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Synthesis of Some Pyrimidine and Fused Pyrimidine Derivatives with Antimicrobial and Anticancer Activities

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ABSTRACT

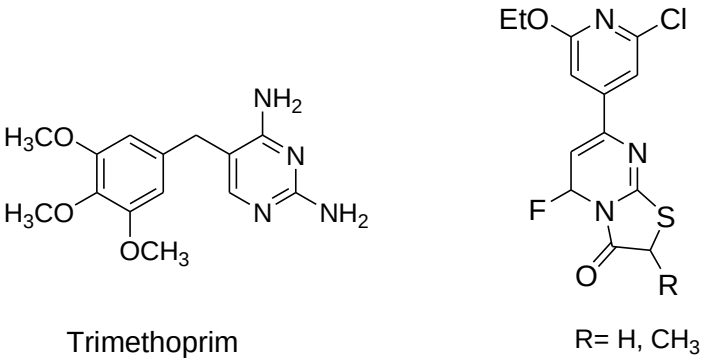
A new series of pyrimidines and fused pyrimidines derivatives including differently substituted benzylidenes, heterocycles such as pyrazole ring and fused ring systems such as triazolo [4,3-a] pyrimidine, pyrido [2,3-d] pyrimidine and pyramido [4,5-d] pyrimidine systems is reported. The synthetic routes were adopted via formation of substituted pyrimidine core structure in simple and efficient procedures. The chemical structures of new compounds were fully characterized by using complete spectral analyses data. The new compounds were evaluated for their anti-cancer and antimicrobial activities. Among the tested compounds, compounds 7i, 7h and 11c exhibited the best activity against different cancer cell lines. Compounds 8c, 11c and 14b showed potent activity as anti-microbial agents compared to standard drugs.

Keywords: Pyrimidine, Triazolo [4,3-a] pyrimidine, pyrido [2,3-*d*] pyrimidine and pyrimido [4,5-*d*] pyrimidine.

INTRODUCTION

Pyrimidine derivatives are an important class of nitrogen heterocycles that have attracted more attention in the last decades. Due to their utilities as a precursor for the construction of condensed heterocyclic systems, they represent an interesting pharmacophore for pharmaceutical products [1-6]. Pyrimidine derivatives have diversified activities such as antiviral [7], antitumor [8], antifolate [9], antibacterial [10], antifungal [11], CNS active [12], diuretic [13], uricosuric [14], diabetogenic, analgesic [15], anti-inflammatory [16], antioxidant [17], bronchodilator [13], antihistaminic and cardiac agents [18]. Currently, bacterial and fungal resistance is a major challenge problem in treatment of infectious diseases [19]. New strains of microbes have been reported to threaten millions of people around the world every year [20]. Several pyrimidine derivatives have long ago been identified as potent bactericidal and fungicidal agents, among which trimethoprim (Proloprim®) has been successfully used in the treatment of urinary tract infections (Figure 1) [21].

In addition, thiazolo [3,2-*a*] pyrimidin-3 (5*H*)-one derivatives, as a fused pyrimidine system, were reported to possess potent antibacterial activity against *S. typhi* and fair sensitivity towards *Escherichia coli* and *Staphylococcus aureus* (Figure 1) [22,23].



Trimethoprim

R= H, CH_3

Figure 1: Anti-microbial agents: trimethoprim and thiazolopyrimidine

Moreover, pyrimidine derivatives display usefulness in the fields of chemotherapy. Pyrimidine-based antimetabolites structurally related to the endogenous substrates as 5-Fluorouracil **(**5-FU**)**, 5-thiouracil and the uracil-based hydroxyamides were early recognized as effective therapies for cancer (Figure 2) [24-26]. It was reported that the chemotherapeutic efficacy of pyrimidine derivatives is related to their ability to inhibit vital enzymes responsible for DNA biosynthesis as dihydrofolate reductase (DHFR) [27], thymidylate synthetase (TSase), thymidine phosphorylase (TPase) and reverse transcriptase (RTase) [28]. Further, pyrimidine and fused pyrimidine derivatives show biologically important activities because of their structural resemblance to purine-pteridine systems [29]. Due to their severe toxicity and limited selectivity, the search for more potent and selective anticancer agents is highly interested.

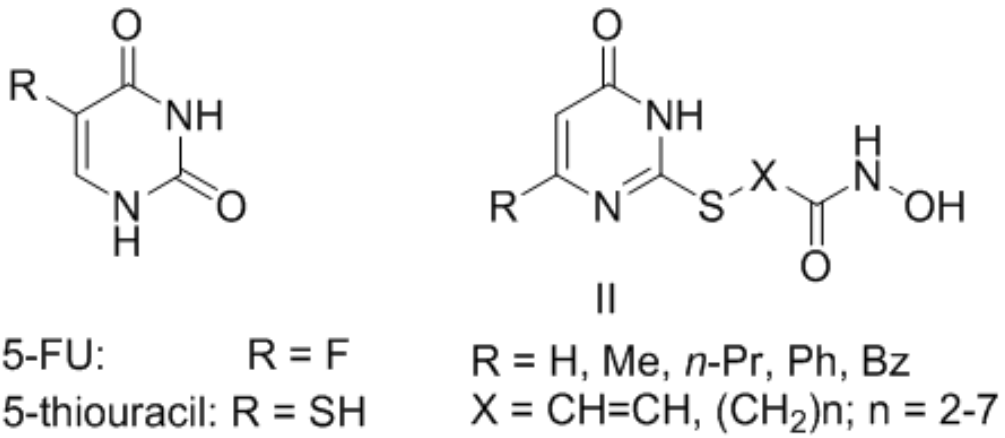


Figure 2: Anticancer agents

Structural variations of the prepared compounds would be employed to trace possible changes in activity caused by changing the nature of substituents at C^2 of the pyrimidine cornerstone. Substituents were chosen to cover diverse functionalities as hydrogen bond donor, acceptor, and rigidity. Our work was designed to encompass synthetic features to achieve the compounds of interest.

In this paper, we synthesized a new series of pyrimidines and fused pyrimidines derivatives *via* formation of fully substituted pyrimidine core structure through multi-component like reaction. Our objective was then directed to examine these compounds as antimicrobial and anticancer agents.

MATERIALS AND METHODS

Experimental

General: Melting points were determined in open-glass capillaries using an electro thermal melting point apparatus (Stuart Scientific, Model SMP1, UK) and were uncorrected. Infrared spectra (IR) were recorded, using KBr discs, υ (cm^{-1}), on a Nicollet Infrared Spectrophotometer IR 470 at the Faculty of science, Assiut University. Nuclear magnetic resonance spectra, ^{1}H-NMR wastaken using the following apparatus: Bruker Avance III apparatus 400 MHz; Central Laboratory Unit, Faculty of Pharmacy, Cairo and Bruker DRX 400 MHz, Central Laboratory Unit, Zagazig University, Egypt for ^{1}H and 100 MHz for ^{13}C-NMR. Chemical shifts are expressed as δ values (ppm) using tetramethylsilane (TMS) as internal reference. Signals are indicated by the following abbreviations: *s*=singlet, *d*=doublet, *t*=triplet, *q*=quartet, *m*=multiplet, and *br*=broad. Mass spectra (MS) were run on a gas chromatograph/mass spectrometer Shimadzu GCMS-Qp2010 plus, single quad (70 ev), Regional Centre for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo. Elemental analyses were run on Vario EL III German CHN Elemental analyser model, Regional Centre for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo (Figures S1-S16).

General procedure for the synthesis of compounds 7a-j and 8a-h: A solution of the 4-amino-2-hydrazinylpyrimidine-5-carbonitrile (2) (150 mg, 1 mmol) or 4-amino-2-hydrazinylpyrimidine-5-carboxamide (4) (168 mg, 1 mmol) in EG (2 ml) was treated with the equimolar amount of the appropriate benzaldehyde or acetophenone derivative in EG (2 ml) containing few drops gl. acetic acid. The reaction mixture was heated up at 120ºC for 30-60 min then left to cool to RT. The separated products were filtered, washed with petroleum ether, dried and crystallized from EtOH. Yields, melting points IR, ^{1}H-NMR (DMSO-d_6), ^{13}C-NMR (DMSO-d_6) and microanalysis of products 7a-j and 8a-h are depicted below:

4-Amino-2-(2-benzylidenehydrazinyl)pyrimidine-5-Carbonitrile (7a): Yield: (87%); M.p. 154-156ºC; IR (KBr): 3455-3343 (NH_2, NH), 3127 (CH, Ar.), 2214 (C≡N), 1637 (C=N), 1654, 1595 (C=C) cm^{-1}; ^{1}*H*-NMR (400 MHz, DMSO-d_6): 11.62 (s, 1H, NH, D_2O exchanged), 8.58 (s, 1H, C*H*), 8.35 (s, pyrimidine-C^6-*H*), 7.99 (NH_2, D_2O exchanged), 7.82-7.55 (m, 5Hs, Ar-Hs); ^{13}*C*-NMR (DMSO-*d*6): 163.82, 162.83, 160.50, 144.36, 135.24, 129.91, 129.25, 127.15, 117.41, 80.92. Anal. calc. for $C_{12}H_{10}N_6$ (238.25): C 60.50, H 4.23, N 35.27; found: C 60.40, H 4.22, N 35.27.

4-Amino-2-(2-(4-chlorobenzylidene)hydrazinyl) pyrimidine -5-carbonitrile (7b): Yield: (90%); M.p. 197-199ºC; IR (KBr): 3516-3397 (NH_2, NH), 2230 (C≡N), 1655 (C=N), 1597 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 11.39 (s, I*H*,N*H)*, 8.36 (s, pyrimidine-C^6-*H*), 8.15 (s, 1H, N=C*H*), 7.67-7.65 (d, 2H, Ar-Hs, *J*=8 Hz), 7.51 (br, NH_2, D_2O exchanged), 7.47 (d, 2*H*s, Ar-*H*s, *J*=8 Hz); ^{13}C-NMR (DMSO-d_6): 163.30, 162.34, 159.94, 142.44, 133.68, 128.83, 128.20, 116.84, 80.92. Anal. calc. for $C_{12}H_9ClN_6$ (272.69): C 52.85, H 3.33, N 30.82; found: C 52.85, H 3.31, N 30.70. **MS:** m/z (% relative abundance): m/z: 272.06 (33%), 274.05 (11%), 273.06 (6%), 43.01(100).

4-Amino-2-(2-(4-(dimethylamino) benzylidene) hydrazinyl) pyrimidine-5-carbonitrile (7c): Yield: (81%); M.p. 133-135ºC; IR (KBr): 3396-3155 (NH_2, NH), 2208 (C≡N), 1654 (C=N), 1585 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 11.02 (s, 1*H*, N*H*), 8.26 (s, pyrimidine-C^6-*H*), 8.00 (s, H, C*H*), 7.44 (d, 2Hs, Ar-Hs, *J*=8 Hz), 7.36 (brs, 2H, NH_2, D_2O exchanged), 6.69 (d, 4Hs, Ar-*H*s, *J*=8 Hz), 2.85 (d, 6Hs, 2CH_3); ^{13}C-NMR (DMSO-d_6): 163.51, 162.41, 160.90, 148.45, 137.94, 131.39, 128.40, 122.44, 117.08, 81.35, 13.97. Anal. calc. for $C_{14}H_{15}N_7$ (281.32): C 59.77, H 5.37, N 34.85; found: C 59.72, H 5.33, N 34.86.

4-Amino-2-(4-(4-nitrobenzylidene)hydrazinyl)pyrimidine-5-carbonitrile (7d): Yield: (92%); M.p. 167-169ºC; IR (KBr): 3469-3340 (NH_2, NH), 2222 (C≡N), 1651 (C=N), 1601 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 10.22 (s, IH, N*H*), 8.61 (s, 1H, N=C*H*), 8.35 (s, pyrimidine-C^6-H), 7.70 (d, 2Hs, 2Hs, Ar-Hs, J=8 Hz), 7.55 (d, 2Hs, 2Hs, Ar-Hs, J=8 Hz), 7.45 (h, 2H, D_2O exchanged NH_2); ^{13}C-NMR (DMSO-d_6): 162.51, 160.30, 145.57, 128.51, 122.61, 117.68, 80.91. Anal. calc. for $C_{12}H_9N_7O_2$ (283.25): C 50.88, H 3.20, N 34.62; found: C 50.77, H 3.19, N 34.73.

4-Amino-2-(4-(2-nitrobenzylidene)hydrazinyl)pyrimidine-5-carbonitrile (7e): Yield: (92%); M.p. 167-169ºC; IR (KBr): 3469-3340 (NH_2, NH), 2222 (C≡N), 1651 (C=N), 1601 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 11.63 (s, 1H, N*H*), 8.55 (s, 1H, N=C*H*), 8.37 (s, pyrimidine-C^6-H), 8.05 (d, 2Hs, 2Hs, Ar-Hs), 7.45 (s, 2H, D_2O exchanged NH_2), 7.58-7.56 (m, 3Hs, Ar-Hs); ^{13}C-NMR (DMSO-*d6*): 163.83, 162.97, 160.47, 148.22, 139.23, 134.03, 130.44, 129.71, 128.21, 125.20, 80.91. Anal. calc. for $C_{12}H_9N_7O_2$ (283.25): C 50.88, H 3.20, N 34.62; found: C 50.77, H 3.19, N 34.73.

4-Amino-2-(2-(4-methoxybenzylidene) hydrazinyl) pyrimidine-5-carbonitrile (7f)*:* Yield: (76%); M.p. 143-14ºC; IR (KBr): 3464-3297 (NH_2, NH), 2210 (C≡N), 1639 (C=N), 1589 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-*d*6): 11.34 (s, 1H, D_2O exchanged, NH), 8.32 (s, 1H, N=C*H*), 811 (s, pyrimidine-C^6-H), 7.59 (d, 2Ar-Hs, *J* = 8.4 Hz), 7.45 (s, 2Hs, D_2O exchanged, NH_2), 7.00 (d, 2Ar-Hs, *J* = 8.4 Hz), 3.79 (OCH_3). ^{13}C-NMR (DMSO-d_6): 163.75, 162.73, 162.65, 160.85, 160.44, 144.42, 128.70, 127.82, 114.76, 80.93, 55.77. Anal. calc. for $C_{13}H_{12}N_6O$ (268.27): C 58.20, H 4.51, N 31.33; found: C 58.00, H 4.53, N 31.20.

4-Amino-2-(2-benzylidenehydrazinyl)pyrimidine-5-carboxamide (7g): Yield: 77%; M.p. 178-180ºC; IR (KBr): 3461, 3422 ($2NH_2$), 3312 (NH), 1672 (C=O), 1643 (C=N), 1600 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6): 11.28 (s, 1H, D_2O exchanged, N*H*), 8.32 (s, 1H, N=CH), 8.14 (s, pyrimidine-C^6 H), 7.67 (brs, D_2O exchanged, NH_2), 7.61 (d, 2Hs, Ar-Hs, *J* = 8.4 Hz), 7.55 (s, D_2O exchanged, NH_2), 7.32-7.31 (m, 3Hs, Ar-Hs); ^{13}C-NMR (DMSO-d_6): 169.34, 163.91, 160.23, 158.29, 141.51, 134.42, 133.99, 129.13, 128.48, 100.91. anal.calc. for $C_{12}H_{12}N_6O$ (256.26): C 56.24, H 4.72, N 32.79; found: C 56.26, H 4.75, N 32.80.

4-Amino-2-(2-(4-chlorobenzylidene) hydrazinyl) pyrimidine-5-carboxamide (7h): Yield: 83%; M.p. 213-215ºC; IR (KBr): 3488 (NH_2), 3461 (NH_2), 3427 (NH), 1669 (C=O), 1600 (C=N), 1605 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 11.17 (s, 1H, D_2O exchanged, NH), 8.29 (s, 1H, N=C*H*), 8.11 (s, pyrimidine-C^6 *H*), 7.84 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz), 7.75 (s, 4Hs, D_2O exchanged, $2NH_2$), 6.95 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz). Anal. calc. for $C_{12}H_{11}ClN_6O$ (290.71): C 49.58, H 3.81, N 28.91; found: C 49.60, H 3.82, N 28.93. MS: m/z (% relative abundance): m/z: 290.34 (36%), 292.09 (12%), 41.03(100).

4-Amino-2-(2-(4-methoxybenzylidene) hydrazinyl) pyrimidine-5-carboxamide (7i): Yield: 74%; M.p. 156-158ºC; IR (KBr): 3522 (NH_2), 3417 (NH_2), 3331 (NH), 1644 (C=O), 1601 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 10.93(s, 1H, D_2O exchanged, N*H*), 8.51 (s, 1H, N=C*H*), 8.04 (s, pyrimidine-C^6 *H*), 7.77 (s, 2Hs, D_2O exchanged, NH_2), 7.55 (d, 2Hs, Ar-Hs, *J* = 8.4 Hz), 7.10 (s, 2Hs, D_2O exchanged, NH_2), 6.92 (d, 2Hs, Ar-Hs, *J* = 8.4 Hz), 3.73 (s, 3Hs, OCH_3); ^{13}C-NMR (DMSO-d_6): 169.48, 164.01, 160.58, 158.28, 143.00, 142.76, 128.61, 128.41, 128.12, 114.93, 114.45, 100.47, 55.85. Anal. calc. for $C_{13}H_{14}N_6O_2$ (286.26): C 54.54, H 4.93, N 29.35; found: C 54.59, H 4.98, N 29.42.

4-Amino-2-(2-(1-phenylethylidene)hydrazinyl)pyrimidine-5-carbonitrile (8a): Yield: (78%); M.p. 187-189^{o}C; IR (KBr): 3400-3278 (NH_2, NH), 2218 (C≡N), 1647 (C=N), 1588 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 10.20 (s, 1H D_2O exchanged, NH), 8.35 (s, pyrimidine-C^6-H), 7.76 (d, 2Hs, 2Ar-Hs, *J* = 8.1 Hz), 7.47 (D_2O exchanged NH_2), 7.35-7.34 (m, 3Hs, 3Ar-Hs), 2.28 (s, 1H, CH_3); ^{13}C-NMR (DMSO-d_6): 163.62, 162.80, 161.21, 150.21, 139.78, 136.85, 129.56, 129.08, 126.70, 126.62, 117.51, 81.37, 14.31. Anal. calc. for $C_{13}H_{12}N_6$ (252.27): C 61.89, H 4.79, N 33.31; found: C 61.89, H 4.75, N 33.28.

4-Amino-2-(2-(1-p-tolylethylidene)hydrazinyl)pyrimidine-5-carbonitrile (8b): Yield: 71%; M.p. 170-172ºC; IR (KBr): 3422-3281 (NH_2, NH), 2213 (C≡N), 1639 (C=N), 1590 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 10.13 (s, 1H, D_2O exchanged, NH), 8.33 (s, pyrimidine-C^6 H), 7.64 (d, 2Ar-Hs, *J*=8 Hz), 7.45 (br, 2Hs, D_2Oe xchanged, NH_2), 7.17 (d, 2Ar-Hs, *J* =8 Hz), 2.28 (s, 3Hs, CH_3), 2.25 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 163.82, 162.44, 161.21, 150.21, 138.88, 136.25, 129.56, 129.08, 126.70, 126.62, 117.51, 80.97, 21.36, 14.42. Anal. calc. for $C_{14}H_{14}N_6$ (266.30): C 63.14, H 5.30, N 31.56; found: C 63.12, H 5.28, N 31.50.

4-Amino-2-(2-(1-(4-bromophenyl)ethylidene)hydrazinyl)pyrimidine-5-carbonitrile (8c): Yield: 46%; M.p. 208-210ºC; IR (KBr): 3451-3397 (NH_2, NH), 2211 (C≡N), 1636 (C=N), 1587 C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 10.13 (s, 1H, D_2O exchanged, NH), 8.33 (s, pyrimidine-C^6 H), 7.65 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz), 7.44 (s, 2Hs, D_2O exchanged, NH_2), 7.16 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz), 2.24 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 163.81, 162.79, 162.44, 161.20, 150.21, 138.88, 136.25, 129.56, 129.70, 126.70, 117.51, 81.25, 14.42. Anal. calc. for $C_{13}H_{11}BrN_6$ (331.17): C 47.15, H 3.35, N 25.38; found: C 47.12, H 3.36, N 25.42; **MS:** m/z (% relative abundance): 329.95 (38%), 330.96 (42%), 331.93 (28%), 314.88(100).

4-Amino-2-(2-(1-(4-methoxyphenyl) ethylidene) hydrazinyl) pyrimidine-5-carbonitrile (8d): Yield: (79%); M.p. 159-161ºC; IR (KBr): 3421-3280 (NH_2, NH), 2213 (C≡N), 1640 (C=N), 1590 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 10.09 (s, 1H, D_2O exchanged NH), 8.32 (s, pyrimidine-C^6-H), 7.70 (d, 2H Ar-Hs, *J* = 8 Hz), 7.42 (brs, D_2O exchanged, NH_2), 6.91 (*d*, 2H, Ar-Hs, *J* = 8 Hz), 3.78 (s, 3Hs, OCH_3), 2.24 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 163.84, 162.74, 161.18, 160.44, 150.29, 131.46, 128.53, 128.18, 127.84, 113.82, 81.07, 55.82, 14.40. Anal. calc. for $C_{16}H_{16}N_4O$ (280.32): C 68.55, H 5.75, N 19.99; found: C 59.50, H 4.98, N 29.75.

4-Amino-2-(2-(1-phenylethylidene)hydrazinyl)pyrimidine-5-carboxamide (8e): Yield: 67%. M.p 123-125ºC; IR (KBr): 3429 (NH2), 3381 (NH), 1673 (C=O), 1651 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 9.87 (s, 1H, D_2O exchanged, N*H*), 8.54 (s, pyrimidine-C^6 *H*), 7.76 (d, 2Hs, 2Ar-Hs), 7.16-7.13 (m, 4Hs, D_2O exchanged, $2NH_2$), 7.34-7.31(m, 3Hs, Ar-Hs), 2.26 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 169.46, 164.03, 161.16, 158.43, 158.12, 147.98, 139.28, 129.23, 128.89, 128.52, 126.55, 100.84, 14.17. Anal. calc. for $C_{13}H_{14}N_6O$ (270.29): C 57.77, H 5.22, N 3109; found: C 57.83, H 5.20, N 31.14.

4-Amino-2-(2-(1-p-tolylethylidene)hydrazinyl)pyrimidine-5-carboxamide (8f): Yield: (77%); M.p. 171-173ºC; IR (KBr): 3443 (NH_2), 3324 (NH), 1654 (C=O), 1615 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 9.81 (s, 1H D_2O exchanged, N*H*), 8.59 (s, pyrimidine-C^6-*H*), 7.77 (brs, 2Hs, D_2O exchanged, NH_2), 7.66 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz), 7.39 (s, 2Hs, D_2O exchanged, NH_2), 7.16-7.14 (d, 2Hs, 2Ar-Hs, J = 8 Hz), 2.28 (s, 1H, CH_3), 2.23 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 169.43, 164.00, 161.09, 158.29, 157.99, 148.10, 138.51, 136.49, 129.54, 126.45, 126.15, 100.72, 21.34, 14.10. Anal. calc. for $C_{14}H_{16}N_6O$ (284.32): C 59.14, H 5.67, N 29.56; found: C 59.20, H 5.70, N 29.60.

4-Amino-2-(2-(1-(4-bromophenyl)ethylidene)hydrazinyl) pyrimidine-5-carboxamide (8g): Yield: (63%); M.p. 201-203ºC. IR (KBr): 3487, 3476 ($2NH_2$), 3336 (NH), 1668 (C=O), 1606 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 9.90 (s, 1H, D_2O exchanged, N*H*), 8.53 (s, pyrimidine-C^6-*H*), 8.06 (s, 2Hs, D_2O exchanged, NH_2), 7.70 (s, 2Hs, 2Ar-Hs), 7.50 (s, 3Hs, 3Ar-Hs), 7.11 (s, 2Hs, D_2O exchanged, NH_2), 2.23 (s, 1H, CH_3); ^{13}C-

NMR (DMSO-d_6): 169.41, 163.98, 161.05, 158.26, 146.69, 138.42, 131.61, 128.50, 122.36, 101.04, 13.90. Anal. calc. for $C_{13}H_{13}BrN_6O$ (349.19): C 44.72, H 3.75, N 24.07; found: C 44.75, H 3.80, N 24.20.

4-Amino-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)pyrimidine-5-carboxamide (8h): Yield: (59%); M.p. 194-196ºC; IR (KBr): 3457, 3398 ($2NH_2$), 3376 (NH), 1682 (C=O), 1615 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 9.74 (s, 1H, D_2O exchanged, N*H*), 8.51 (s, pyrimidine-C^6-*H*), 7.98 (s, 2Hs, D_2O exchanged, NH_2), 7.71 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz), 7.08 (s, 2Hs, D_2O exchanged, NH_2), 7.91-7.89 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz), 3.74 (OCH_3), 2.22 (s, 1H, CH_3); ^{13}C-NMR (DMSO-d_6): 169.47, 164.02, 161.18, 160.20, 158.40, 158.32, 158.09, 148.08, 131.78, 127.99, 120.97, 100.58, 55.82, 14.10. Anal. calc. for $C_{14}H_{16}N_6O$ (300.13): C 55.99, H 5.37, N 27.98; found: C 55.95, H 4.40, N 27.90.

Synthesis of 4-amino-2-(3,5-dimethyl-1H-pyrazol-1-yl)pyrimidine-derivatives (9a,b): A mixture of 4-amino-2-hydrazinylpyrimidine-5-carbonitrile (2) (150 mg, 1 mmol) or 4-amino-2-ydrazinylpyrimidine-5-carboxamide (4) (168 mg, 1 mmol) and acetyl acetone (100 mg, 1 mmol) in EG (5 ml) was heated for 3 h at 120ºC. The solution was diluted with ice-cold H_2O (5 ml) and refrigerated overnight. The obtained precipitate was filtered, dried and crystallized from EtOH.

4-Amino-2-(3,5-dimethyl-1H-pyrazol-1-yl)pyrimidine-5-carbonitrile (9a): Yield: 63%; M.p. 112-114ºC; IR (KBr): 3477 (NH_2), 2926 (aliph, C-H), 2220 (C≡N), 1632 (C=N), 1585 C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 8.64 (s, pyrimidine-C^6-H), 8.30-7.91 (s, 2H, D_2O exchanged, NH_2), 6.12 (s, 1H, pyrazol H), 2.54 (s, 3Hs, CH_3), 2.16 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 163.88, 163.14, 158.01, 150.68, 143.36, 115.97, 110.86, 86.89, 15.68, 13.94. Anal. calc. for $C_{10}H_{10}N_6$ (214.23): C 56.07, H 4.71, N 39.23; found: C 56.10, H 4.73, N 39.25.

4-Amino-2-(3,5-dimethyl-1H-pyrazol-1-yl)pyrimidine-5-carboxamide (9b): Yield: (65%); M.p. 177-179ºC; IR (KBr): 3543-3417 ($2NH_2$), 1674 (C=O), 1601 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 8.61 (s, pyrimidine-C^6-H), 8.32-8.87 (4Hs, D_2O exchanged, $2NH_2$), 6.08 (s, 1H, pyrazol-H), 2.56 (s, 3Hs, CH_3), 2.17 (s, 3Hs, CH_3). Anal. calc. for $C_{10}H_{12}N_6O$ (332.24): C 51.72, H 5.21, N 36.19; found: C 51.76, H 5.28, N 36.30. MS: m/z (% relative abundance): m/z: 232.03 (100%), 233.11 (16%), 234.09 (2%).

General Procedure for the Synthesis of Compounds 10a,b, 11a-c, 12a-c: A mixture of 4-amino-2-hydrazinylpyrimidine-5-carbonitrile 2 (150 mg, 1 mmol) with equimolar amount of 2-(ethoxymethylene)malono-nitrile, 2-(benzylidene)malono-nitrile, 2-(4-ethoxy-benzylidene) malono-nitrile, 2-(4-(dimethyl-amino) benzylidene) malono-nitrile, Ethyl (2-cyano)-3-phenyl-acrylate or Ethyl 3-(4-chlorophenyl)-2-cyano acrylate in EG (3 ml) containing 3 drops of TEA, heated at 120ºC for 2 h. the reaction monitored by TLC till completed. The solution was diluted with ice-cold H_2O (5 ml) and refrigerated overnight. The obtained precipitate was filtered, dried and crystallized from EtOH afforded 10a,b, 11a-c and 12a-b respectively. Following the same procedure; a mixture of 4-amino-2-hydrazinylpyrimidine-5-carboxamide (168 mg, 1 mmol) with Ethyl 3-(4-chlorophenyl)-2-cyano acrylate (337 mg, 1 mmol) afforded 12c. Yields, melting points and microanalysis of products 10a,b, 11a-c and 12a-c are listed.

4-Amino-2-(5-amino-4-cyano-1H-pyrazol-1-yl)pyrimidine-5-carbonitrile (10a): Yield: (49%); M.p. 212-214ºC; IR (KBr): 3417 (NH_2), 2221 (C≡N), 2216 (C≡N), 1614 (C=C), 1601 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 8.67 (s, 2Hs, D_2O exchanged, NH_2), 8.22 (s, 2Hs, D_2O exchanged, NH_2), 8.09 (s, pyrimidine-C^6-*H*), 7.84 (s, 1H, pyrazole H); ^{13}C-NMR (DMSO-d_6): 163.23, 157.82, 155.00, 143.71, 115.52, 114.83, 87.12, 73.09. Anal. calc. for $C_9H_6N_8$ (226.20): C 47.79, H 2.67, N 49.54; found: C 47.73, H 2.71, N 49.62.

4-Amino-2-(5-amino-4-cyano-1H-pyrazol-1-yl) pyrimidine-5-carboxamide (10b): Yield: 43%; M.p. 143-145ºC; IR (KBr): 3417-3310 ($3NH_2$), 2213 (C≡N), 1644 (C=O), 1601 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 8.38 (s, pyrimidine-C^6 *H*), 8.33-8.18 (m, 4Hs, D_2O exchanged, $2NH_2$), 7.78 (s, 1H, pyrazole-H), 7.49 (s, 2Hs, D_2O exchanged, NH_2). Anal. Calc. for $C_9H_7N_9O$ (257.21): C 42.03, H 2.74, N 49.01; found: C 42.10, H 2.76, N 49.15.

4-Amino-2-(5-amino-4-cyano-3-phenyl-1H-pyrazol-1-yl)pyrimidine-5-carbonitrile (11a)*:* Yield: (67%); M.p. 185-187ºC; IR (KBr): 3562 (NH_2), 3407 (NH_2), 2234 (C≡N), 2212 (C≡N), 1643 (C=N), 1518 C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 8.33 (s, pyrimidine-C^6-H), 7.96 (s, 2Hs, D_2O exchanged, NH_2), 7.82-7.36 (m, 7Hs, $2D_2O$ exchanged NH_2 + Ar-Hs); ^{13}C-NMR (DMSO-d_6): 163.85, 162.82, 162.47, 161.23, 150.10, 139.07, 129.53, 129.13, 128.90, 128.54, 126.72, 117.27, 114.50, 81.38, 73.67. Anal. calc. for $C_{15}H_{10}N_8$ (302.29): C 59.60, H 3.33, N 37.07.

4-Amino-2-(5-amino-4-cyano-3-(4-methoxyphenyl)-1H-pyrazol-1-yl)pyrimidine-5-carbonitrile (11b): Yield: 64%; M.p. 234-236ºC; IR (KBr): 3454 (NH_2), 3293 (NH_2), 2213 (C≡N), 2212 (C≡N), 1638 (C=N), 1596 C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 8.08 (s, pyrimidine-C^6-H), 7.88 (s, 2Hs, D_2O exchanged, NH_2), 7.56, 7.54 (d, 2Ar-Hs, *J* = 8 Hz), 7.41 (h, 2Hs, D_2O exchanged, NH_2), 6.81, 6.79 (d, 2Ar-Hs, *J* = 8 Hz), 3.91 (s, 3Hs, OCH_3); ^{13}C-NMR (DMSO-d_6): 162.45, 160.62, 160.20, 144.22, 128.47, 127.56, 117.27, 114.50, 80.92, 72.08, 55.52. Anal. Calc. for $C_{16}H_{12}N_8O$ (332.32): C 57.83, H 3.64, N 33.72; found: C 57.80, H 3.62, N 33.75.

4-Amino-2-(5-amino-4-cyano-3-(4-(dimethylamino)phenyl)-1H-pyrazol-1-yl)pyrimidine-5-carbonitrile (11c): Yield: 47%. M.p. 267-269ºC; IR (KBr): 3566 (NH_2), 3338 (NH_2), 2217 (C≡N), 2215 (C≡N), 1644 (C=N), 1596 C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 8.14 (s, pyrimidine-C^6-H), 7.83 (s, 2Hs, D_2O exchanged, NH_2), 7.61 (d, 2Hs, 2Ar-Hs, *J* = 8 Hz), 7.48 (s, 2Hs, D_2O exchanged, NH_2), 7.38-7.31(m, 3Hs, 3Ar-Hs), 2.28 (s, 3Hs, CH_3). 2.22 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 163.85, 162.99, 162.54, 160.43, 143.09, 134.26, 134.17, 129.18, 128.99, 128.76, 117.25, 113.82, 80.92, 73.36, 2 1.11, 21.08. Anal. calc. for $C_{17}H_{15}N_9$ (345.36): C 59.12, H 4.38, N 36.50; found: C 59.13, H 4.40, N 36.48. MS: m/z (% relative abundance): m/z: 345.07 (21%), 346.12 (7%), 146.04 (100).

4-Amino-2-(4-cyano-5-hydroxy-3-phenyl-1H-pyrazol-1-yl)pyrimidine-5-carbonitrile (12a): Yield: 55%; M.p. 188-190ºC; IR (KBr): 3454 (OH), 3295 (NH_2), 2216, 2213 (C≡N, C≡N), 1639 (C=N), 1596 C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 11.36 (s, 1H, D_2O exchanged OH), 8.32 (s, pyrimidine-C^6 H), 8.11-7.41 (m, 7Hs, D_2O exchanged, NH_2 and Ar-Hs). Anal. calc. for $C_{15}H_9N_7O$ (303.28): C 59.40, 2.99, 32.33; found: C 59.44, H 2.98, N 32.30.

4-Amino-2-(3-(4-chlorophenyl)-4-cyano-5-hydroxy-1H-pyrazol-1-yl)pyrimidine-5-carbonitrile (12b): Yield: 62%; M.p. 289-291ºC; IR (KBr): 3516 (OH), 3343 (NH2), 2230, 2225 (C≡N), (C≡N), 1654 (C=N), 1598(C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 11.15 (s, 1H, D_2O exchanged OH), 8.08 (s, pyrimidine-C^6 H), 7.56, 7.54 (d, 2Ar-Hs, *J* = 8 Hz), 6.41 (s, 2Hs, $2D_2O$ exchanged, NH_2), 6.93 (d, 2Ar-Hs, *J* = 8 Hz); ^{13}C-NMR (DMSO-6): 163.83, 162.82, 160.44, 142.99, 134.27, 134.16, 129.31, 128.71, 117.34, 112.33, 80.97, 72.10. Anal. calc. for $C_{15}H_8ClN_7O$ (337.72): C 53.35, H 2.39, N 29.03 found; C 53.33 H 2.41, N 29.12.

4-Amino-2-(3-(4-chlorophenyl)-4-cyano-5-hydroxy-1H-pyrazol-1-yl)pyrimidine-5-carboxamide (12c): Yield: (85%); M.p.193-195ºC; IR (KBr): IR 3516 (OH), 3417 (NH_2), 1614 (C=O), 1601 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 11.30 (s, 1H, D_2O exchanged, OH), 8.14 (s, pyrimidine-C^6-*H*), 7.79 (s, 2Hs, D_2O exchanged, NH_2), 7.60 (d, 2Ar-Hs, *J* = 8 Hz), 7.40 (s, 2Hs, D_2O exchanged, NH_2), 7.38-7.31 (m, 3Hs, 3Ar-Hs); ^{13}C-NMR (DMSO-d_6): 169.34, 163.83, 162.82, 160.44, 142.99, 134.27, 134.16, 129.31, 128.71, 117.34, 100.91, 80.96, 73.16. Anal. calc.

for $C_{15}H_{10}ClN_7O_2$ (355.74): C 50.64, H 2.83, N 27.56; found: C 50.70, H 2.82, N 27.49.

Synthesis of 7-(Methylthio)pyrimido[4,5-d]pyrimidin-4(3H)-one (**13a**): A mixture of the hydrazine 4 (1 mmol, 184 mg) and triethyl orthoformate (1 mmol, 138 mg) was heated under reflux in gl. AcOH (2 ml). The reaction was monitored by TLC ($CHCl_3$/MeOH 10: 1). After the reaction was complete (ca. 4 h), the mixture was cooled and poured into ice-cold H_2O. The resulting precipitate was filtered off, dried, and recrystallized from dioxane.

7-(Methylthio)pyrimido[4,5-d]pyrimidin-4(3H)-one (13a): $C_7H_6N_4OS$ (M. w. 194.03) Yield: (87%); M.p. 204-206ºC; IR (KBr): 3374 (NH), 3153 (CH, Ar-Hs), 1664 (C=O) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): 11.72 (s, 1*H*, N*H*, D_2O exchanged), 8.65 (s, 1*H*, C*H*), 8.84 (s, pyrimidine-C^6-*H*), 2.63 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 177.68, 169.56, 162.78, 157.70, 114.04, 96.45, 24.01.

Synthesis of 2-methyl-7-(methylthio)pyrimido[4,5-d]pyrimidin-4(3H)-one (**13b**): 4-Amino-2-(methylthio)pyrimidine-5-carbonitrile (1) (1 mmol, 166 mg) was refluxed in Ac_2O (2 ml) for 4 h. The reaction was monitored by TLC ($CHCl_3$/MeOH 10: 1). After the reaction was complete, the mixture was cooled and poured into ice-cold H_2O. The resulting precipitate was filtered off, dried, and recrystallized from EtOH.

2-Methyl-7-(methylthio)pyrimido[4,5-d]pyrimidin-4(3H)-one (13b): $C_8H_8N_4OS$ (M. w 208.04) Yield: (95%); M.p. 284-286ºC; IR(KBr): 3374 (NH), 3153 (CH, Ar-Hs), 1663 (C=O) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): 11.21 (s, I*H*, N*H*, D_2O exchanged), 8.93 (s, pyrimidine-C^6-*H*), 2.56 (s, 3Hs, S-CH_3), 2.18 (s, 3Hs, CH_3); ^{13}C-NMR (DMSO-d_6): 176.72, 169.19, 162.17, 157.06, 112.01, 95.81, 23.35, 14.22.

5-Amino-2-ethoxy-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (**13c**): In dry flask, sodium metal (25 mg, 1.1 mmol) was dissolved in 5 ml absolute EtOH, (113 mg, 1 mmol) of ethyl cyanoacetate and (1 mmol, 166 mg) of 4-amino-2-(methylthio) pyrimidine-5-carbonitrile (1) was refluxed. The reaction was monitored by TLC (Hex/EA 3: 1). After the reaction was completed (ca. 5 h), the mixture was cooled, neutralized with AcOH and poured into ice-cold H_2O. The resulting precipitate was filtered off, dried, and recrystallized from EtOH.

5-Amino-2-ethoxy-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (13c): Yield: (45%); M.p 231-233ºC; IR (KBr): 3374 (NH), 3213 (NH_2), 3153 (CH, Ar-Hs), 2219 (C≡N), 1676 (C=O) cm^{-1}; ^{1}*H*-NMR (400 MHz, DMSO-d_6): 8.96 (s, pyrimidine-C^6-*H*), 8.57 (s, 1*H*, N*H*, D_2O exchanged), 7.75 (s, 2*H*, NH_2, D_2O exchanged), 4.21 (q, 2Hs, CH_2), 1.27 (t, 3Hs, *J* = 8 Hz, CH_3).

Synthesis of 7-chloro-5-(4-chlorophenyl)-3-aryl-[1,2,4]triazolo[4,3-]pyrimidine-6-carbonitrile (14a,b): A mixture of 4-(4-chlorophenyl)-2-hydrazinyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6**)** (389 mg, 1.49 mmol), $POCl_3$ (0.4 ml, 4 mmol), *N*,*N*-dimethylaniline (0.2 ml, 1.58 mmol) and equimolar amount of benzoic acid derivatives was heated at 60°C for 2 h. After completion of the reaction as indicated by TLC, the mixture was left to cool to RT, poured onto ice-cold H_2O and the formed precipitate was filtered, dried and crystallized from EtOH. Yields, melting points and microanalysis of products are listed.

7-Chloro-5-(4-chlorophenyl)-3-phenyl-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (14a): Yield: 1.98 g (95%); M.p. 284-286ºC; IR (KBr): 2076 (CH, Ar), 2223 (C≡N), 1649 (C=N), 1605 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 7.58-7.33 (m, 9Hs, Ar. Hs). Anal. calc. for $C_{18}H_9Cl_2N_5$ (366.20): C 59.04, H 2.48, N 19.12; found: C 59.10, H 2.54, N 19.18. MS: m/z (% relative abundance): m/z: 366.12 (23%), 367.31 (27%), 368.34 (6%), 100.52(100).

7-Chloro-5-(4-chlorophenyl)-3-(4-nitrophenyl)-[1,2,4] triazolo[4,3-a] pyrimidine-6-carbonitrile (14b): Yield: (46%). M.p. 236-238ºC; IR (KBr): 2106 (CH, Ar), 2225 (C≡N), 1649 (C=N), 1604 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 7.98-7.06 (8Hs, Ar. Hs). Anal. calc. for $C_{18}H_8C_{l2}N_6O_2$ (411.20): C 52.58, H 1.96, N 20.44; found: C 52.60, H 1.94, N 20.48.

Synthesis of 5-(Aryl)-3-phenyl-7-(pyrrolidin-1-yl)-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile derivatives (15a,b): A mixture of 7-chloro-5-(4-chlorophenyl)-3-aryl-[1,2,4] triazolo [4,3-a] pyrimidine-6-carbonitrile (2.86 mmol), pyrolidine (2.88 mmol) and anhydrous K_2CO_3 (0.4 g, 5.76 mmol) in EtOH (15 ml) was heated under reflux for 5 h. It was then cooled and filtered and the filtrate was concentrated to half its volume. The obtained precipitate was filtered and crystallized from EtOH-H_2O (3: 1 v/v).

5-(4-Chlorophenyl)-3-phenyl-7-(pyrrolidin-1-yl)-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (15a): Yield: (87%); M.p. 211-213ºC; IR (KBr): 3106 (CH, Ar), 2966 (CH, aliphatic), 2225 (C≡N), 1649 (C=N), 1604 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 7.86-7.32 (m, 9Hs, 9Ar-Hs), 3.62-3.50 (m, 4Hs, 2CH_2 pyrolidine), 1.68-1.66 (m, 4Hs, pyrolidine Hs). Anal. calc. for $C_{22}H_{17}ClN_6$ (400.86): C 65.92, H 4.27, N 20.96; found: 65.98, H 4.34, N 20.99.

5-(4-Chlorophenyl)-3-(4-nitrophenyl)-7-(pyrrolidin-1-yl)-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (15b): Yield: (75%); M.p. 266-268ºC; IR (KBr): 3106 (CH, Ar), 2966 (CH, aliphatic), 2225 (C≡N), 1649 (C=N), 1604 (C=C) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 7.54-7.40 (m, 8Hs, Ar-Hs), 3.65-3.52 (m, 4Hs, pyrolidine), 1.68-1.64 (m, 4Hs, pyrolidine Hs). Anal. calc. for $C_{22}H_{16}ClN_7O_2$ (445.86): C 59.26, H 3.62, N 21.99; found: C 59.36, H 3.72, N 21.91; MS: m/z (% relative abundance): 445.14 (83%), 446.16 (49%), 447.14 (28%), 444.13 (100).

Anticancer activity

Thirteen synthesized compounds were chosen by National Cancer Institute (NCI) to investigate the ant proliferative activity according to the protocol of the drug assessment branch. The prepared compounds were added at single concentration of 10^{-5} M and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers.

Results for each compound were reported as the growth percentage of the treated cells which are evaluated spectrophotometrically and compared to that of the untreated control cells.

Antimicrobial activity

Using the diffusion agar technique (at regional center of mycology and biotechnology, Alazhar University, Cairo, Egypt) the synthesized compounds were screened for their antifungal activity against *Aspergillus flavus* (RCMB0020002) and *Candida albicans* (RCMB005003 (1) ATTC10231). The antibacterial activity investigated against Gram positive bacteria; *Staphylococcus aureus* (RCMB010010) and *Bacillus subtilis* (RCMB015(1) NRRLB-543) and Gram negative bacteria; *Salmonella typhimurium* (RCMB006 (1)ATCC14028**)** and *Escherichia coli* (RCMB010052)ATCC25955. The test done on nutrient agar medium (bacteria) [30] and molt extract (fungi). The sterile medium (15 ml) in each petri-plate was uniformly smeared with cultures of Gram positive, Gram negative bacteria or Fungi. Sterile discs of 6 mm diameter were placed in the petriplates, to which (10 mg/ml, 100 µl/disc, DMSO as a solvent) of different synthesized compounds were added.

Ketoconazol [31] and gentamicin [32] were selected as antifungal antibacterial positive control respectively for comparison. For each treatment; three replicates were maintained. The plates were incubated at 37ºC ± 2ºC for 24 h and the zone of inhibition was determined. MIC investigated by the same previous technique used in the screening on the same organisms compared to the same controls.

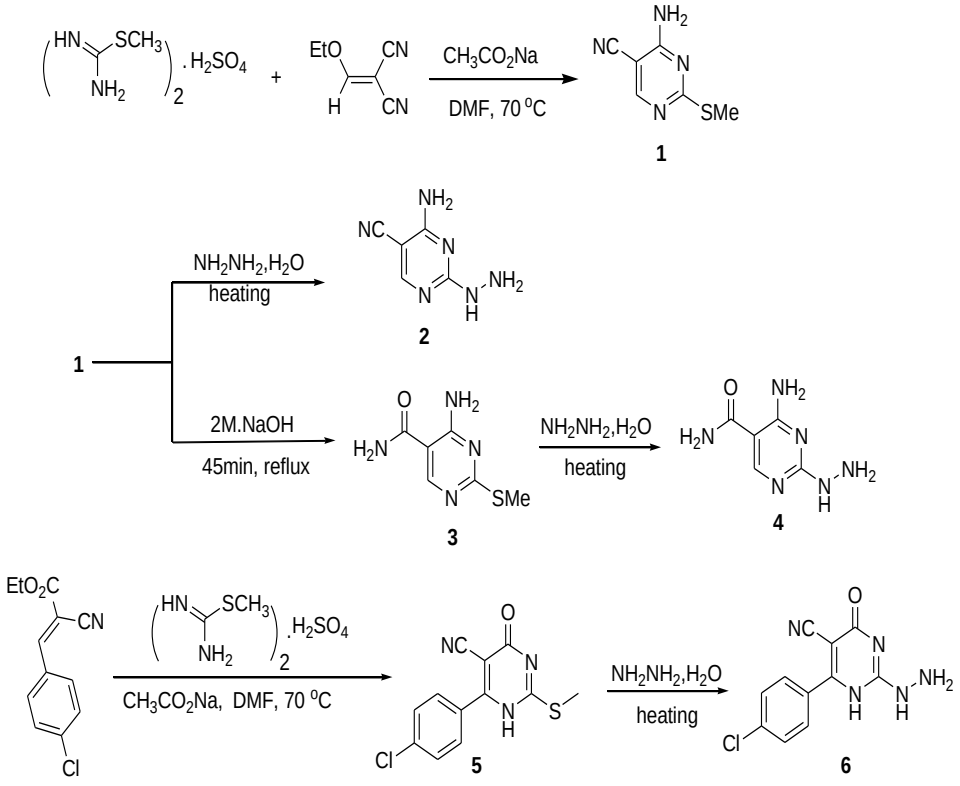
RESULTS AND DISCUSSION

Chemistry

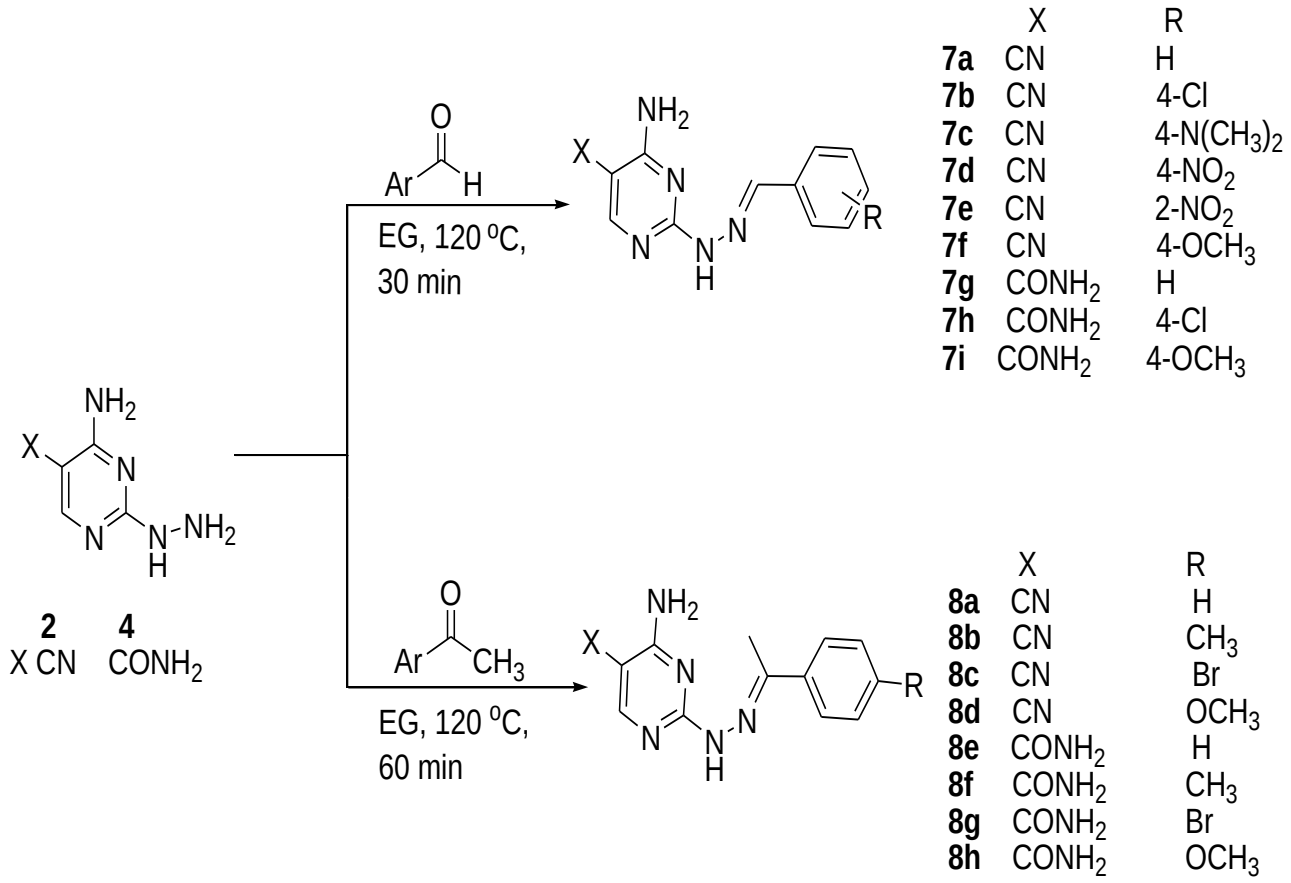
The requisite intermediates 2, 4, 6 were prepared according to procedures depicted in Scheme 1. Heating of *S*-methylisothiourea sulfate with 2-(ethoxymethylene) malononitrile [33] in the presence of anhydrous sodium acetate in DMF as a solvent to afford 4-amino-2-(methylthio)pyrimidine-5-carbonitrile 1 [34]. Basic hydrolysis of compound 1 generated the corresponding carboxamide derivative 3 [35]. Compounds 1 and 3 were treated with hydrazine hydrate to furnish the corresponding pyrimidyl hydrazine analogs 2 and 4 respectively [36]. In similar way, reaction of ethyl 3-(4-chlorophenyl)-2-cyano acrylate [37] with *S*-methylisothiourea sulfate in presence of anhydrous sodium acetate in hot DMF afforded 4-(4-chlorophenyl)-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile 5 [38]. Displacing of the methyl thio group with hydrazine hydrate afforded 4-(4-chlorophenyl)-2-hydrazinyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile 6 (Scheme 1) [39]. Compounds 2, 4, and 6 are the key intermediates and building blocks for the newly synthesized compounds in this study. Having in hand the pyrimidpyl hydrazine as a key intermediate, our attention was directed to install substituent at C2. Condensation of compound 2 with aromatic aldehydes including benzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, 2-nitrobenzaldehyde and 4-methoxybenzaldehyde in ethylene glycol (EG) in presence of few drops of glacial acetic acid at 120^{o}C afforded the corresponding hydrazones 7a-g (Scheme 2).

Similarly, compounds 7g-i were obtained from its precursor 4, (Scheme 2). Reaction of acetophenone, 4-methyacetophenone, 4-bromoacetophenone and 4-methoxyacetophenone with pyrimidyl hydrazine 2 or 4 led to the formation of compounds 8a-d and 8e-h respectively (Scheme 2). Cyclo condensation of hydrazine derivative with 1,3-dielectrophiles has been extensively used for the preparation of pyrazole heterocycles [40]. In this regard, heating the hydrazine derivatives 2 and 4 with 1,3-dielectrophile such as acetyl acetone in EG containing a few drops of glacial acetic acid afforded 2-pyrazolyl pyrimidine derivatives 9a,b. On the other hand, reaction of the hydrazine derivatives 2 and 4 with 2-(ethoxymethylene) malononitrile in EG containing a few drops of triethylamine (TEA) afforded the 2-pyrazolyl pyrimidine derivatives 10a,b. Additionally, 2-pyrazolyl pyrimidine bearing phenyl moiety 11a-c and 12a-c was generated (Scheme 3). In order to extend our strategy, different fused pyrimidine derivatives were explored (Scheme 4). The pyrimido-pyrimidinone 13a was prepared by the reaction of compound 3 with triethyl orthoformate in boiling AcOH [41].

Boiling compound 2 in acetic anhydride for 3 h generated also pyrimido-pyrimidinone, compound 13b (Scheme 4). The previous reaction can be rationalized by a Dimroth rearrangement which is an isomerization involving an O/N-translocation through a ring-opening ring-closure sequence [42]. Moreover, treatment of compound 1 with ethyl cyano acetate in boiling EtOH in presence of EtONa furnished pyrido-pyrimidinone compound 13c. Then, we thought to examine the pyrimidine fusion system in different place. The hydrazine derivative 6 was treated with $POCl_3$ *N*,*N*-dimethylaniline and equimolar amount of benzoic acid or 4-nitrobenzoic acid to afford chlorinated triazolopyrimidine 14a,b. Compounds 14a, b were subjected to nucleophilic substitution reaction at the halogen of imine functionality with pyrolidine moiety in refluxing EtOH in the presence of K_2CO_3 to producetriazolo [4,3-*a*] pyrimidine-6-carbonitrile derivatives 15a,b.



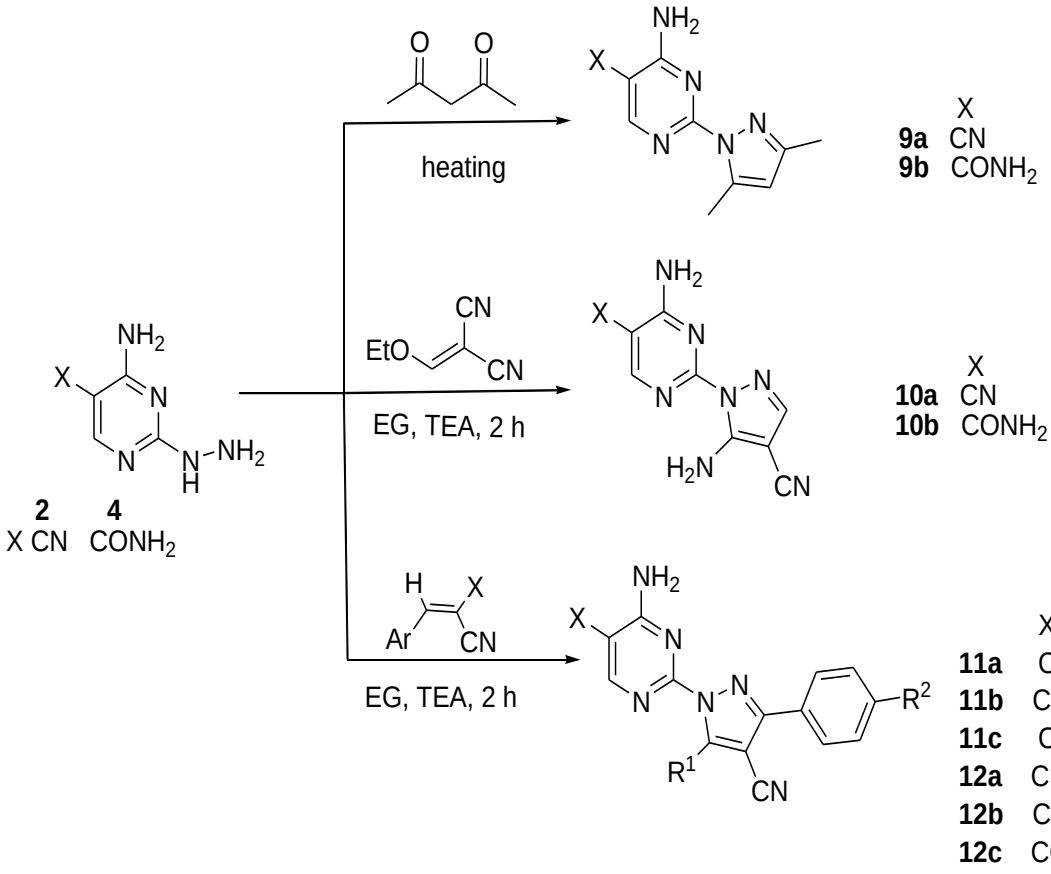
Scheme 1: Synthesis of key intermediates hydrazinyl 4, 6



	X	R
7a	CN	H
7b	CN	4-Cl
7c	CN	4-N(CH3)2
7d	CN	4-NO2
7e	CN	2-NO2
7f	CN	4-OCH3
7g	CONH2	H
7h	CONH2	4-Cl
7i	CONH2	4-OCH3

	X	R
8a	CN	H
8b	CN	CH3
8c	CN	Br
8d	CN	OCH3
8e	CONH2	H
8f	CONH2	CH3
8g	CONH2	Br
8h	CONH2	OCH3

Scheme 2: Synthesis of benziylidine compounds; EG (ethyelene glycol)

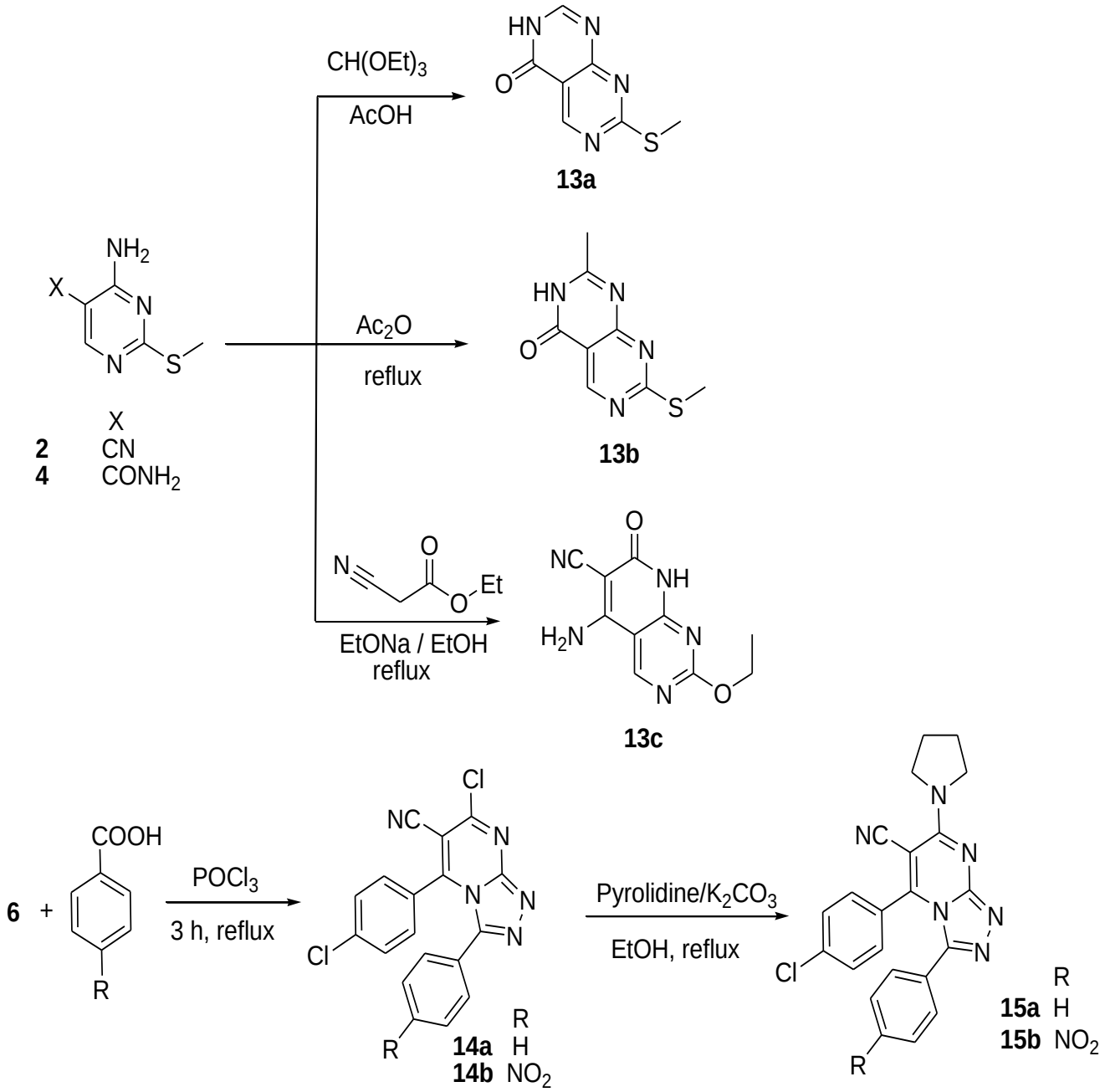


	X
9a	CN
9b	CONH2

	X
10a	CN
10b	CONH2

	X	R1	R2
11a	CN	NH2	H
11b	CN	NH2	OCH3
11c	CN	NH2	N(CH3)2
12a	CN	OH	H
12b	CN	OH	Cl
12c	CONH2	OH	Cl

Scheme 3: Synthesis of Pyrimidine teathered to pyrazole miety



Scheme 4: Synthesis of fused pyrimidine architecture

Biology

Anticancer activity: Thirteen synthesized compounds were chosen by National Cancer Institute (NCI) to investigate the anti-proliferative activity according to the protocol of the drug assessment branch, Bethesda, USA, (http://www.dtp.nci.nih.gov).

Table 1: Summarize the received data: Percentage growth inhibition (GI %) of *in vitro* subpanel tumor cell lines at 10 µM concentration of the selected compounds [a] (-=GI<10%) (nt: not tested)

Subpanel cancer cell Lines	%Growth Inhibition (GI%)[a]												
	7g	7h	7a	7i	8b	8c	8e	9a	10b	11b	11c	12a	12c
Leukemia													
CCRF-CEM	-	-	-	15.18	-	-	-	-	-	-	-	-	-
K-562	-	-	16.48	33.6	17.31	19.52	-	-	-	12.15	11.56	-	11.5
MOLT-4	11.76	19.83	18.13	-	-	-	-	-	-	13.35	-	-	27.38
RPMI-8226	-	-	-	23.16	23.17	-	16.13	-	-	14.21	11.39	-	29.5
SR	-	23.24	-	-	26.34	12.89	-	-	-	18.62	18.07	-	27.2

Non-small cell lung cancer													
A549/ATCC	17.46	29.21	12.78	-	-	-	-	-	-	-	-	-	-
EKVX	-	-	-	-	-	-	-	-	-	-	-	-	-
HOP-62	-	-	-	12.57	-	11.77	-	-	-	-	-	-	-
HOP-92	10.08	-	-	32.52	-	-	-	-	-	-	-	-	-
NCI-H226	-	-	-	-	-	-	-	-	-	-	-	-	14.24
NCI-H23	11.79	19.89	-	-	14.5	-	-	-	-	-	16.9	-	12.74
NCI-H322M	11.25	31.17	20.53	21.81	-	-	25.02	-	-	-	-	-	18.11
NCI-H460	-	14.81	-	15.13	-	-	-	-	-	-	-	-	-
NCI-H522	-	25.12	17.23	16.42	-	-	17.13	15.94	18.18	-	-	-	-
Colon cancer													
COLO 205	-	-	-	-	-	-	-	-	-	-	-	-	-
HCC-2998	-	-	-	-	-	-	-	-	-	-	-	14.37	-
HCT-116	-	21.98	11.61	-	-	-	-	-	-	-	-	-	-
HCT-15	13.5	17.25	-	-	-	-	-	-	-	-	-	-	-
HT29	-	24.37	-	-	-	-	14.11	-	-	-	-	-	-
KM12	-	-	-	-	11.1	-	-	-	-	21.26	24.7	-	24.82
SW-620	-	-	-	-	-	-	-	-	-	-	27.08	-	-
CNS cancer													
SF-268	21.44	29	15.13	-	13.71	-	11.95	-	-	-	35.16	-	25.18
SF-295	-	-	-	-	-	-	-	-	-	-	-	-	-
SF-539	-	-	-	-	-	-	-	-	-	-	-	-	-
SNB-19	-	18.54	12.92	-	-	-	-	-	-	-	-	-	-
SNB-75	14.5	10.91	16.44	-	-	-	-	-	-	13.38	13.22	-	18.13
U251	-	-	-	-	-	-	-	-	-	-	-	-	-
Melanoma													
LOX IMVI	-	12.62	-	-	-	-	-	-	-	-	-	-	-
MALME-3M	-	-	-	-	16.47	-	-	-	-	-	-	-	-
M14	-	34.76	21.39	13.91	-	-	-	-	-	-	22.2	-	-
MDA-MB-435	-	13.57	-	-	-	-	-	-	-	-	-	-	-
SK-MEL-2	-	30.04	23.84	28.18	-	-	-	-	-	15.41	-	-	-
SK-MEL-28	-	-	-	-	-	-	-	-	-	-	-	-	-
SK-MEL-5	14.06	-	-	-	-	-	-	-	-	-	-	-	-
UACC-257	10.84	34.4	-	-	-	-	-	15.33	-	-	-	-	-
UACC-62	-	32.86	-	-	-	-	-	-	-	-	-	-	-
Ovarian cancer													
IGROV1	-	31.13	19.88	21.03	14.48	-	-	-	-	-	15.06	-	23.55
OVCAR-3	-	-	-	-	-	-	-	-	-	-	-	-	-
OVCAR-4	11.02	21.45	12.71	-	-	-	-	-	-	-	12.11	-	-
OVCAR-5	-	-	-	-	-	-	-	-	-	-	-	-	-
OVCAR-8	-	-	-	-	-	-	-	-	-	-	-	-	-
NCI/ADR-RES	-	-	-	-	-	-	-	-	-	-	-	-	-
SK-OV-3	-	23.53	14.06	20.37	-	-	-	-	-	-	-	-	-
Renal cancer													
786-0		16.19	-	-	-	-	-	-	-	-	-	-	-
A498	22.38	31.21	15.23	50.36	14.62	-	11.72	-	19.14	15.77	13.77	-	15.46
ACHN	-	15.05		10.13	-	-	-	-	-	-	21.21	-	-

XF 393	-	14.74	16.74	17.31	-	-	-	-	-	-	-	-	-
SN12C	-	-	-	-	-	-	-	-	-	-	-	-	-
TK-10	11.92	37.93	26.57	27.9	-	-	17.28	-	-	-	-	-	-
UO-31	15.93	36.11	26.68	29.71	12.71	-	17.61	-	11.97	13.63	28.61	-	18.48
Prostate cancer													
PC-3	-	23.54	-	24.62	-	-	-	-	-	-	-	-	-
DU-145	-	24.89	-	-	-	-	-	-	-	-	-	-	14.28
Breast cancer													
MCF7	17.75	24.84	13.27	-	13.26	-	13.71	-	-	15.47	-	-	12.97
MDA-MB-231/ATCC	-	-	-	-	-	-	-	-	-	-	-	-	-
HS 578T	-	-	-	-	18.02	-	-	-	-	-	-	-	-
BT-549	-	-		-	-	-	-	-	-	-	23.98	-	-
T-47D	14.47	-	13.47	16.6	12.81	-	-	-	-	-	-	-	-
MDA-MB-468	12.39	-	-	-	-	-	-	-	-	-	-	-	11.75

Among the selected compounds, compound 7i exhibited best activity against leukemia, renal cancer and non-small lung cancer (Table 1). Compound 7h represent anticancer activity against ovarian cancer, breast cancer and melanoma. Compound 11c showed the best activity against colon cancer and CNS cancer. These active compounds belong to non-fused pyrimidine architecture with tethered to pyrazole moiety or aryl hydrazonyl moiety.

Antimicrobial activity: Using the diffusion agar technique [38] the following compounds were tested as antimicrobial agents against the illustrated organisms Table 2.

Table 2: Antimicrobial activity of the synthesized compounds expressed as size of the inhibition zone (mm/mg sample)

	Fungi		**Gram (+) bacteria**		**Gram (-) bacteria**	
	Asperigillus Flavus	***Candida albicans***	***Staphylococcus aureus***	***Bacillus subtilis***	***Salmonella typhi***	***Escherichia coli***
7f	-	-	17	16	18	-
7h	-	-	-	-	-	-
8a	-	-	25	21	24	25
8b	-	-	33	25	26	23
8c	-	-	31	28	23	26
8d	-	-	22	10	12	13
8e	-	-	-	-	-	-
8f	-	-	30	26	24	23
8g	-	-	-	-	-	-
8h	-	-	-	-	-	-
9a	-	-	13	15	17	-
9b	-	-	28	27	25	22
10a	-	-	-	-	-	-
10b	-	-	27	26	25	24
11a	-	-	-	-	-	-
11b	-	-	29	33	30	24
11c	-	-	8	-	10	-
12a	-	-	30	24	23	27
12b	-	-	11	-	8	-
12c	9	-	-	12	10	17
14a	15	16	-	10	8	21
14b	15	16	14	-	-	-
15a	-	-	19	19	-	-

15b	-	-	-	-	-	-
CONTROL	16	20	24	26	27	30
	Ketoconazol		Gentamicin		Gentamicin	

Compounds 14a, 14b 8b, 8c, and 11c exhibited the best antifungal activity and antibacterial compounds among the synthesized compounds. So, these compounds were further screened to determine their MIC (minimum inhibitory concentration µg/ml).

Table 3: MIC of the highest active antifungal and antibacterial compounds

	species	**MIC tested cpds**			**control**
		12c	**14a**	**14b**	**ketoconazol**
Fungi	*A. flavus*	-	-	1250	16
	C. albicans	2500	1250	625	20
		8b	**8c**	**11c**	gentamicin
Grame (+) bacteria	*S. aureus*	78.13	19.53	39.06	24
	B. subtilis	19.53	39.06	19.53	26
Grame (-) bacteria	*S. typhimurium*	78.13	78.13	156.25	27
	E. coli	39.06	19.53	39.06	30

From the data represented in Table 3, compound 14b was the most active compounds against *C. albicans* with MIC=625 µg/ml in comparison to ketoconazole (MIC=20 µg/ml). Compound 8c was the most active compound against *S. aureus and E. coli* with MIC=19.53 µg/ml in comparison of gentamicin with MIC=24 µg/ml and 30 µg/ml respectively. Compounds 11c was the most active compound against *B. subtilis* with MIC=19.53 µg/ml in comparison of gentamicin with MIC=26 µg/ml.

CONCLUSION

A new series of pyrimidine and fused pyrimidine derivatives was synthesized. These pyrimidyl derivatives were constructed from the key intermediates pyrimidyl hydrazine (2,4,6). The varieties of target compounds include differently substituted benzylidene functionalities, or planar aromatic heterocycles such as pyrazole ring. Fused ring systems such as triazolo [4,3-a] pyrimidine, pyrido [2,3-d] pyrimidine and pyrimido [4,5-d] pyrimidin systems. The synthetic courses were adopted to prepare pyrimidines and fused pyrimidines derivatives *via* formation of substituted pyrimidine core structure in very efficient and simple reactions. The structures of the synthesized compounds were assigned based on different spectroscopic techniques. The new compounds were evaluated for their antibacterial and antifungal activity compared to gentamicin and ketoconazol as standard drugs. Compound 14b was the most active compounds against *C. albicans* with *MIC*=625 µg/ml in comparison to ketoconazole (*MIC*=20 µg/ml). Compound 8c was the most active compound against *S. aureus and E. coli* with *MIC*=19.53 µg/ml in comparison of gentamicin with *MIC*=24 µg/ml and 30 µg/ml respectively. Compounds 11c was the most active compound against *B. subtilis* with *MIC*=19.53 µg/ml in comparison of gentamicin *with MIC=26 µg/*ml. Moreover, some compounds were selected and screened for their anticancer activity by National Cancer Institute (NCI), and exhibited moderate anticancer activity. From the obtained results, Compounds 7h, 7i and 12d revealed a significant anticancer activity and they are the most active compounds.

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SUPPLEMENTARY DATA

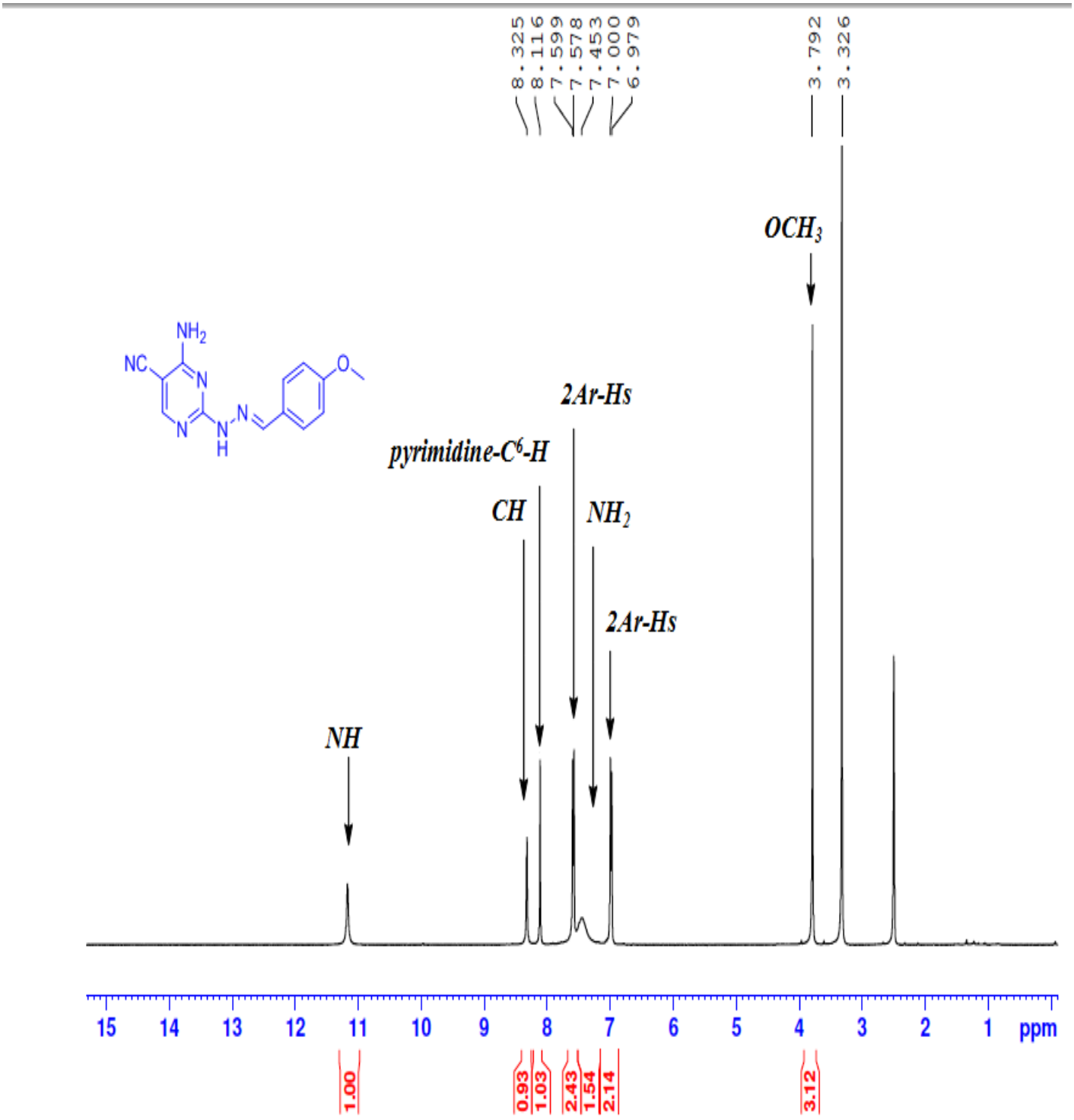

Figure S1: ^{1}H-NMR spectra of copound IXf

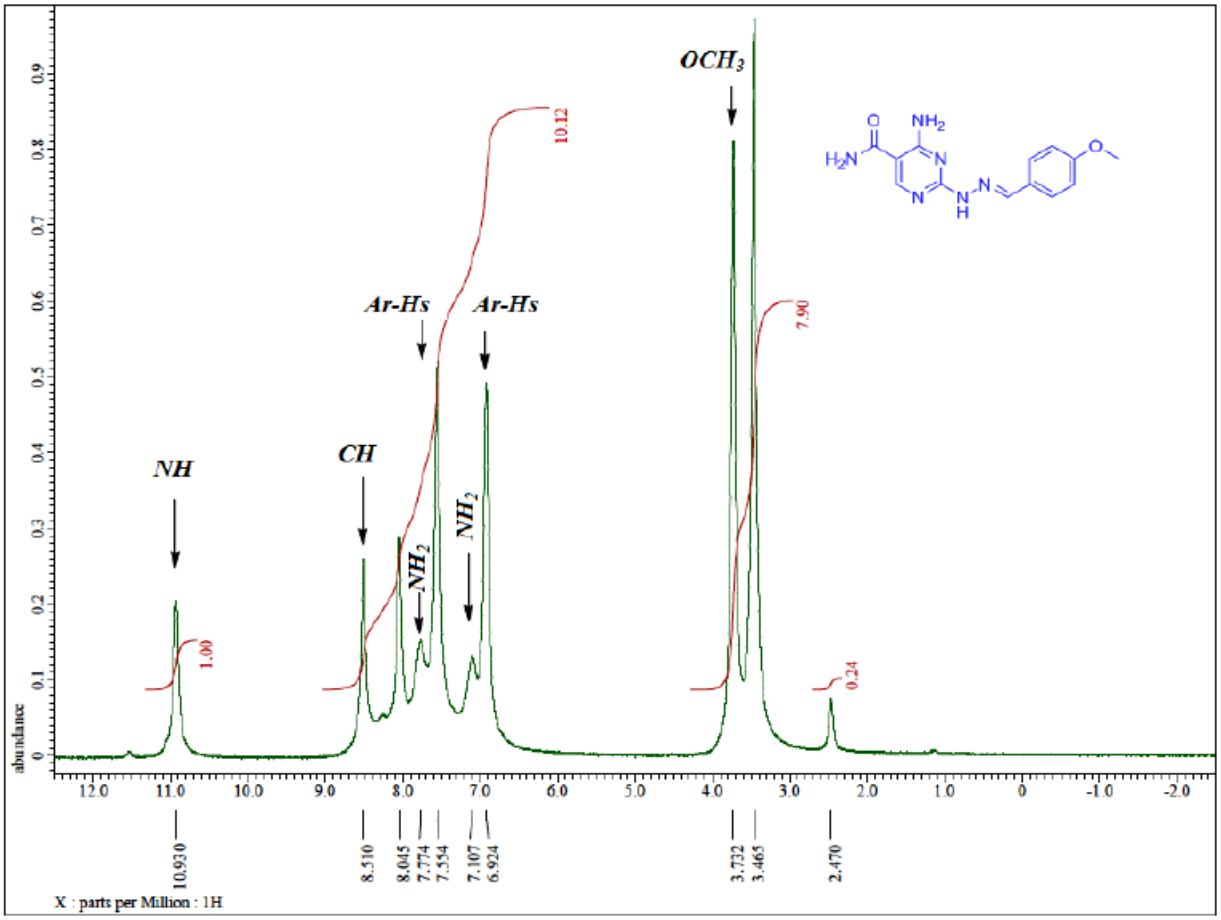

Figure S2: ^{1}H-NMR spectra of copound IXj

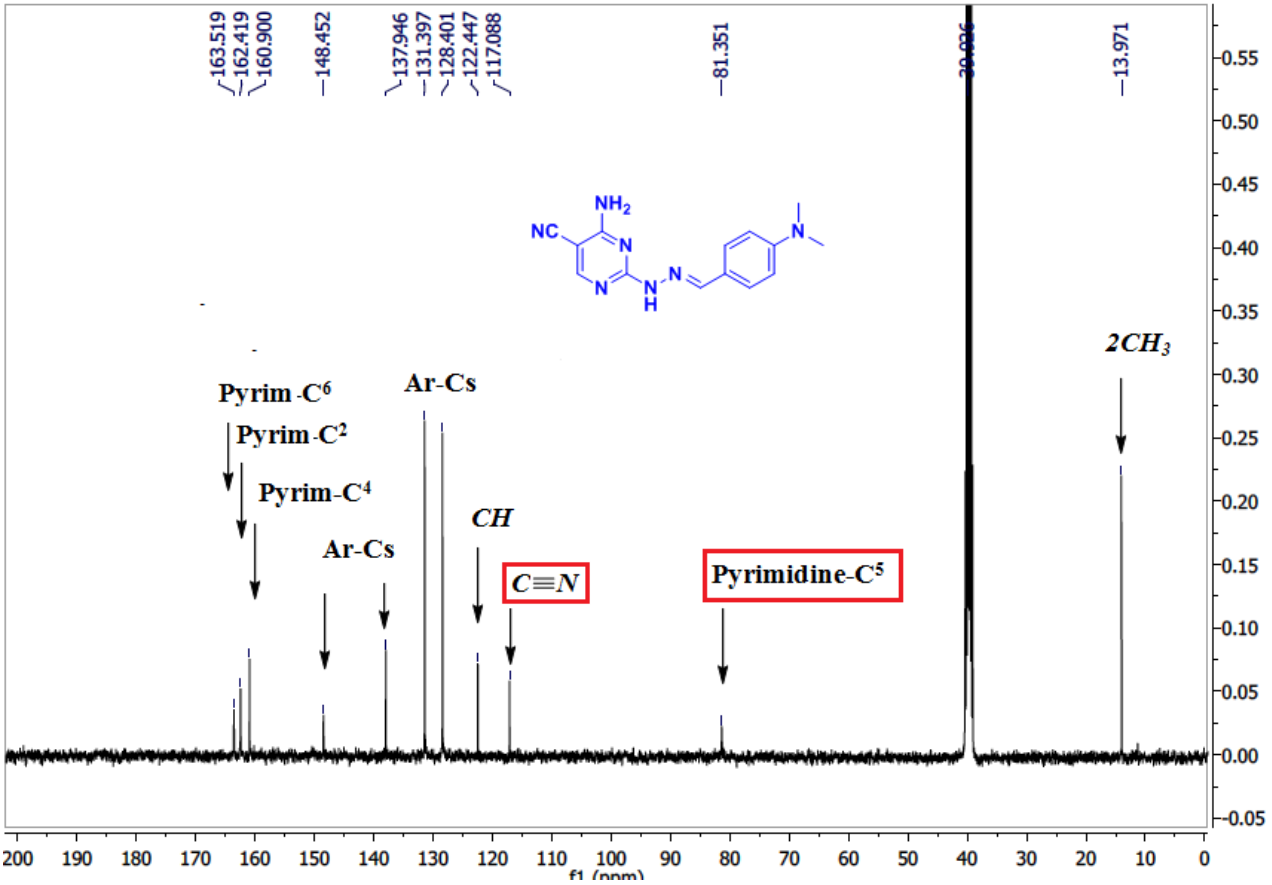

Figure S3: ^{13}C-NMR spectra of compound IXc

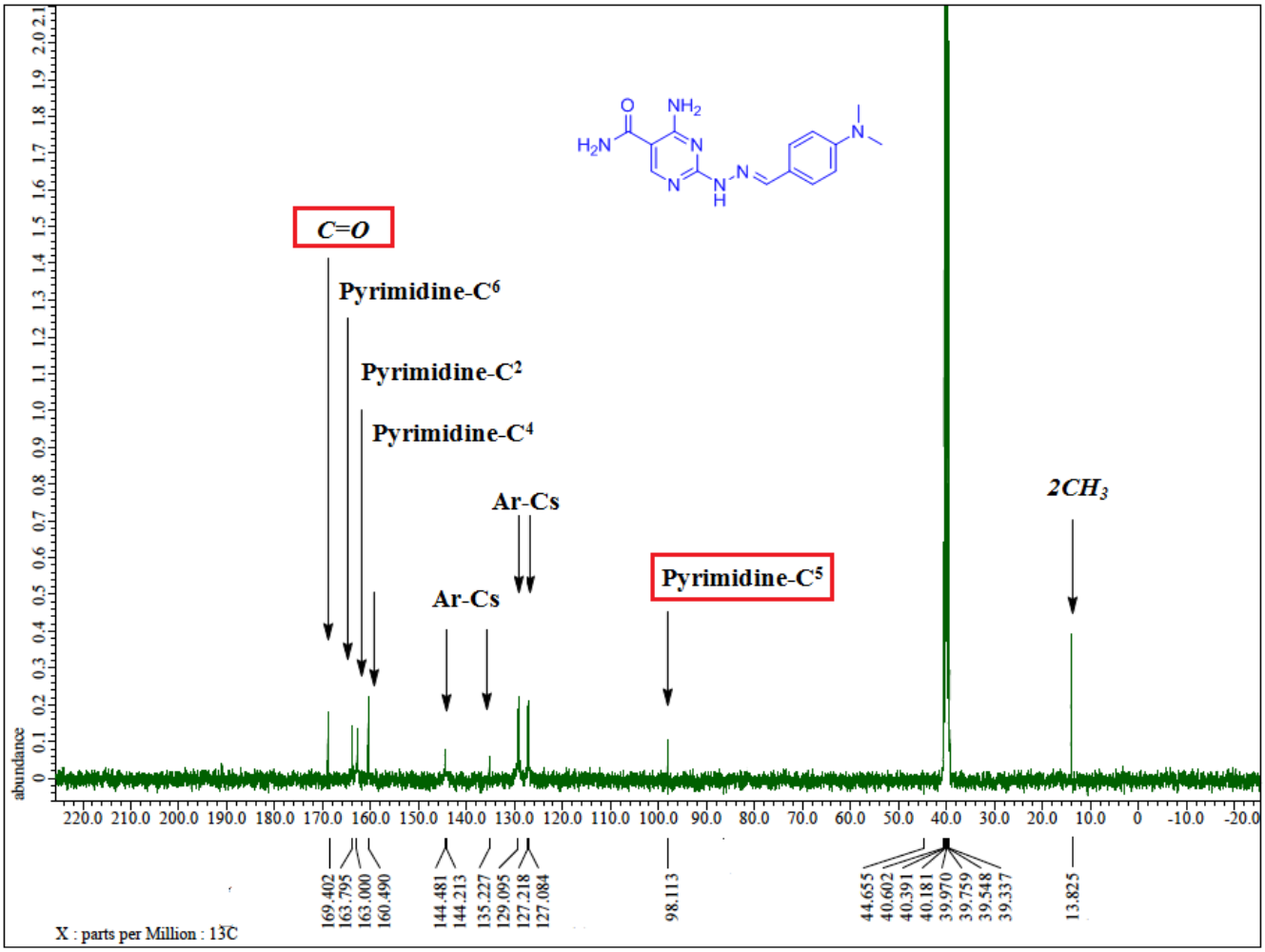

Figure S4: ^{13}C-NMR spectra of Ixi

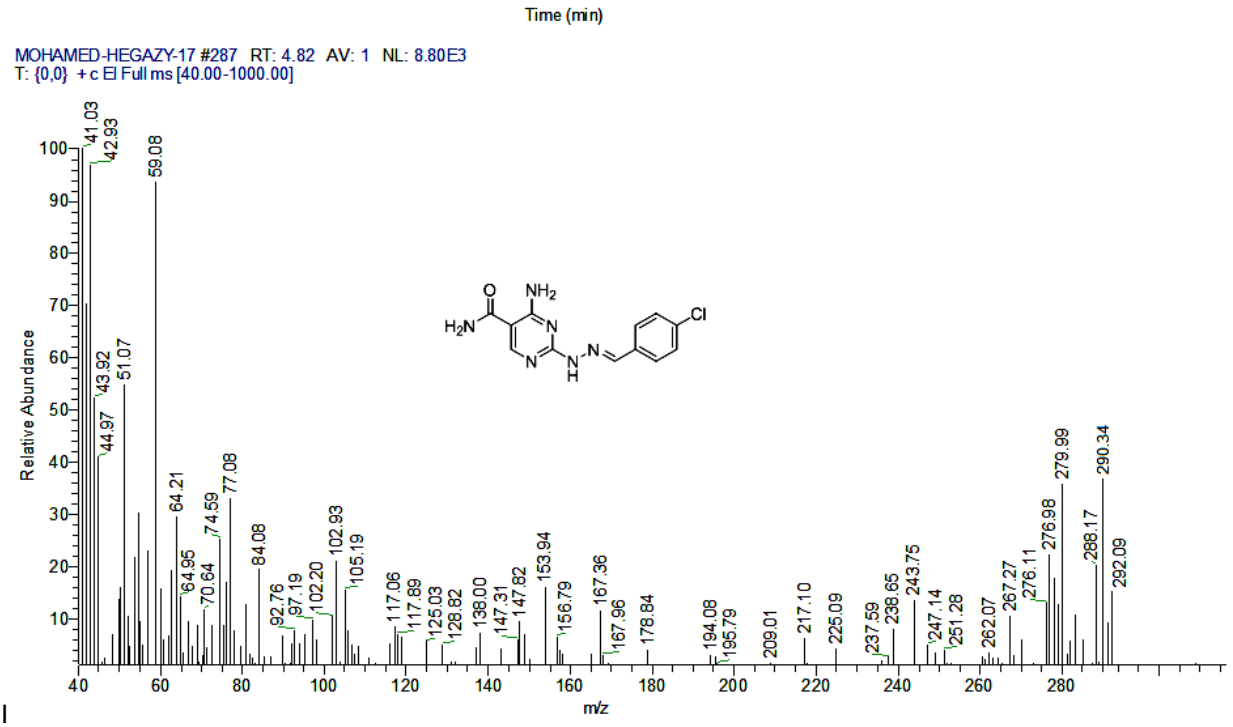

Figure S5: Mass spectrum of compound IXh

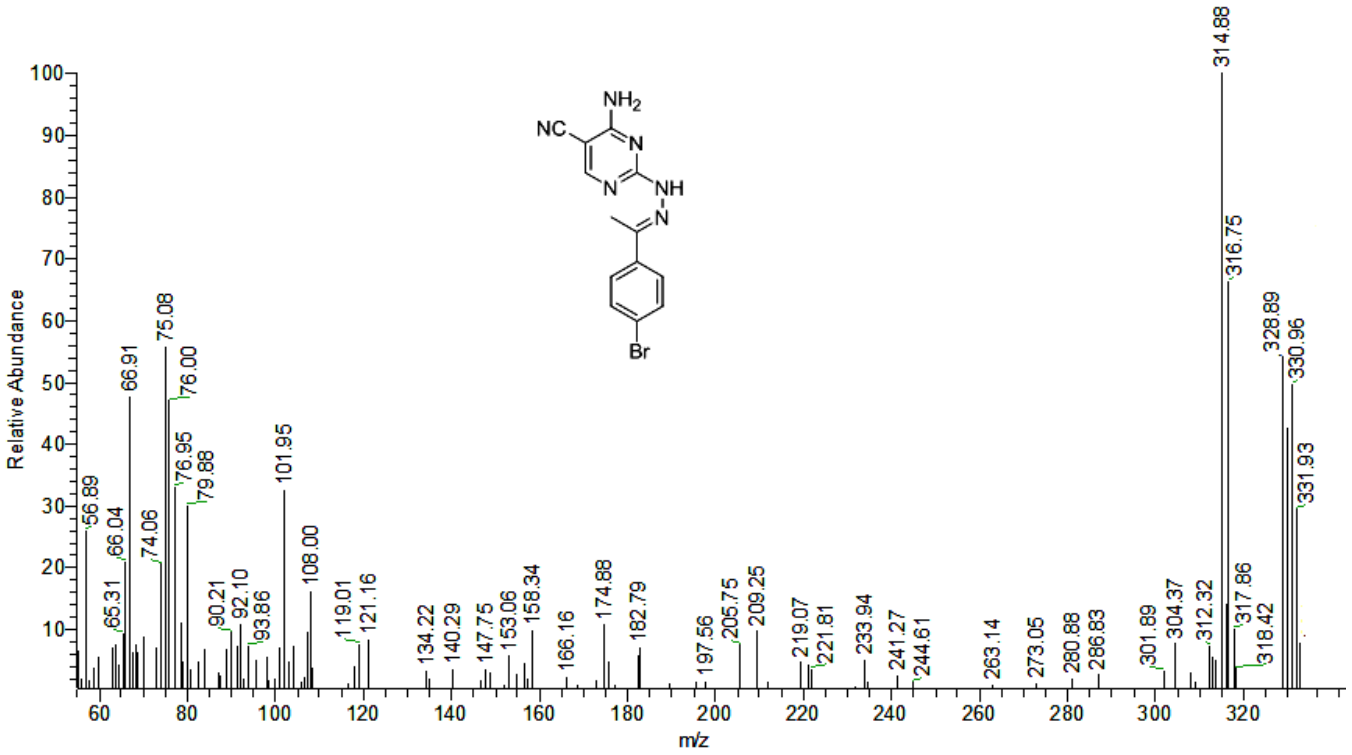

Figure S6: Mass spectra of compound Xc

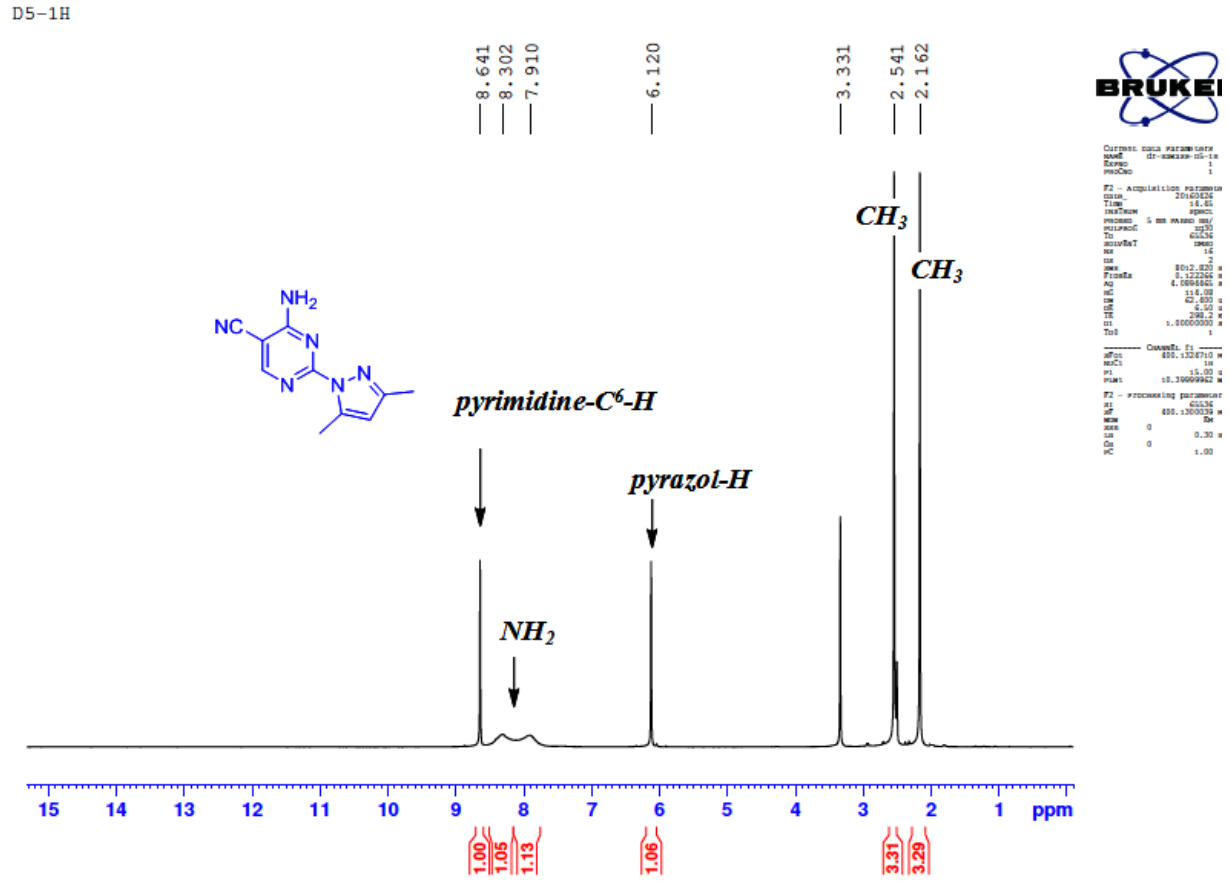

Figure S7: ^{1}H-NMR spectrum of Xia

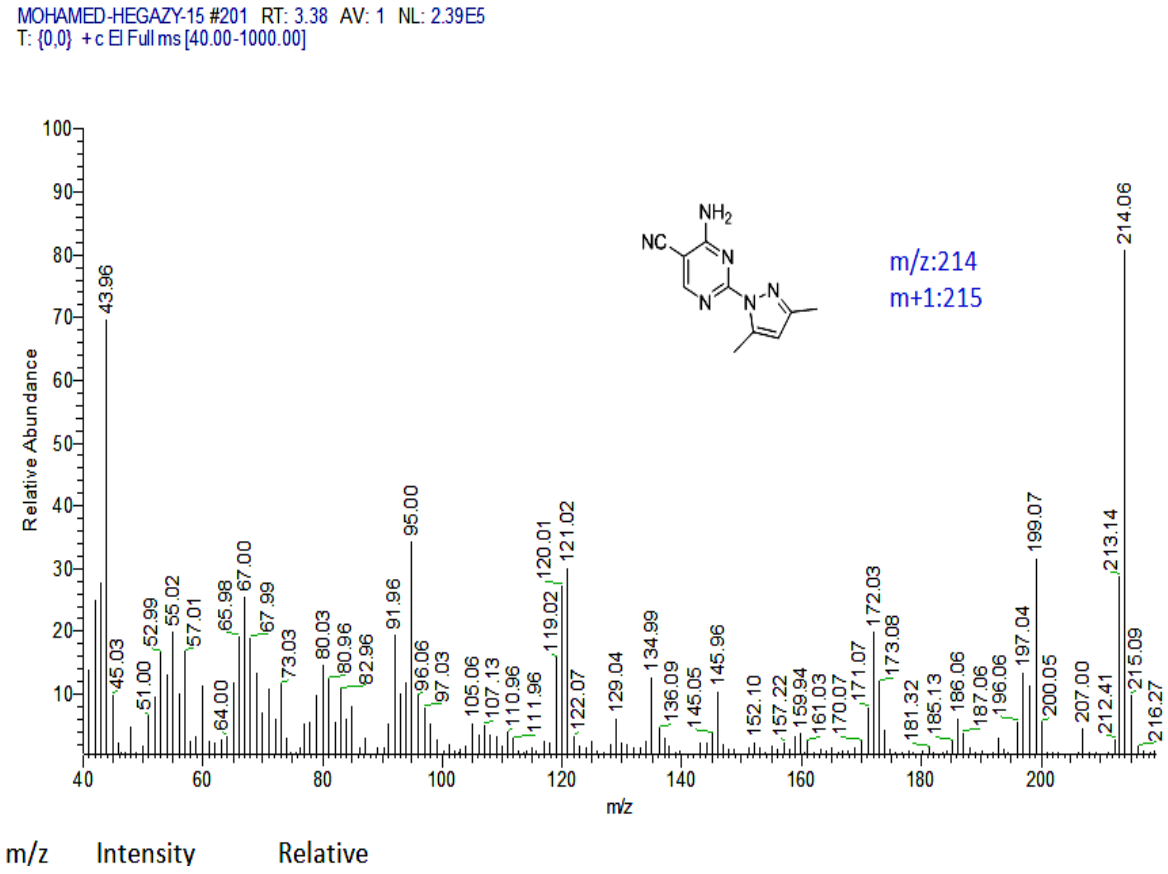

Figure S8: Mass spectra of compound Xia

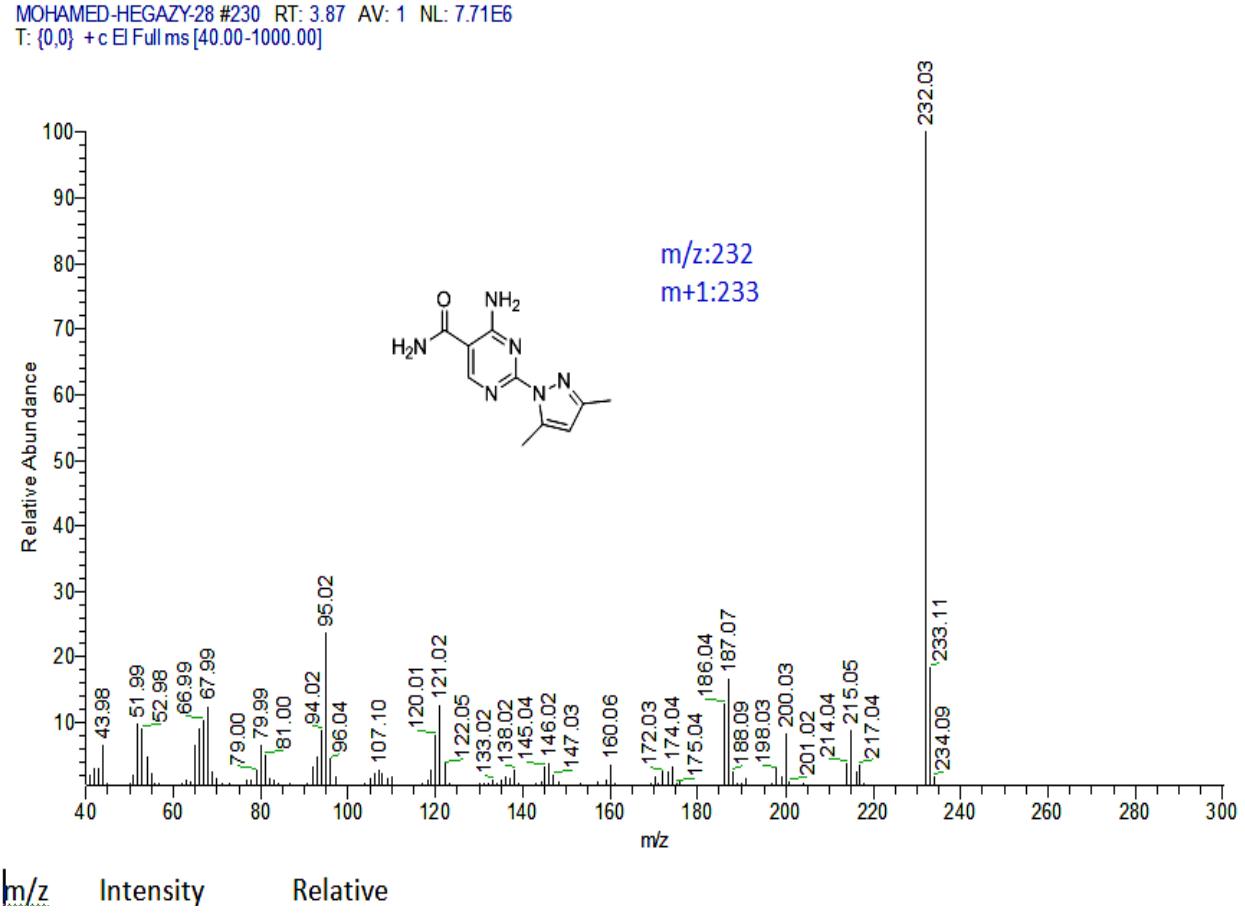

Figure S9: Mass spectra of compound Xb

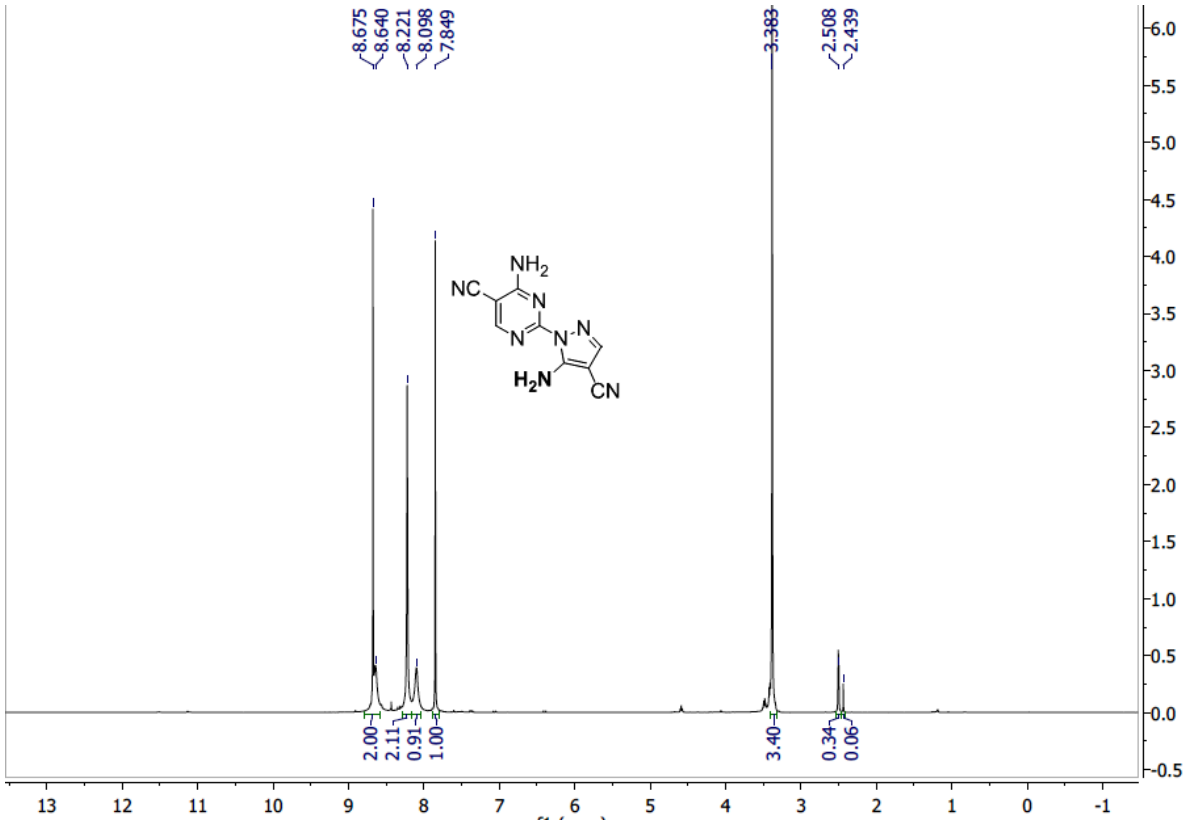

Figure S10: ^{1}H-NMR spectrum of XIIa

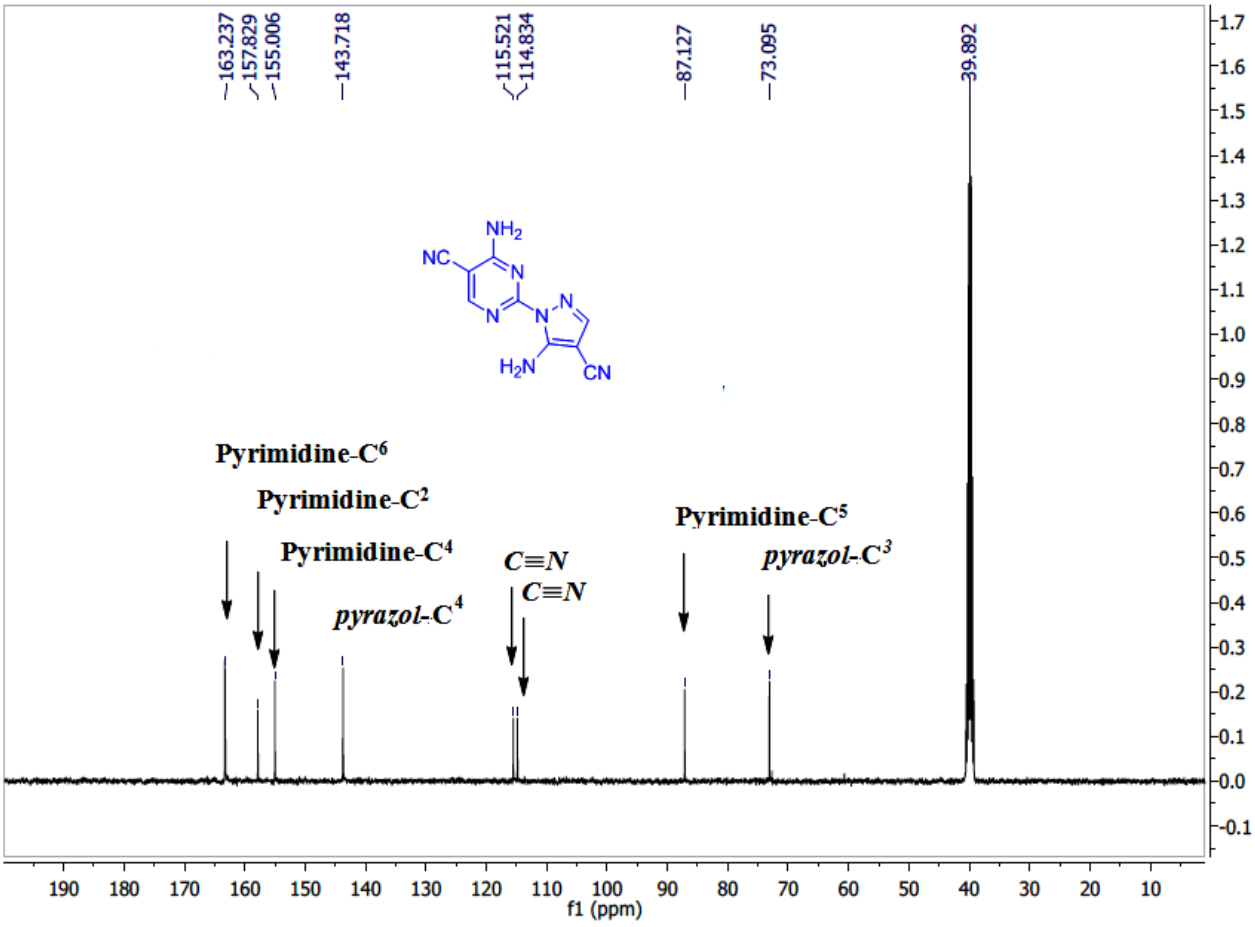

Figure S11: ^{13}C-NMR for XIIa

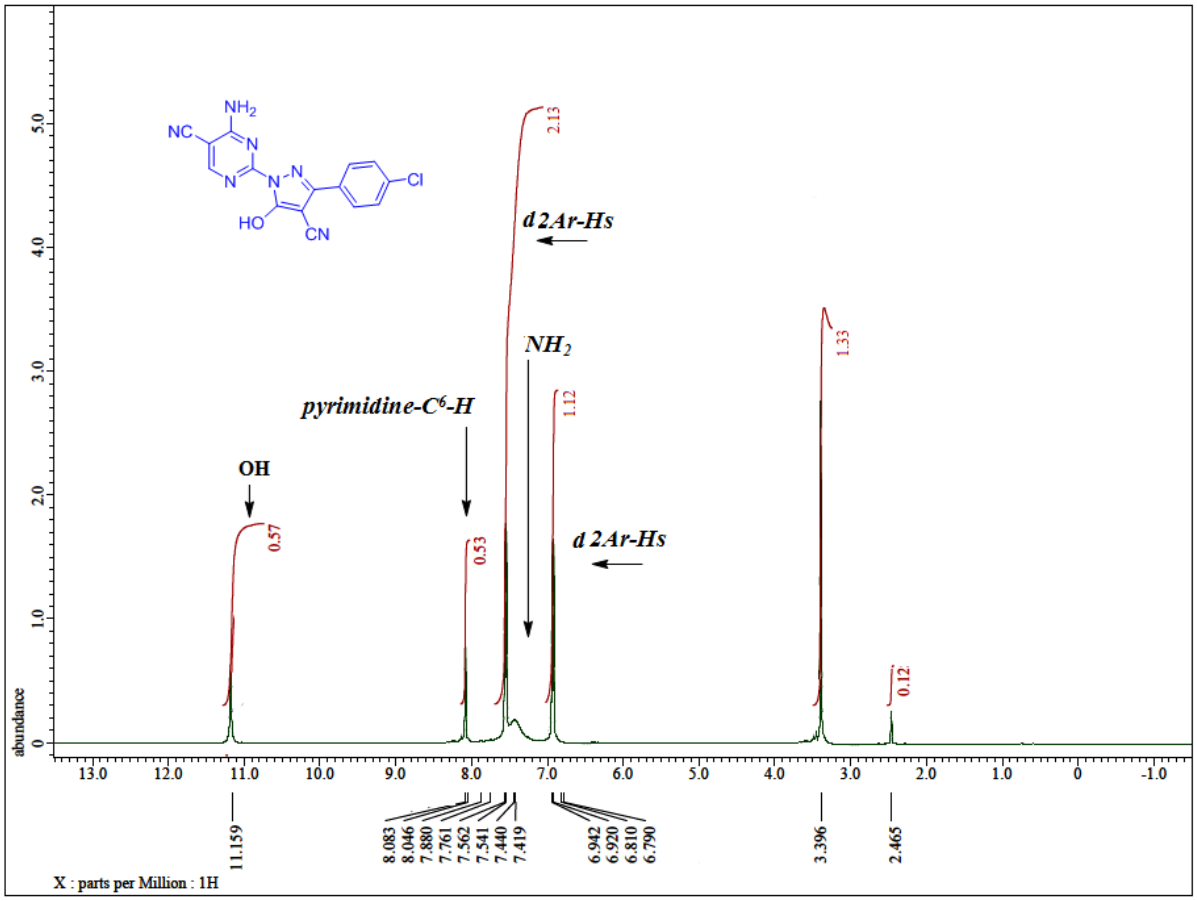

Figure S12: 1H-NMR of XIVb

Figure S12: ^{13}C-NMR for compound XIIIa

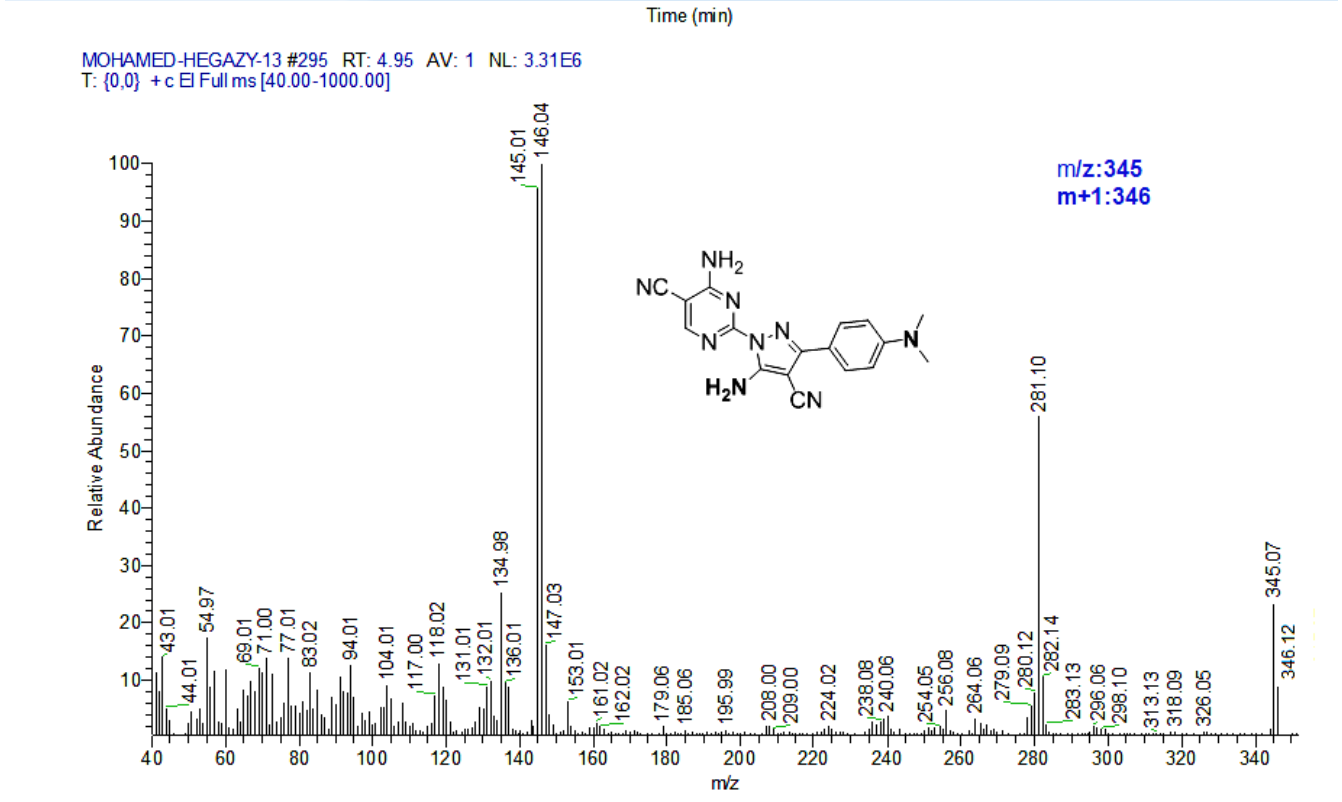

Figure S13: Mass spectra of compound XIIIc

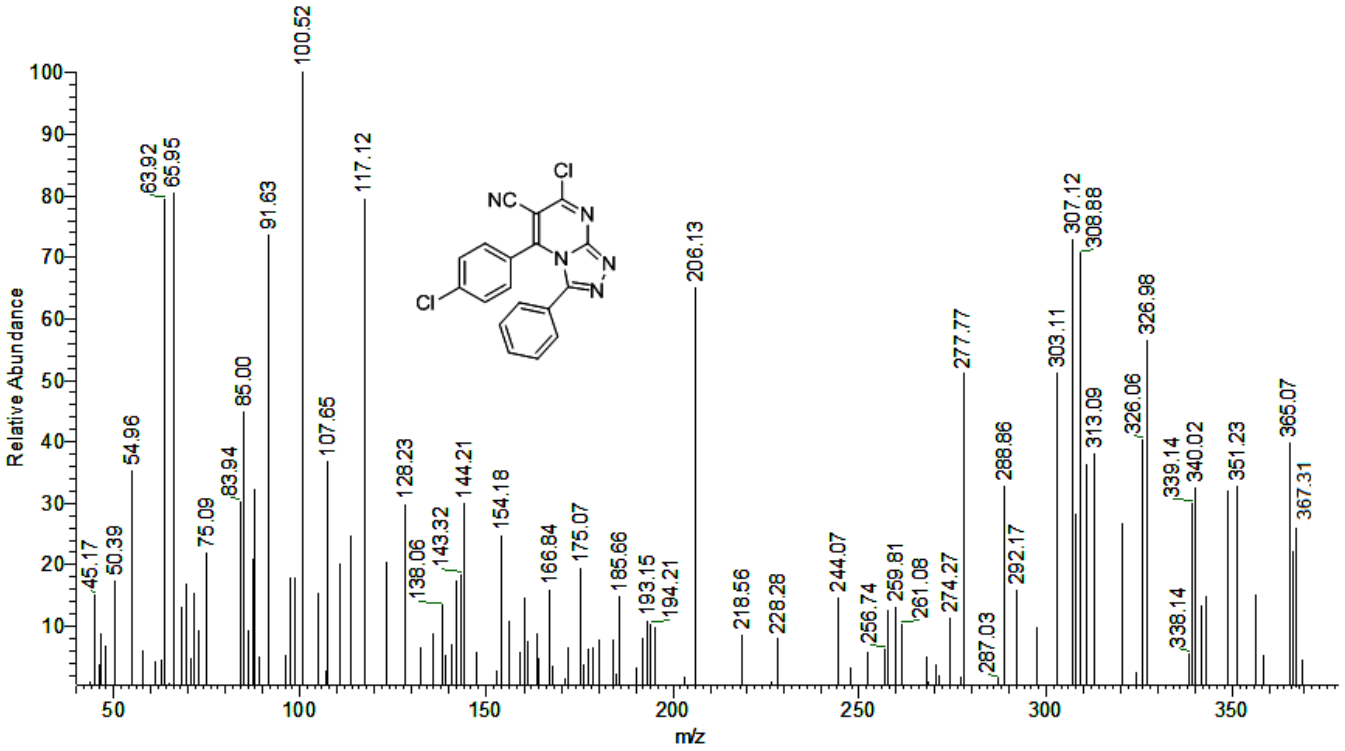

Figure S14: Mass spectra of compound XVIIa

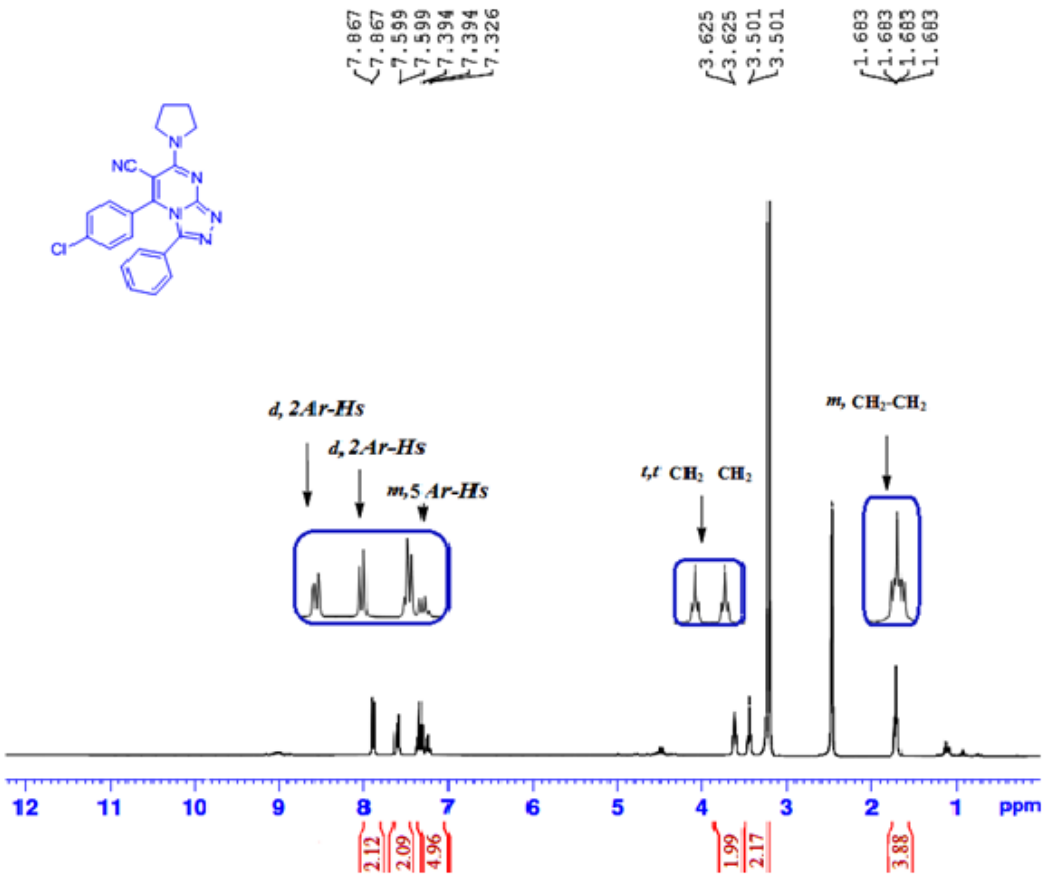

Figure S15: ^{1}H-NMR spectrum of XVIIIa

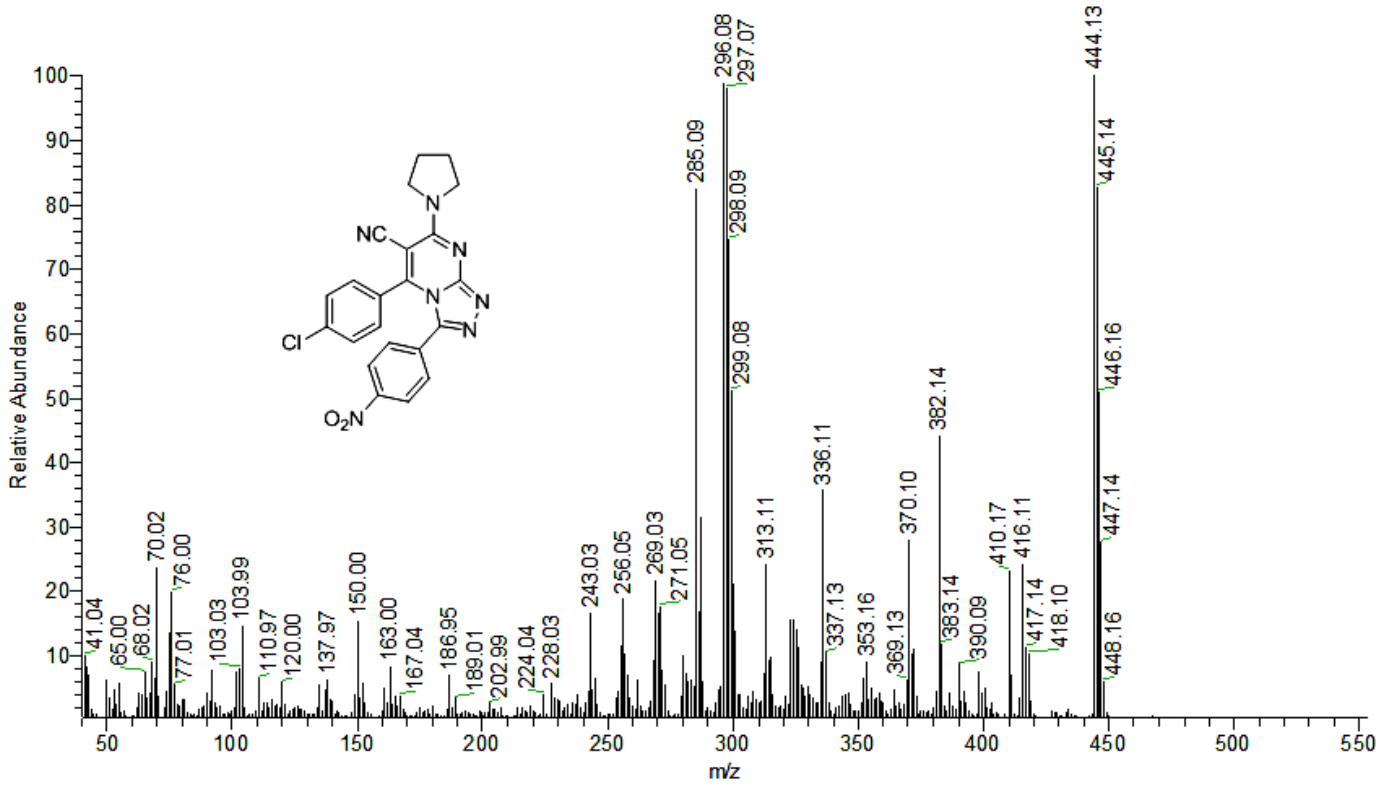

Figure S16: Mass spectra of compound XVIIIb