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Novel Pyrimidine Derivatives from 2,5-Dichloro-3-acetylthienyl Chalcones as Antifungal, Antitubercular and Cytotoxic Agents: Design, Synthesis, Biological Activity and Docking Study

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Twenty novel pyrimidine derivatives were synthesized from 2,5-dichloro-3-acetylthienyl chalcones by reacting with guanidine HCl in presence of KOH and ethanol under reflux for 6 h. Their structural characterizations were evaluated by ATR-FTIR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy. They were also screened for antifungal, antitubercular and cytotoxicity activities. They were displayed good antifungal activity (MIC = 32-125 µg/mL) against *Aspergillus niger* and *Candida tropicalis* fungal species except compound **15** with 4"-pyridinyl moiety (MIC = 8.00 µg/mL) being more potent. Compound **5** with 2",4"-dichlorophenyl moiety was shown with good antitubercular activity (MIC = 6.2 µg/mL) against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB) stain. They have also tested for *in vitro* cytotoxicity activity against DU-145 prostate cancer cell lines. In which the compound **15** with 4"-pyridinyl moiety (IC_{50} = 2.0 ± 0.1 µg/mL) and compound **17** with 2"-pyrrolyl moiety (IC_{50} = 6.0 ± 0.1 µg/mL) possess highly potent antiprostate cancer properties. The molecular docking was done with the crystalline structure of mitochondrial 2-enoyl thioester reductase Etr1p/Etr2p heterodimer from *Candida tropicalis* fungal species with compound **15** (-7.80 kcal/mol) and shown greater binding affinity than fluconazole (-7.60 kcal/mol). Docking was performed with protein crystalline structure (PDB ID: 2WEE) of *Mycobacterium tuberculosis* H37Rv (MTB) stain and among all, compound **5** was exhibited good binding affinity (-6.90 kcal/mol), compared to pyrazinamide (-4.10 kcal/mol). The protein crystalline structure of a mutant androgen receptor (AR) ligand-binding domain (LBD) (PDB file: 1GS4) was tested with compounds **15** and **17** (-7.60 and -8.20 kcal/mol). They were exhibited good binding properties compared to methotrexate (-5.10 kcal/mol). Hence, these novel pyrimidine compounds are as lead compounds as antifungal, antitubercular and cytotoxic agents.

Keywords: 2,5-Dichloro-3-acetylthiophene, Novel pyrimidines, Antifungal activity, Antitubercular activity, Cytotoxicity.

INTRODUCTION

Chalcones belongs to a group of compounds with two aromatic rings connected by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids exhibiting a wide spectrum of biological activities. Chalcones are widely distributed in natural ferns to higher plants and most of them are polyhydroxylated in the aryl rings [1].

The presence of a reactive α,β-unsaturated keto (also called as 1,3-diaryl-2-propen-1-one) functional group is partly responsible for their biological activity. These chalcones are utilized as intermediates in the synthesis of various five, six and seven membered heterocyclic compounds like pyrimidines, pyrazoles, isoxazoles, 1,5-benzothiazepines and other heterocyclic systems. Chalcones were obtained by various schemes of synthesis reported in the literature in which many techniques and schemes are reported for their synthesis of pyrimidines. Amongst all, aldol condensation and Claisen-Schmidt condensation are most widely adapted [2-4].

Pyrimidine **(a)** is the most important member of all the six-membered heterocyclic compounds containing nitrogen among pyridines, quinolines and indoles as this ring system occurs widely in living organisms. Purines, uric acid, alloxan, barbituric acid and a group of antimalarial and antibacterial agents also contain the pyrimidine ring. The chemistry of pyrimidine and its derivatives have been reported since the past so many decades due to their diverse pharmacological activities. Pyrimidine and purine are the two-nitrogen containing hetero-

cyclic aromatic compounds as parents of nitrogenous bases found in DNA that constitute a key structural unit of nucleic acids, even though pyrimidine itself does not exist in nature. Both pyrimidine and purine are planar and this flat shape is very important when it must be considered in the structure of nucleic acids [5]. In terms of their chemistry, pyrimidine and purine resemble pyridine. They are weak bases and relatively unreactive toward electrophilic aromatic substitution. There is an important structural difference between pyrimidine derivatives that bear hydroxyl (–OH) group and those with amino ($–NH_2$) group.

Literature review emphasized on various synthetic routes to prepare new pyrimidines and evaluated for their biological activities such as antifungal, anti-inflammatory, antitubercular, antioxidant and anticancer activities. New pyrimidines were synthesized from 3-aminoacetophenone and aromatic aldehyde derived chalcone derivatives upon reacting with urea, thiourea and guanidine to give respective pyrimidine derivatives. It has also been reported in same paper that another series of pyrimidine derivatives from sulfadiazine and screened for an antibacterial activity against Gram-positive and Gram-negative bacteria. The emphasis was much on synthesis rather than biological activity [6].

In two separate reviews, various synthetic schemes were reported to prepare simple pyrimidine derivatives and possess antifungal, antihyperlipidemic, antineoplastic, antihistaminic agents and acetyl choline esterase (AchE) inhibitors [7]. Most pyrimidines were synthesized from a starting material of chalcones or modified chalcones using either urea, thiourea or guanidine as a coupling agent to synthesize new pyrimidines. Antitubercular, antioxidant, anti-inflammatory, anticonvulsant, antimicrobial, antifungal, antiplasmodial and anticancer activities were established [8].

A reaction scheme with substituted chalcones were prepared by the reaction with anthracene-9-carbaldehyde with different ketones in presence of aqueous ethanolic KOH solution. These substituted chalcones were treated with guanidine carbonate containing aqueous NaOH to produce corresponding 2-amino-4,6-diarylpyrimidines and possess antifungal and antibacterial activities [9]. Imidazolyl chalcones were used in a reaction with guanidine hydrochloride to synthesize pyrimidine derivatives and evaluated for their antibacterial and antifungal activities [10].

Chalcones were synthesized by the reaction of 3-acetyl, 2,5-dimethyl furan with aromatic and heteroaromatic aldehydes to give substituted 3-acetyl, 2,5-dimethyl furan chalcones. Pyrimidines were prepared by the reaction of modified chalcone with guanidine in presence of aqueous ethanol and compounds were evaluated for its anticancer activity against DU-145 prostate cancer cell lines [11].

The synthesis of chalcone was reported and performed by condensing 2-(4-carboxyphenylazo)acetoacetate with aromatic aldehydes to produce modified chalcones and these chalcones were treated with urea in ethanolic KOH to form a series of pyrimidine derivatives. They were also screened for antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ stain using microplate alamar blue assay [12].

4-Acetyl pyridine was treated with a substituted benzaldehyde to synthesize new chalcones and pyrimidine derivatives are produced by the reaction of new chalcones with guanidine and urea in ethanolic KOH. These pyrimidine derivatives have been screened for antitubercular activity by microplate alamar blue assay (MABA) against *Mycobacteria tuberculosis*. These compounds were also assessed for antioxidant, antibacterial and anti-inflammatory activity [13].

Novel mercaptopyrimidines were synthesized and reported to possess antimicrobial and antitubercular activities. Thienyl chalcones were treated by selective cyclization with thiourea to yield mercaptopyrimidines [14]. Chalcones of 2-acetyl-5-bromo furan are reacted with guanidine hydrochloride in absolute ethanol and KOH and refluxed in water to produce desired pyrimidine derivatives and possess anti-inflammatory and cytotoxic activities [15].

From the literature, it has been clearly emphasized that pyrimidine derivatives from either chalcones or modified chalcones continued to exhibit a spectrum of biological activities and attract considerable pharmacological significance. The synthesis of novel 2,5-dichloro-3-acetyl thienyl chalcone derivatives were reported in our recent studies. The resultant thienyl chalcone derivatives have exhibited potential antifungal, antitubercular and antiprostate cancer activities [16].

EXPERIMENTAL

Melting points were recorded with METTLER melting point apparatus on electrotherm capillary tube and are uncorrected. Reaction rates were performed by thin-layer chromatography (TLC) on silica gel 60 GF_{254} coated glass plates and the spots were visualized by exposure to iodine vapours or monitored under UV-lamp set at 254 nm for few seconds. ATR-FTIR infrared spectra were obtained by using Nicolet™ iS5 FTIR Spectrophotometer (Thermo-scientific, Madison, Wisconsin, USA) with iD5 ATR accessory featuring a diamond crystal located in ν (cm^{-1}) Postgraduate Laboratory, Faculty of Pharmaceutical Sciences, UCSI University. Nuclear magnetic resonance (^1H NMR and ^{13}C NMR) spectra were recorded on a Bruker 300 MHz spectrophotometer, College of Science and Technology, Andhra University by using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent. The data were recorded as chemical shifts expressed in δ (ppm) relative to tetramethylsilane (TMS) as internal standard. Microanalytical data (C, H, N, O, F and S) were performed at the microanalytical unit, College of Science and Technology, Andhra University, Visakhapatnam, India.

4-(2-Amino-6-(2,5-dichlorothiophen-3-yl)pyrimidin-4-yl)phenol (1): To a solution of 1-(2',5'-dichloro-3'-thienyl)-3-(4''-hydroxyphenyl)-2-propen-1-one (0.005 mol) and guanidine hydrochloride (0.005 mol) in absolute ethanol (20 mL), alcoholic potassium hydroxide (0.3 mL) was added drop wise at room temperature. The reaction mixture was refluxed for 6 h at ambient temperature and the solvent was evaporated completely. The reaction mixture was poured into ice-cold water and the solid that separated out was filtered, dried and purified by column chromatography with ethyl acetate/hexane and crystallized from chloroform to give compounds **1-20**.

Compound **1** was analyzed for $C_{14}H_9N_3OS$, m.p.: 188 °C yielding 77 %, well supported by its molecular ion $[M^+H]^+$ at

m/z 340 in its mass spectrum. The ATR-FTIR spectrum (cm^{-1}) **1** showed the characteristic absorption bands at 3348 (NH_2), 1628 (C=N), 1580 (C=C), 856 (C-Cl), 1052 (C-S), 3200 (-OH). The ^1H NMR spectrum (400 MHz, $CDCl_3$) of **1** exhibited a singlet at δ 7.26 integrating for one proton due to the C-5-H of the pyrimidine. The spectrum also revealed a singlet at δ 5.31 for the protons of the $–NH_2$ groups at position 2 of the pyrimidine. The other singlet at δ 4.75 accounted for the proton of the –OH group present on the phenyl ring at position 6 of the pyrimidine ring. Two doublets at δ 7.43 and 7.88 integrating for 4 protons due to the C-3''-H, C-5''-H, C-2''-H and C-6''-H of the phenyl ring. The spectrum also showed the characteristic protons of the 2,5-dichlorothiophene ring at δ 7.11 (1-H, d, *J* = 7.0 Hz, C-4'-H). The ^{13}C NMR spectrum of **1** accounted for all the carbons whose resonances appeared at the following δ values: 163.74 (C-2-NH_2), 163.66 (C-4), 164.31 (C-6), 95.03 (C-5), 131.21 (C-1''), 128.92 (C-2'' and C-6''), 130.41 (C-3'' and 5''), 134.33 (C-4''), 160.43 (C-2'), 109.52 (C-3'), 106.24 (C-4') and 119.66 (C-5'). The results of elemental analysis were also in close accord with those of calculated values. Based on the above spectral data the structure of the compound **1** was confirmed as 4-(2-amino-6-(2,5-dichlorothiophen-3-yl)-pyrimidin-4-yl)phenol.

4-(4-Chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (2): Yield 89 %, m.p.: 210 °C, m.w. 357, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3342 (NH_2), 1628 (C=N), 1580 (C=C), 856 (C-Cl), 1052 (C-S); ^1H NMR ($CDCl_3$): 7.30 (1H, s, C-5-H), 5.34 (2H, s, C-2-NH_2), 7.98 (2H, d, *J* = 7 Hz, C-2''-H and C-6'-H), 7.44 (2H, d, *J* = 7 Hz, C-3''-H and C-5''-H), 7.15 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{14}H_8N_3SCl_3$: C, 47.05, H, 2.24, N, 11.76, S, 8.96, Cl, 29.83. Found: C, 47.01, H, 2.02, N, 11.46, S, 8.97, Cl, 29.81.

4-(4-Methoxyphenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (3): Yield 86 %, m.p.: 144 °C, m.w. 353, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3345 (NH_2), 1625 (C=N), 1590 (C=C), 1165 (OCH_3), 1142 (C-S), 864 (C-Cl), ^1H NMR ($CDCl_3$): 7.68 (1H, s, C-5-H), 4.90 (2H, s, C-2-NH_2), 3.88 (3H, s, C-4''-OCH_3), 8.00 (2H, d, *J* = 7 Hz, C-2''-H and C-6''-H), 7.02 (2H, d, *J* = 7 Hz, C-3''-H and C-5''-H), 7.18 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{15}H_{11}N_3OSCl_2$: C, 51.15, H, 3.15, N, 11.93, S, 9.06, Cl, 20.11. Found: C, 50.95, H, 3.00, N, 11.82, S, 8.97, Cl, 20.05.

4-(4-Dimethylaminophenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (4): Yield 72 %, m.p.: 173 °C, m.w. 366, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3338 (NH_2), 1633 (C=N), 1588 (C=C), 1185 ($N(CH_3)_2$), 1160 (C-S), 867 (C-Cl), 864 (C-Cl), ^1H NMR ($CDCl_3$): 7.27 (1H, s, C-5-H), 5.39 (2H, s, C-2-NH_2), 3.09 (6H, s, C-4''-N (CH_3)2), 8.00 (2H, d, C-2''-H and C-6''-H), 6.74 (2H, d, C-3''-H and C-5''-H), 7.14 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{16}H_{14}N_4SCl_2$: C, 52.61, H, 3.86, N, 15.34, S, 8.74, Cl, 19.39. Found: C, 52.25, H, 3.52, N, 15.11, S, 8.71. Cl, 19.32.

4-(2,4-Dichlorophenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (5): Yield 97 %, m.p.: 102 °C, m.w. 472, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3348 (NH_2), 1635 (C=N), 1582 (C=C), 850 (C-Cl), 1067 (C-S), 868 (C-Cl), ^1H NMR ($CDCl_3$): 7.07 (1H, s, C-5-H), 5.47 (2H, s, C-2-NH_2), 8.55 (1H, d, *J* = 2 Hz, C-3''-H), 8.07 (1H, d, *J* = 7 Hz, C-5''-H), 8.77 (1H, d, *J* = 7 Hz, C-6''-H), 7.80 (1H, d, *J* = 6.5 Hz, C-3'-H), 7.11 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{14}H_7N_3SCl_4$: C, 42.99, H, 1.80, N, 10.74, S, 6.78, Cl, 30.08. Found: C, 52.25, H, 3.52, N, 15.11, S, 6.77. Cl, 29.98.

4-(9-Anthracenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (6): Yield 81 %, m.p.: 235 °C, m.w. 423, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3356 (NH_2), 1636 (C=N), 1582 (C=C), 1099 (C-S), 862 (C-Cl), ^1H NMR ($CDCl_3$): 7.27 (1H, s, C-5-H), 5.69 (2H, brs, C-2-NH_2), 7.14-7.59 (9H, m, Ar-H), 7.62 (1H, d, *J* = 6.5Hz, C-3'-H), 7.17 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{22}H_{13}N_2SCl_2$: C, 62.57, H, 3.10, N, 9.95, S, 7.57, Cl, 16.78. Found: C, 62.12, H, 2.91, N, 9.71, S, 7.53. Cl, 16.71.

4-(4-Methylphenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (7): Yield 81 %, m.p.: 187 °C, m.w. 337, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3350 (NH_2), 1630 (C=N), 1580 (C=C), 1077 (C-S), 760 (C-Cl), ^1H NMR ($CDCl_3$): 7.31 (1H, s, C-5-H), 5.34 (2H, s, C-2-NH_2), 2.14 (3H, s, C-4''-CH_3), 7.98 (2H, d, *J* = 7 Hz, C-2''-H and C-6''-H), 7.44 (2H, d, *J* = 7 Hz, C-3''-H and C-5''-H), 7.50 (1H, d, *J* = 6.5 Hz, C-3'-H), 7.14 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{15}H_{11}N_3SCl_2$: C, 53.58, H, 3.30, N, 12.50, S, 9.50, Cl, 21.07. Found: C, 53.28, H, 3.15, N, 12.22, S, 9.48. Cl, 21.01.

4-(Phenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (8): Yield 85 %, m.p.: 219 °C, m.w. 333, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3340 (NH_2), 1630 (C=N), 1575 (C=C), 1082 (C-S), 866 (C-Cl), ^1H NMR ($CDCl_3$): 7.34 (1H, s, C-5-H), 5.35 (2H, s, C-2-NH_2), 7.62 (2H, m, C-2'' and 6''-H), 7.44-7.52 (3H, m, C-3'', 4'' and 5''-H), 8.04 (1H, d, *J* = 6.5 Hz, C-3'-H), 7.13 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{14}H_9N_3SCl_2$: C, 52.19, H, 2.82, N, 13.04, S, 9.61, Cl, 21.32. Found: C, 51.99, H, 2.41, N, 12.94, S, 9.49. Cl, 21.28.

4-(4-Fluorophenyl)-6-(2,5-dichlorothiophen-3-yl)-pyrimidin-2-amine (9): Yield 94 %, m.p.: 221 °C, m.w. 341, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3335 (NH_2), 1630 (C=N), 1575 (C=C), 1120 (C-F), 1055 (C-S), 861 (C-Cl), ^1H NMR ($CDCl_3$): 7.30 (1H, s, C-5-H), 5.17 (2H, brs, C-2-NH_2), 7.62 (2H, d, *J* = 7 Hz, C-2'' and C-6'' H), 7.47 (2H, d, *J* = 7 Hz, C-3'' and C-5'' H), 7.74 (1H, d, *J* = 6.3 Hz, C-3'H), 7.14 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{14}H_8N_3SFCl_2$: C, 49.43, H, 2.37, N, 12.32, S, 9.38, Cl, 20.82. Found: C, 49.11, H, 2.05, N, 12.02, S, 9.39. Cl, 20.90.

4-(3,4-Dimethoxyphenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (10): Yield 80 %, m.p.: 182 °C, m.w.: 383, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3414 (NH_2), 1641 (C=N), 1519 (C=C), 1145 (-O-CH_3), 1073 (C-S), 862 (C-Cl), ^1H NMR ($CDCl_3$):7.30 (1H, s, C-5-H), 5.22 (2H, brs, C-2-NH_2), 3.93 (3H, s, -OCH_3), 3.98 (3H, s, -OCH_3), 7.04 (1H, s, C-2''H), 6.94 (1H, d, *J* = 8 Hz, C-5''H), 7.46 (1H, d, *J* = 8 Hz, C-6''H), 7.59 (1H, d, *J* = 6.5 Hz, C-3'H), 7.13 (1H, d, *J* = 7.0 Hz, C-4'H). Anal. calcd. for $C_{16}H_{13}N_3O_2SCl_2$: C, 50.27, H, 3.43, N, 10.99, S, 8.36, Cl, 18.54. Found: C, 50.01, H, 3.12, N, 10.44, S, 8.39. Cl, 18.35.

4-(3,4,5-Trimethoxyphenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (11): Yield 85 %, m.p.: 194 °C, m.w. 413, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3361 (NH_2), 1602 (C=N), 1572 (C=C), 1120 (-O-CH_3), 1070 (C-S), 864 (C-Cl), ^1H NMR ($CDCl_3$): 7.29 (1H, s, C-5-H), 5.21 (2H, s, -NH_2), 3.90 (3H, s,

-OCH_3), 3.98 (6H, s, =2X-OCH_3), 7.28 (2H, s, C-2"-H and C-6"-H), 7.16 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{17}H_{15}N_3O_3SCl_2$: C, 49.52, H, 3.67, N, 10.26, S, 7.75, Cl, 17.19. Found: C, 49.12, H, 3.48, N, 10.01, S, 7.71. Cl, 17.10.

4-(3-Nitrophenyl)-6-(2,5-dichlorothiophen-3-yl)-pyrimidin-2-amine (12): Yield 95 %, m.p.: 155 °C, m.w.: 368, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3335 (NH_2), 1635 (C=N), 1575 (C=O), 1510 (N=O, asymmetric), 1330 (N=O, symmetric), 1092 (C-S), 862 (C-Cl) ^{1}H NMR ($CDCl_3$): 7.39 (1H, s, C-5-H), 5.18 (2H, brs, C-2-NH_2), 8.90 (1H, d, *J* = 2 Hz, C-2"-H), 8.49 (1H, m, C-4"-H), 7.65 (1H, d, *J* = 7.0 Hz, C-5"-H), 8.34 (1H, d, *J* = 8 Hz, C-6"-H), 7.16 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{14}H_8N_4O_2SCl_2$: C, 45.79, H, 2.20, N, 15.26, S, 7.75, Cl, 17.19. Found: C, 45.33, H, 2.04, N, 15.12, S, 7.71. Cl, 17.10.

4-(4-Nitrophenyl)-6-(2,5-dichlorothiophen-3-yl)-pyrimidin-2-amine (13): Yield 86 %, m.p.: 144 °C, m.w.: 368, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3413 (NH_2), 1512 (N=O, asymmetric), 1335 (N=O, symmetric), 1605 (C=N), 1083 (C-S), 762 (C-Cl) ^{1}H NMR ($CDCl_3$): 7.37 (1H, s, C-5-H), 5.25 (2H, brs, C-2-NH_2), 8.32 (2H, d, *J* = 8 Hz, C-2" and 6"-H), 8.19 (2H, d, *J* = 8 Hz, C-3" and 5"-H), 7.16 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{14}H_8N_4O_2SCl_2$: C, 45.79, H, 2.20, N, 15.26, S, 7.75, Cl, 17.19. Found: C, 45.33, H, 2.04, N, 15.12, S, 7.71. Cl, 17.10.

4-(Pyridin-3-yl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (14): Yield 76 %, m.p.: 156 °C, m.w. 324, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3335 (NH_2), 1633 (C=N), 1572 (C=C), 1122 (C-S), 868 (C-Cl), ^{1}H NMR ($CDCl_3$): 7.35 (1H, s, C-5-H), 5.21 (2H, brs, C-2-NH_2), 9.24 (2H, C-2" and C-6"H), 8.71 (1H, d, *J* = 8 Hz, C-4"-H), 8.32 (1H, d, *J* = 7.0 Hz, C-5"-H), 7.15 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{13}H_8N_4SCl_2$: C, 48.31, H, 2.49, N, 17.34, S, 9.88, Cl, 21.91. Found: C, 48.01, H, 2.05, N, 17.34, S, 9.86. Cl, 21.89.

4-(Pyridin-4-yl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (15): Yield 92 %, m.p.: 176 °C, m.w.: 324, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3338 (NH_2), 1635 (C=N), 1570 (C=C), 1101 (C-S), 864 (C-Cl), ^{1}H NMR ($CDCl_3$): 7.37 (1H, s, C-5-H), 5.21 (2H, brs, C-2-NH_2), 7.89 (2H, d, *J* = 8 Hz, C-2" and 6"H), 8.75 (2H, d, *J* = 8 Hz, C-3" and 5"-H), 7.15 (1H, d, *J* = 7.0 Hz, C-4'-H). Anal. calcd. for $C_{13}H_8N_4SCl_2$: C, 48.31, H, 2.49, N, 17.34, S, 9.88, Cl, 21.91. Found: C, 48.01, H, 2.05, N, 17.34, S, 9.86. Cl, 21.89.

4-(Pyridin-3-yl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (16): Yield 72 %, m.p.: 146 °C, m.w.: 324, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3332 (NH_2), 1638 (C=N), 1566 (C=C), 1112 (C-S), 864 (C-Cl), ^{1}H NMR ($CDCl_3$): 7.37 (1H, s, C-5-H), 5.09 (2H, brs, C-2-NH_2), 7.46 (2H, d, *J* = 6 Hz, C-3" and 5"-), 7.13 (2H, d, *J* = 7.0 Hz, C-4' and 4"-H), 7.75 (1H, d, *J* = 6 Hz, C-6"-H). Anal. calcd.for $C_{13}H_8N_4SCl_2$: C, 48.31, H, 2.49, N, 17.34, S, 9.88, Cl, 21.91. Found: C, 48.01, H, 2.05, N, 17.34, S, 9.86. Cl, 21.89.

4-(2-Pyrrolyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (17): Yield 81 %, m.p.: 187 °C, m.w.: 337, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3328 (NH_2), 1638 (C=N), 1572 (C=C), 1117 (C-S), 874 (C-Cl), ^{1}H NMR ($CDCl_3$): 7.35 (1H, s, C-5-H), 5.18 (2H, brs, C-2-NH_2), 4.78 (1H, s, -NH), 7.48 (1H, d, *J* = 6 Hz, C-3"), 7.18 (2H, d, *J* = 7.0 Hz, C-4' and 4"-H), 7.86 (1H, d, *J* = 6 Hz, C-5"-H). Anal. calcd. for $C_{15}H_{11}N_3SCl_2$: C, 46.32, H, 2.26, N, 13.46, S, 9.49, Cl, 21.07. Found: C, 45.95, H, 2.11, N, 13.11, S, 9.39. Cl, 20.98.

4-(2-Thienyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (18): Yield 65 %, m.p.: 174 °C, m.w.: 329, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3335 (NH_2), 1048 (C-S), 1570 (C=C), 1093 (C-S), 871 (C-Cl), ^{1}H NMR ($CDCl_3$): 7.37 (1H, s, C-5-H), 5.09 (2H, brs, C-2-NH_2), 7.46 (1H, d, *J* = 6 Hz, C-3"), 7.13 (2H, d, *J* = 7.0 Hz, C-4' and 4"-H), 7.75 (1H, d, *J* = 6 Hz, C-5"-H). Anal. calcd. for $C_{12}H_7N_3S_2Cl_2$: C, 43.91, H, 2.15, N, 12.80, S, 19.45, Cl, 21.58. Found: C, 43.42, H, 1.98, N, 12.35, S, 19.39. Cl, 21.43.

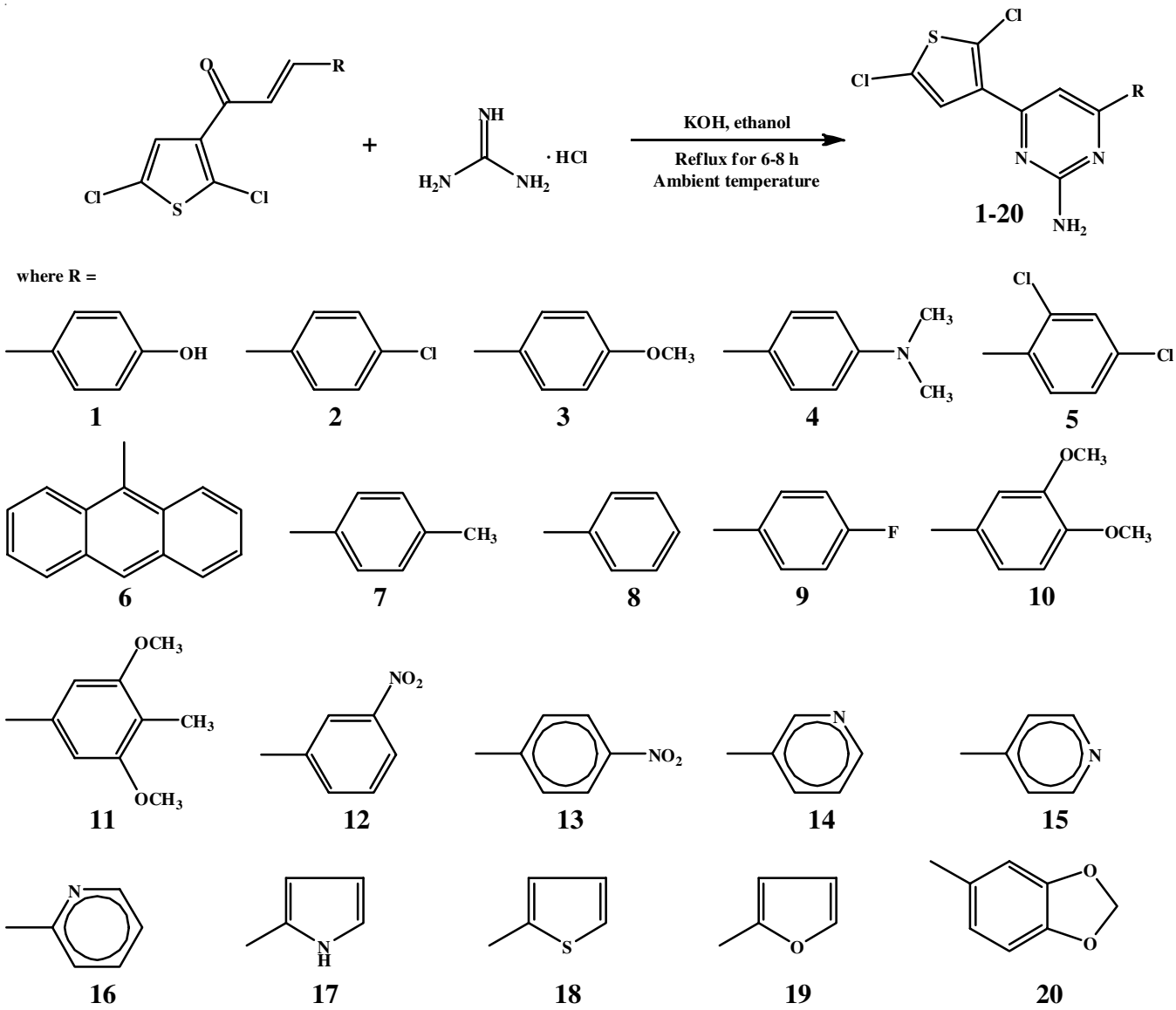
4-(2-Furyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (19): Yield 56 %, m.p.: 199 °C, m.w.: 313, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3329 (NH_2), 1125 (C-O), 1573 (C=C), 1095 (C-S), 878 (C-Cl), ^{1}H NMR ($CDCl_3$): 7.34 (1H, s, C-5-H), 5.12 (2H, brs, C-2-NH_2), 7.49 (1H, d, *J* = 6 Hz, C-3"), 7.17 (2H, d, *J* = 7.0 Hz, C-4' and 4"-H), 7.78 (1H, d, *J* = 6 Hz, C-5"-H). Anal. calcd. for $C_{12}H_7N_3OSCl_2$: C, 46.17, H, 2.26, N, 13.46, S, 10.22, Cl, 22.68, O, 5.11. Found: C, 45.95, H, 2.11, N, 13.12, S, 10.11. Cl, 22.43. O, 5.01.

4-(3,4-Methylenedioxyphenyl)-6-(2,5-dichlorothiophen-3-yl)pyrimidin-2-amine (20): Yield 72 %, m.p.: 194 °C, m.w.: 367, ATR-FTIR (KBr, ν_{max}, cm^{-1}): 3338 (NH_2), 1133 (C-O), 1576 (C=C), 1086 (C-S), 866 (C-Cl), ^{1}H NMR ($CDCl_3$): 7.41 (1H, s, C-5-H), 5.14 (2H, brs, C-2-NH_2), 5.72 (2H, s, -O-CH2-O-), 7.46 (1H, d, *J* = 6 Hz, C-2" and 6"), 7.13 (2H, d, *J* = 7.0 Hz, C-4' and 6"-H), 7.75 (1H, d, *J* = 6 Hz, C-5"-H). Anal. calcd. for $C_{15}H_9N_3O_2SCl_2$: C, 49.19, H, 2.48, N, 11.47, S, 8.72, O, 8.72, Cl, 19.34. Found: C, 49.01, H, 2.25, N, 11.21, S, 8.65. O, 8.59, Cl, 19.01.

Molecular docking studies: Molecular docking is an important indicator in structural molecular biology and computer-aided drug design. The primary objective of ligand-protein docking is to predict and interpret the predominant and most reliable binding mode(s) of a ligand with a known protein of its three-dimensional structure. It has been discussed in the literature about the background and theory of molecular docking software, which covers the usage of some of the most-cited docking software [17]. Molecular docking was utilized to perform a virtual screening of all pyrimidine derivatives to propose structural hypotheses of how various functional groups of ligands bind effectively and inhibit the target proteins of candida fungal species, *Mycobacterium tuberculosis* H37Rv (MTB) stain and human androgen receptor (ARccr) derived from an androgen-independent prostate cancer to correlate their results, which are essential for lead optimization.

RESULTS AND DISCUSSION

In our proposed investigation, it was extended to synthesize some novel pyrimidines from 2,5-dichloro-3-acetylthienyl chalcones, which has highly reactive dielectrophilic ketovinyl side chain to condense with guanidine hydrochloride in presence of KOH to produce twenty novel pyrimidine derivatives (**1-20**) at room temperature and refluxed for 6 h (**Scheme-I**). The spectral characterizations were carried out on the synthesized pyrimidine derivatives using suitable IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis data.



Scheme-I: Synthesis of pyrimidine derivatives from 2,5-dichloro-3-actyl thiopene chalcone reagents and conditions: (i) KOH, ethanol (ii) reflux for 6-8 h (iii) ambient temperature

All novel pyrimidine derivatives (**1-20**) were exhibited characteristic absorption bands in the IR spectra (cm^{-1}) in between 3400-3300 for amino-(-NH_2), 1650-1600 (C=N of pyrimidine), 1580-1560 (C=C) and at other regions of the spectrum depending upon the specific substituents present in each compound. ^1H NMR spectra of the aminopyrimidines was shown characteristic resonance signal for amino protons between δ 5.0-5.5 ppm. Peaks were clearly shown in the spectra accounting for the aromatic protons and for the different substituent protons in between the corresponding regions of the spectrum. ^{13}C NMR spectra of the compound **1** exhibited the characteristic peaks for the carbons of the pyrimidine ring 163.74 (C-2-NH_2), 163.66 (C-4), 164.31 (C-6), 95.03 (C-5), apart from the peaks corresponding to the other carbons. The mass spectra were obtained by positive mode ionization method revealed the $[M+H]^+$ ions, whereas the spectra were obtained by EI method, revealed the molecular ion. Similarly, spectral and chemical compositions for all other derivatives were confirmed and the results were within ± 0.4 % of the calculated values statistically. All compounds were identified for their structural requirements by spectral and elemental analysis.

Biological activity: They were screened for their antifungal, antitubercular and *in vitro* cytotoxicity activities. They were also correlated with ligand interaction and binding affinity by molecular docking studies based on the importance of various structure-activity relationship parameters especially on the ring stability, change in aromatic or heterocyclic nucleus, polarity, electronegativity of an element in heterocycles, inductive and steric effects accordingly on the type of individual substituted pyrimidine.

Antifungal activity: These pyrimidine derivatives (**1-20**) were screened for antifungal activity against two fungal species *Candida tropicalis* and *Aspergillus niger* by Tube-dilution method [18]. The results of antifungal activity of novel pyrimidines are represented in the Fig. 1.

Most of the compounds were exhibited poor activity against both fungal species. Some of the pyrimidines were inactive, whereas the others exhibited action only at higher MIC. How-

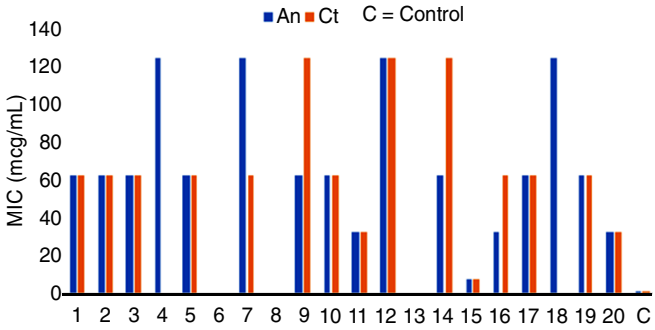


Fig. 1. Antifungal activity of new pyrimidine derivatives against *Aspergillus niger* (An) and *Candida tropicalis* (Ct)

ever, the compound **15** is shown potential antifungal activity (MIC= 8.0 µg/mL) for both species against fluconazole as control (MIC = 1.0 µg/mL). This was clearly indicated the importance of α,β-unsaturated carbonyl system of chalcones and aminopyrimidine with 4"-pyridinyl moiety for antifungal activity and as the activity is lessened with other substituted aromatic and heteroaromatic nuclei on the aminopyrimidine nucleus.

Molecular docking studies revealed that compound **15** has been exhibited with good binding affinity (-7.80 kcal/mol) compared to its fluconazole (-7.60 kcal/mol) when it is formed a complex with mitochondrial 2-enoyl thioester reductase Etr1p/Etr2p heterodimer (PDB: 1N9G) from *Candida tropicalis* species as shown in 3D structure in Fig. 2.

The ligand interaction and binding affinity of **15** was slightly higher than fluconazole among all compounds screened for docking. This clearly signifies that **15** has potential antifungal activity particularly candida fungal species.

Antitubercular activity: Antitubercular activity of all pyrimidines derivatives (**1-20**) was conducted against *M. tuberculosis* $H_{37}Rv$ strain by broth dilution assay [19-22]. Among all pyrimidines derivatives, the compound **5** containing 2",4"-dichlorophenyl moiety was shown 50 % activity (MIC = 6.2 µg/mL) compared to pyrazinamide (MIC = 3.12 µg/mL), whereas compounds **9** (MIC = 12.5 µg/mL) and **11** (MIC = 12.5 µg/mL) were exhibited 50 % activity compared to compound **5**. Some compounds have observed with relatively low activity (MIC values 25-100 µg/mL) whereas other pyrimidines (**1**, **6** and **17**) are inactive (Fig. 3).

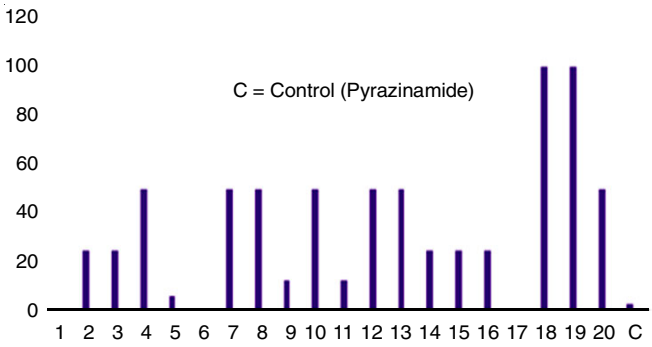


Fig. 3. Antitubercular activity of novel pyrimidine derivatives

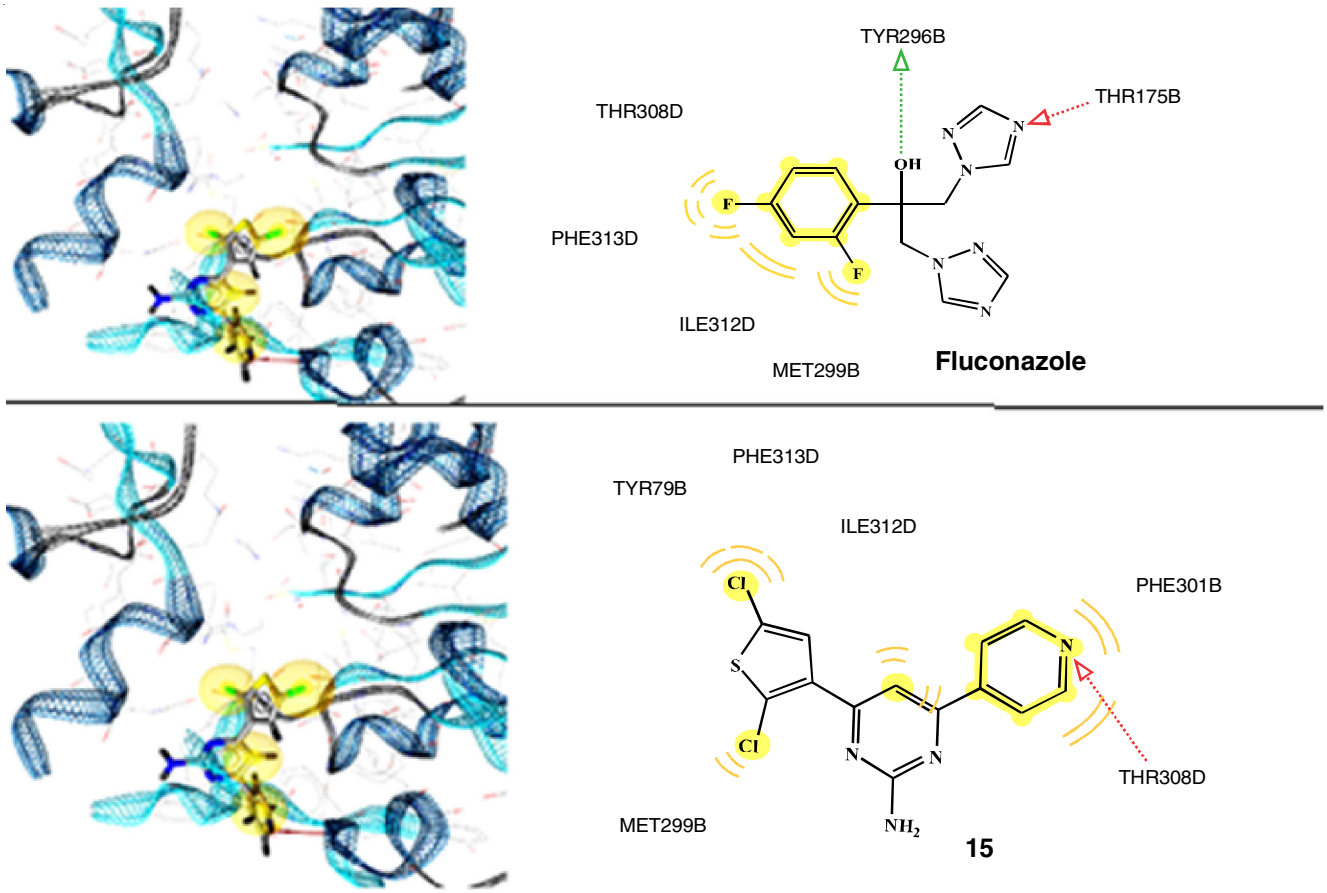


Fig. 2. Docking of compound **15** and fluconazole complex with mitochondrial 2-enoyl thioester reductase Etr1p/Etr2p heterodimer (PDB: 1N9G)

A structure-activity-relationship (SAR) study was indicated clearly on the cyclization of chalcone linkage to aminopyrimidine group decreased the antitubercular activity. Some compounds are less active due to polar aminopyrimidine group, which has less ability to penetrate into the lipophilic core barrier of the cell wall structure, function and biogenesis of *Mycobacterium tuberculosis*.

Docking was done with crystalline protein structure of Rv0371c (PDB ID: 2WEE) *Mycobacterium tuberculosis* (MTB) stain and all pyrimidine derivatives **1-20** were performed. Among them, **5**, **9** and **11** were exhibited much higher binding affinity (-6.90, -6.70, to-6.70 kcal/mol) compared to pyrazinamide (-4.10 kcal/mol) as a standard target molecule as shown in Fig. 4.

These correlations of MIC values and docking studies were indicated to consider nonpolar functional groups on aminopyrimidine nucleus of compound **5**. Structurally modified compound would be promising lead compound as potential antitubercular agent against both drug sensitive and drug resistant strains of *M. tuberculosis*. Further investigation is needed to ascertain their potency, safety and efficacy.

Anticancer activity: All pyrimidine derivatives **(1-20)** were evaluated for their *in vitro* cytotoxicity activity DU-145 human prostate cancer cell line [17] by MTT cell proliferation assay [23,24]. Methotrexate is one of the most effective anticancer agents used as reference drug. The half maximal inhibitory concentration (IC_{50}) was estimated and results are summarized in Table-1.

Data presented as mean ± SD (n = 3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1 % DMSO. The control cells were treated with culture medium containing 0.1 % DMSO.

These IC_{50} values referred to the drug concertation that produced a measured inhibitory response of a specific biological or biochemical function. The drug concentration is noted for total growth inhibition and killing of 50 % the cells. Among all compounds, IC_{50} values were categorized and grouped into an cytotoxicity activity criteria based on their IC_{50} values as shown in Table-2.

TABLE-1
in vitro ANTICANCER SCREENING OF NOVEL PYRIMIDINE DERIVATIVES AGAINST DU-145 HUMAN PROSTATE CANCER LINE

Compound	R	IC_{50} (µg/mL)
1	4"-Hydroxyphenyl	121 ± 2
2	4"-Chlorophenyl	46 ± 2
3	4"-Methoxyphenyl	112 ± 2
4	4"-Dimethylaminophenyl	42 ± 2
5	2",4"-Dichlorophenyl	40 ± 2
6	9"-Anthracenyl	89 ± 2
7	4"-Methylphenyl	80 ± 2
8	Phenyl	93 ± 1
9	4"-Fluorophenyl	46 ± 2
10	3",4"-Dimethoxyphenyl	90 ± 2
11	3",4",5"-Trimethoxyphenyl	60 ± 2
12	3"-Nitrophenyl	52 ± 2
13	4"-Nitrophenyl	49 ± 2
14	3"-Pyridinyl	18 ± 1
15	4"-Pyridinyl	2 ± 0.1
16	2"-Pyridinyl	12 ± 1
17	2"-Pyrrolyl	6 ± 0.2
18	2"-Thienyl	32 ± 2
19	2"-Furfuryl	41 ± 2
20	3",4"-Methylenedioxyphenyl	66 ± 2
	Methotrexate	5 ± 0.2

Data presented as mean ± SD (n = 3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1 % DMSO. The control cells were treated with culture medium containing 0.1 % DMSO.

TABLE-2
ACTIVITY CRITERIA FOR SCREENING COMPOUNDS BASED ON THEIR IC_{50} VALUES

Cytotoxicity activity criteria	IC_{50} values (µg/mL)
Low	> 90
Moderate	30-70
Potent	< 30
Highly potent	< 10

Compounds **1**, **3**, **6**, **7**, **8** and **10** were displayed less anticancer activity ($IC_{50} \geq 90 \pm 1$ µg/mL) and compounds **2**, **4**, **5**, **9**, **11**, **12**, **13**, **19** and **20** were exhibited moderate anticancer

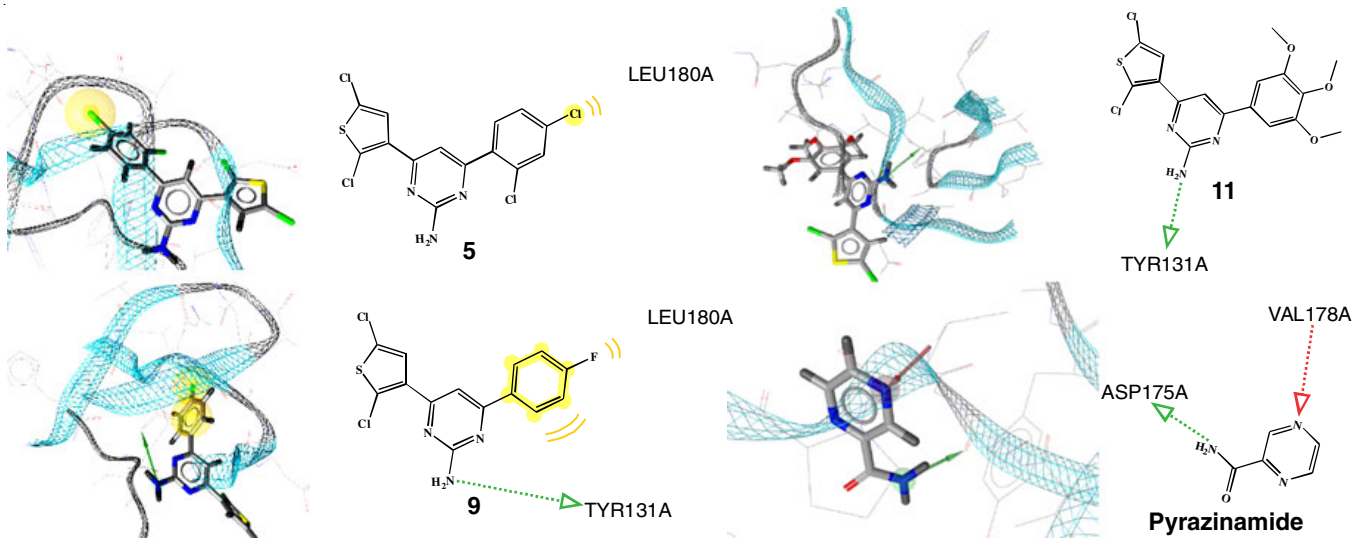


Fig. 4. Docking of compounds **5**, **9**, **11** complexes with a crystal structure of Rv0371c of *M. tuberculosis* $H_{37}Rv$ (MTB) stain (PDB ID: 2WEE)

activity (30 1 μg/mL ≤ IC_{50} ≤ 66 ± 2 μg/mL). Compounds **14** and **16** were demonstrated with potent antiprostate cancer activity (IC_{50} =18 ± 1 μg/mL and IC_{50} = 12± 2 μg/mL). Compounds **15** (IC_{50} = 2 ± 0.1 μg/mL) and compound **17** (IC_{50} = 6 ± 0.2 μg/mL) were shown highly potent antiprostate cancer activity as shown in Fig. 5.

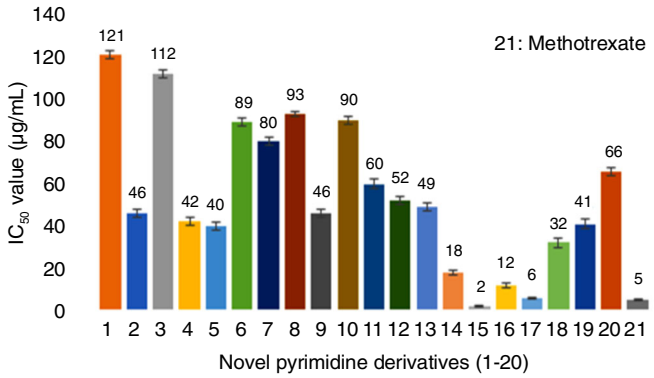
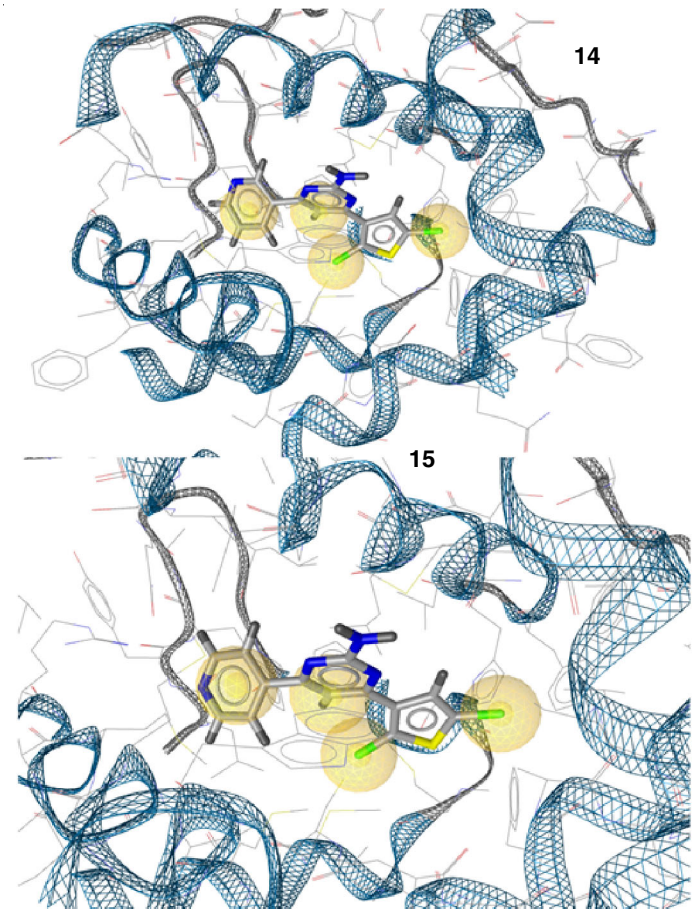


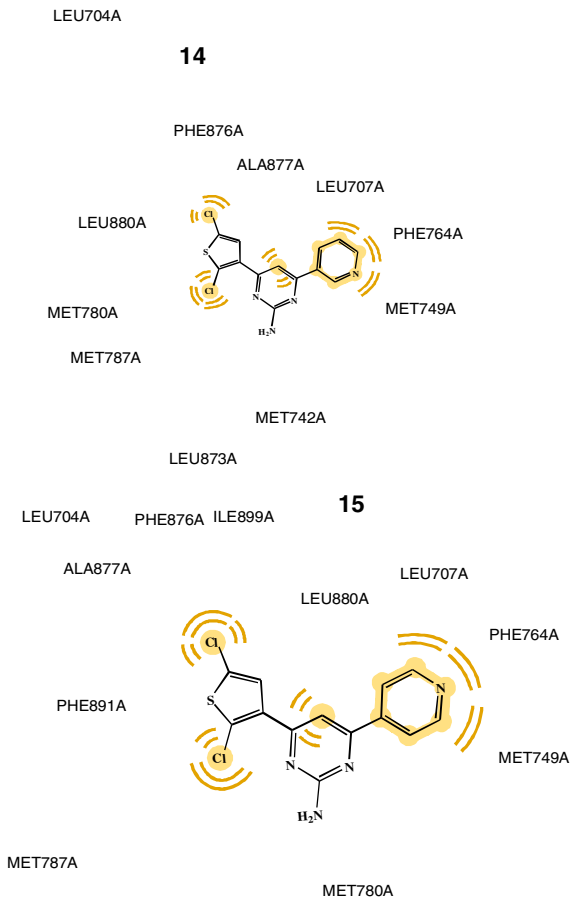
Fig. 5. *in vitro* Anticancer screening of novel pyrimidine derivatives against DU-145 human prostate cancer lines

Molecular docking studies were performed to predict the binding affinity and binding orientations of all pyrimidine derivative with crystalline protein structure of human androgen receptor (ARccr) derived from an androgen-independent prostate cancer (PDB ID: 1GS4). Ligands were prepared by default settings and applied to other parameters. Rigid docking was performed during these calculations.

These binding affinities were correlated with *in vitro* cytotoxic activity (IC_{50} values) against DU-145 prostate cancer cell lines. Compound **15** with 4"-pyridinyl and compound **17** with 2"-pyrrollyl groups at 4th position of 2-aminopyrimidine ring possess highly potent cytotoxicity (IC_{50} = 2.0 ± 0.1 μg/mL and 6.0 ± 0.2 μg/mL) also compared with methotrexate (IC_{50} = 5.0 ± 0.2 μg/mL) as a control. Compounds **14** with 3"-pyridinyl and compound **16** with 2"-pyridinyl groups on 4-position of 2-aminopyrimidine ring were exhibited potent cytotoxic activity (IC_{50} = 18 ± 2 μg/mL; 12 ± 1 μg/mL). The crystalline structure of human androgen receptor (ARccr) derived from an androgen-independent prostate cancer (PDB ID: 1GS4) protein was chosen to execute molecular docking studies of all pyrimidine derivatives using licensed Ligand scout 4.1 series software. Among them, compounds **14**, **15**, **16** and **17** were exhibited much better binding affinity (-7.70, -7.60, -8.00 and -8.20 kcal/mol) compared with methotrexate (-5.10 kcal/mol) as shown in Fig. 6.

Structure activity relationship (SAR) studies were interpreted by introducing heterocyclic ring with nitrogen at 4-position on aminopyrimidine ring with good cytotoxicity activity comparatively replaced with unsubstituted or substituted aromatic ring or heteroaromatic ring at the same position. This clearly signifies that pyrimidine with 4"-pyridinyl (compound **15**) or 2"-pyrrolyl (compound **17**) groups mimic purine base at cellular level and these similarities in structural features of nitrogen containing heterocyclic compounds. Their gene specific cytotoxicity suppresses certain types of cancer cells. Activity is enhanced at cellular level with similarity in structural features nitrogen containing heterocyclic compounds.





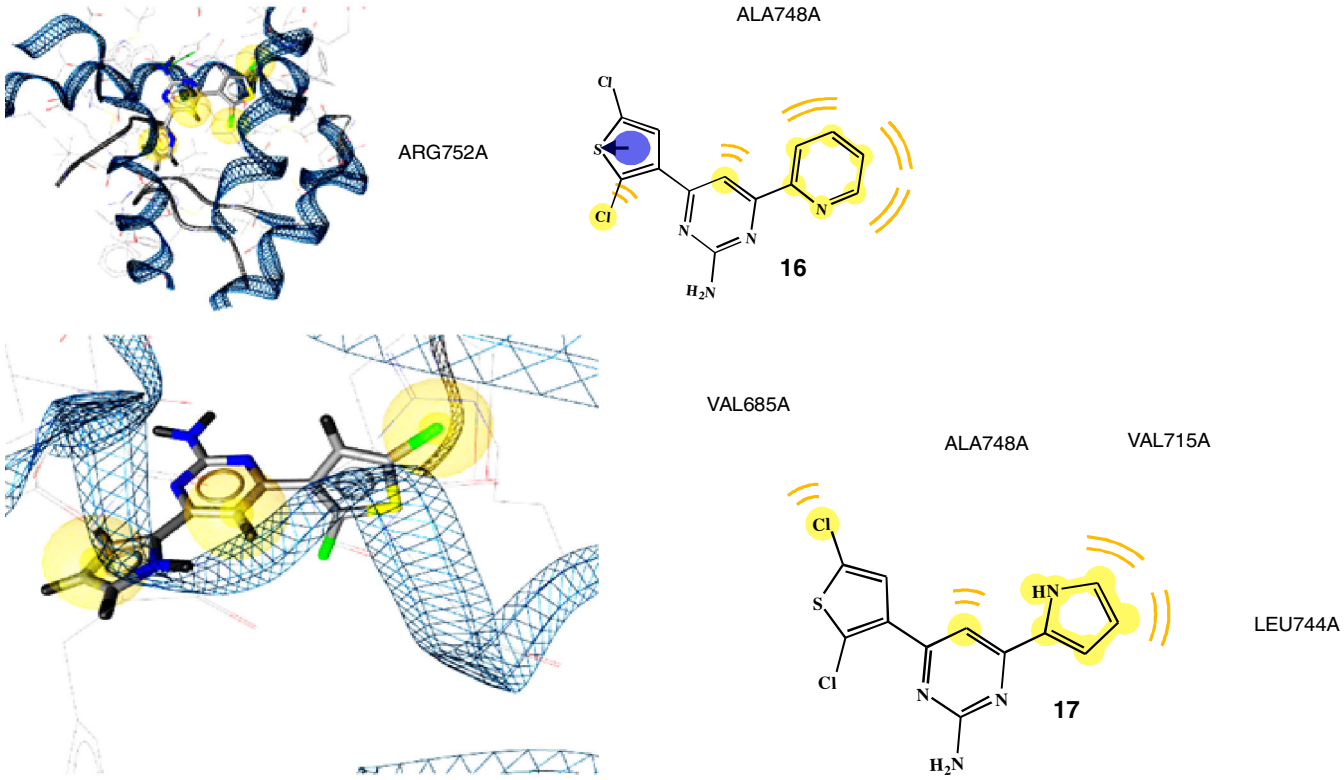


Fig. 6. Docking of compounds **14**, **15**, **16**, **17** complexes with a crystal structure of human androgen receptor (ARccr) derived from an androgen-independent prostate cancer (PDB ID: 1GS4)

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CONFLICT OF INTEREST

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

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