Hz), 7.70 (dd, 1H, Ar-H, *J*=8Hz, 4Hz), 7.71 (s, 1H, CH), 8.06 (d, 1H, Ar-H, *J*=8Hz); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 28.50, 34.06, 46.01, 105.12, 111.05, 115.09, 120.11, 121.02, 122.07, 125.49, 128.18, 129.11, 130.82, 135.50, 143.31, 149.26, 150.35, 151.39, 153.19, 154.05, 159.11; MS m/z (%): 388 $[M]^+$ (22%); Anal. Calcd for $C_{21}H_{16}N_4O_4$: C, 64.94; H, 4.15: N, 14.43; O, 16.48; Found: C, 64.28; H, 4.25; N, 14.85; O, 16.12%.

*6.2.5. 1,3-dimethyl-9-((5,7-dimethyl-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3e**).* Colourless crystal; Yield 84%; m.p: 277-280 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1715 (C=O of lactone), 1703 (C=O of theophylline), 1672 (C=O of theophylline), 1548 (C=N diazine of theophylline); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ 2.40 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 3.36 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 5.37 (s, 1H, Ar-H), 5.86 (s, 2H, CH_2), 6.96 (s, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 7.67(s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): δ 21.23, 24.61, 27.92, 29.91, 49.44, 106.59, 111.49, 114.42, 116.56, 130.28, 135.04, 141.49, 143.07, 149.06, 151.54, 152.17, 155.10, 155.17, 160.08; MS m/z (%): 366 $[M]^+$ (100%); Anal. Calcd for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95: N, 15.29; O, 17.47; Found: C, 62.56; H, 4.35; N, 15.30; O, 17.45%.

*6.2.6. 1,3-dimethyl-9-((6-methoxy-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3f**).* Colourless crystals; Yield 80%; m.p: 275-277 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1729 (C=O of lactone), 1697(C=O of theophylline), 1659 (C=O of theophylline), 1548 (C=N diazine of theophylline); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ 3.38 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 5.71 (s, 2H, CH_2), 5.78 (s, 1H, Ar-H), 7.05 (d, 1H, Ar-H,

J=2.8Hz), 7.18 (dd, 1H, Ar-H, *J*=8.8Hz, 2.8Hz), 7.33(d, 1H, Ar-H, *J*=9.2Hz), 7.67 (s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): δ 28.12, 30.07, 46.23, 56.09, 106.40, 106.91, 113.74, 117.39, 118.79, 119.94, 141.64, 148.18, 149.18, 149.35, 151.65, 155.37, 156.52, 160.30; MS m/z (%): 368 $[M]^+$ (42%); Anal. Calcd for $C_{18}H_{16}N_4O_5$; C, 58.69; H, 4.38; N, 15.21; O, 21.72; Found: C, 58.20; H, 4.48; N, 15.11; O, 21.74%.

*6.2.7. 1,3-dimethyl-9-((6-chloro-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3g**)*. Colourless crystals; Yield 75%; m.p: 278-280 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1726 (C=O of lactone), 1708 (C=O of theophylline), 1666 (C=O of theophylline), 1551 (C=N diazine of theophylline); ^{1}H NMR (400 MHz, DMSO-d_6 , δ ppm) δ 3.17 (s, 3H, CH_3), 3.44 (s, 3H, CH_3), 5.76 (s, 1H, Ar-H), 5.78 (s, 2H, CH_2), 8.01 (d, 1H, Ar-H, *J*=2.4Hz), 7.69 (dd, 1H, Ar-H, *J*= 8.8Hz, 2.4Hz), 7.45 (d, 1H, Ar-H, *J*=9.2Hz), 8.14 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) δ 27.01, 29.21, 46.19, 106.74, 113.67, 119.02, 124.49, 129.42, 132.74, 139.75, 144.12, 149.32, 150.19, 151.04, 152.21, 155.03, 159.39; MS m/z (%): 372 $[M]^+$ (100%), 374 [M+2] (33%); Anal. Calcd for $C_{17}H_{13}ClN_4O_4$: C, 54.78; H, 3.52; Cl, 9.51: N, 15.03; O, 17.17; Found: C, 54.68; H, 3.50; Cl, 9.23; N, 15.05; O, 17.52%.

*6.2.8. 1,3-dimethyl-9-((6-bromo-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3h**)*. Colourless crystals; Yield 73%; m.p: 276-280 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1727 (C=O of lactone), 1709 (C=O of theophylline), 1665 (C=O of theophylline), 1551(C=N diazine of theophylline); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ 3.18 (s, 3H, CH_3), 3.44 (s, 3H, CH_3), 5.76 (s, 1H, Ar-H), 5.77 (s, 2H, CH_2), 7.39 (d, 1H, Ar-H, *J*=9.2Hz), 7.80 (dd, 1H, Ar-H, *J*=8.8Hz, 2.8Hz), 8.11 (d, 1H, Ar-H, *J*=2.8Hz), 8.12 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) δ 27.03, 29.12, 46.09, 105.22, 113.24, 117.11, 119.21, 120.12, 129.22, 132.23, 144.11, 145.13, 150.21, 151.16, 153.12, 154.11, 160.02; MS m/z (%): 416 $[M]^+$ (100%), 418 [M+2] (100%); Anal. Calcd for $C_{17}H_{13}BrN_4O_4$: C, 48.94; H, 3.14; Br, 19.15: N, 13.43; O, 15.34; Found: C, 45.06; H, 3.24; Br, 19.75; N, 13.13; O, 15.32%.

*6.2.9. 1,3-dimethyl-9-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3i**)*. Colourless crystals; Yield 74%; m.p: 250-255 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1721 (C=O of lactone), 1701 (C=O of theophylline), 1655 (C=O of theophylline), 1504 (C=N diazine of theophylline), 3411(OH of Coumarin); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ 3.13 (s, 3H, CH_3), 3.41 (s, 3H, CH_3), 5.75 (s, 2H, CH_2), 5.95 (s, 1H, Ar-H), 7.17 (d, 1H, Ar-H, *J*=8.8Hz, 2Hz), 7.28 (s, 1H, Ar-H), 7.41 (d, 1H, Ar-H, *J*=8.8Hz), 8.17 (s, 1H, CH), 3.65

(s, 1H, Ar-OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.06, 30.10, 46.40, 102.81, 106.67, 111.38, 113.56, 126.21, 143.75, 149.13, 151.67, 154.91, 155.25, 157.52, 160.38, 161.72, 162.01; MS m/z (%): 354 $[M]^+$ (72%); Anal. Calcd for $C_{17}H_{14}N_4O_5$: C, 57.63; H, 3.98; N, 15.81; O, 22.58; Found: C, 57.67; H, 3.78; N, 15.15; O, 21.52%.

*6.2.10. 1,3-dimethyl-9-((6-tert-butyl-2-oxo-2H-chromen-4-yl)methyl)-1H-purine-2,6(3H,9H)-dione (**3j**).* Colourless crystals; Yield 84%; m.p: 200-202 ^oC; IR (KBr) (υ_{max}/cm^{-1}): 1725 (C=O of lactone), 1706 (C=O of theophylline), 1664 (C=O of theophylline), 1550 (C=N diazine of theophylline); ^{1}H NMR (400 MHz, DMSO-d_6 , δ ppm) δ 1.30 (s, 9H, CH_3), 3.32 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 5.68 (s, 2H, CH_2), 5.86 (s, 1H, Ar-H), 7.36 (d, 1H, Ar-H, *J*=8.8Hz), 7.71 (dd, 1H, Ar-H, *J*=8.8Hz, 2.8Hz), 7.74 (d, 1H, Ar-H, *J*=2.8Hz), 8.17 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) δ 28.03, 29.99, 31.37, 34.85, 46.24, 106.84, 112.96, 116.28, 117.23, 119.23, 130.54, 141.77, 148.07, 149.03, 150.01, 151.58, 151.69, 155.27, 160.37; MS m/z (%): 394 $[M]^+$ (100%); Anal. Calcd for $C_{21}H_{22}N_4O_4$: C, 63.95; H, 5.62: N, 14.20; O, 16.23; Found: C, 63.90; H, 5.22; N, 14.22; O, 16.35%.

6.3. Docking study

Surflex-Dock was used to investigate detailed intermolecular interactions between the ligand and the target protein. Three dimensional structure information on the target protein was taken from the PDB entry 4FDO. The proteins were prepared for docking by adding polar hydrogen atom with Gasteiger-Huckel charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program (Tripos Inc., St. Louis, USA) and its energy-minimized conformation was obtained with the help of the Tripos force field using Gasteiger-Huckel [28] charges and molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0.[29] and other miscellaneous parameters were assigned with the default values given by the software.

6.4. Anti-tubercular assay

Initially all the compounds **3**(**a-j**) were screened against M. tuberculosis H_{37}Rv (ATCC-27294) at a concentration of 7.8 µg/mL using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting >90% inhibition were further taken for re-test against MtbH_{37}Rv at lower concentrations to evaluate the minimum inhibitory concentration in the MABA. The

experiments were carried out in triplicate using a 96-well plate; to each well 100 µL of Middlebrook 7H9 broth was added and a serial dilution of the compound was made directly on the plate using a two-fold serial dilution method. Isoniazid was included as a positive drug control. The plates were sealed with parafilm and incubated at 37 °C for 5 days. After this, 25 µL of a freshly prepared 1: 1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. Inhibition of bacterial growth was indicated by blue color in the wells while a pink color indicated as growth.

6.5. Antimicrobial assay

Two bacteria viz., *S. aureus* (Gram-positive) and *E. coli*, *S. typhi* (Gram-negative) and one fungi *Candida albicans* were used to screen the antimicrobial activity of synthesized compounds by evaluating the minimum inhibitory concentration of the all the compounds. All the synthesized compounds **3(a-j)** were screened for their potential in-vitro antimicrobial activity in triplicate. Minimum inhibitory concentration (MIC) was determined by broth micro dilution method using 96 well microplate (Andrews, 2001). The microbial inoculum was prepared from 24 h broth cultures using 0.5 McFarland standards. The test compounds **3(a-j)** were dissolved in DMSO at 1 mg/mL, and were then diluted in tryptone broth to achieve a twofold serial dilution of the stock samples (concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.6 7.8 and 3.9 µg/mL). The inoculums (100 µL) were added to each well and a final volume 200 µL was obtained in each well. A number of wells were reserved in each plate for positive and negative controls. Tetracyclin and nystatin was used as positive control for bacteria and fungi respectively. The wells containing 100 µL of sterile broth and 100 µL of inoculum was used as a negative control. The microplates were incubated at 37 ^{o}C for 24 h. After that, resazurin (30 µL) in aqueous solution (0.01%) was added to the microplates, to indicate the microorganism viability (Palomino et al., 2002). The MIC was determined as the lowest concentration of the compound capable of inhibiting microorganism growth.

7. Conclusions

In conclusion, various substituted C-4 bridged coumarin-thyophilline hybrids **3(a-j)** were synthesised and evaluated for their anti-microbial and anti-tubercular properties. Single crystals x-ray confirmed the proposed structure of the synthesised compounds. Compound **3a** showed highest anti-TB activity, whereas **3a, 3f** and **3j** showed excellent anti-bacterial activity. Further **3a, 3b** and **3f** proved good candidates as anti-fungal agents. *In silico* molecular docking shows the hydrogen bond interactions and further supports the obtained

results. These compounds can act as lead compounds for design and synthesis of potent anti-microbial and anti-tubercular drugs in future.

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Appendix A. Supplementary data

IR, NMR and Mass spectral data of new compounds **3**(**a-j**).

Crystallographic data had been deposited with the Cambridge Crystallographic Data Centre as supplementary publication for the compounds (**3c, 3d** and **3e**) with CCDC No. 1588777, 1506761 and 1589963 respectively. This data can be obtained free of charge via the URL http://26 www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223 336033; e-mail: deposit@ccdc.cam.ac.uk)

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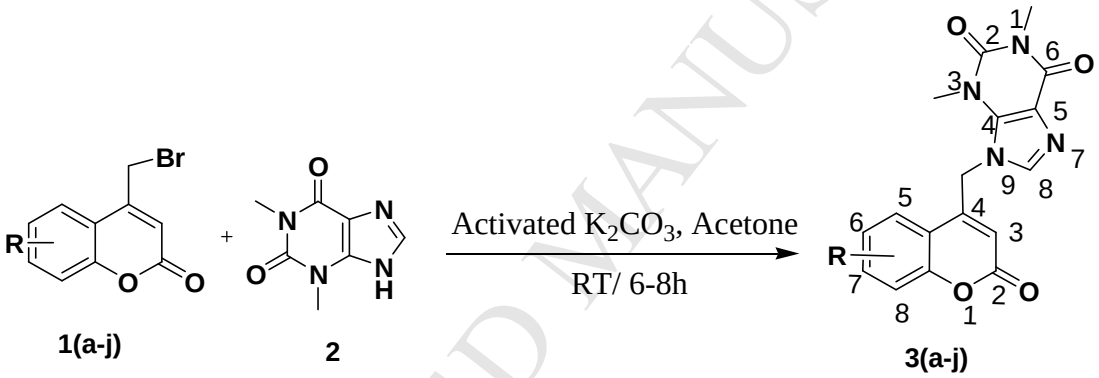


R = a) 6-CH_3, b)7-CH_3, c) 5,6-benzo, d)7,8-benzo, e) 5,7-diCH_3, f) 6-OCH_3, g) 6-Cl, h) 6-Br, i) 7-OH, j) 6-ter but.

Scheme 1. Schematic representation for the synthesis of the desired compounds.

Tables

Table 1. Crystal data, Data collection and Structure refinement of compound **3c**.

Empirical formula	$C_{21}H_{16}N_4O_4$
Formula weight	388.38
Temperature/K	297.49
Crystal system	monoclinic

Space group	C2/c
a/Å	19.8675(9)
b/Å	8.3781(3)
c/Å	22.2513(8)
α/°	90
β/°	108.902(3)
γ/°	90
Volume/$Å^3$	3504.0(2)
Z	8
$\rho_{calc}g/cm^3$	1.472
μ/mm^{-1}	0.105
F(000)	1616.0
Crystal size/mm^3	0.21 × 0.19 × 0.17
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.784 to 56.632
Index ranges	$-26 \leq h \leq 26$, $-11 \leq k \leq 11$, $-29 \leq l \leq 29$
Reflections collected	72910
Independent reflections	4356 [R_{int} = 0.0460, R_{sigma} = 0.0161]
Data/restraints/parameters	4356/0/264
Goodness-of-fit on F^2	1.176
Final R indexes [I>=2σ (I)]	R_1 = 0.0527, wR_2 = 0.1569
Final R indexes [all data]	R_1 = 0.0720, wR_2 = 0.1773
Largest diff. peak/hole / e $Å^{-3}$	0.66/-0.70
CCDC No.	**1588777**

Table 2. Crystal data, Data collection and Structure refinement of compound **3d**.

Empirical formula	C_{21} H_{16} N_4 O_4
Formula weight	388.38
Temperature	293(2) K
Crystal system	Monoclinic
Space group	P 21/n

a/Å	10.8667(16)Å
b/Å	13.567(2)Å
c/Å	12.4117(18)Å
α/°	90°
β/°	104.347(4)°
γ/°	90(6)°
Volume/Å^3	1772.8(5)
Z	4
ρ_{calc}g/cm^3	1.455
Crystal size/mm^3	0.22 × 0.15 × 0.12
Absorption coefficient	0.859mm^{-1}
F(000)	808
Crystal form	Prism, colourless
Radiation source	fine-focus sealed tube
Radiation type	Cu *Kα*
Radiation monochromator	Graphite
Criterion for observed reflection	$I > 2\sigma(I)$

Data collection

Diffractometer	Bruker SMART CCD area-detector
Data collection method	ω- χ scans
Absorption correction	multi-scan
2Θ range for data collection/°	5.318 to 64.651
Index ranges	-11<=h<=12, -15<=k<=15, -14<=l<=13
Reflections collected / unique	12825 / 2908 [R(int) = 0.0291]
Completeness to theta	97.2 %
Max. and min. transmission	T_{max} = 1.000, T_{min} = 0.790

Refinement

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2908 / 0 / 262
Goodness-of-fit on F^2	1.071
Final R indices [I>2σ(I)]	R1 = 0.0427, wR2 = 0.1178

R indices (all data)	R1 = 0.0442, wR2 = 0.1196
(Δ/σ)max	< 0.001
Extinction coefficient	0.039(4)
Largest diff. peak and hole	0.236 and -0.238 e.Å^{-3}
CCDC No.	**1506761**

Table 3. Crystal data, Data collection and Structure refinement of compound **3e**.

Empirical formula	$C_{19}H_{18}N_4O_4$
Formula weight	366.37
Temperature/K	298.99
Crystal system	triclinic
Space group	P-1
a/Å	7.9181(6)
b/Å	8.4627(8)
c/Å	14.2273(13)
α/°	97.649(5)
β/°	90.998(5)
γ/°	117.355(4)
Volume/Å^3	835.86(13)
Z	2
ρ_{calc}g/cm^3	1.456
μ/mm^{-1}	0.105
F(000)	384.0
Crystal size/mm^3	0.25 × 0.23 × 0.21
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.49 to 49.984
Index ranges	$-9 \leq h \leq 9$, $-10 \leq k \leq 10$, $-16 \leq l \leq 16$
Reflections collected	24267
Independent reflections	2935 [R_{int} = 0.0743, R_{sigma} = 0.0410]
Data/restraints/parameters	2935/0/248

Goodness-of-fit on F^2	1.492
Final R indexes [I>=2σ (I)]	R_1 = 0.0987, wR_2 = 0.3420
Final R indexes [all data]	R_1 = 0.1191, wR_2 = 0.3535
Largest diff. peak/hole / e $Å^{-3}$	0.55/-0.43
CCDC No.	**1589963**

Table 4. In vitro anti-tubercular screening data of the compounds **3(a-j)** against MtbH_{37}Rv.

Compounds	R	% inhibition at a concentration of 7.8 µg/mL	MIC[a] values µg/mL
3a	**6CH_3**	**96**	**0.12**
3b	7CH_3	92	1.95
3c	5,6-Benzo	92	1.95
3d	7,8-Benzo	16	ND
3e	**5,7-diCH_3**	**94**	**0.97**
3f	6-OCH_3	56	ND
3g	6-Cl	92	3.9
3h	6-Br	92	3.9
3i	7-OH	15	ND
3j	**6-ter but**	**96**	**0.48**
Isoniazid	--	100	0.06

[a]Minimum inhibitory concentration against the H_{37}Rv strain of Mtb (µg/mL).

N.D – not determined.

Table 5. Minimum inhibition concentration of Compounds **3(a-j)** using standard antibiotics for antimicrobial activity.

Compounds	R	Micro-organisms used for Antimicrobial Activity (MIC values µg/mL)			
		Gram positive	Gram negative		*Candida albicans*
		Staphylococcus aureus	***Escherichia coli***	***Salamonella typhi***	
3a	6-CH_3	3.9	3.9	7.8	31.3
3b	7-CH_3	62.5	31.3	125	31.3

3c	5,6-Benzo	7.8	7.8	15.6	62.5
3d	7,8-Benzo	15.6	15.6	62.5	62.5
3e	5,7-diCH_3	15.6	125	250	250
3f	6-OCH_3	7.8	7.8	7.8	31.3
3g	6-Cl	31.3	31.3	62.5	125
3h	6-Br	15.6	31.3	31.3	125
3i	7-OH	15.6	125	31.5	62.5
3j	6-ter but	7.8	3.9	7.8	62.5
Tetracyclin	--	3.9	3.9	3.9	--
Amphoterecin B	--	--	--	--	0.97

Table 6. Surflex Docking score (kcal/mol) of the coumarin derivatives **3**(**a-j**)

Entry	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	ChemScore[g]
3a*	**6.27**	**-1.33**	**2.05**	**-118.030**	**-55.890**	**-219.991**	**-22.030**
3b	5.63	-1.20	1.99	-114.194	-58.178	-213.297	-22.486
3c	5.29	-1.42	1.16	-115.041	-8.974	-211.385	-21.843
3d	5.10	-2.18	1.05	-116.011	-40.860	-229.890	-31.307
3e	5.78	-1.48	3.06	-91.620	-10.523	-181.545	22.486
3f	4.78	-1.70	2.49	-101.345	-30.188	-188.143	-23.770
3g	5.15	-2.37	0.93	-122.356	-35.416	-228.685	-17.088
3h	4.40	-0.69	3.09	-80.910	-23.043	-140.315	-22.243
3i	5.83	-0.57	2.11	-91.388	-10.490	-150.021	-19.977
3j	5.34	-2.30	2.18	-100.623	2.279	-203.667	-22.427

*Asterisk indicates compound with higher 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.

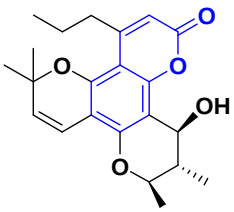
[d] D-score for charge and van der Waals interactions between the protein and the ligand.

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

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

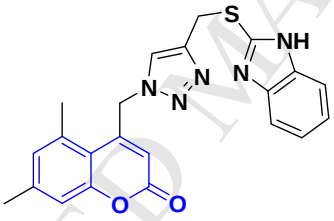
[g]Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.

Figures



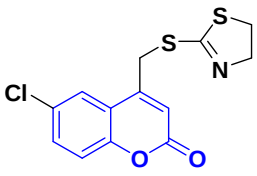
MIC= 3.13µg/mL

A



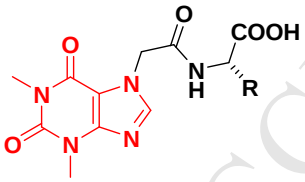
MIC= 3.8µmol/mL

B



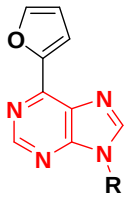
MIC= 0.09µg/mL

C



R= H, 6.78µg/mL
Me, 0.65µg/mL
*i*Pr, 5.93µg/mL
*i*Bu, 5.69µg/mL
2-CH2-indole, 0.47µg/mL

D

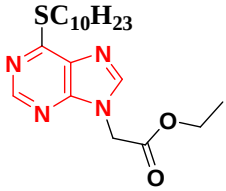


R= H, 6.25µg/mL
CH_3, 6.25µg/mL
CH_2Ph, 3.13µg/mL
CH_2CH_2Ph, 6.25µg/mL

E

MIC_{90} = 3.13µg/mL

F



MIC_{90} = 1.56µg/mL

G

Figure 1. Structurally related anti-tubercular coumarins and purines.

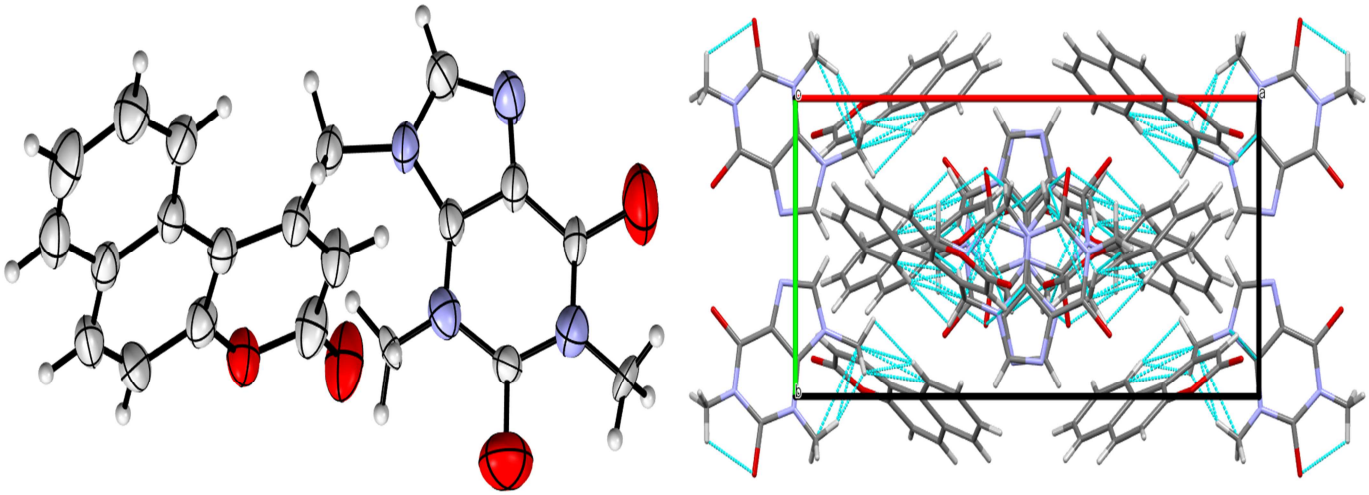


Figure 2. ORTEP and packing diagram for compound **3c.**

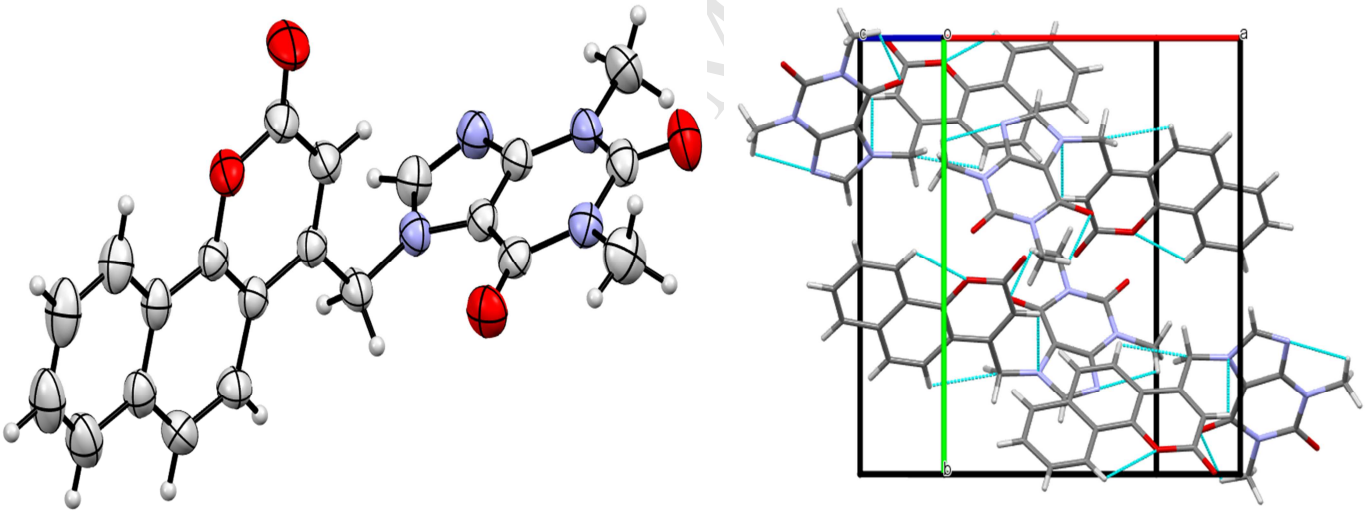


Figure 3. ORTEP and packing diagram for compound **3d.**

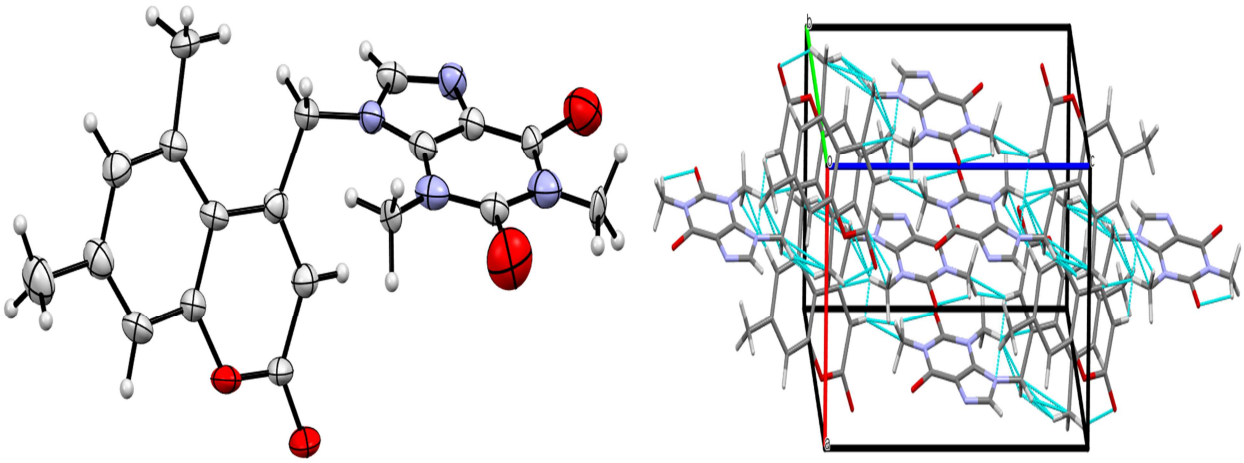


Figure 4. ORTEP and packing diagram for compound **3e.**

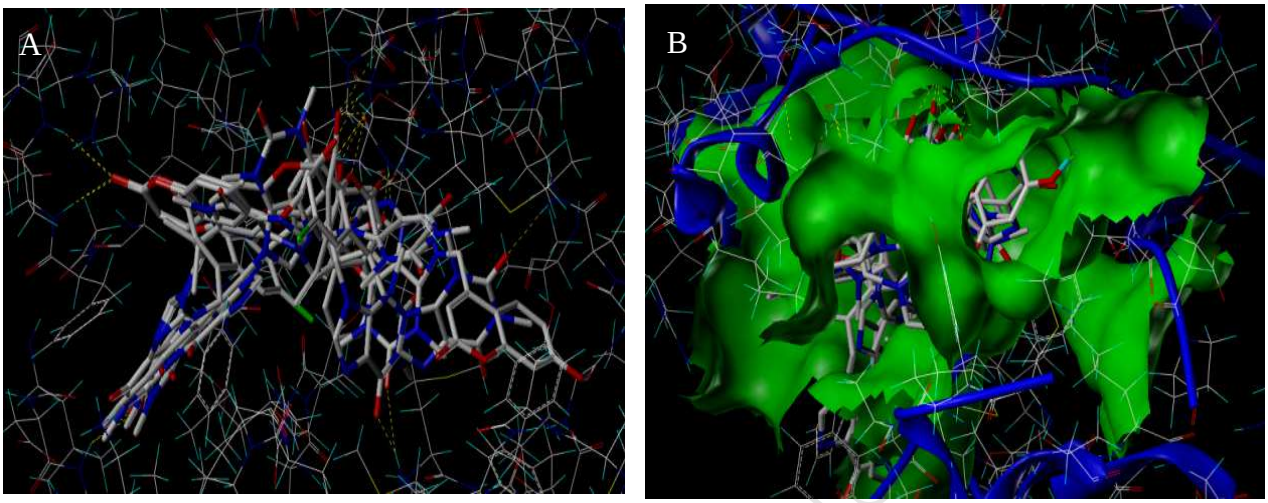


Figure-5: Docked view of all the compounds **3(a-j)** at the active site of the enzyme PDB ID: 4DQU.

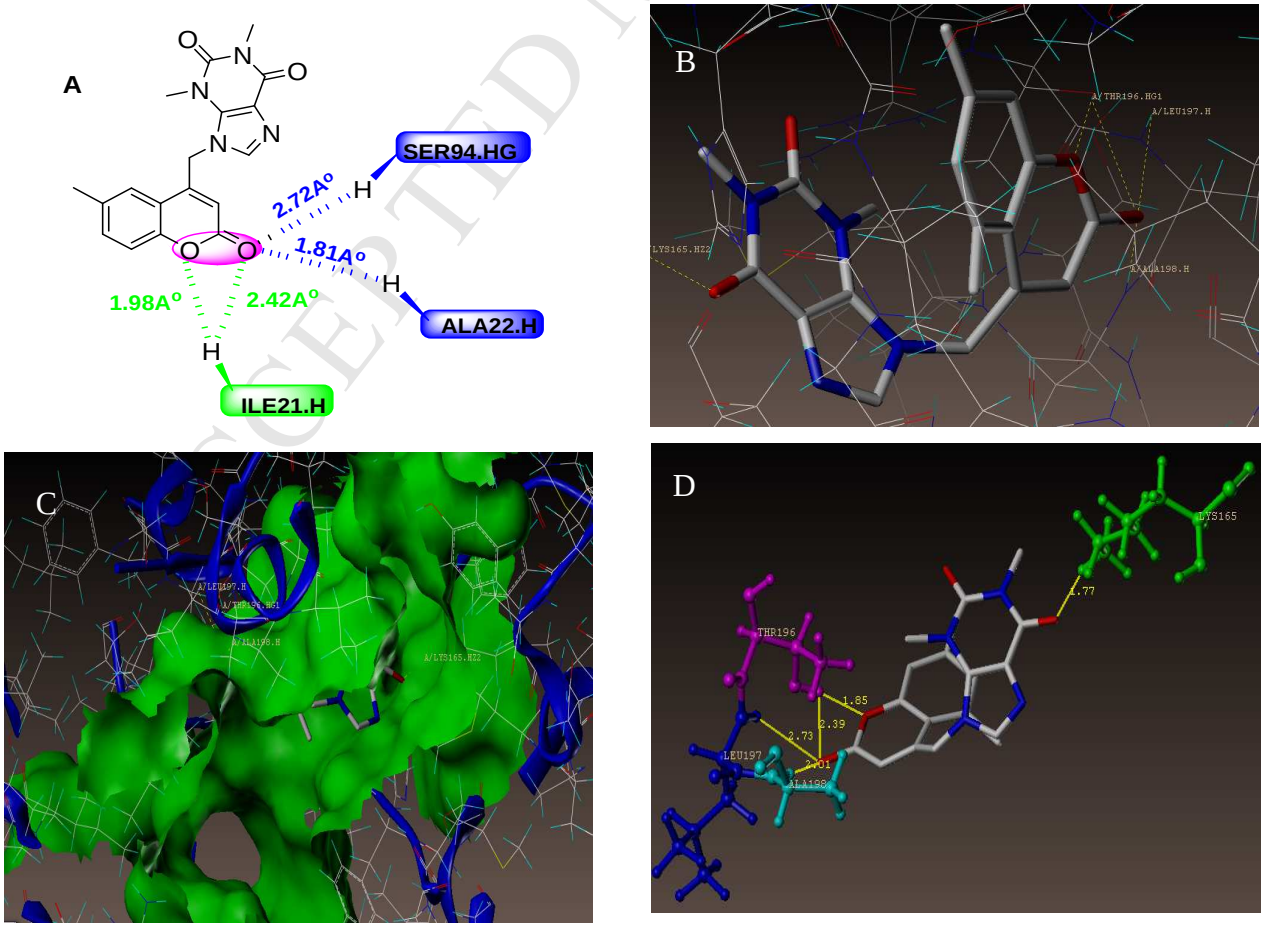


Figure-6: Docked view of compound (**3a**) at the active site of the enzyme PDB: 4DQ

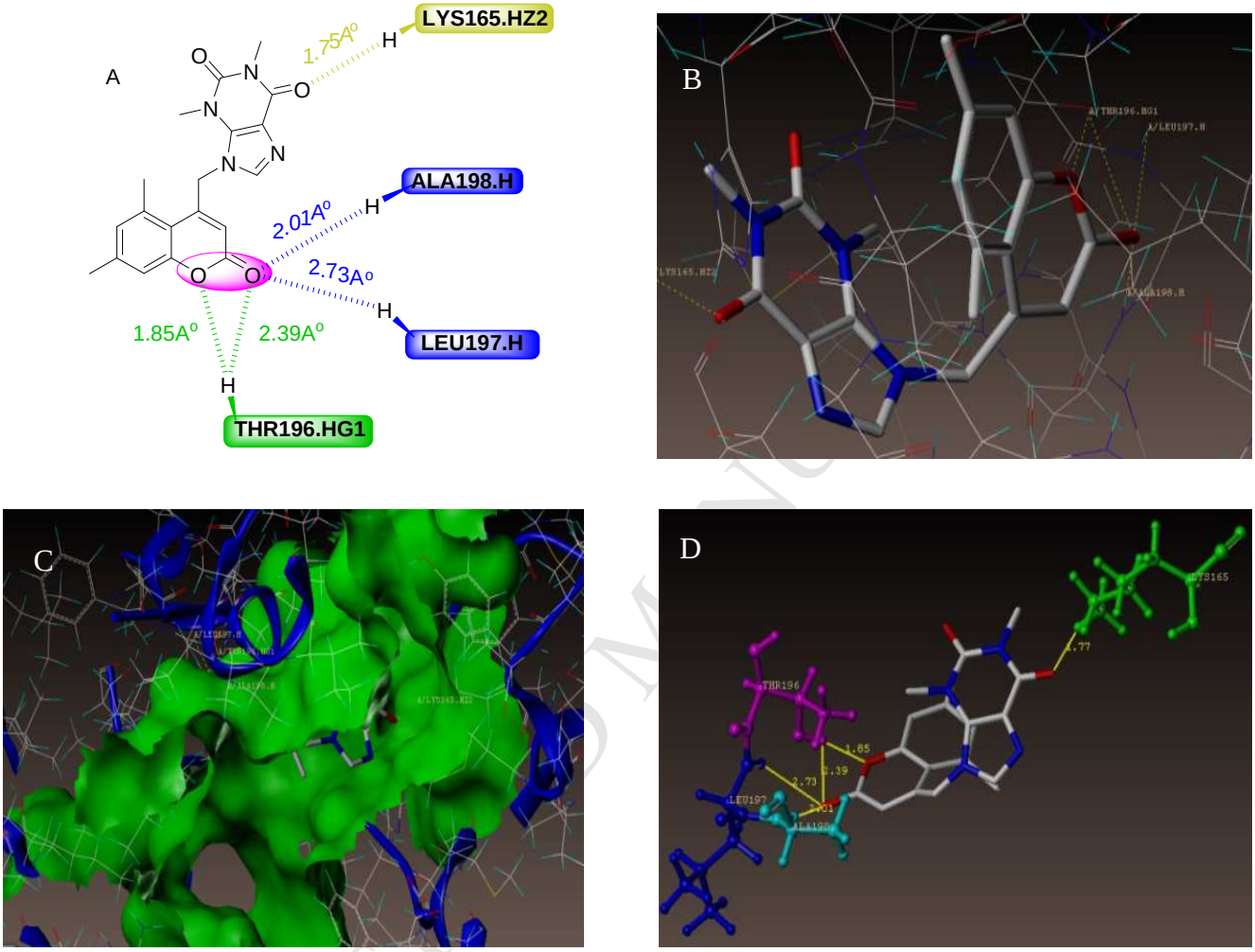


Figure-7: Docked view of compound (**3e**) at the active site of the enzyme PDB: 4DQU.

Synthesis of coumarin-theophylline hybrids as a new class of anti-tubercular and anti-microbial agents

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Highlights

- A series of novel Coumarin-Theophylline hybrids were prepared.
- The structures of **3c, 3d, 3e** were confirmed by single-crystal X-ray diffraction.
- Compound **3a** exhibited the highest anti-TB activity against $MtbH_{37}Rv$.
- Most compounds displayed good anti-tubercular and antimicrobial activity.
- Molecular docking studies have shown good binding interactions and are in good agreement with *in vitro* anti-TB.