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SYNTHESIS, ANTIBACTERIAL, ANTIFUNGAL ANTITUBERCULAR ACTIVITIES AND MOLECULAR DOCKING STUDIES OF NITROPHENYL DERIVATIVES

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ABSTRACT

Infectious diseases are one of the common problems encountered globally. Even though there are a number of drugs available in the market for the treatment of infectious diseases, still many new molecules are required due to the problems with the existing drugs. Hence we prepared a series of chalcones (A1-A10) containing nitrophenyl moieties by Claisen-Schmidt condensation reaction. All the ten compounds were characterized by IR, NMR, Mass spectroscopy and elemental analysis. The compounds were further screened for antitubercular activity employing MABA assay and antibacterial and antifungal activities by cup plate method against selected tubercular, bacterial and fungal organisms. The compound A9 displayed potent antitubercular activity with MIC 11.02±0.030, whereas the compound A3 showed superior antibacterial and antifungal activities. Further, molecular docking studies were performed on Thymidine Kinase (TMP) of *Mycobacterium tuberculosis* by employing AU autodocker. The docking scores were in association with the *in vitro* MABA results and also identified the potent nature of A9 with the docking score -6.7. This study helped to identify novel nitrophenyl derivatives against thymidylate kinase.

KEYWORDS: ***Claisen-Schmidt condensation; Cup plate method; MABA assay; Thymidine Kinase; Molecular docking.***

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INTRODUCTION

Microorganisms, both useful and harmful are a part and parcel of human life. The harmful ones cause the infectious diseases. Commonly encountered infectious diseases are caused by bacterial and fungal organisms. Drugs exist for the treatment of bacterial and fungal infections, but with limitations. Some of the usual difficulty with the current therapeutic agents is resistance and undesirable side effects. This opens the door for the research in developing novel molecules to overcome resistance with good safety profiles. Tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis*, is a global problem affecting many. According to 2014 report by the WHO, incidences, prevalence, and mortality rates of tuberculosis have globally declined [1]. However, in 2016, India estimated 2.79 million new cases of tuberculosis and 0.9 million deaths caused by the disease were reported [2]. Furthermore, the emergence of multidrug-resistant and extensively drug-resistant strains led to a growing need for effective novel agents in the fight against tuberculosis. Unfortunately, there is a dire the need to design and synthesize novel drugs of potent, selective, shorter length treatments with less toxic antimicrobial drugs to fight against this lethal disease[3,4,5]. Chalcones refer to an extensive group of compounds occurring in plants. They are prominent plant secondary metabolites that are found in dietary components including fruits, vegetables, olive oil, tea and red wine. Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds. A few changes in their structure have offered a high degree of diversity that has proven useful for the development of new medicinal agents having improved potency and lesser toxicity and good pharmacological actions. Over 20 years, chalcones became an object of continued interest in research in Pharmaceutical chemistry. . Nowadays several chalcones are used to treat the inflammatory conditions [6, 7], cancer[8] viral infections[9, 10], cardiovascular diseases, parasitic infections, gastritis, microbial infections[11,12] and convulsions[13]. In addition, they are also used as food additives and cosmetic formulation ingredients. Motivated by the existing studies on the biological properties of chalcones, in the present work we synthesized and evaluated a series of chalcones containing 4-nitrophenyl scaffold for their antibacterial, antifungal and antitubercular activities.

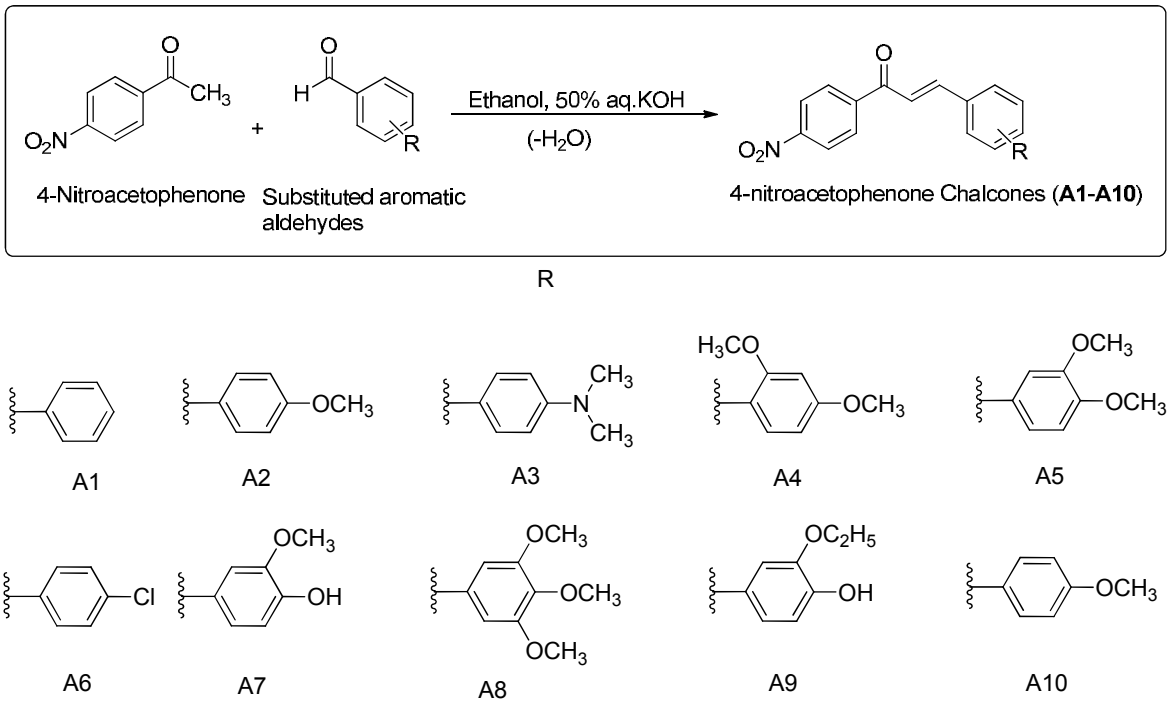
MATERIALS AND METHODS

Chemistry

All the chemicals used were of analytical grade 98% and purchased from SD Fine and Himedia. 4-Nitroacetophenone (99%) was obtained from Aldrich Chemical Co. Silica gel-G for TLC (Merck) was used as stationary phase and ethyl acetate: hexane (2: 8) as mobile phase to check the purity of the compounds using thin layer chromatography. UV light (254 nm) and iodine vapours were used to visualize the spots. Melting points were determined in open capillaries by using Boitus melting point apparatus, expressed in °C, and were uncorrected. The IR spectra were recorded using Bruker Vertex 80v spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 400 MHz and chemical shifts are given in units as parts per million, downfield from tetramethylsilane (TMS) as the internal standard. MS spectra were recorded on Agilent LC-MS spectrometer and elemental analyses were carried out using a Carlo Erba 1108 elemental analyzer for C, H, and N.

Synthesis of 4-nitrophenyl derivatives

4-nitroacetophenone (0.005 moles) in equimolar quantities and the appropriate aryl aldehydes (0.005 moles) were dissolved in ethanol (7.5 mL) and simultaneous stirring was carried out. To this mixture, 7.5mL of 50% aqueous KOH was added drop wise and the reaction mixture was left for 24 h at room temperature [14]. Later, it was acidified with a mixture of hydrochloric acid and water (1:1), which resulted in the precipitation of the target compounds. The chalcones were then filtered under vacuum, washed with water, dried, and recrystallized from ethanol (Scheme 1).



Scheme 1

Synthetic scheme for the synthesis of 4-nitroacetophenone chalcones (A1–A10)

1-(4'-Nitrophenyl)-3-Phenyl-2-propene-1-one A1

Yield : 80%; m.p. 82°C; R_f = 0.6 (20% Ethyl acetate in Hexane); IR (KBr, cm^{-1}): 1659.40 (-C=O-), 1580.23 (-CH=CH), 1326.95 (Ar-NO_2 sym) 1510.17 (Ar-NO_2 asym), 3049.40 (Ar-CH stretching); ^{1}H NMR (400MHz, $CDCl_3$, ppm): 7.83 (1H, d, *J* =16, Ar-CH=) 8.14 (1H, d, *J* =8.8, -CO-CH=), 7.43-7.46 (m, C- 2', 3', 4', 5', 6', Ar-H) , 8.33-8.35 (m, C- 2", 3", 5", 6", Ar-H); MS (m/z, %): 254.07 (M + 1, 99.11); Anal. Calcd for: $C_{15}H_{11}O_3N$: C, 71.13; H, 4.37; O, 18.94; N, 5.53; Found: C, 71.14; H, 4.34; O, 18.97; N, 5.56.

1-(4'-Nitrophenyl)-3-(4"- methoxyphenyl)-2-propene-1-one A2

Yield : 79%; m.p. 84°C; R_f = 0.7 (20% Ethyl acetate in Hexane); IR (KBr cm^{-1}): 1681.09 (-C=O-), 1574.11 (-CH=CH)**,** 1335.18(Ar-NO_2 sym), 1508.78(Ar-NO_2asym), 1072.60 (Aryl alkyl ether sym C-O-C), 1247.42 (aryl alkyl ether asym C-O-C; ^{1}H-NMR (400 MHz, $CHCl_3$ ppm), : 3.88 (^{1}H, d, *J* =5.4 CO-CH=), 6.96-6.94 (m, C- 2', 3', 5', 6', Ar-H), 8.33-8.31 (m, C - 2", 3'', 5", 6"- Ar-H); MS (m/z, %): 253.08 (M + 1, 98.81); Anal. Calcd for: $C_{15}H_{13}NO_4$: C, 67.84; H, 4.63; O, 22.59; N, 4.94; Found: C, 67.84; H, 4.59; O, 22.61; N, 4.94.

1-(4'-Nitrophenyl)-3-(4"-dimethylaminophenyl)-2-propene-1-one A3

Yield: 85%; m.p. 110°C; R_f =0.4 (20% Ethyl acetate in Hexane); IR (KBr cm-1) : 1654 (-C=O-), 1581.08 (-CH=CH-), 1327.47 (Ar-NO2 sym), 1522.38 (Ar-NO2 asym),1020-1250 (aliphatic amines C-N), 842 (aromatic nitro compounds C-N); ^{1}H-NMR(400 MHz, $CHCl_3$ ppm), : 3.06 (6H, s, Ar-N $(CH_3)_2$), 6.68 (1H, d, *J*=8.8, -CO-CH=), 7.64 (1H, d, *J* =8.8, Ar-CH=), 7.79-8.33 (m, C-2", 3", 5", 6". Ar-H); MS (m/z, %): 253.08 (M + 1, 98.81); Anal. Calcd for: $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; O, 16.20; N, 9.45; Found: C, 68.91; H, 5.40; O, 16.21; N, 9.45.

1-(4'-Nitrophenyl)-3-(2", 4"-dimethoxyphenyl)-2-propene-1-one A4

Yield: 86%; m.p. 103°C; R_f = 0.7 (25% Ethyl acetate in Hexane); IR (KBr cm^{-1}) : 1647.84 (-CO-stretching), 1577.91 (-C=C-), 1303.09 (Ar-NO_2sym), 1514.52 (Ar-NO_2asym) ,1026 (aryl alkyl ether sym C-O-C), 1206.42 (aryl alkyl ether asym C-O-C); ^{1}H-NMR(400 MHz, $CHCl_3$ ppm), δ : 3.89 (3H, s, 2'-OCH_3), 3.91 (3H,s,3'-OCH_3), 6.56 (1H, d, *J* = 2.4 CO-CH=), 7.57 (1H, d, *J* =8.4, Ar-CH=), 6.48-6.51 (m, C-2', 3', 4', 5', 6', Ar-H) 8.04-8.12 (M, C - 2", 3", 5",6".Ar-H); MS (m/z, %) : 313.1 (M + 1, 99.54);

Anal. Calcd for: $C_{17}H_{15}NO_5$: C, 65.17; H, 54.83; O, 25.53; N, 4.47; Found: C, 65.17; H, 4.79; O, 25.55; N, 4.47.

1-(4'-Nitrophenyl)-3-(3", 4"-dimethoxyphenyl)-2-propene-1-one A5

Yield: 75%; m.p. 220°C; R_f = 0.6(25% Ethyl acetate in Hexane); IR (KBr cm^{-1}) : 1659.09 (C=O-), 15538.05 (-CH=CH-), 1026 (aryl alkyl ether sym C-O-C), 1205 (aryl alkyl ether asym C-O-C), 1319.12 (Ar-NO2 sym), 1516.88 (Ar-NO2 asym); ^{1}H-NMR (400 MHz, $CHCl_3$ ppm), δ : 3.94 (3H, s, 3'- OCH_3), 3.96 (3H, s, 4'- OCH_3), 6.93 (1H, d, *J* = 8.4, -CO-CH=), 7.75 (1H, d, *J* = 15.6, Ar-CH=), 7.16-7.35 (m, C-2', 3', 4', 5', 6', Ar-H); MS (m/z, %): 313.1 (M + 1, 98.14); Anal. Calcd for: $C_{17}H_{15}NO_5$: C, 65.17; H, 54.83; O, 25.53; N, 4.47; Found: C, 65.17; H, 4.79; O, 25.55; N, 4.47.

1-(4'-Nitrophenyl)-3-(4"-chlorophenyl)-2-propene-1-one A6

Yield: 78%; m.p. 152°C; R_f = 0.3 (25% Ethyl acetate in Hexane); IR (KBr cm^{-1}) :1663.29 (-C=O-), 1588.55 (-CH=CH-), 1330.04 (Ar-NO2 Sym), 1518.59 (Ar-NO2 Asym), 1092.08 (chlorobenzene), 1023.33 (C-N stretching for Ar-NO_2), 849.11 (C-N stretching for Ar-NO_2); ^{1}H-NMR(400 MHz, $CHCl_3$ ppm), δ: 7.58 (1H, d, *J* = 8, Ar-CH=), 7.78 (1H, d, *J* = 16, -CO-CH=), 8.13-8.1 (m, C -2', 3', 5', 6', Ar-H), 8.34-8.37 (m, C- 2'', 3'', 4'', 6'', Ar-H); MS (m/z, %): 287.2 (M + 1, 99.58); Anal. Calcd for: $C_{15}H_{10}ClNO_3$: C, 62.62; H, 3.50; O, 16.68; N, 4.87; Found: C, 62.60; H, 3.47; O, 16.69; N, 4.86.

1-(4'-Nitrophenyl)-3-(3"methoxy-4"hydroxy phenyl)-2-propene-1-one A7

Yield: 79%; m.p. 148°C; R_f = 0.5 (25% Ethyl acetate in Hexane); IR (KBr cm^{-1}) : 3103.85 (OH), 1684.74 (C=O-), 1562.70 (-CH=CH), 1338.31 (Ar NO2-sym), 1516.30 (Ar NO2-Asym), 1070.43 (aryl alkyl ether sym C-O-C), 1241.60 (aryl alkyl ether asym C-O-C); ^{1}H-NMR (400 MHz, $CHCl_3$ ppm), δ: 2.69 (3H, s, 3'-OCH_3), 8.35 (2H, s, *J* =5.2, Ar-CH=), 2.691 (3H, s, *J* = 5.4 -CO-CH=), 7.284 (m, C-2', 5', 6'-Ar H), 8.328-8.306 (m, C - 2", 3", 5", 6" -Ar H); MS (m/z, %): 299.08 (M + 1, 99.27); Anal. Calcd for: $C_{16}H_{13}NO_5$: C, 62.13; H, 4.20; O, 26.73; N, 4.68; Found: C, 62.13; H, 4.20; O, 25.88; N, 453.

1-(4'-Nitrophenyl)-3-(3"ethoxy-4"hydroxy phenyl)-2-propene-1-one A8

Yield: 65%; m.p. 139°C; R_f = 0.6 (25% Ethyl acetate in Hexane); IR (KBr cm^{-1}) : 1618.29 (-C=O-), 1588.27 (-CH=CH), 1338.31 (Ar-NO_2sym), 1516.30 (Ar NO_2-asym), 1240.20 (ethoxy), 3614.57 (OH); ^{1}H-NMR (400 MHz, $CHCl_3$ ppm), □ : 7.28 (1H, d, *J* = 5.2 Ar-CH=), 7.28 (m,C-2",5",6" –Ar-H), 8.133-8.11 (m, C - 2", 3", 5", 6" –Ar-H); MS (m/z, %): 313.1 (M + 1, 97.82); Anal. Calcd for: $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; O, 25.53; N, 4.47; Found: C, 65.17; H, 4.79; O, 25.55; N, 4.47.

1-(4'-Nitrophenyl)-3-(3",4",5"-trimethoxyphenyl)-2-propene-1-one A9

Yield: 79%; m.p. 149°C; R_f = 0.4 (25% Ethyl acetate in Hexane); IR (KBr cm^{-1}):1686.35 (-C=O-), 1556.31 (-CH=CH-) , 1339.86 (Ar NO_2-sym), 1516.57 (Ar NO_2-asym), 1072.07 (aryl alkyl ether sym C-O-C), 1246.82 (Aryl Alkyl ether asym C-O-C); ^{1}H-NMR(400 MHz, $CHCl_3$ ppm), □ : 3.08 (3H, s, 3'-OCH_3), 3.38 (3H, s, 4'-OCH_3), 3.55 (3H, s, 5'-OCH_3), 7.8 (1H, s, *J* = 25.2 –CO-CH=), 7.37 (1H, s, *J* = 25.2 Ar-CH=), 6.440-6.363 (m, C - 2', 6' -Ar H), 8.171- 8.120 (m, C - 2", 3", 5", 6", Ar-H); MS (m/z, %): 343.11 (M + 1, 99.79); Anal. Calcd for: $C_{18}H_{17}NO_6$: C, 62.97; H, 4.99; O, 27.96; N, 4.08; Found: C, 62.97; H, 4.95; O, 27.98; N, 4.08.

2.2.11. 1-(4'-Nitrophenyl)-3-(4"-hydroxyphenyl)-2-propene-1-one A10

Yield: 86%; m.p. 132°C; R_f = 0.3 (25% Ethyl acetate in Hexane);IR (KBr cm-$^{-1}$) : 1646.92 (-CO-), 1568.44 (-CH=CH), 1315.80 (Ar-NO_2sym), 1509.76 (Ar NO_2Asym), 3614.42 (hydroxy benzene); ^{1}H-NMR (400 MHz, $CHCl_3$ ppm), □ : 6.7 (2H,d,4'-OH), 7.57 (1H, d, *J* = 5.4 Ar-CH=), 7.28 (1H, d, *J*, = 5.4 –CO-CH=), 6.713-6.691 (m, C - 2', 3', 5', 6'-Ar H), 8.12-8.10 (m, C - 2", 3", 5", 6" -Ar H); MS (m/z, %): 269.07 (M + 1, 98.80); Anal. Calcd for: $C_{15}H_{11}NO_4$: C, 66.91; H, 4.12; O, 23.77; N, 5.20; Found: C, 66.91; H, 4.08; O, 23.79; N, 5.202.

Antimicrobial studies

The antimicrobial activity was performed against six antimicrobial strains. The organisms selected include the Gram-positive bacteria: *Bacillus subtilis* (NCIM-2079), *Staphylococcus aureus* (NCIM-2079), Gram-negative bacteria: *Escherichia coli* (NCIM-2065), and *Proteus vulgaris* (NCIM-2027), Fungi:

*Aspergillus niger (*MTCC 5889) and *Candida albicans (*MDCC-227*)*. Before performing the antimicrobial assay, the glassware and the media were sterilized and the antibiotic solution (standard) and A1-A10 samples were kept ready. Meanwhile, composition of the nutrient agar medium was prepared: (Peptone 0.5%, Meat extract 0.3%, Sodium chloride 0.5%, Agar 2%, distilled water to make up to 100 ml, and pH adjusted to 7.2). The weighed quantities of peptone, meat extract and sodium chloride were dissolved in 1000 ml of distilled water and the pH of the medium was adjusted to 7.2. The agar got dissolved and the medium was distributed in boiling tubes each containing 25 ml. The media and sterile water required were sterilized by autoclaving at 121°C temperature and 15 lbs/sq. inch pressure for 20 minutes. Petri plates, test tubes, pipettes and borer required for experiment were sterilized by dry heat sterilization using hot air oven. 18 hour old cultures of respective organisms were taken and sterile water was used for making the suspension of these organisms. 0.5 ml of this suspension was used as inoculum and pour plate technique was conducted. The inoculated agar medium was poured into sterile 10 cm diameter petri dishes. Medium in the plates was allowed to solidify. The solutions of the test compounds in concentrations of 0.1 µg/ml were prepared in DMSO. The cups of 5 mm diameter were prepared using borer in the corresponding medium. In each plate, 5 wells were prepared. Three cups are of test compounds, one of standard compound and other one was used as control in each cup samples were poured and then plates were left for 45 minutes in a refrigerator for diffusion. After incubation for 18 hours at 37 ^{o}C, the plates were examined for inhibition zones. The experiments were done thrice and the average diameter of the zones of inhibition was recorded.

Antitubercular activity

The synthesized novel nitrophenyl chalcone compounds were estimated by MABA as analytical method[15]. The efficacy of the compounds was competed with the standard first line drugs. The activity was done thrice. The MIC µg/mL was defined as lowest drug concentration which prevented the color change from pink to blue and the percentage inhibition values was calculated by comparing the absorbance values of the control and test samples apart from 10 compounds.

Molecular Docking studies

All ten chalcones were synthesized and then the structure were drawn by using the Chem3D ultra 10.0 (Cambridge software). After that, the modelled structure was copied to Chem3D Ultra 10.0 to create a 3D model which was subjected to energy minimization using molecular mechanics (MM2). The minimization was executed until the root-mean-square gradient value reached a value lower than 0.001 kcal/mol. This type of energy minimized structures was considered for molecular docking studies. The corresponding MDL MOL files were prepared using Chem3D Ultra 10.0 integral options (save as /MDL MOL). The mol files were converted into PDB files, opened in AUTODOCK tools. Then Goseiger charges and torsion angle were added to the molecules and finally saved as PDBQ.

STATISTICAL ANALYSIS

The data obtained was analysed using spss statistical 20 Software. Student's "t" test was used for analysis of comparison. The data was presented as mean ± standard deviation (SD). Probability value (P) of less than 0.05 and was considered statistically significant.

RESULTS AND DISCUSSION

Chemistry

Base-catalyzed Claisen-Schmidt condensation of various substituted aromatic aldehydes with 4-Nitroacetophenone led to the synthesis of target compounds (A1-A10). All the compounds were recrystallized using ethanol as solvent. The yield of the compounds after recrystallization ranged from 65 to 95%. The compounds were characterized with the help of elemental analysis and spectroscopic data. The results of elemental analysis were within ±0.4% of the calculated values. All the compounds showed absorption bands and peaks characteristic to chalcones in their IR, ^{1}H NMR, ^{13}C NMR (for A3 and A9) and mass spectra.

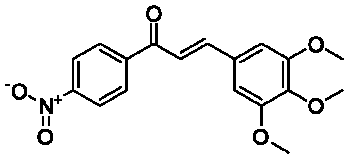


Figure 1
Structure of compound A9

The IR spectrum of A9 illustrated intense carbonyl band (C=O) of chalcones at 1686.35 and strong stretching band at 1581.22 accounting for vinyl (CH=CH) double bond. In addition, the other absorption bands were seen at wave numbers 1339.86 (NO_2-sym), 1516.57 (NO_2-asym), 1072.07 (C-O-C) and 1246.82 (C-O-C) respectively. Two important doublet peaks at 7.37 ppm and 7.81 ppm with the coupling constant value J =16 Hz in the ^{1}H NMR spectrum of A9 confirmed the creation of chalcone scaffold with trans geometry. The other peaks seen are three singlets corresponding to the three methoxyl groups at chemical shift 3.08, 3.38, 3.55 and two multiplets around 6.36-6.44 and 8.12-8.17 for six aromatic protons. The ^{13}C NMR spectra of compound A9 demonstrated three crucial peaks at 189.9, 120.2 and 146.1 corresponding to the three carbons of the propenone moiety. The other ^{13}C peaks include 55.6 (3"-OCH_3), 62.1 (4"-OCH_3), 55.1 (5"-OCH_3), 102.6 (C-2" and C-6"), 125.6 (C-3' and C-5'), 131.4 (C-2' and C-6'), 138.4 (C-4"), 145.2 (C-4"), 154 (C-3" and C-5") and 155.6 (C-4'). The mass spectrum of A9 recorded in positive mode has given an [M + 1] peak at m/z 343. 11. Based on the above spectral data, the compound A9 was predicted to be as *(E)*-1-(4'-Nitrophenyl)-3-(3",4",5"-trimethoxyphenyl)-2-propene-1-one (Figure 1).

Antimicrobial activity

Despite the availability of many antimicrobial agents available in the market, there is an essential need to evaluate newer molecules for their antimicrobial property because the existing molecules are not sufficient to overcome infections due to antimicrobial resistance. Hence, the synthesized compounds (A1-A10) were tested to study their antimicrobial behaviour against four bacterial strains-*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and two fungal strains-*Aspergillus niger and Candida albicans* at 0.1% concentration. Ciprofloxacin and Ketoconazole were used as standard drugs for antibacterial and antifungal activities respectively. The results are summarized in Table 1 and the activity of some of the compounds was found to be superior to the standard drugs. The study showed that the compounds exhibited varying degree of activities (Figure 2). Most of the compounds were more active than ciprofloxacin against Gram negative *Escherichia coli* and *Proteus vulgaris*. Among others, A8 containing 3",4",5"-trimethoxyphenyl scaffold showed highest activity with a zone of inhibition of 5.2 and 8.13 mm. A3 demonstrated tremendous activity against *Proteus vulgaris* and A5 with 4"-dimethylaminophenyl and 3",4"-dimethoxyphenyl functionalities also demonstrated tremendous activity against *Proteus vulgaris*. Among the ten compounds, A9 exhibited superior activity against Gram positive bacterium and fungal strains indicating that the presence of 3"-ethoxy-4"-hydroxy phenyl substitution is essential for the activity of nitrophenyl derivatives against the above strains. A9 displayed no activity against the gram negative bacterial strains. A8 and A3 holding 3",4",5"-trimethoxyphenyl and 4"-dimethylamino phenyl moieties demonstrated highest activity against *Proteus vulgaris* with a zone of inhibition of 8.13 and 8.06 mm respectively. The zone of inhibition of A1 and A7 containing phenyl and 3"-methoxy-4"-hydroxyphenyl moieties was 5.3 mm against *E.coli* and were the most active compounds against this organism. It is surprising to note that A9 and A10 containing the electron releasing groups showed poor activity against *E.coli* despite the potent activity of A7. The other compounds were more active than ciprofloxacin with the zone of inhibition ranging from 2.1 – 4.16 mm against *E.coli*. The antifungal activity of the nitrophenyl derivatives is more against *Candida albicans* than *Aspergillus niger*. Two compounds A5 and A9 had shown excellent antifungal activity than fluconazole against both *Aspergillus niger* and *Candida albicans* and the activity of A9 was more than A5 against *Aspergillus niger*. The zone of inhibition of A5 containing 3",4"-dimethoxyphenyl skeleton was 3.5 and 3.3 mm respectively against both these organisms whereas A9 with 3"-ethoxy-4"-hydroxyphenyl moiety shown a zone of inhibition of 5.16 and 5 mm. A7 exhibited nearly equal activity (3.2 mm) to fluconazole (3.15 mm) against *Aspergillus niger* and excellent activity against *Candida albicans* which is equal to the

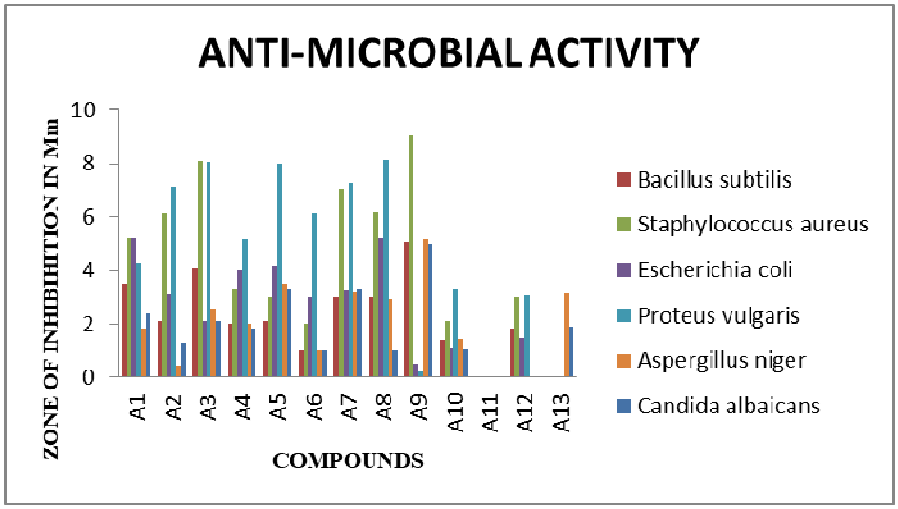
activity of A5. A1, A3 exhibited better activity than fluconazole against *Candida albicans.* The SAR studies indicate that the presence of electron releasing groups is essential for the antifungal activity.

Table 1.
Results for Antimicrobial Activity with zone of inhibition in mm

Sample codes	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Aspergillus niger*	*Candida albicans*
A1	3.5±0.3	5.2±0.24	5.2±0.3	4.26±0.17	1.8±0.52	2.4±0.28
A2	2.1±0.12	6.1±0.12	3.1±0.12	7.16±0.108	0.43±0.08	1.3±0.14
A3	4.1±0.12	8.1±0.12	2.1±0.2	8.06±0.03	2.5±0.35	2.1±0.22
A4	2±0.08	3.33±0.20	4.0±0.08	5.16±1.08	2.03±0.10	1.8±0.44
A5	2.1±0.14	3.0±0.21	4.16±0.2	8±0	3.5±0.22	3.3±0.26
A6	1±0.4	2.03±0.04	3±0	6.1±0.07	1±0.07	1±0.07
A7	3±0.80	7.0±0.08	3.23±0.2	7.3±0.18	3.2±0.17	3.3±0.27
A8	3±0.04	6.16±0.02	5.2±0.3	8.13±0.16	2.9±0.10	1.0±0.10
A9	5.1±0.16	9.03±0.04	0.5	0.2	5.16±0.14	5.0±0.05
A10	1.4±1.92	2.1±0.08	1.1±0.12	3.26±0.26	1.43±0.04	1.03±0.04
Control (A11)	--	--	--	--	--	--
Ciprofloxacin (A12)	1.80±0.04	3.0±0.08	1.48±0.08	3.06±0.04	---	--
Ketoconazole (A13)	--	--	--	--	3.15±0.04	1.9±0.08

Values are means ± SD; (n=3) P≤0.05 when compared with control

Figure 2
Results of the antimicrobial activity



Antitubercular activity

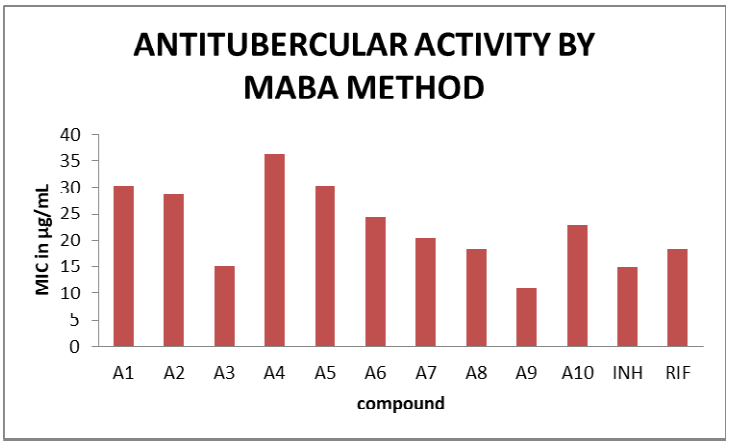
The antitubercular potency results of the ten compounds (A1-A10) are shown in table 2 with a pictorial representation in figure 3. Among the ten compounds, three compounds viz., A3, A8 and A9 shown with potent activity. Compounds A9 (MIC = 11 µg/mL) and A3 (MIC = 15 µg/mL) containing 3"-ethoxy-4"-hydroxy phenyl and 4-"-dimethylamino phenyl moieties possess activity greater than the standard drug Rifampin. A9 is even more than potent than Isoniazid whereas A3 is less active. A8 containing the 3",4",5"-trimethoxy phenyl scaffold has equal potency as Rifampin. The other compounds were less active than the standard drugs.

Table 2
Anti-tuberculosis activity (MIC µg/mL) of compounds (A1-A10)

Compounds	MIC µg/mL± SD*
A1	30.22±0.018
A2	28.70±0.011
A3	15.05±0.020
A4	36.26±0.06
A5	30.22±0.016
A6	24.54±0.073
A7	20.47±0.024
A8	18.22±0.025
A9	11.02±0.030
A10	22.88±0.007
Isoniazid	14.25±0.008
Rifampin	18.27±0.04

Note: the values were expressed as the mean of three repetitions

Figure 3
Graph plotted with compounds vs. MIC in µg/mL for anti-tubercular Activity by MABA Method



Molecular docking studies

The inhibitors of TMP kinase of *Mycobacterium tuberculosis* cause mutation in the TMP binding site in the protein ligand complex. This is due to addition of an intriguing high value of the electric field to the vicinity of the phosphate group of TMP and the putative binding site of the gamma phosphate group of ATP[16]. The mutation leads to alter the cell membrane of Mycobacterium tuberculosis.Molecular docking was performed to investigate the binding modalities of 4-Nitrophenyl chalcone derivatives against TMP kinase. Results suggested that all 4-Nitrophenyl chalcone analogs could conveniently occupy mostly in the loop between helix alpha 9 and strand beta 5, common to all ligands binding site of TMPKs as shown in Fig.2. Binding energies for these compounds were in the range of 5.3-6.7 kcal/mol. It was observed that all 4-Nitrophenyl chalcone compounds afforded stronger binding energies than of the 5-TMP found in the co-crystallized structure there by suggesting promising inhibitory effect for this set of compounds. The observed TMPK's binding energies for these compounds are in accordance with their antitubercular activity by Microplate Alamar Blue Assay , in which the more potent compounds as seen (A1, A3 and A9) displayed huge binding energies (6.6, 6,6 and 6.7 kcal/mol) and interaction with TMPKs along with engaging in hydrophobic bond lengths were seen to be (ASN100(3.4), ASN100(3.4), ASN100(3.2), SER99(3.4), ASP9(3.5), ARG160(3.5); SER99(3.4) ASN100(3.3), ASN100(3.0), ASN100(3.4), ARG95(3.5), TYR39(3.3), ARG160(3.4); ARG160(3.2), ARG160(3.5), ARG160(3.5), ASP94(2.8)) respectively. Among 10 compounds, 7 molecules exhibited mild binding energies with more interaction as shown in table 3 and figure 4. Particularly, the lowest active compounds A5, A6, A7 and A10 showed

the highest binding energy ranging from 6.0 to 6.5 kcal/mol. Interestingly, docking poses of these compounds revealed that they could also occupy the ATP binding site of alpha-helix that is adjacent to the compounds binding site of beta helical. These findings suggest that 4-Nitrophenyl chalcone compounds could plausibly act as dual site inhibitors of alpha- and beta-helical complexes. Molecular modelling analysis of the crystal structure revealed that the co-crystallized 5-TMP interacts with beta-helical structure via the formation of hydrogen bonds involving a methoxyl group to ASP9, ARG160, SER99, ASN100 and engaging in hydrophobic interactions with TYR39 as shown in fig 6. The ATP binds alpha-helical via the formation of hydrogen bonding network between the phosphate moiety to ASP69.

Validation of docking protocol

Co-crystallized ligands, 5-TMP was re-docked to their target proteins: $C_{10}H_{15}N_2O_8P$ kinase, as to validate the docking protocol. The covalent bonds between Thymidine-5-phosphate active site were disrupted and re-docked via non-covalent docking.Thymidine-5-phosphate could be re-docked to their respective target proteins with a root mean squared deviation (RMSD) of 0.697 Å and 1.+7 Å respectively.

Figure 4

Compounds A1 to A10 into the active site of TMPKs. The molecule in bold colours is the ball and stick model of the ligand with the active site residues. Dotted yellow color is interaction with white color label amino acid residues with different color sticks are represented as TMPKs.

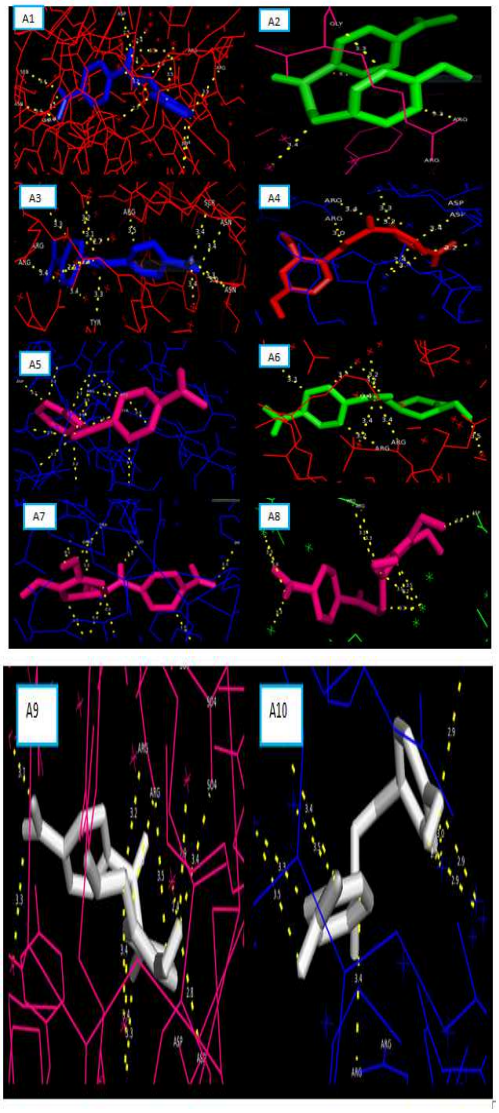


Table 3
Dock scores of the compounds determined through molecular docking studies

Compound codes	Dock score	Interactions
A1	-6.6	ASN100(3.4), ASN100(3.4), ASN100(3.2), SER99(3.4), ASP9(3.5), ARG160(3.5)
A2	-5.3	GLY159(3.3),ARG160(3.3)
A3	-6.6	SER99(3.4) ASN100(3.3), ASN100(3.0), ASN100(3.4), ARG95(3.5), TYR39(3.3), ARG160(3.4)
A4	-5.7	ASP94(3.4), ARG160(3.4), ARG160(3.0)
A5	-6.0	ARG160(3.2), ARG160(3.4)
A6	-6.5	ARG160(3.5), ARG160(3.3), ARG160(3.2), ASP9(3.2), TYR39(3.3)
A7	-6.5	ARG160(3.5), ARG160(3.2), GLY159(3.4), HIS53(3.3)
A8	-5.4	ARG160(3.3), ASP94(3.4), ARG160(3.3)
A9	-6.7	ARG160(3.2), ARG160(3.5), ARG160(3.5), ASP94(2.8)
A10	-6.4	ARG160(3.4)

CONCLUSION

In the current study, out of 10 compounds synthesized and analysed for antitubercular potency, one nitrophenyl derivatives exhibited potent antitubercular activity. This scaffold can be useful as a starting point to derive more nitrophenyl derivatives against thymidylate kinase. Further, *invitro* studies need to be carried out to determine the molecule-enzyme interaction against thymidylate kinase.

AUTHORS CONTRIBUTION STATEMENT

Afzal Basha Shaik designed the study. Afzal Basha Shaik and Lagu Surendra Babu collected the data from archives and performed the synthesis, characterization, biological and computational studies. Yejella Rajendra Prasad supervised the project. Afzal Basha Shaik and Lagu Surendra Babu contributed to the interpretation of the results and writing of the manuscript. Yejella Rajendra Prasad corrected and provided critical feedback about the research and manuscript. All authors read and approved the final manuscript.

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CONFLICTS OF INTERESTS

Conflict of interest declared none.

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