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MCF-7 Human Breast Adenocarcinoma Anticancer and Antimicrobial, *in silico* Docking and ADME Prediction Studies of Furan Moiety Containing Substituted 2-Aminopyrimidine Derivatives

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A series of 4(5-(4-chlorophenyl)furan-2-yl)-6-phenylpyrimidin-2-amine derivatives **(5a-h)** were synthesized from 2-(4-chlorophenyl)-5-styrylfuran **(3a-h)** with guanidine nitrate in absolute ethanol under conventional method and evaluated for their *in vitro* anticancer, antimicrobial activities and *in silico* studies. The chemical structure of the furan moiety containing substituted amino pyrimidine derivatives **(5a-h)** were elucidated from spectroscopic analysis like infrared, ^{1}H & ^{13}C NMR spectral data and CHN analysis. *in silico* docking studies were predicted for the synthesized compounds **(5a-h)** using bacterial protein 1UAG and *in silico* ADME predictions were also carried for the synthesized compounds **(5a-h)**. The *in vitro* anticancer study was carried the compound **5b** by MMT assay. Compound **5b** shows the LC_{50} value of 120.15 ± 0.003 µg/mL. *in vitro* Antimicrobial activities were screened for the compounds **(5a-h)** using different strains. Compound **5h** has electron withdrawing group in benzene ring substituted in the *para* position showed good antimicrobial activity against all the bacterial strains and fungal strains. *in silico* studies, compound **5h** shows excellent docking score (-9.7 kcal/mol) compared with ciprofloxacin (-7.8 kcal/mol).

Keywords: *in vitro* Anticancer, Molecular docking studies, *in silico* ADME prediction, Antimicrobial activity, Spectral studies.

INTRODUCTION

Cancer results from the uncontrolled spread and growth of abnormal cells and is the second major global cause of death [1]. With changes in the people's lifestyle and the aging of global population, cancer mortality inevitably increases [2,3]. Recently, the World Health Organization (WHO) reported that in 2018, 9.6 million people died due to cancer. By 2030, this number can increase to 15 million. Studies have provided numerous novel anticancer drugs and have confirmed their efficacy in treating specific cancers. However, the cancer mortality rate remains high because of cancer drug resistance [4,5]. Hence, the development effective, novel and safe chemotherapeutic agents to treat cancer is still urgent [6].

Recently, the development of new antimicrobial agents gives an additional option for the cure of different bacterial and fungal infections, which affect millions of people worldwide [7]. Antibiotic resistance emerged as a major problem in infectious disease management. However, in clinical practice, for most antibacterial agents, for a minimum of one bacterial pathogen, resistance was reported. For the production of different classes of heterocyclic compounds, such as oxazoles, pyrazoles, thiophenes, isoxazoles and pyrimidines [8,9], the derivatives of chalcones are considered the key starting materials. Heterocyclic rings play a crucial role in medicinal chemistry and serve as the main intermediates for the construction of crucial therapeutic agents [10]. The pyrimidine and its derivatives are known as one of the *N*-containing heterocyclic compounds, which give various biological and pharmacological activities [11] such as antiviral [12], antioxidant [13], antibacterial [14], anticancer [15], antimicrobial, anticonvulsion and anti-inflammatory activities [16,17]. Literature review shows that amino moiety at the second position of carbon amino pyrimidine ring intensifies the biologically potential of pyrimidines derivatives.

Molecular docking is an optimization problem, which describes the 'optimum-fit' orientation of ligands binding to target proteins, and is employed to establish the structures of intermolecular complexes formed among two or more mole-

cules. The protein ligand interaction is a sought-after topic due to its medicinal applications. Ligands are small molecules, interacting with the protein-binding site [18]. Molecular docking principally applied in modern drug discovery for determining drug-receptor interactions and is a computational method [19-21]. For many years, QSAR developed models to predict the interesting physio-chemical properties of ADMET [22]. These properties include aqueous solubility [23], partition coefficient, blood brain barrier (BBB) penetration [24], absorption and permeability [25], metabolism, plasma protein binding [26,27], excretion [28], hERG inhibition [29], physiologically based pharmacokinetic (PBPK) modelling, P-glycoprotein (P-gp) efflux and toxicity [30]. Additionally, homology and pharmacophore modelling were conducted to improve toxicity and metabolism predictions [31,32]. Thus, pharmaceuticals are seeking to understand the ADMET process to obtain the advantage of early discovery. The objectives are to predict, which compounds pass the test to be used as drugs at the early stage of the process perhaps even before the synthesis of compounds. Numerous softwares are developed to investigate the toxicity and properties of absorption, distribution, metabolism and elimination (ADME)-based organisms [33-36]. For online software swiss ADME online tool, we must predict ADME properties.

In this study, herein we report the synthesis of amino pyrimidine derivatives **(5a-h)** from 2-(4-chlorophenyl)-5-styrylfuran chalcone reacting with guanidine nitrate in the presence of a strong base. The skeleton structure of amino pyrimidine derivatives was unambiguously assigned using infrared, ^{1}H & ^{13}C NMR and CHN analysis. The *in silico* molecular docking and *in silico* ADME prediction were performed for the compounds (**5a-h**). The *in vitro* anticancer study was conducted only for compound **5b**. The *in silico* study was performed by bacterial protein 1UAG. The antimicrobial study was done for synthesized compounds (**5a-h**).

EXPERIMENTAL

Melting points were determined on a MELT-TEMP melting point apparatus and are uncorrected, Infrared spectra were recorded neat on a Shimadzu FT-IR spectrometer. ^{1}H & ^{13}C NMR spectra were recorded on a BRUCKER 400 MHz NMR spectrometer in $CDCl_3$with TMS as an internal standard at room temperature. All the reactions were monitored by TLC, which was carried out on Merck silica gel coated on plates. Laboratory grade chemicals and solvents available commercially in high grade purity were used. All the synthesized compounds were identified by physical properties, IR, NMR data and Elemental analysis. Yields reported are isolated yields unless indicated otherwise. By adopting literature procedure, 2-(4-chlorophenyl)-5-styrylfuran chalcones (**3a-h**) were synthesized [37].

Synthesis of 4-(5-(4-chlorophenyl)furan-2-yl)-6-phenylpyrimidin-2-amine derivatives (5a-h): By adopting the literature procedure, compounds (**5a-h**) were synthesized [38]. The solution of 2-(4-chlorophenyl)-5-styrylfuran derivatives (**3a-h**) 0.01 mol in ethanol (60 mL) was added with 0.01 mol of NaOH and 0.50 M of guanidine nitrate and refluxed for 10-12 h. The reaction mixture was then cooled and poured in to crushed ice and kept aside for overnight. The resulting precipitate of 4-(5-(4-chlorophenyl)furan-2-yl)-6-arylpyrimidine-2-amine was filtered dried and recrystallized from ethanol. Thin layer chromatography was performed to check the purity of the synthesized compounds using the ratio of petroleum ether and ethyl acetate (9:1 ratio).

4-(5-(4-Chlorophenyl)furan-2-yl)-6-phenylpyrimidine-2-amine (5a): Yield 57%; m.p.: 152-154 ºC; yellow solid: Elemental analysis calcd. (found) % of $C_{20}H_{14}N_3OCl$: C, 69.01 (69.07); H, 4.03 (4.06); N, 12.07 (12.08); Cl, 10.19 (10.19); O, 4.60 (4.60): IR (KBr, ν_{max}, cm^{-1}): 3290.56 (NH_2), 1560.41 (C=N), 1355.96 (C-N), 3151.89 (Ar-CH), 2916.37 (aliph. CH). ^{1}H NMR ($CDCl_3$) 400 MHz δ ppm: 7.08 (H5 of pyrimidine), 6.73 (H3′ of furan ring), 6.88 (H4′ of furan ring), 7.23-8.20 (Ar-H). ^{13}C NMR 100 MHz δ ppm: 163.39 (C2 of pyrimidine), 160.92 (C4 of pyrimidine ring), 99.23 (C5 of pyrimidine), 155.05 (C6 of pyrimidine), 153.56 (C2′ of furan ring), 108.02-129.02 (Ar-C), 132.87, 130.16 (*ipso*-carbons).

4-(5-(4-Chlorophenyl)furan-2-yl)-6-(4-flurophenyl)-pyrimidin-2-amine (5b): Yield 52%; m.p.: 112-114 ºC; yellow solid. Elemental analysis calcd. (found) % of $C_{20}H_{13}N_3OClF$: C, 65.61 (65.57); H, 3.55 (3.58); N, 11.48 (11.49); Cl, 9.69 (9.69); O, 4.37 (4.37). IR (KBr, ν_{max}, cm^{-1}): 3287.31 (NH_2), 3098.77 (Ar-CH), 2953.02 (aliph. CH), 1545.13 (C=N), 1346.71 (C-N). ^{1}H NMR ($CDCl_3$) 400 MHz δ ppm: 7.03 (H5 of pyrimidine), 6.73 (H3′ of furan ring), 6.91 (H4′ of furan ring), 7.03-7.98 (Ar-H). ^{13}C NMR ($CDCl_3$) 100 MHz δ ppm: 163.74 (C2 of pyrimidine), 160.41 (C4 of pyrimidine), 98.34 (C5 of pyrimidine), 156.21 (C6 of pyrimidine), 153.93 C2′ of furan ring, 109.27-125.31 (Ar-C), 138.92, 140.12 (*ipso*-carbons).

4-(4-Bromophenyl)-6-(5-(4-chlorophenyl)furan-2-yl)-pyrimidine-2-amine (5c): Yield 56%; m.p.: 128-130 ºC; dark yellow solid. Elemental analysis calcd. (found) % of $C_{20}H_{13}N_3O$ BrCl: C, 56.25 (56.30); H, 3.05 (3.07); N, 9.84 (9.85); Br, 18.73 (18.73); Cl, 8.31 (8.31); O, 3.75 (3.75). IR (KBr, ν_{max}, cm^{-1}): 3318.09 (NH_2), 3067.81 (Ar-CH), 2927.07 (aliphatic CH), 1567.32 (C=N), 1357.09 (C-N). ^{1}H NMR ($CDCl_3$) 400 MHz δ ppm: 7.11 (H5 of pyrimidine), 6.80 (H3′ of furan ring, 6.96 (H4′ of furan ring), 7.33-8.13 (Ar-H). ^{13}C NMR ($CDCl_3$) 100 MHz δ ppm: 163.83 (C2 of pyrimidine), 160.52 (C4 of pyrimidine), 98.29 (C5 of pyrimidine), 156.93 (C6 of pyrimidine), 153.67 (C2′ of furan ring), 112.83-126.92 (Ar-C), 140.23, 140.96 (*ipso*-carbons).

4-(5-(4-Chlorophenyl)furan-2-yl)-6-*p*-tolylpyrimidine-2-amine (5d): Yield 62%; m.p.: 154-156 ºC; dark Yellow solid. Elemental analysis calcd. (found) % of $C_{21}H_{16}N_3OCl$: C, 69.64 (69.71); H, 4.42 (4.46); N, 11.60 (11.61); Cl, 9.79 (9.80); O, 4.42 (4.42). IR (KBr, ν_{max}, cm^{-1}): 3302.17 (NH_2), 3091.23 (Ar-CH), 2991.29 (aliph. CH), 1567.32 (C=N), 1355.72 (C-N). ^{1}H NMR ($CDCl_3$) 400 MHz δ ppm: 7.09 (H5 of pyrimidine), 6.83 (H3′ of furan ring, 6.87 (H4′ of furan ring), 7.12-8.04 (Ar-H), 2.39 (methyl protons). ^{13}C NMR (CDC_{l3}) 100 MHz δ ppm: 163.68 (C2 of pyrimidine), 160.17 (C4 of pyrimidine), 99.31 (C5 of pyrimidine), 156.29, (C6 of pyrimidine), 152.87 (C2′ of furan ring), 109.94-124.86 (Ar-C), 138.21, 139.02 (*ipso*-carbons).

4-(5-(4-Chlorophenyl)furan-2-yl)-6-(4-methoxyphenyl)-pyrimidine-2-amine (5e): Yield 60%; m.p.; 126-128 ºC; yellow

solid. Elemental analysis calcd. (found) % of $C_{21}H_{16}N_3O_2Cl$: C, 66.69 (66.76); H, 4.23 (4.27); N, 11.11 (11.12); Cl, 9.38 (9.38); O, 8.46 (8.47). IR (KBr, ν_{max}, cm^{-1}): 3289.38 (NH_2), 3058.03 (Ar-CH), 2924.59 (aliph. CH), 1589.03 (C=N), 1358.93 (C-N). ^1H NMR ($CDCl_3$), 400 MHz, δ ppm: 7.05 (H5 of pyrimidine), 6.77 (H3′ of furan ring), 6.83 (H4′ of furan ring), 7.21-8.01 (Ar-H), 3.78 (methoxy protons). ^{13}C NMR ($CDCl_3$) 100 MHz δ ppm: 163.77 (C2 of pyrimidine), 160.47 (C4 of pyrimidine), 99.28 (C5 of pyrimidine), 156.92 (C6 of pyrimidine), 153.20 (C2′ of furan ring), 114.83-125.43 (Ar-C), 135.65, 136.18 (*ipso*-carbons).

4-(4-Biphenyl)-6-(5-(4-chlorophenyl)furan-2-yl)pyrimidine-2-amine (5f): Yield 62%; m.p.: 162-164 ºC; yellow solid. Elemental analysis calcd. (found) % $C_{28}H_{18}N_3OCl$: C, 73.63 (73.67); H, 4.26 (4.28); N, 9.90 (9.91); Cl, 8.33 (8.36); O, 3.76 (3.77). IR (KBr, ν_{max}, cm^{-1}): 3303.03 (NH_2), 3102.09 (Ar-CH), 2948.62 (aliph. CH), 1553.06 (C=N), 1351.29 (C-N). ^1H NMR ($CDCl_3$) 400 MHz δ ppm: 7.01 (H5 of pyrimidine), 6.79 (H3′ of furan ring), 6.85 (H4′ of furan ring), 7.03-7.98 (Ar-H). ^{13}C NMR ($CDCl_3$) 100 MHz δ ppm. 163.58 (C2 of pyrimidine), 160.28 (C4 of pyrimidine), 99.87 (C5 of pyrimidine), 156.73 (C6 of pyrimidine), 153.11 (C2′ of furan ring), 124.45-129.41 (Ar-C), 132.64, 133.87 (*ipso*-carbons).

4-(4-Chlorophenyl)-6-(5-(4-chlorophenyl)furan-2-yl)-pyrimidine-2-amine (5g): Yield 66%; m.p.: 114-116 ºC; yellow solid. Elemental analysis calcd. (found) % of $C_{20}H_{13}N_3OCl_2$: C, 62.98 (62.84); H, 3.40 (4.05); N, 10.55 (10.99); Cl, 8.91 (8.55); O, 4.18 (4.19). IR (KBr, ν_{max}, cm^{-1}): 3342.71 (NH_2), 3054.70 (Ar-CH), 2962.17 (aliph. CH), 1559.14 (C=N), 1361.69 (C-N). ^1H NMR ($CDCl_3$), 400 MHz, δ ppm: 7.12 (H5 of pyrimidine), 6.71 (H3′ of furan ring), 6.91 (H4′ of furan ring), 7.11-8.03 (Ar-H). ^{13}C NMR ($CDCl_3$) 100 MHz δ ppm: 163.91 (C2 of pyrimidine), 160.49 (C4 of pyrimidine), 99.13 (C5 of pyrimidine) 156.91 (C6 of pyrimidine), 153.88 (C2′ of pyrimidine), 118.34-125.87 (Ar-C), 134.45, 134.98 (*ipso*-carbons).

4-(5-(4-Chlorophenyl)furan-2-yl)-6-(4-nitrophenyl)-pyrimidine-2-amine (5h): Yield 62%; m.p.: 144-146 ºC; yellow solid. Elemental analysis calcd. (found) % of $C_{20}H_{13}N_4OCl$: C, 61.21 (61.16); H, 3.31 (3.34); N, 14.28 (14.26); Cl, 9.04 (9.03); O, 12.24 (12.22). IR (KBr, ν_{max}, cm^{-1}): 3301.89 (NH_2), 3082.19 (Ar-CH), 2989.77 (aliph. CH), 1558.06 (C=N), 1357.08 (C-N). ^1H NMR ($CDCl_3$) 400 MHz δ ppm: 7.02 (H5 of pyrimi-dine), 6.81 (H3′ of furan ring), 6.86 (H4′ of furan ring), 7.11-8.12 (Ar-H). ^{13}C NMR ($CDCl_3$), 100 MHz, δ ppm: 163.29 (C2 of pyrimidine), 160.27 (C4 of pyrimidine), 99.62 (C5 of pyrim-idine, 156.38 (C6 of pyrimidine), 153.71 (C2′ of furan ring), 119.03-124.49 (Ar-C), 134.76, 134.98 (*ipso*-carbons).

Computational studies

Molecular docking studies: Molecular docking studies were carried out for synthesized for 4-(5-(4-chlorophenyl)-furan-2-yl)-6-aryl pyrimidine derivatives (**5a-h**) by bacterial protein by Auto dock version 4.2.5.1 docking software. The reference method was adopted for the docking study [39].

ADME prediction: ADME properties were predicted for all the compounds (**5a-h**) using Swissadme online software. This software tool provides information about molecular weight (m.w), hydrogen bond acceptor (Hy-A), hydrogen bond donor (Hy-D), octanol-water partition coefficient (log P_{ow}), solubility (log S), skin permeation (log Kp), total polar surface area (TPSA), molar refractivity (M.Ref) and bioavailability score. From these parameters, we have to understand the ADME property of any drug or organic molecule [40,41].

Biological studies

Antimicrobial screening: Antimicrobial screening was conducted by following the literature survey method [42]. All the synthesized compounds were investigated using agar cup diffusion techniques in DMSO by employing 1 mg/mL solution. *S. pyogenes* and *S. aureus* were used as the Gram-positive bacteria and *P. aeruginosa* and *E. coli* were used as the Gram-negative bacteria. Moreover, *Candida albicans* was employed as representative fungi. In 1 mL of 6 h broth culture, each sterile molten agar (at 45 ºC) was received. Then, in sterile petri dishes, seeded agar was poured. In agar solution, cups (8 mm diameter) were cut. Each cup contained 0.1 mL of 1 mg/mL solution of test compounds. Subsequently, the plates were incubated for 24 h and, for *C. albicans*, for 48 h at 37 ºC. For all the organisms, a control with DMSO without test compounds was incubated. Ciprofloxacin and clotrimazole were employed as the standard antibacterial and antifungal references, respectively.

***in vitro* Anticancer activity:** MCF-7 (human breast adenocarcinoma) cells were procured from National Centre for Cell Sciences (NCCS), Pune, India, and stored in Dulbecco's modified Eagles medium, DMEM (Sigma-Aldrich, USA). The cell lines were cultured in 25 cm^2 tissue culture flask having DMEM supplemented with 10% FBS, sodium bicarbonate (Merck, Germany), L-glutamine and an antibiotic solution containing streptomycin (100 µg/mL), penicillin (100 U/mL), and amphoteracin B (2.5 µg/mL). The cultured cell lines were stored in a humidified 5% CO_2 incubator at 37 ºC. The cell viability was evaluated by analyzing the cells through inverted phase contrast microscopy and using the MTT assay method.

Cells seeding in 96 well plate: The confluent monolayer of cells (2 days old) were trypsinized. In a 10% growth medium, the cells were suspended; in a 96-well tissue culture plate, 100 µL cell suspension (5×10^4 cells/well) was seeded and incubated in the humidified 5% CO_2 incubator at 37 ºC.

Preparation of compound stock: The sample (1 mg) was weighed and then dissolved in 1 mL of DMEM by using a cyclomixer. Through a 0.22 µm Millipore syringe filter, the sample solution was filtered to ensure sterility.

Anticancer evaluation: After 24 h, the growth medium was eliminated. Each compound was freshly prepared in 5% DMEM with five serial dilutions through two-fold dilution (6.25, 12.50, 25, 50, 100 µg/mL in 500 µL of 5% DMEM). Each concentration (100 µL) was added to respective wells in triplicates and the resulting mixture was incubated in the humidified 5% CO_2 incubator at 37 ºC. The control cells were stored.

Anticancer assay by direct microscopic observation: After 24 h of treatment, the entire plate was analyzed through inverted phase contrast tissue culture microscopy (Olympus CKX41 with Optika Pro5 CCD camera). The results were obtained as images. The changes detected in the cell morphology, such

as shrinking or rounding, vacuolisation and granulation in the cell cytoplasm, were considered cytotoxicity indicators [43].

Anticancer assay by MTT method: In 3 mL PBS, 15 mg of MTT (Sigma, M-5655) was completely dissolved and sterilized through filter sterilization. After 24 h of incubation, the sample was removed from wells, and to all test and cell control wells, 30 µL of the reconstituted MTT solution was added. The plate was shaken gently, and then, was incubated in the humidified 5% CO_2 incubator at 37 ºC for 4 h. After incubation, the supernatant was separated, and 100 µL of MTT solubilization solution (DMSO, Sigma-Aldrich, USA) was added to it. The wells were gently mixed through pipetting up and down to solubilize formazan crystals. Absorbance was measured using a microplate reader at 540 nm [44,45].

The percentage of growth inhibition was calculated using the formula:

$$\text{Viability (\%)} = \frac{\text{Mean OD of samples}}{\text{Mean OD of control group}} \times 100$$

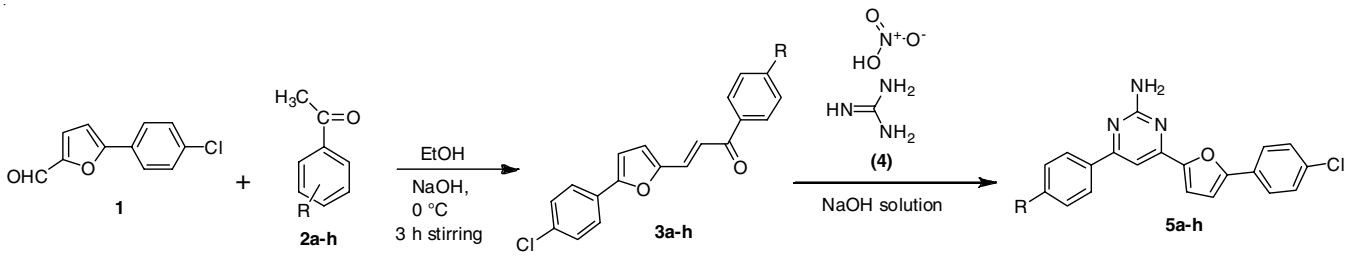
RESULTS AND DISCUSSION

The synthetic strategies adopted to obtain the intermediate and target compounds are shown in **Scheme-I**. The key intermediate chalcones (**3a-h**) were synthesized in excellent yields by condensing 5-(4-chlorophenyl)furan-2-carbaldehyde (**1**) with the appropriate substituted acetophenone in presence of NaOH under Claisen-Schmidt reaction conditions. These chalcones were subjected to cycloaddition condensation reactions using guanidine nitrate in the presence of 20% NaOH to give the corresponding 4-(5-(4-chlorophenyl)furan-2-yl)-6-arylpyrimidine-2-amine derivatives (**5a-h**) in good yields.

The chemical structure of aminopyrimidine compounds were elucidated using infrared, ^1H & ^{13}C NMR spectral studies and CHN analysis. The FT-IR spectrum of compound **5a** shows a strong absorption frequency at 3290.56 cm^{-1} and the presence of NH_2 group of pyrimidine moiety. The strong absorption frequency at 1560.41 cm^{-1} is due to the presence of C=N in pyrimidine moiety. The C-N stretching frequency gives sharp absorption at 1355.96 cm^{-1}. The absorption at 3151.89 and 2916.37 cm^{-1} is due the presence of aromatic and aliphatic CH stretching frequency, respectively. The structure of the furan bearing aminopyrimidines were further confirmed by ^1H NMR spectra. ^1H NMR spectrum of compound **5a** gives the signal at 7.08 ppm is attributed to H_5 proton of pyrimidine moiety. The 3′ proton and 4′ proton of furfuran ring moiety shows the signal at 6.73 and 6.88 ppm, respectively. The aromatic protons appear at 7.23 ppm to 8.20 ppm. The ^{13}C NMR spectrum of compound **5a** shows the ^{13}C resonance at in the most downfield region of 163.69 ppm, which is attributed to C-2 of pyrimidine ring. The ^{13}C resonance in the downfield (shielded) region of 160.92 ppm is due to the presence of C-4 carbon of pyrimidine ring. The ^{13}C resonance at 99.23 ppm is attributed to C-5 carbon of pyrimidine ring. The ^{13}C resonance at 155.05 ppm is attributed to C-6 of pyrimidine moiety. The aromatic carbons appear in the region of 108.02 ppm to 129.02 ppm. The ^{13}C resonance at 153.56 ppm is unambiguously assigned to C-2′ of furfuran ring. The *ipso*-carbons appeared at 132.87 and 130.16 ppm.

Computational studies

***in silico* Molecular docking studies:** The *in silico* studies were predicted for aminopyrimidine derivatives (**5a-h**) using bacterial protein 1UAG. The bacterial protein 1UAG was involved in the cell wall synthesis mechanism. This protein was downloaded from Protein Data Bank file. From the results of *in silico* studies (Table-1), compound **5h** shows excellent docking score (-9.7 kcal/mol) compared with ciprofloxacin (-7.8 kcal/mol).



R= 2a = 3a = 5a = H; R = 2b = 3b = 5b = F; R = 2c = 3c = 5c = Br; R = 2d = 3d = 5d = CH_3
R = 2e = 3e = 5e = OCH_3; R = 2f = 3f = 5f = phenyl; R = 2g = 3g = 5g = Cl; R = 2h = 3h = 5h = NO_2

Scheme-I: Synthetic pathway for the compounds **5a-h**

TABLE-1
MOLECULAR DOCKING RESULTS FOR AMINO PYRIMIDINE DERIVATIVES (**5a-h**) USING BACTERIAL PROTEIN (1UAG)

Compd.	R	Docking score	H-B interaction	Hydrophobic interaction
5a	H	-8.8	–	ARG: 302, LEU: 263, LEU: 333
5b	F	-9.0	–	LEU: 263, ARG: 302, LEU: 333
5c	Br	-9.0	–	ARG: 302, LEU: 263, LEU: 333
5d	CH_3	-8.5	–	LEU: 333, ARG: 302, LEU: 263
5e	OCH_3	-8.2	–	LEU: 333, ARG: 302, LEU: 263
5f	C_6H_5	-8.7	ASN A: 268, LEU A: 299	PRO A: 300, ARG A: 302
5g	Cl	-8.9	–	LEU A: 263, ARG A: 302, LEU A: 333
5h	NO_2	-9.7	ARG A: 302, LYS A: 319, LYS A: 115	PRO A: 142
Std. drug	Ciprofloxacin	-7.8	LEU: 416, SER: 415, HIS: 183, LYS: 115, LYS: 319	LYS: 319,PHE: 422

Compound **5h** (electronegativity group, 4-NO_2) has three conventional hydrogen bond interactions with the amino residues, which are ARG: 302, LYS: 319 and LYS: 115 and this compound shows a hydrophobic interaction with amino residue PRO: 142. From the results of docking studies, eight aminopyrimidine derivatives show high docking score (kcal/mol) compared with ciprofloxacin. Rest of the amino pyrimidine derivatives' interactions are given in Table-1. The 2D and 3D images of compound **5h** are shown in Fig. 1. The procedures were adopted by literature survey method [46].

***in silico* ADME prediction study:** Numerous biologically active compounds cannot meet clinical standards due to insufficient ADME parameters. Thus, synthesized compounds (**5a-h**) were studied through computation to evaluate ADME properties. The number of rotational bonds (n-ROTB), the polar surface area (TPSA), Lipinski's rule of five and the molecular weight (MW) were calculated with the Swissadme online property calculation toolkit [47]. All the synthesized compounds exhibited excellent percentage absorption (Table-2). Furthermore, no compound trespassed Lipinski's rule of five and hence exhibited potential series applications to improve the compound having drug-like properties. Compounds, which are to be produced as orally active drugs, should not exhibit >1 violation of the following criteria: molecular weight ≤ 500, logP (octanol-water partition coefficient) ≤ 5, number of hydrogen bond donors ≤ 5 and number of hydrogen bond acceptor ≤ 10 [48]. All the synthesized compounds showed the acceptable solubility values of 64.94-110.76 [49]. Compound **5f** exhibited the highest solubility (-6.82) among other compounds of the series. Compound **5e** showed a good drug-likeness score (3.31), while compound **5a** had a good drug score (0.70). All the synthesized compounds together compiled, for orally active drugs, a standard. Therefore, the proposed compounds can advance as candidates for oral drugs. The results indicated that the prepared compounds satisfy computational assessment criteria and thus, provide a framework that is pharmacologically active and should be considered for further potential applications. The drug-likeness model score (a combined effect, given by a numerical value, of the pharmacokinetics pharmacodynamics and physio-chemical properties of compounds) was computed through swissadme software (http://www.swissadme.com) for the eight prepared compounds.

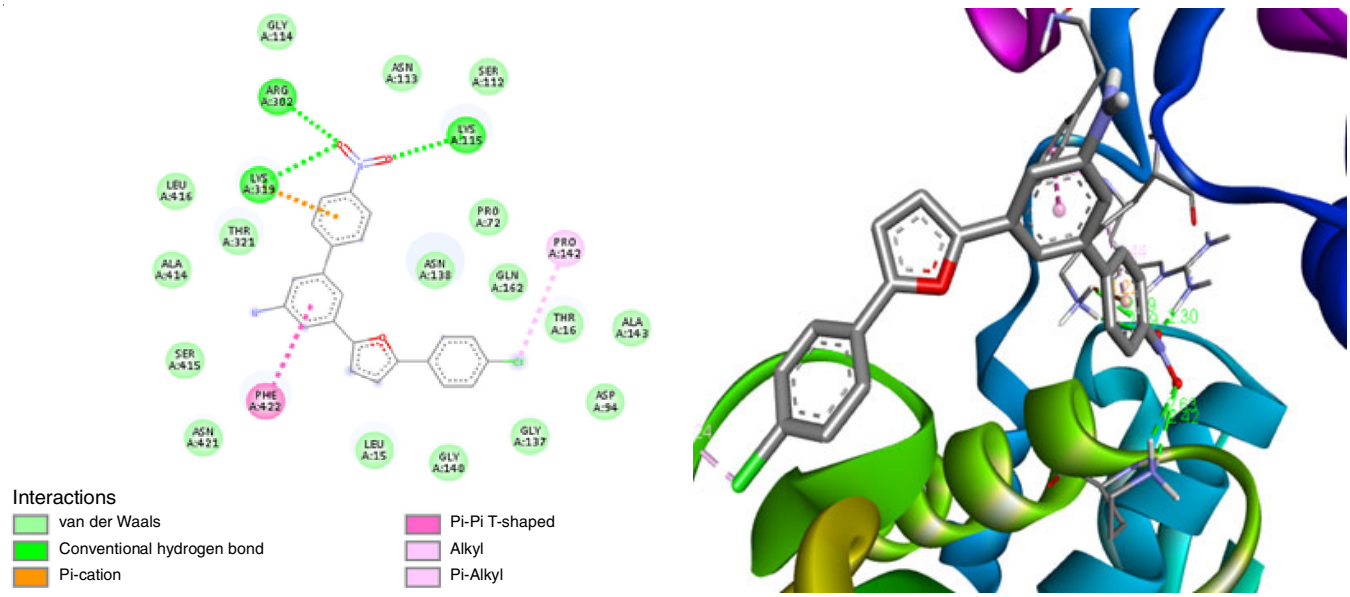


Fig. 1. 2D and 3D images of the compound **5h**

TABLE-2
ADME PROPERTIES FOR GOOD ORAL BIOAVAILABILITY OF THE COMPOUNDS **5a-h**

Compd.	%ABS	TPSA	n-ROTB	m.w.	log P	n-ON	n-OHNH	n-heavy atoms	Solubility	M.R	Drug likeness	Drug Score
5a	86.6	64.94	3	347.80	4.87	3	1	25	-6.38	100.02	3.14	0.70
5b	86.6	64.94	3	365.79	4.97	4	1	26	-6.69	99.98	2.26	0.66
5c	86.6	64.94	3	426.70	5.60	3	1	26	-7.21	107.72	1.16	0.51
5d	86.6	64.94	3	361.83	5.21	3	1	26	-6.72	104.99	1.68	0.61
5e	83.42	74.17	4	377.83	4.80	4	1	27	-6.40	106.51	3.31	0.69
5f	86.6	64.94	4	397.86	6.07	3	1	31	-7.98	125.46	0.77	0.47
5g	86.6	64.94	3	382.25	5.48	3	1	26	-7.11	105.03	3.12	0.61
5h	70.79	110.76	4	406.83	3.67	5	1	28	-6.69	108.84	-2.06	0.44

%ABS: percentage absorption, TPSA: topological polar surface area, n-ROTA: number of rotational bonds, m.w.: molecular weight, log P: logarithm of partition coefficient of compound between n-octanol and water, n-ON: number of hydrogen bond acceptor, n-OHNH: number of hydrogen bond donors, n-heavy atoms: numberof heavy atoms, M.R: molar refractivity.

Pharmacokinetics' and drug-likeness prediction by Swiss ADME: The pharmacokinetics' and drug-likeness prediction of the synthesized compounds (**5a-h**) were carried out by Swiss ADME online tool and the data's are given Tables 3 and 4. According to pharmacokinetic properties, most of the synthesized compounds showed high gastrointestinal absorption, except compounds **5f** and **5h**, these two compounds low gastrointestinal absorption. In blood brain barrier (BBB), all the compounds have no permeability. However, all of them (**5a-h**) showed inhibition to cytochrome P450 isomers (CYP1A2 and CYP2C19). Similarly, cytochrome P450 isomers (CYP-C2D6 and CYP3A4) inhibit all the synthesized compounds except compound **5f**. Skin permeation values *i.e.* log K_p values appeared in the range of -4.48 to -5.57. The drug-likeness prediction was also conducted depending on the selected Lipinski's, Ghose, Veber and bioavailability score. All the synthesized compounds (**5a-h**) were obey the Lipinski's rule of five these (**5a-h**) compounds zero violations. In Ghose filter, only five compounds **5a, 5e, 5f, 5g** and **5h** were heeled others have one violation. All of them accept the veber rules there is no violation. Most of the synthesized compounds probably accept the Muggee and Egan rules. All the compounds (**5a-h**) have the similar bioavailability score of 0.55 (Table-4). Medicinal Chemistry Properties also carried out by swissadme software. In these study, they have no alert in pains and brenk but in compound **5h** showed one violation, In leadlikeness properties, all the synthesized compounds (**5a-h**) showed two violations like molecular weight > 350 and XLOGP3 > 3.5 (Table-4). All the compounds have the synthetic ability value between 3.18-3.54 (Table-4). From these values of synthetic ability of

TABLE-3
PHARMAKOKINETICS FOR THE SYNTHESIZED COMPOUNDS **5a-h**

Compd.	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYPC2D6 inhibitor	CYP3A4 inhibitor	log K_p skin permeation (cm/s)
5a	High	No	Yes	Yes	Yes	No	Yes	Yes	-5.18
5b	High	No	Yes	Yes	Yes	No	Yes	Yes	-5.22
5c	High	No	Yes	Yes	Yes	No	Yes	Yes	-5.17
5d	High	No	Yes	Yes	Yes	No	Yes	Yes	-5.01
5e	High	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.38
5f	Low	No	Yes	Yes	Yes	No	No	No	-4.48
5g	High	No	Yes	Yes	Yes	No	Yes	Yes	-4.94
5h	Low	No	No	Yes	Yes	Yes	Yes	Yes	-5.57

TABLE-4
DRUGLIKENESS AND MEDICINAL PROPERTY FOR THE SYNTHESIZED COMPOUNDS **5a-h**

Compd.	Druglikeness						Medicinal chemistry			
	Lipinski's	Ghose	Veber	Egan	Muegge	Bio availability	PAINS	Brenk	Leadlikeness	Synthetic accessibility
5a	Yes: 0 violation	Yes	Yes	Yes	Yes	0.55	0-alert	0-alert	No: 1violation XLOGP3 > 3.5	3.18
5b	Yes: 0 violation	No: 1 violation WLOGP > 5.6	Yes	Yes	Yes	0.55	0-alert	0-alert	No: 2 violation MW > 350 XLOGP3 > 3.5	3.18
5c	Yes: 0 violation	No: 1 violation WLOGP > 5.6	Yes	No: 1 violation WLOGP > 5.88	No: 1 violation XLOGP 3 > 5	0.55	0-alert	0-alert	No: 2 violationMW > 350 XLOGP3 > 3.5	3.21
5d	Yes 0-violation	No: 1 violation WLOGP > 5.6	Yes	Yes	Yes	0.55	0-alert	0-alert	No: 2 violation MW > 350 XLOGP3 > 3.5	3.29
5e	Yes 0-violation	Yes	Yes	Yes	Yes	0.55	0-alert	0-alert	No: 2 violation MW > 350 XLOGP3 > 3.5	3.21
5f	Yes: 0 violation	Yes	Yes	No: 1 violation WLOGP > 5.88	No: 1 violation XLOGP3 > 5	0.55	0-alert	0-alert	No: 2 violation MW > 350 XLOGP3 > 3.5	3.54
5g	Yes: 0 violation	Yes	Yes	No: 1 violation WLOGP > 5.88	No: 1 violation XLOGP3 > 5	0.55	0-alert	0-alert	No: 2 violation MW > 350 XLOGP3 > 3.5	3.18
5h	Yes 0-violation	Yes	Yes	Yes	Yes	0.55	0-alert	1-alert NO_2 Gp	No: 2 violation MW > 350 XLOGP3 > 3.5	3.25

the compounds (**5a-h**) is obeyed the medicinal chemistry property.

Biological studies

***in vitro* Antimicrobial activity:** Biological screening was performed for the synthesized amino pyrimidine derivatives (**5a-h**) against the Gram-positive and Gram-negative bacterial strains. These were *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa* and fungal strain of *C. albicans* was done by agar disk diffusion method. The antimicrobial activity results indicated that compounds **5b**, **5c**, **5f**, **5g** and **5h** have good antimicrobial activity as compared to standard drugs ciprofloxacin (antibacterial) and clotrimazole (antifungal), the other compounds showing better activity (Table-5). The Gram-positive antibacterial activity of compound **5h** shows moderate activity against *S. aureus* and compounds **5h**, **5b** and **5g** show excellent activity against *S. pyogenes*. The Gram-negative antibacterial activity of the compounds **5b**, **5c**, **5f**, **5g** and **5h** is most potent against *E. coli* while compound **5f** shows excellent activity against *P. aeruginosa* [50]. The antifungal activity results indicate that compound **5h** is found to be most effective against *C. albicans*. Finally, the most active synthesized aminopyrimidine derivative **5h** may be considered as a lead compound and these compounds have good antimicrobial agents.

Comparison of SAR studies: According to the biological studies and *in silico* studies, the SAR of the aminopyrimidine derivatives (SAR) can be concluded as synthesized compound **5h** was found to be the most potent antibacterial agent against *S. pyogenes*, *E. coli* and *P. aeruginosa* as well as a good antifungal agent against *C. albicans*. From the *in silico* studies, compound **5h** is found to be the most active molecule which has the maximum number of HB interaction like three and one hydrophobic interaction network among the aminopyrimidine derivatives. Electron-withdrawing group (fluoro and chloro) on benzene ring of compounds **5b** and **5g** increases the potential of antibacterial property against *S. pyogenes* and *E. coli*. Electron withdrawing groups in the *para* position (bromo) on benzene ring of compound **5c** increases the potential of antibacterial

TABLE-5
in vitro ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **5a-h**

Compound	Bacterial strains				Fungal strain
	S. aureus	*S. pyogenes*	*E. coli*	*Ps. aeruginosa*	*C. albicans*
5a	14	12	16	11	13
5b	21	19	21	20	15
5c	19	18	18	17	11
5d	15	13	10	13	10
5e	16	11	11	12	10
5f	18	15	19	13	10
5g	21	19	18	17	18
5h	24	23	19	24	19
Ciprofloxacin/Clotrimazole	26	19	17	22	24

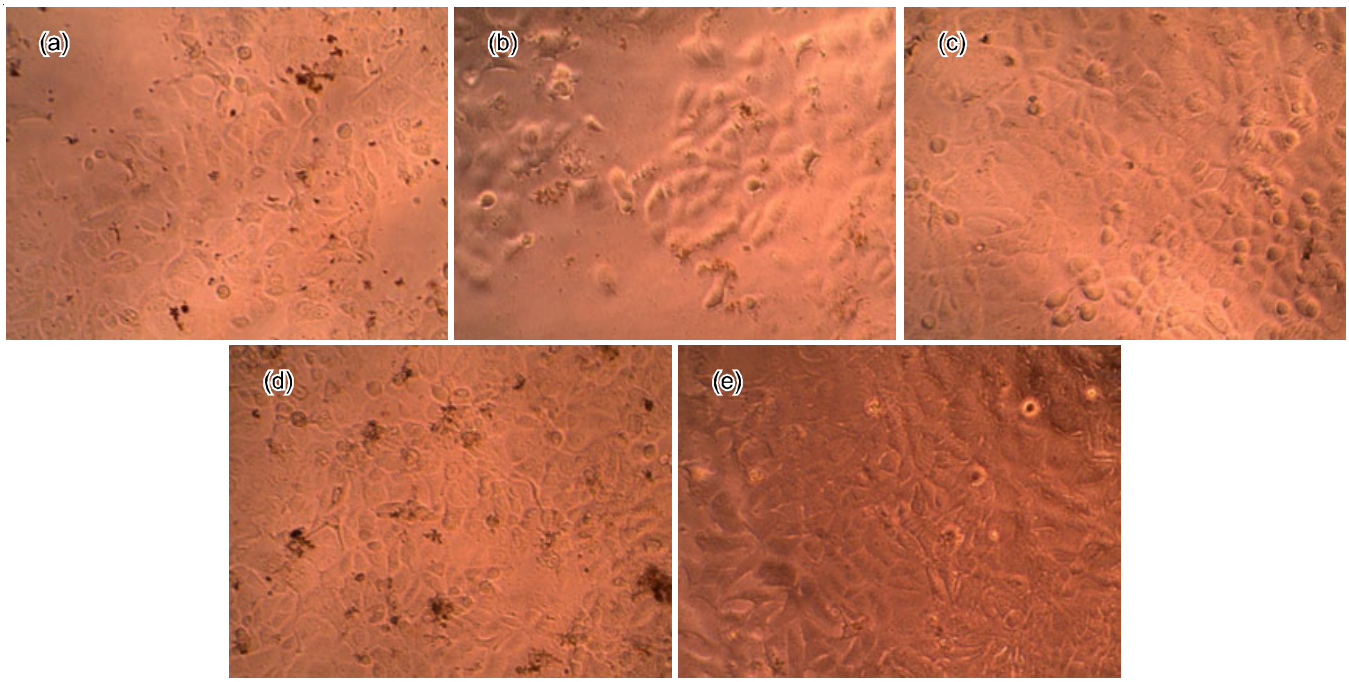


Fig. 2. *in vitro* Anticancer activity of compound **5b**. MCF7 cells were incubated for 48 h in presence of (a) 6.25 µg/mL (b) 12.5 µg/mL (c) 25 µg/mL (d) 50 µg/mL (e) 100 µg/mL

property against *E. coli*. The above mentioned results showed that different structural requirements are needed for a compound to be effective against different targets molecules.

***in vitro* anticancer activity:** Mammary gland (MCF-7), a human cancer cell line, was employed to estimate the *in-vitro* anticancer activity of the synthesized compound **5b** under various concentrations (6.25, 12.50, 25, 50, 100 µg/mL). The anticancer results (Fig. 2) indicated that pyrimidine derivatives exhibit high anticancer potential against the human breast cancer cell line; especially, compound **5b** provides high activity at 6.25 µg/mL and the LC_{50} of compound **5b** is 120.15 ± 0.003 µg/mL.

Conclusion

The structurally diverse compounds (**5a-h**) were synthesized using the cyclization method. The synthesized compounds were characterized by spectral data and found in good agreement with the assigned molecular structures of the target compounds. Also compound **5b** was carried for their *in vitro* anticancer activity with the good LC_{50} value of 120.15 ± 0.003 µg/mL. The *in vitro* biological studies of synthesized compounds indicated that compound **5h** exhibited excellent activity against antibacterial and antifungal strains. The *in silico* docking studies were carried for compounds **5a-h** and the results indicated that compound **5h** was the most active compound and had the maximum HB interaction (three). Also, it had one hydrophobic interaction network among the amino pyrimidine derivatives. The *in silico* ADME predictions were carried out for eight aminopyrimidine derivatives. These results confirmed that Lipinski's rule of five were obeyed for the aminopyrimidine derivatives. So, it is concluded that compounds (**5a-h**) are suitable to be drugs subjected to further clinical tests.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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