

# SYNTHESIS, SPECTRAL CHARACTERIZATION, INSILCOMOLECULAR DOCKING STUDIES AND *IN- VITRO* ANTIMICROBIAL STUDIES OF 5-(4- SUBSTITUTED PHENYL)-3-(THIOPHEN-2-YL)-4, 5- DIHYDROPIRAZOLE-1-CARBOHYDROAMIDE DERIVATIVES

## Abstract

Using the melting point, Infra-red,  $1\text{-D}^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data, a new series of pyrimidines were synthesized and characterized. In vitro biological activities of all the synthesized compounds are tested. Also the same compound is tested by zone of inhibition against set of bacterial and fungal strains; by this the compound 2a tested against *S.Aureus*, *S.Pyogenes*, *E.Coli*, 4b against *P.Aeruginosa*, and this shows an excellent antibacterial activity. Similarly the compound 2c can show inhibition against *C.albicans*. And also all the compounds were undergone in-silico molecular docking predictions. The *insilico* studies were inspected by Bacterial protein 1UAG. They show good docking score than the standard drug.

**Keywords:** Substitute acetophenone, thiophene-2-carbaldehyde, antimicrobial activity, molecular docking, 1UAG.

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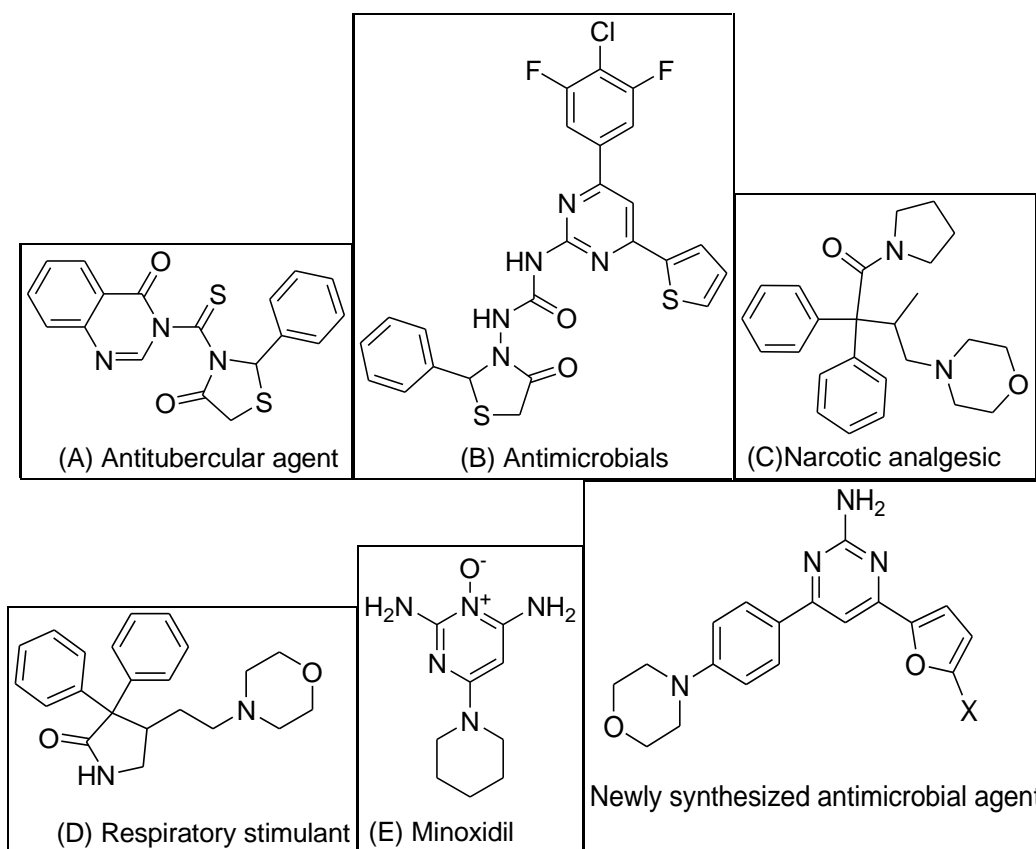
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## I. INTRODUCTION

In current days an extensive variety of microorganisms like bacteria, viruses, protozoa and fungi are becoming rebellious to drugs that have to utilize cure infections. This resistance is a major hurdle to treatment of infectious diseases worldwide [1]. Microbial infections are a rising problem in contemporary medicine and the use of antibiotics is habitual across the world. Accordingly, there is an urgent need to widen antimicrobial agents, which have a broad spectrum of activity against the repellent microorganism [2]. The obligatory to have number of microbial agents with upgrade potency. The area of organic chemistry developed and referred to harmonize of bioactive compounds. One of N containing compounds; pyrimidine evidently acquired significant importance mature to their varied biological properties and therapeutically importance. The most rudimentary nucleus present in the nucleic acid is named as pyrimidine, which is correlated with a large number of biological activities. The marketed drugs likes anti-atherosclerotic nifedipine, anti-histaminic thonzylamine, anti-anxiolytic buspirone, antihypertensive minoxidil and prazosin, anti-psoriatic enzamide, and other medically pertinent compounds which contains the familiar substituted aminopyrimidine. A bit of remarkable biological activity of Pyrimidine derivatives includes adenosine receptor antagonists [3], kinase inhibitors [4], analgesic [5], anti-inflammatory [5], inhibitors of cyclin-dependent kinase 1 and 2 [6], calcium channel antagonist [7], anti-histaminic [8] and antitubercular [9] activities. Different N-functionalized morpholines are showing assuring various pharmacological activities. They were reported to exert a number of important physiological activities such as antidiabetic [10], antiemetic [11], platelet aggregation inhibitors, anti-hyperlipoproteinemics [10] bronchodilators, growth stimulants [12] and antidepressants [13]. These were also used for inflammatory diseases, pain, migraine and asthma [14]. Tridemorph, morpholine derivatives was also used as antifungal agent [15]. 4-Phenyl morpholine derivatives were outline to possess anti-inflammatory [16] and central nervous system [17] activities. Some of pyrimidine's moiety containing clinically beneficial compounds reveals strong TB (A) and anti-microbials (B) from scheme 1. Here, few of the clinically essential drugs carry morpholine moiety. Morpholine assimilated compounds are dextromoramide, (C) a narcotic analgesic and the respiratory stimulant is doxapram HCl, (D). Doxapram used in the treatment of respiratory depression following anaesthesia [18]. Minoxidil [E] was a very good antihypertensive vasorelaxation (reduce blood pressure) and also used for medication of hair growth for men and women and was marketed under the trade name of Rogaine.



**Scheme 1:** Some of the synthetic compounds having the core pyrimidine and morpholine nuclei with therapeutic activities

Structure based drug designing (SBDD) and Ligand based drug designing (LBDD) techniques are employed as important drug discovery tools in rational drug designing process [19]. Molecular docking is the advanced computational used techniques in SBDD to obtain optimized conformation of Ligand-receptor interaction and to study their relative orientation through the minimized energy free system [20]. Computer aided drug designing (CADD) is fast, economical modernized technique that gives valuable, accurate and deep understandings of experimental findings and new suggestions for molecular structures to be synthesized [21]. In continuation of our interest in synthesizing structurally diverse biologically active heterocycles [22-27], we report now the synthesis of pyrimidine derivatives and the biological and insilico studies.

## II. EXPERIMENTAL METHOD

**1. Chemicals and Instruments:** All chemical were bought from sigma Aldrich INDIA. Using open capillary method, the determination of melting point will be done. TLC has been checked for formation of compounds regularly and spots were seen by iodine. For neat liquid compounds FT-IR spectra was taken and for KBr pellets for solids on a Shimadzu spectrum RXI FT-IR.

- 2. Making of TLC Plates & Column Chromatography:** Using silica gel pre camouflaged glass plates the Analytical TLC was carry out (Merck, Germany). Silica gel was prepared with 30g wasin 100ml of water. Then the mixtureof solution was coated on the glass plates and dries in air for 3 hours and kept in an oven for another one hour before using the plate. Using silica gel for the Column chromatography was carry out
- 3. Equipments and Analytical Instruments:** Melting points determination was carried open capillary method. FT-IR-8400 instrument is using for IR spectra. The uncorrected IR was recorded on a Shimadzu and Perkin Elmer transform spectrometer. Bruker Avance 400 NMR instrument was using for  $^1\text{H}$  NMR spectra and  $^{13}\text{C}$  NMR spectra.
- 4. Percentage of Product:** Using recrystallization procedures purification of All the harmonized compounds will be done. Using TLC method, the cleanness of the compound was examined. The percentage of the compound calculated using the equation.

$$\% \text{ Yield} = \frac{\text{Weight of the product obtained}}{\text{Theoretical weight}} \times 100$$

- 5. Common Procedure For The Preparation Of Chalcone Derivatives:** (1a-1c) Take a beaker and add one mole of thiophen-2-carbaldehyde and one mole of various substituted acetophenone followed by the addition of 30ml of ethanol containing 2g of NaOH pellets. Using magnetic stirrer, the mixtures were stirring well for 30 minutes in a cold-water immersion, after it was poured into the crushed ice containing 500ml beaker and this reaction mixture was kept overnight at room temperature. The chalcones were obtained out as solid. Then it was recovered and crystallization was done using ethanol. Using  $\text{CHCl}_3$  as a solvent the goodness of the compound was checked by TLC method
- 6. Common Procedure (2a-2c):** Thiosemicarbazide (0.001 mmol) and 2% sodium hydroxide solution of 10 ml was added in to a different mixture of chalcone (0.001mol) in ethanol (40 ml). Then the mixture was warmed up, then refluxed for 16 hours. Then the process gets completed, that mixture was streamed into squashed ice and hold on to overnight at normal temperature. After this reaction it was filtered by using filter paper and dried. After that it gets recrystallized by using ethanol. Using TLC method, the purity of the resulted compound was checked; in this reaction utilized solvent is  $\text{CHCl}_3$ .
- 7. Antimicrobial Activity Assay:** There is a discrete evaluation of harmonized compounds done with a panel of Gram twins bacteria pathogen, and fungi. Antimicrobial tests were conducted using the agar well-diffusion method [28-30]. After the media had cooled and solidified, well (6 mm in diameter) were made in the solidified agar, before microbial inoculums was uniformly spread using a sterile cotton swab on a sterile Petri dish containing agar nutrient (NA) medium, or Sabouraud dextrose agar (SDA) media for bacteria and fungi, respectively. The 1 mg of the product dissolved in 1 mL of dimethylsulfoxide (DMSO) to obtain quantity of 100  $\mu\text{L}$  of the evaluated compound

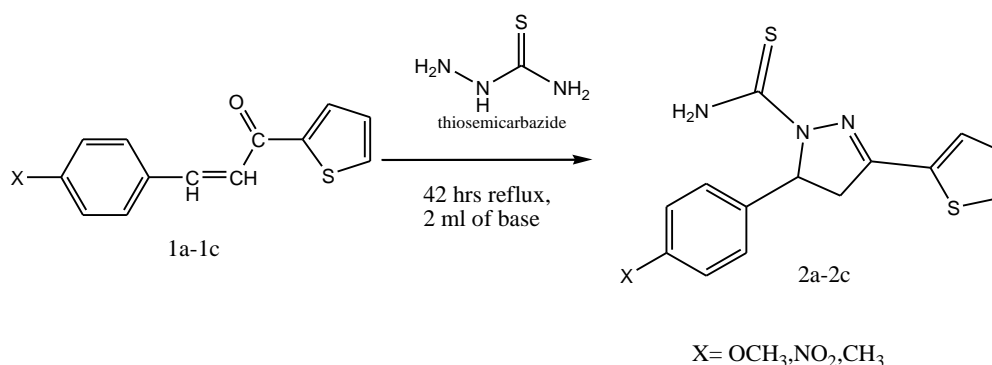
solution. The hatching of immunized plates was done for 24h at 37°C for bacteria and yeast, and 48h at 28°C for fungi. The tested compounds were dissolved with the help of DMSO for the preparation of negative controls. The utilized standards of bacteria and fungi is named as Ciprofloxacin (1mg/mL), Clotrimazole(1mg/mL) respectively. After the procedure of incubation, the calculation of the zone of inhibition against the tested microorganisms is help to evaluate the antimicrobial activity. By adopting the literature precedent, the antimicrobial activity procedure was followed [31].

## 8. Computational study

**Molecular Docking Study:** Docking done with using 1UAG by Auto dock version, the reference method was followed for the docking study [32].

## III. RESULTS AND DISCUSSION

**1. Synthetic Chemistry:** Due to the in-depth scientific studies, the formation of the chemistry of chalcones is occur throughout the world. The main focused area of interest is synthesis and biodynamic activity of chalcones. In chalcones, aliphatic three carbon chain connected with two aromatic rings. Chalcone carries a good python so that diversity of novel heterocyclic with good pharmaceutical profile can be outlined. Harmonization of Thiophen chalcone from different substituted acetophenone reacted with thiophen-2-carbaldehyde in the presence of ethanol containing NaOH solution. The thiophen chalcone were then condensed with hydroxylamine hydrochloride to give carbothioamidederivatives. To generate specific compound like trans E-isomer, the Claisen-Schmidt condensation method is used.



**Scheme 2:** Synthetic route for the target compounds

From the IR spectrum information gathered for (1a-1c) compound are; the carbonyl compounds has shown the stretching at 1690-1750  $\text{Cm}^{-1}$ , the Aromatic C-H compounds stretches at 3000-3100  $\text{Cm}^{-1}$  and Aliphatic C-H bond stretching is found at 2900-3000  $\text{Cm}^{-1}$ . The (2a-2c) compound shows IR spectra at different stretching frequency including C=N Stretching about 1600-1650  $\text{Cm}^{-1}$ , the N-H bond Stretches about 3350-3500  $\text{Cm}^{-1}$ . The Ar C-H bond stretches about 3000-3100  $\text{Cm}^{-1}$ , the C=C Stretches about 1450-1600  $\text{Cm}^{-1}$ , Aromatic ring stretches at 600-800  $\text{Cm}^{-1}$ . The  $^1\text{H}$  NMR of **1a** exhibit that

the proton H-5 in the pyrimidine moiety gives a singlet peak at 6.16 ppm. The NH<sub>2</sub> proton of pyrimidine ring gives broad singlet peak at 5.13 ppm. The protons H-3, H-4 & H-5 gives peaks at 6.85 ppm and 6.96 ppm. The protons present in the aromatic ring gives peaks within the range of 7.08-8.02 ppm. On taking the <sup>13</sup>C NMR of **1a** compound exhibit the peaks at 165.30 and 163.22 ppm, these are the peaks for C=N in the pyrimidine moiety. The peaks appeared in the down field region at 152.90 ppm is the presence of C-N in pyrimidine moiety. The resonance at 100.60 ppm is of C-5 carbon of pyrimidine. The peak 156.61 ppm in the down field region is the resonance of C-2 carbon in the furan ring. The furan ring carbon signals are found at 108.69 is C-3, 112.80 is C-4 and 144.44 ppm is C-5. The Aromatic carbon in the compound gave the resonance in the range of 113.32-130.87 ppm. From the above discussed characteristics of the compound assigned from spectroscopic studies, the skeletal structure of the particular compound is confirmed. The spectral results of the compounds are discussed in the tables below.

**Table 1: IR Stretching Frequency of (1a-1c)**

Compounds	C=O	Ar-CH	Aliphatic-CH	Ar ring	Ar C=C
1a	1653.07	3030.30 3058.23	2932.89 2963.75	691.51,736.84 767.70	1449.57
1b	1655.96	3066.85 3180.40	2925.17 2962.79	687.65,734.91 768.67	1410.02
1c	1676.21	3068.27	2924.21	692.47,758.06 814.21	1400.38

**Table 2: IR Stretching Frequency of (2a-2c)**

Compounds	N-H	C=N	Ar-CH	Ar ring
2a	3349.53,3473.95	1572.05	3048.62	694.40,760.79 833.28
2b	3404.51	1588.45,1681.04	3029.34 3054.41	765.77, 819.78 838.11
2c	3408.36 3553.03	1587.48, 1680.07	3030.30, 3058.34 3076.54	695.37,765.77 841.96

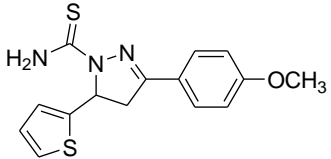
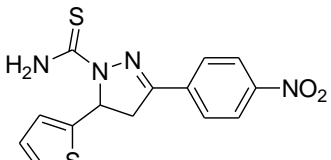
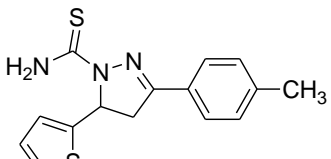
**Table 3: Physical characterizations of the synthesized compounds 2a-2c**

Entry	MF	MW	% of Yield	Colour	MP <sup>0</sup> C
1a	C <sub>14</sub> H <sub>12</sub> O <sub>2</sub> S	244.31	83	Yellow	112
1b	C <sub>13</sub> H <sub>9</sub> NO <sub>3</sub> S	259.28	78	Brown	104
1c	C <sub>14</sub> H <sub>12</sub> OS	228.31	80	Yellow	101
2a	C <sub>15</sub> H <sub>15</sub> N <sub>3</sub> OS <sub>2</sub>	317.43	75	Yellow	179
2b	C <sub>14</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub>	332.40	68	Brown	184
2c	C <sub>15</sub> H <sub>15</sub> N <sub>3</sub> S <sub>2</sub>	301.43	72	Yellow	195

#### IV. MOLECULAR DOCKING STUDIES

- 1. In silico Activity:** The cell wall synthesis mechanism is done by with the help of IUAG protein. a good docking score and good interaction is displayed by the recently prepared compounds. Mainly (compound 2b) exhibiting a good docking score (-6.7 kcal/mol) related to another 2 compounds. Another compound docking scores are specified by reducing order -6.3, -6.0 kcal/mol of 2c, 2a. There is an interaction will be occurred between compound 2b and IUAG by forming conventional hydrogen bonding at ARG A: 302, LYS A: 319 and LYS A: 115. the conventional hydrogen bond at VAL A: 335, GLY A: 337 will be also formed by compound 2c. Compounds 2a forms conventional hydrogen bonding at ASN A: 421. The interaction of ALA A: 414 and the alkyl, pi-alkyl will give the compound 2b. Compound 2c forms alkyl, pi-alkyl relation at LEU A: 333, LEU A: 330, LEU A: 339, VAL A: 364. Binding score value of the prepared compounds are listed in the Table-4. The 2D and 3D images of the harmonized compounds are given in Table-5.



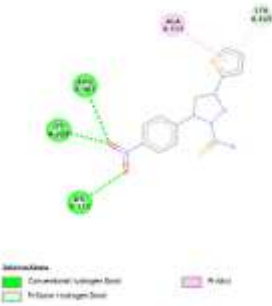


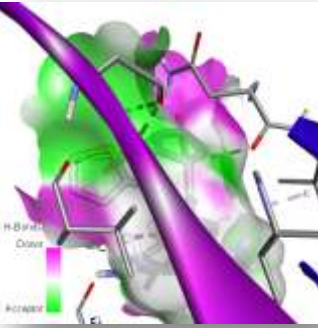
**Table 4: Docking Binding Score values of the synthesized compounds 2a-2c**

S.No	Compounds structure	Compounds	Binding affinity value (kcal/mol)	CHB interaction	Alkyl & pi - Alkyl interaction	Other interactions
1.		2a	-6.0	ASN A: 421	-	SER A: 415, GLU A: 423
2.		2b	-6.7	ARG A: 302, LYS A: 319, LYS A: 115	ALA A: 414	SER A : 415
3.		2c	-6.3	VAL A: 335 GLY A: 337	LEU A: 333, LEU A: 330, LEU A: 339, VAL A: 364	ASN A: 363

(' - ') which is indicates that there is no bond interaction



**Table 5: The 2d and 3D images of the 2a-2c**

Compounds	2D Image	3D Image
2a		
2b		
2c		

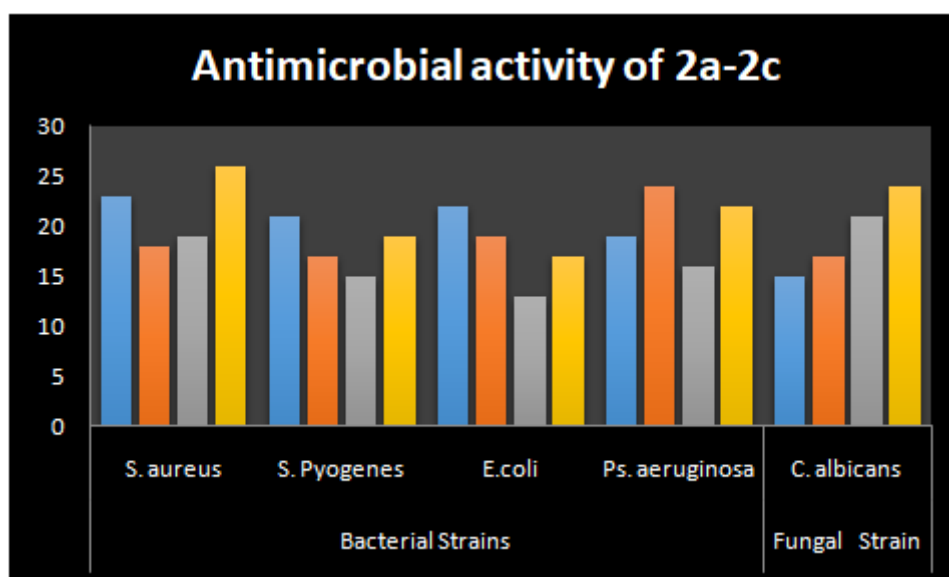
## 2. Biology

**Antimicrobial Study:** Using disc diffusion method, the novel pyrimidine derivatives 2a-2c was tested for their antibacterial activity against microbial strains and the results are exhibited in Table-6. The *S. Pyogenes* showed by compound 4a very excellent manner. As well as the *E. Coli* exhibits favorable zone with *S. Aureus* and *P. Aeruginosa*. The another excellent zone of inhibition showed by compound 4b against *E. Coli* and *P. Aeruginosa* and also favorable zone with *S. Aureus* and *S. Pyogenes*. The compound 2c showing good zone of inhibition opposed to *S. Aureus*, *S. Pyogenes*, *E. Coli* and *P. Aeruginosa*. when correlate with

the standard drug ciprofloxacin some of the harmonized compound **2a-2c** the **2a** presenting best zone of inhibition. according to antifungal studies the excellent zone of inhibition showed by 2c against *C.albicanand* also when correlate with the standard drug Clotrimazole the compound **2a** and **2b** displays good zone of inhibition.

**Table 6: Antimicrobial activity screening of the compounds (2a-2c) at 10mg/ml**

Entry	<i>Bacterial Strain</i>				<i>Fungal Strain</i>
	<i>S.Aureus</i>	<i>S.Pyogenes</i>	<i>E.Coli</i>	<i>P.aeruginosa</i>	<i>C.albicans</i>
<b>4a</b>	23	21	22	19	15
<b>4b</b>	18	17	19	24	17
<b>4c</b>	19	15	13	16	21
Ciprofloxin/ Clotrimazole	26	19	17	22	24



**Figure 1:** *in vitro* antimicrobial microbial activity of 2a-2c by disc diffusion method

## V. CONCLUSION

In summary, new and most relevant synthetic way of a few amino pyrimidine derivatives were attained. The construction of the recently developed compounds was entrenched. The target molecules were assessed for their antimicrobial activity against Gram-twins' bacteria as well as fungal stain. The conclusion shows that the synthesized compounds 4a-4c displayed a sufficient restrictive growth of Gram-twins' bacteria. Mainly, there is a magnificent zone of inhibition (23mm in diameter) of Compound 2a occur against *S. Aureus*. and also this compound 2a shows magnificent zone of inhibition (21 mm in diameter) against *S.Pyogenes* as well as the compound shows outstanding zone of inhibition against *E.Coli* (22 mm in diameter) when compared with the standard drug Ciprofloxacin. There is a better antibacterial activity occur by compound 2a against all the compounds. According to fungal

study declare that the compound 4c shows good zone of inhibition (21 mm in diameter) against the fungal stain *C.albican*, because of the thiophene ring contains a CH<sub>3</sub> present in their 5<sup>th</sup> position. The docking study was take place with bacterial protein and Breast cancer protein. All the compound exhibits best binding activity scores.

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