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SYNTHESIS OF ANTIMICROBIAL, ANTI- INFLAMMATORY, ANTIOXIDANT OF NEW PYRIMIDINE DERIVATIVE

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ABSTRACT

A series of novel pyrimidine analogs were synthesized in good yield from 6-amino-4-aryl-2-oxo-pyrimidine-5-carbonitrile (1a-d). The synthesized compounds were characterized using various spectral studies, including FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. Newly synthesized and 2-(substituted-pyrazolyl) pyrimidine derivatives were assessed in vitro for their cytotoxic activities against three cancerous cell lines: colorectal carcinoma (HCT-116), mammary gland breast cancer (MCF-7), and hepatocellular carcinoma (HEPG-2), as well as normal fibroblasts (W138). The results indicated that compounds 3b, 10b, and 10c exhibited the highest cytotoxic activities, with IC_{50} values very close to those of the reference drug (doxorubicin) across all studied cancerous cell lines, while also demonstrating good safety effects on the normal human lung fibroblast cell line. Furthermore, all the synthesized compounds were examined for their antimicrobial activity against two Gram-positive bacteria (Staphylococcus aureus and Bacillussubtilis), one Gram negative bacterium (Escherichia coli) and two fungal species (Candida albinos and Aspergillus flatus). The antimicrobial results of the synthesized compounds, when compared with the reference drugs ampicillin and clotrimazole, revealed that compounds 3a, 3b, 3d, 4a-d, 9c and 10b exhibited excellent antimicrobial activities. Moreover, membrane stabilization or anti-hemolytic activity was employed as a method to study the in vitro anti-inflammatory activity of the prepared heterocyclic compounds. Antioxidant activities were also assessed by measuring the percentage of free radical scavenging. Compounds 4b, 10c and 11a-c demonstrated strong anti-hemolytic and antioxidant effects, which can be attributed to their ability to protect red blood cells from hemolysis.

Keywords:. Pyrimidine, colorectal carcinoma, antimicrobial, in vitro anti-inflammatory, antioxidant.

1. INTRODUCTION

The bioactive properties of heterocyclic compounds, especially those containing pyrimidine are well known. Pyrimidines have a rich history, dating back to their discovery as constituents of nucleic acids and their current use in AIDS chemotherapy. Allegan, a drug known for its diabetogenic effects in animals, along with three important nucleic acid constituents, uracil, thymine, and cytosine are pyrimidine derivatives. Vitamins such as thiamine, riboflavin, and folic acid also contain the pyrimidine ring. The pyrimidine derivative baritone is the first barbiturate hypnotic sedative and anticonvulsant.

Pyrimidine derivatives are also considered promising antineoplastic, antifolates, CNS active agents, cardiac agents, antihistaminic agents, analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), metabolic electrolytes and anticancer agents. Moreover, pyrimidine derivatives have been reported as anti-Alzheimer's agents , antiangiogenic agents, anticonvulsant agents , antidiabetic agents , antihepatitis agents , anti-inflammatory agents, antimalarial agents antimicrobial agents, antioxidant agents, antiparkinsonian agents, antiprotozoal agents antithyroid agents، antitubercular agents, antiviral agents, human urea transport protein (ut-b) inhibitors, immunosuppressant's and antiepileptic drugs due to their anticonvulsant activity.

The development of new protocols for the synthesis of pyrimidines has advanced significantly in recent years. To enhance their chemical and biological properties, several research teams have created pyrimidine derivatives with modified molecular structures 6-Amino-4-aryl-2-oxo-1,2- dihydropyrimidine-5-carbonitrile derivatives were used as starting materials for the synthesis of new pyrimidine and pyramid[4,5-d]pyrimidine derivatives. 6-Amino-4-aryl-2-oxo-1, 2-dihydropyrimidine-5-carbonitrile derivatives, were synthesized by the direct condensation of substituted aromatic aldehydes and urea with malononitrile in the presence of different catalysts, such as potassium carbonate, ammonium chloride under solvent-free conditions, phosphorous pentoxide, and in the absence of both solvent and catalyst using the Ball Mill method.

In recent years, a large number of new pyrimidine compounds have been created for their anticancer properties. The structure-activity relationship (SAR) of pyrimidine derivatives as anticancer agents over the past ten years is discussed. Topoisomerase IIα is often overexpressed in different types of tumors cells, especially in the G2/M phase of the cell cycle. Its inhibition leads to DNA double-strand breaks and apoptosis. Pyrimidine derivatives are capable of inhibiting the

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activity of topoisomerase IIα and intercalating DNA. Derivatives of pyrimidine can reduce the number of cells in the proliferation (S) and G2/M phases. Pyrimidines exhibit pro- apoptotic properties.

In the field of medicinal chemistry research, pyrimidine and pyramids [4, 5-d] pyrimidine hold a distinguished position due to their therapeutic and synthetic significance. Pyrimidine derivatives are valuable leads for drug discovery because of their important role in cellular processes. Finding new and powerful antibacterial medications is imperative to combat bacterial resistance and develop effective treatments, as harmful bacteria continuously evolve defense mechanisms against existing antibacterial. On the other hand, cytotoxic medications can inhibit the rapid growth and division of cancer cells. However, these cytotoxic drugs can also adversely affect other rapidly proliferating cells in the body, such as those in hair follicles and the intestinal lining. Consequently, treatment can damage a large number of healthy cells along with cancer cells. For these reasons, this study was conducted to synthesize novel pyrimidine and pyramids [4, 5-d] pyrimidine derivatives that possess antibacterial and cytotoxic properties with high safety for normal cells. The antioxidant and anti-inflammatory properties of the synthetic compounds were also assessed.

2. RESULTS AND DISCUSSION

Chemistry 6-Amino-4-aryl-2-oxo-1,2-dihydropyrimidine-5-carbonitrile derivatives 1a-dwere produced in good yield by reacting the respective aromatic aldehydes with malononitrile and urea in the presence of absolute ethanol and potassium carbonate. The elemental analysis and spectral data verified the chemical structure of compounds 1a-d. The IR spectra of 1a revealed regions of absorption at 3493, 3399, and 3177 cm−1 for (NH and NH2); 2217 cm−1 for (C ≡ N); and the presence of a carbonyl group (C = O) at 1693 cm−1. The1H-NMR spectrum of 1a showed singlet signals at δ 6.42 and δ 10.53 ppm for NH2, NH protons, which are D2O exchangeable, and a multiple signal at δ 7.33–7.45 ppm for aromatic protons. Furthermore, the mass spectrum of 1a showed a molecular ion peak at m/z 212 (M+, 35.64%) corresponding to its molecular formula C11H8ON4. The reaction of compounds 1a-d with POCl3 under reflux for 3 h furnished 6-amino-4-aryl-2-chloro-pyrimidine-5-carbonitrile 2a-d (Fig. 1). the chemical structures of compounds 2a-d were confirmed by spectral data and elemental analy- sis. The IR spectrum of 2a showed the absence of both (C = O) and (NH) groups and the appearance of absorp- tion bands at 3597 and 3474 cm−1 for (NH2), at 3070 cm−1 for (C-H aromatic), and absorption bands at 2206,

1632, 1464 and 766 cm−1for (C ≡ N), (C = C), (C = N) and (C-Cl), respectively. On the other hand, the1H-NMR spectrum of 2a showed a singlet signal at δ 7.15 ppm for (NH2) protons (D2O exchangeable) and a multiplet signal at δ 7.56–7.58 ppm for aromatic protons.

The reactions of compounds 2a-d with a mixture of acetic anhydride and acetic acid in the presence of a few drops of concentrated H2SO4 yielded 5-aryl-7-chloro-2-methyl-pyrimido[4,5-d]pyrimidine-4(3 H)-one 3a-d

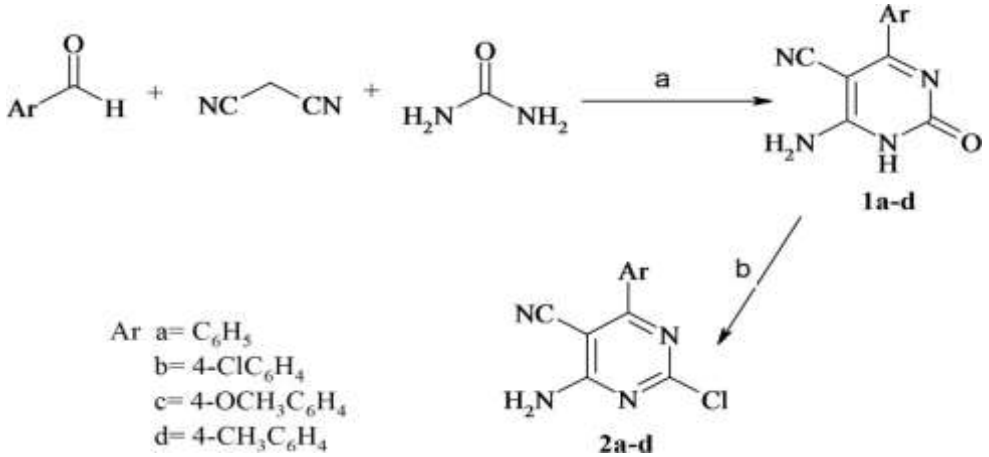

Fig. 1. Synthesis of compounds **1a-d**, **2a-d**. Reagents and conditions: **(a)** Potassium carbonate (0.1 mol), abs. EtOH (100 ml), reflux, 24 h. **(b)** $POCl_3$ (20 ml), reflux, 3 h.

(Fig. 2**).** the reaction sequence involved the acetylation of the amino group followed by hydrolysis of the cyano group and then cyclization and the formation of compounds **3a-d**. The IR spectrum of compound **3b** revealed the absence of a cyano group and the appearance of stretching bands at 3451, 2924 and 2853 cm^{-1} for (NH), aliphatic (C-H), and absorption bands at 1660 and 1602 cm^{-1} for (C = O), (C = C), 1491 cm^{-1} for (C = N) and 835 cm^{-1} for (C-Cl). The ^{1}H-NMR spectrum of **3b** showed a singlet signal at δ 2.43 ppm for (CH_3), and a singlet at δ 11.21 ppm for the (NH) proton (D_2O exchangeable). The mass spectrum of **3b** showed molecular ion peak at m/z 307 (M^+, 27%) corresponding to its molecular formula $C_{13}H_8N_4OCl_2$. Compounds **2a-d** were refluxed

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with hydrazine hydrate in the presence of absolute ethanol to afford 4-amino-6-aryl-5-cyano-2-hydrazino-Pyrimidine **4a-d**. The IR spectrum of compound **4a** showed absorption bands at 3444, 3323, and 3219 for the NH and NH_2 groups. Additionally, absorption bands at 3037, 2204, 1609 and 1565 cm^{-1}were observed for aromatic (C-H), (C ≡ N), (C = C) and (C = N), respectively. The ^{1}H-NMR spectrum of **4a** indicated that singlet signals were present at δ 4.27, 6.78 and 11.89 ppm for (NH_2-NH), (NH_2) and (NH), respectively, which are D_2O exchangeable, and a multiplet signal at δ 7.47–7.59 ppm was attributed to aromatic protons.

The reaction of a mixture of **2a-d** with ethanol amine in the presence of absolute ethanol under reflux for 3 h afforded **5a-d** which was confirmed by elemental and spectral analysis. The IR spectrum of compound **5a** showed strong absorption bands at 3518 cm^{-1} for (OH), 3443 cm^{-1} for (NH), 3322, 3217 cm^{-1} for (NH_2), 2924 cm^{-1} for (C-H aliphatic), 2204 cm^{-1} for (C ≡ N), 1609 cm^{-1} for (C = C) and 1564 $cm^{-1}$for (C = N). The^{1}H-NMR spectrum of compound **5a** showed two triplet signals at δ 3.48 and 3.51 ppm for (CH_2-N and CH_2-O), respectively. Additionally, its ^{1}H-NMR data revealed the presence of three singlet signals at δ 4.75, 7.19 and 7.69 ppm for the OH, NH_2 and NH protons, respectively (D_2O exchangeable). The aromatic protons of **5a** resonated as a multiplet signal at δ 7.21–7.48 ppm. The mass spectrum of compound **5a** showed a molecular ion peak at m/z 255 (M^+, 16%) corresponding to its molecular formula $C_{13}H_{13}ON_5$. Compounds **2a-d** reacted with methyl amine solution in the presence of absolute ethanol by heating under reflux to give 4-amino-6-aryl-2- (methylamine)-pyrimidine-5-carbonitrile **6a-d (**Fig. 2**)**. the chemical structure was confirmed by spectroscopic

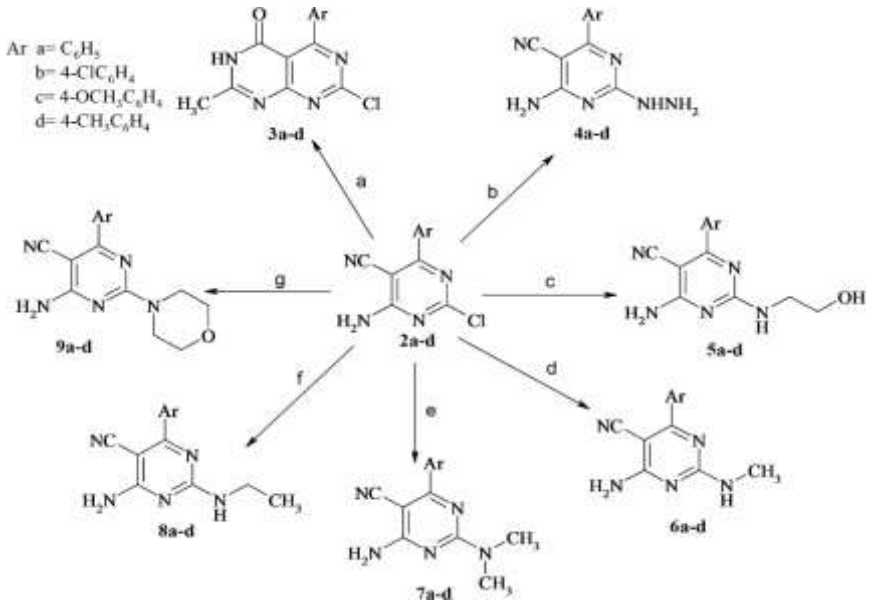

Fig. 2. Synthesis of compounds **3a-d** to **9a-d**. Reagents and conditions: **(a)** Acetic anhydride (10 ml), acetic acid (10 ml), conc. H_2SO_4 (2 drops), reflux, 20 h. **(b)** Hydrazine hydrate (5 ml), abs. EtOH (20 ml), reflux, 3 h. **(c)** Ethanol amine (5 ml), abs. EtOH (20 ml), reflux, 3 h. **(d)** Methyl amine (5 ml), abs. EtOH (20 ml), reflux, 3 h. **(e)** Dimethyl amine (5 ml), abs. EtOH (20 ml), reflux, 3 h. **(f)** Ethyl amine (5 ml), abs. EtOH (20 ml), reflux, 3 h. **(g)** Morpholine (5 ml), abs. EtOH (20 ml), reflux, 3 h.

Analysis where the IR spectrum of **6c** showed absorption bands at 3469, 3379 and 3365 cm^{-1} for NH and NH_2; at 2927 and 2864 cm^{-1} for aliphatic C-H; and at 2201, 1627 and 1557 $cm^{-1}$for (C ≡ N), (C = C) and (C = N), respectively. Furthermore, the ^{1}H-NMR spectrum of **6c** revealed three singlet signals at δ 2.89, 3.85 and 6.93 ppm for CH_3-N, OCH_3 and NH_2(D_2O exchangeable), a doublet doublet at δ 7.09–7.45 ppm for aromatic protons and a singlet signal at δ 7.95 ppm for the NH proton (D_2O exchangeable). Moreover, the reaction of **2a-d** with a dimethyl amine solution in refluxing absolute ethanol gave **7a-d** in good yield. The IR spectrum of **7b** showed characteristic absorption bands at 3488, 3374 cm^{-1} for (NH_2), 2921 cm^{-1} for (C-H aliphatic), 2205 cm^{-1} for(C≡N), 1611 cm^{-1} for (C=C), 1579 cm^{-1} for (C =N) and at 775 $cm^{-1}$for (C-Cl). The^{1}H-NMR spectrum of **7b** showed singlet signals at δ 3.24 ppm for (CH_3-N-CH_3), at δ 7.10 ppm for (NH_2) (D_2O exchangeable) and a doublet doublet at δ 7.53–7.64 ppm for (aromatic protons). ^{13}C-NMR for **7b** showed signals at δ 39.32 ppm for ($2CH_3$), 80.45 ppm for (C-CN), 116.68 ppm for (C≡N), 129.17, 131.09, 134.82, 135.24, 159.70, 160.39 and 161.21 ppm for seven types of aromatic carbons.

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The reaction of compounds **2a-d** with an ethyl amine solution in the presence of absolute ethanol afforded pyrimidine derivatives **8a-d**. The IR spectrum of **8a** presented stretching absorption bands at 3489, 3378, and 3336 cm^{-1} for NH and NH_2, respectively, and absorption bands at 2974, 2930, 2204, 1622 and 1562 cm^{-1} for (C-Haliphatic), (C≡N), (C=C) and (C=N), respectively. On the other hand, the ^{1}H-NMR spectrum of **8a** showed a triplet signal at δ 1.12–1.16 ppm for (CH_3-CH_2-N), a quarter signal at δ 3.42–3.50 ppm for (N-CH_2-CH_3) protons, a singlet signal at δ 6.88 ppm for (NH) proton (D_2O exchangeable) and a multiplet signal at δ 7.29–7.61 ppm for (NH_2) (D_2O exchangeable) and aromatic protons. The mass spectrum of compound **8a** showed a molecular ion peak at m/z 239 (M^+, 39.50%), corresponding to its molecular formula $C_{13}H_{13}N_5$. In addition, the reaction of **2a-d** with morphine in the presence of absolute ethanol yielded pyrimidine derivatives **9a-d**.

The chemical structure was confirmed by spectral analysis where the **IR** spectrum of **9b** presented characteristic absorption bands at 3395 and 3311 cm^{-1} for (NH_2), at 2977, 2903, and 2856 cm^{-1} for (C-H aliphatic), 2209 cm^{-1} for (C≡N), 1647 cm^{-1} for (C=C), 1575 cm^{-1} for (C=N), 1300 cm^{-1} for (C-O) and 1270 cm^{-1} for (C-N) of morphine, and an absorption band at 782 cm^{-1} for (C-Cl). Furthermore, the ^{1}H-NMR spectrum of **9b** showed two triplet signals at δ 3.70–3.74 ppm for (CH_2-N-CH_2) and 3.75–3.79 ppm for (CH_2-O-CH_2), a singlet signal at δ 7.09 ppm attributed to (NH_2) (D_2O exchangeable), and a doublet at δ 7.55–7.65 ppm for aromatic protons. ^{13}C-NMR for **9b** showed signals at δ 48.15 ppm for (CH_2-N-CH_2), at δ 66.39 ppm for (CH_2-O-CH_2), at δ 81.93 ppm for (C-CN), at δ 116.37 ppm for (C≡N) and at δ 129.23 (2 C), 131.12 (2 C), 134.50, 135.43, 160.14, 161.09 and 161.13 ppm for seven types of aromatic carbons.

The reaction of compounds **4a-c** with malononitrile in refluxing absolute ethanol afforded compounds **10a-c** (Fig. 3), and the reaction sequence involved nucleophilic attack on cyano groups and then cyclization. The IR spectrum of **10b** showed absorption bands at 3430, 3342, 3235 and 3201 cm^{-1} for NH_2, 2208 cm^{-1} for C≡N, 1630 cm^{-1} for C=C, 1579 m^{-1} for C=N and 780 cm^{-1} for C-Cl. The ^{1}H-NMR spectrum of **10b** showed a singlet signal at δ 6.59 ppm for the pyrazine proton and a multiplet signal at δ 7.46–7.67 ppm for three NH_2 protons.

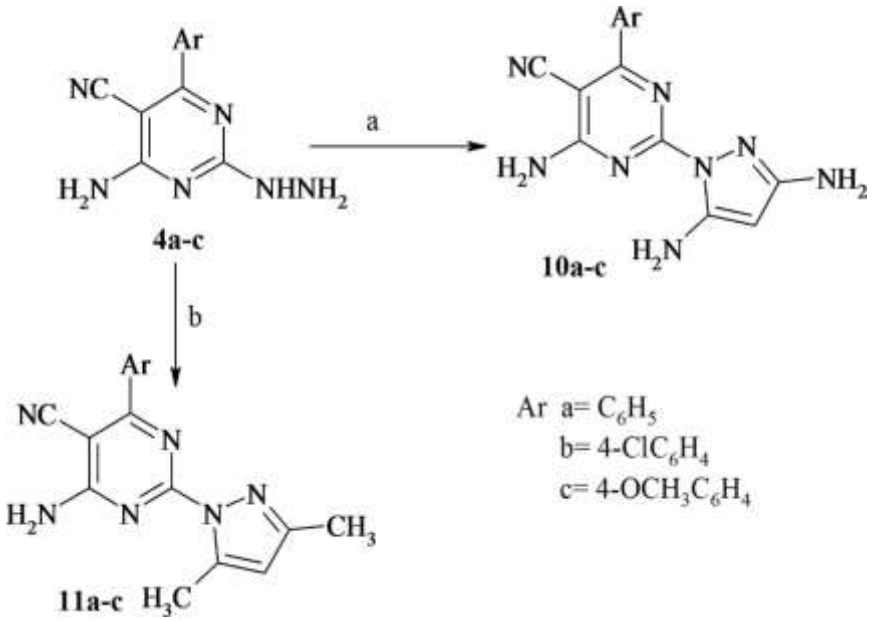

Fig. 3. Synthesis of compounds **10a-c**, **11a-c**. Reagents and conditions: **(a)** Malononitrile (0.79 g, 0.012 mol), abs. EtOH (20 ml), reflux, 3 h. **(b)** Acetyl acetone (1.2 g, 0.012 mol), abs. EtOH (20 ml), two drops of conc. HCl, reflux, 3 h.

(D_2O exchangeable) and aromatic protons. Finally, the reaction of compounds **4a-c** with acetyl acetone in refluxing absolute ethanol and drops of concentrated HCl afforded pyrimidine derivatives **11a-c**. The chemical structure was confirmed by spectral analysis, where the IR spectrum of **11c** showed absorption bands at 3429 and 3338 cm^{-1} for (NH_2); 2997, 2954, and 2933 cm^{-1} for (C-H aliphatic); 2209 cm^{-1} for (C≡N); 1629 cm^{-1} for (C=C); and 1577 cm^{-1} for (C=N). The ^{1}H-NMR spectrum of **11c** exhibited singlet signals at δ 2.44 and 3.17 ppm for two methyl protons and a singlet signal at δ 3.82 ppm for OCH_3 protons. Additionally, singlet signals at δ 6.34 and 7.31 ppm were attributed to pyrazine proton and NH_2 protons (D_2O exchangeable), whereas doublet at δ 7.52–7.69 ppm were attributed to aromatic protons.

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3. ANTIMICROBIAL ACTIVITY

The entire synthesized compounds were tested for their antimicrobial activity against two Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), one Gram-negative bacterium (Escherichia coli), and two fungi (Candida albicans and Aspergillus flavus). The results presented in Table 2 showed that, compounds **3a**, **3b**, **3d**, **4a-d**, **9c** and **10b** had strong antimicrobial effects against all the tested microorganisms compared to the reference drugs. Some compounds exhibited moderate antimicrobial activity, such as **1a**, **1b**, **1d**, **2a**, **3c**, **5b**, **5d**, **7a**, **7d**, **8a**, **9b** and **11b**. The remaining compounds showed weak properties, except compounds **2b**, **8b**, **8d** and **9d** which demonstrated no activity against any of the tested microorganisms. The potent antimicrobial activities of compounds **4a-d** can be attributed to their chemical structure, particularly the presence of a good donating hydrazino group. By comparing the antimicrobial results of the **4a-d** series with their structures, it was observed that the presence of electron-donating groups in the para position of the phenyl group (OCH_3, CH_3) increased the inhibition activity against all the tested bacteria and fungi compared to the presence of electron-withdrawing groups (Cl).

ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY

Membrane stabilization, or anti-hemolytic activity, has been used as a method to study in vitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane. Its stabilization implies that the tested compound may well stabilize lysosomal activities. Considerable progress has been made in recent years in relating aging to oxidation in biological cells. Reactive oxygen species (ROS), which cause oxidation in biological cells, are basically involved in the detoxification of invading organisms and chemicals. Additionally, ROS initiate lipid peroxidation in healthy cells leading to diverse pathologies such as Alzheimer's disease, atherosclerosis, diabetes, Parkinson's disease, and many other diseases. Thus, reducing the rates of these life-linacting metabolic processes by the use of newly synthesized chemical compounds is a great goal for many studies.

4. EXPERIMENTAL

Chemistry: Using a Sturat melting point apparatus, all melting points were measured in capillary tubes and were uncorrected. Using a Bruker Tensor 37 FT-IR, Perkin-Elemer FT/IR spectrophotometer (Alexandria University), and Thermo Scientific (Nicolet iS10 FT-IR Spectrometer, USA) (Mansoura University), the IR spectra were recorded on KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were acquired in DMSO-d_6 as the solvent using JEOL ECA-500 II (500 MHz) and Bruker (400 MHz) (Mansoura University and Kefir El-Sikh University), and tetramethylsilane (TMS) was used as an internal reference standard. A Vario III CHN analyzer (at the Regional Center for Mycology and Biotechnology, Al-Azhar University) was used to perform elemental analysis. On the Thermo Scientific ISQ LT (AL-Azhar University), mass spectra were recorded. All reactions were monitored and verified by TLC with normal hexane: ethyl acetate was used as a mobile phase, and silica gel plates 60 Fwere used to examine the spots under a UV lamp with a wavelength of 254 nm. The chemical reagents used for synthesis were purchased from Fluka, Sigma, and Aldrich.

General procedure for the Preparation of compounds 1a-d

Derivatives of 6-amino-4-aryl-2-oxo-1, 2-dihydropyrimidine-5-carbonitrile were synthesized by refluxing the corresponding substituted aromatic aldehyde (0.1 mol), malononitrile (0.1 mol) and urea (0.1 mol) with K_2CO_3 (0.1 mol) in absolute ethanol (100 ml) for 24 h. The reaction mixture was added to cold water, followed by acidification with acetic acid (diluted), and then the formed precipitate was washed with water, stirred well, filtered, dried and recrystallized from ethanol.

6-Amino-2-oxo-4-phenyl-1,2-dihydropyrimidine-5-carbonitrile 1a Color: yellow; yield: 89%; m.p: 180–182 °C (reported: 179–181 °C); IR (KBr) cm^{-1}: 3493, 3399 and 3177 (NH, NH_2), 2217 (C≡N), 1693 (C=O), 1574 (C=C), 1449 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 6.42 (s, 2 H, NH_2 (D_2O exchangeable)), 7.33–7.45 (m, 5 H, ArH), 10.53 (s, 1 H, NH (D_2O exchangeable)); mass spectrum (m/z, %): 212 (M^+, 35.64), 188 (74.23), 160 (100), 122 (74.66) and 77 (82); Anal. Calcd for $C_{11}H_8ON_4$ (212): C, 62.26; H, 3.77; N, 26.41. Found: C, 62.12; H, 4.06; N, 26.69.

Table 1. Antimicrobial activities of the synthesized compounds. • NA → No activity.

	Gram-negative bacteria		Gram-positive bacteria				Fungi			
	E. coli		S. aureus		Bacillus subtilis		C. Albicans		A. flavus	
	Diameter of inhibition	%	Diameter of	%	Diameter of	%	Diameter of inhibition zone	%	Diameter of	% Activit

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Compound	zone (mm)	Activity index	inhibition zone (mm)	Activity index	inhibition zone (mm)	Activity index	(mm)	Activity index	inhibition zone (mm)	y index
1a	10	57.7	14	63.6	16	69.6	19	70.4	18	72
1b	14	53.8	13	59.1	16	69.6	19	70.4	17	68
1c	6	23.1	9	40.9	11	47.8	14	51.8	12	48
1d	10	38.5	11	50	14	60.9	18	66.7	16	64
2a	11	42.3	13	59.1	15	65.2	20	74.1	18	72
2b	NA	----	NA	----	NA	----	NA	----	NA	----
2c	9	34.6	10	45.4	12	52.2	16	59.2	15	60
2d	7	26.9	9	40.9	11	47.8	16	59.2	16	64
3a	18	69.2	17	77.3	19	82.6	22	81.5	20	80
3b	19	73.1	18	81.8	20	86.9	23	85.2	22	88
3c	11	42.3	10	45.4	13	56.5	14	51.8	13	52
3d	17	65.4	17	77.3	19	82.6	23	85.2	21	84
4a	21	80.8	20	90.9	21	91.3	24	88.9	22	88
4b	19	73.1	19	86.4	20	86.9	23	85.2	22	88
4c	22	84.6	20	90.9	21	91.3	24	88.9	23	92
4d	22	84.6	21	95.4	23	100	25	92.6	23	92
5a	4	15.4	5	22.7	7	30.4	13	48.1	11	44
5b	13	50	12	54.5	15	65.2	19	70.4	17	68
5c	NA	----	NA	----	4	17.4	10	37	7	28
5d	16	61.5	15	68.2	18	78.3	22	81.5	20	80
6a	NA	----	NA	----	NA	----	4	14.8	3	12
6b	NA	----	NA	----	NA	----	7	25.9	5	20
6c	NA	----	NA	----	3	13	6	22.2	4	16
6d	5	19.2	8	36.4	9	39.1	15	55.5	14	56
7a	12	46.1	11	50	14	60.9	17	63	16	64
7b	NA	----	NA	----	NA	----	8	29.6	5	20
7c	9	34.6	10	45.4	13	56.5	18	66.7	17	68
7d	16	61.5	15	68.2	16	69.6	20	74.1	18	72
8a	15	57.7	15	68.2	18	78.3	20	74.1	18	72
8b	NA	----	NA	----	NA	----	NA	----	NA	----
8c	NA	----	NA	----	NA	----	3	11.1	NA	----
8d	NA	----	NA	----	NA	----	NA	----	NA	----
9a	5	19.2	7	31.8	8	34.8	13	48.1	11	44
9b	10	38.5	12	54.5	15	65.2	18	66.7	19	76
9c	17	65.4	17	77.3	19	82.6	21	77.8	19	76
9d	NA	----	NA	----	NA	----	NA	----	NA	----
10a	3	11.5	7	31.8	8	34.8	12	44.4	9	36
10b	20	76.9	19	86.4	20	86.9	23	85.2	22	88

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10c	8	30.8	10	45.4	12	52.2	18	66.7	12	48
11a	NA	----	NA	----	6	26.1	11	40.7	8	32
11b	10	38.5	12	54.5	15	65.2	19	70.4	17	68
11c	NA	----	3	13.6	7	30.4	11	40.7	9	36
Ampicillin	26	100	22	100	23	100	----	----	----	----
ClOtrimazole	----	----	----	----	----	----	27	100	25	100

Table 2. The Anti-inflammatory activity (R.B.C. Membrane stabilization %) and antioxidant activities (free radical scavenging %) of the synthesized compounds.

Compound	R.B.Cs membrane stabilization (%)	Free Radical Scavenging (%)
1a	34.09 ± 0.87	22.10 ± 0.74
1b	22.50 ± 1.35	14.54 ± 0.97
1c	30.08 ± 2.01	11.85 ± 0.41
1d	35.27 ± 1.48	41. 75 ± 1.56
2a	58.91 ± 0.83	51.28 ± 1.22
2b	56.33 ± 1.28	50.03 ± 1.79
2c	63.20 ± 0.57	60.38 ± 1.85
2d	39.42 ± 1.23	18.52 ± 0.29
3a	50.86 ± 0.92	34.50 ± 0.51
3b	58.78 ± 1.61	38.97 ± 0.67
3c	61.24 ± 2.12	40.54 ± 1.30
3d	44.75 ± 1.65	36.18 ± 1.07
4a	64.82 ± 1.77	61.02 ± 1.16
4b	69.33 ± 1.42	71.34 ± 0.86
4c	65.74 ± 1.33	53.42 ± 0.73
4d	60.05 ± 2.24	49.31 ± 0.68
5a	45.89 ± 1.90	39.75 ± 0.92
5b	52.35 ± 1.18	48.11 ± 0.64
5c	57.20 ± 0.86	40.99 ± 0.81
5d	60.19 ± 1.65	42.15 ± 0.75
6a	18.50 ± 0.34	11.40 ± 0.48
6b	32.40 ± 0.82	9.66 ± 0.58
6c	24.18 ± 0.71	8.22 ± 0.34
6d	18.92 ± 0.43	10.36 ± 0.59
7a	66.85 ± 0.75	54.19 ± 1.26
7b	64.28 ± 1.83	58.48 ± 0.96
7c	51.24 ± 1.37	48.62 ± 1.17
7d	58.70 ± 0.42	52.33 ± 0.72
8a	32.44 ± 0.76	36.75 ± 0.83
8b	57.31 ± 1.18	44.32 ± 1.08
8c	50.04 ± 1.66	42.88 ± 0.73

8d	48.44 ± 1.07	43.05 ± 0.79
9a	51.28 ± 1.39	45.02 ± 1.31
9b	30.51 ± 0.77	14.46 ± 0.34
9c	28.13 ± 0.53	18.23 ± 0.78
9d	63.42 ± 0.80	55.24 ± 1.37
10a	55.83 ± 0.46	50.98 ± 0.66
10b	52.37 ± 0.94	46.75 ± 0.49
10c	72.62 ± 2.01	77.56 ± 1.80
11a	70.05 ± 1.90	69.86 ± 1.33
11b	69.38 ± 1.70	70.15 ± 0.84
11c	67.32 ± 1.44	66.17 ± 1.19
Reference drugs	Aspirin (acetylsalicylic acid) 76.33 ± 1.89	Ascorbic acid 81.36 ± 1.72

6-Amino-4-(4-chlorophenyl)−2-oxo-1,2-dihydropyrimidine-5-carbonitrile 1b

Color: white; yield: 80%; m.p: 163–165 °C (reported: 162–164 °C); IR (KBr) cm^{-1}: 3475, 3424 and 3363 (NH, NH_2), 3107 (C-H aromatic), 2206 (C ≡ N), 1674 (C = O), 1624 (C = C), 1559 (C = N), 701 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 6.40 (s, 2 H, NH_2 (D_2O exchangeable)), 7.39–7.54 (dd, 4 H, ArH, J = 51, 7.6 Hz), 10.43 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{11}H_7ON_4Cl$ (246.45): C, 53.56; H, 2.84; N, 22.72. Found: C, 53.39; H, 2.99; N, 22.59.

6-Amino-4-(4-methoxyphenyl)−2-oxo-1,2-dihydropyrimidine-5-carbonitrile 1c

Color: yellow; yield: 82%; m.p: 157–159 °C (reported: 156–157 °C); IR (KBr) cm^{-1}: 3377, 3312 and 3190 (NH, NH_2), 2921, 2851 (C-H aliphatic), 2201 (C ≡ N), 1653 (C = O), 1605 (C = C), 1561 (C = N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.83 (s, 3 H, OCH_3), 6.43 (s, 2 H, NH_2 (D_2O exchangeable)), 7.11–7.54 (dd, 4 H, ArH, J = 203.5, 8.5 Hz), 10.57 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{12}H_{10}O_2N_4$ (242): C, 59.50; H, 4.13; N, 23.14. Found: C, 59.74; H, 4.02; N, 23.30.

6-Amino-4-(4-methylphenyl)−2-oxo-1,2-dihydropyrimidine-5-carbonitrile 1d

Color: brown; yield: 85%; m.p: 150–152 °C (reported: 148–150 °C); IR (KBr) cm^{-1}: 3471, 3338 and 3209 (NH, NH_2), 3027 (C-H aromatic), 2918, 2850 (C-H aliphatic), 2201 (C ≡ N), 1659 (C = O), 1543 (C = C), 1513 (C = N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.37 (s, 3 H, CH_3), 6.62 (s, 2 H, NH_2 (D_2O exchangeable)), 7.21–7.37 (dd, 4 H, ArH, J = 60, 4 Hz), 10.37 (s,1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{12}H_{10}ON_4$ (226): C, 63.71; H,4.42; N, 24.77. Found: C, 63.60; H, 4.58; N, 24.90.

General procedure for the Preparation of compounds 2a-d

Compounds **1a-d** (0.05 mol) were refluxed with $POCl_3$ (20 ml) for 3 h. The reaction mixture was allowed to cool, and then poured into ice-cold water. The precipitate formed was filtered, dried and recrystallized from ethanol.

4-Amino-2-chloro-6-phenylpyrimidine-5-carbonitrile 2a

Color: dark brown; yield: 56%; m.p: 294–296 °C; IR (KBr) cm^{-1}: 3597, 3474 (NH_2), 3070 (C-H aromatic), 2206 (C ≡ N), 1632 (C = C), 1464 (C = N), 766 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 7.15 (s, 2 H, NH_2 (D_2O exchangeable)), 7.56–7.58 (m, 5 H, ArH); Anal. Calcd for $C_{11}H_7N_4Cl$ (230.45): C, 57.28; H, 3.04; N, 24.30.
Found: C, 57.15; H, 3.11; N, 24.40.

4-Amino-2-chloro-6-(4-chlorophenyl)pyrimidine-5-carbonitrile 2b

Color: brown; yield: 59%; m.p: 292–294 °C; IR (KBr) cm^{-1}: 3448, 3409 (NH_2), 2204 (C ≡ N), 1570 (C = C), 1490 (C = N), 723 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 7.05 (s, 2 H, NH_2 (D_2O exchangeable)), 7.60–7.68 (dd, 4 H, ArH, J = 35, 10 Hz); Anal. Calcd for $C_{11}H_6N_4Cl_2$ (265): C, 49.81; H, 2.26; N, 21.13. Found: C, 49.96; H, 2.11; N,

21.29.

4-Amino-2-chloro-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile 2c

Color: brown; yield: 54%; m.p: 274–276 °C; IR (KBr) cm^{-1}: 3388, 3229 (NH_2), 3083 (C-H aromatic), 2918, 2847 (C-H aliphatic), 2214 (C≡N), 1608 (C=C), 1577 (C=N), 793 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.81 (s, 3 H, OCH_3), 6.51 (s, 2 H, NH_2 (D_2O exchangeable)); 7.00–7.34 (dd, 4 H, ArH, J = 120, 8 Hz); Anal. Calcd for $C_{12}H_9N_4ClO$ (260.45): C, 55.29; H, 3.46; N, 21.50. Found: C, 55.40; H, 3.35; N, 21.55.

4-Amino-2-chloro-6-(4-methylphenyl)pyrimidine-5-carbonitrile 2d

Color: pale brown; Yield: 61%; m.p: 284–286 °C; IR (KBr) cm^{-1}: 3483, 3416 (NH_2), 3028 (C-H aromatic), 2923, 2860 (C-H aliphatic), 2202 (C≡N), 1635 (C=C), 1554 (C=N), 815 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.38 (s, 3 H, CH_3), 6.12 (s, 2 H, NH_2 (D_2O exchangeable)), 7.13–7.27 (dd, 4 H, ArH, J = 44, 12 Hz); Anal. Calcd for $C_{12}H_9N_4Cl$ (244.45): C, 58.91; H, 3.68; N, 22.91. Found: C, 58.61; H, 3.59; N, 23.08.

General procedure for the Preparation of compounds 3a-d

The corresponding derivatives of 5-aryl-7-chloro-2-methyl-pyrimido[4,5-d]pyrimidine-4(3 H)-one **3a-d** were prepared via the reaction of **2a-d** (0.01 mol) with a mixture of acetic acid (10 ml) and acetic anhydride (10 ml) with 2 drops of concentrated H_2SO_4. The mixture was refluxed for 20 h, allowed to cool, poured into ice-cold water, filtered, and recrystallized from DMF.

7-Chloro-2-methyl-5-phenyl-pyrimido[4,5-d]pyrimidine-4(3 H)-one 3a

Color: brown; yield: 59%; m.p: 192–194 °C; IR (KBr) cm^{-1}: 3467 (NH), 3060 (C-H aromatic), 2925 (C-H aliphatic), 1655 (C=O), 1599 (C=C), 1494 (C=N), 700 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.43 (s, 3 H, CH_3),7.07–7.37 (m, 5 H, ArH), 12.34 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{13}H_9N_4OCl$ (272.45): C,57.26; H, 3.30; N, 20.55. Found: C, 57.40; H, 3.26; N, 20.67.

7-Chloro-2-methyl-5-(4-chlorophenyl)-pyrmido[4,5-d]pyrimidine-4(3 H)-one 3b

Color: reddish brown; yield: 52%; m.p: 200–202 °C; IR (KBr) cm^{-1}: 3451 (NH), 2924, 2853 (C-H aliphatic), 1660 (C=O), 1602 (C=C), 1491 (C=N), 835 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.43 (s, 3 H, CH_3), 7.31–7.37 (m,4H, ArH), 11.21 (s, 1 H, NH (D_2O exch angeable)); Mass spectrum (m/z,%): 307 (M^+, 27), 299 (53), 243 (62), 180 (84), 149 (100) and 77 (37); Anal. Calcd for $C_{13}H_8N_4OCl_2$ (307): C, 50.81; H, 2.61; N, 18.24. Found: C, 51.09; H, 2.71; N, 18.45.

7-Chloro-2-methyl-5-(4-methoxy-phenyl)pyrimido[4,5-d] pyrimidine-4(3 H)-one 3c

Color: dark brown; yield: 57%; m.p: 222–224 °C; IR (KBr) cm^{-1}: 3450 (NH), 2918, 2849 (C-H aliphatic), 1703 (C=O), 1645 (C=C), 1606 (C=N), 721 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.36 (s, 3 H, CH_3), 3.82 (s,3 H, OCH_3), 7.35–7.45 (dd, 4 H, ArH, J = 32, 8 Hz), 12.27 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{14}H_{11}N_4O_2Cl$ (302.45): C, 55.55; H, 3.64; N, 18.52. Found: C, 55.41; H, 3.79; N, 18.39.

7-Chloro-2-methyl-5-(4-methyl phenyl)pyrimido[4,5-d] pyrimidine-4(3 H)-one 3d Color: reddish brown; yield: 54%; m.p: 247–249 °C; IR (KBr) cm^{-1}: 3448 (NH), 2923, 2852 (C-H aliphatic), 1656 (C=O), 1599 (C=C), 1491 (C=N), 856 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.39 (s, 3 H, CH_3), 2.43 (s, 3 H, CH_3), 7.19–7.26 (dd, 4 H, ArH, J = 20, 8 Hz), 12.47 (s, 1 H, NH, (D_2O exchangeable)); Anal. Calcd for $C_{14}H_{11}N_4OCl$ (286.45): C, 58.65; H, 3.84; N, 19.55. Found: C, 58.70; H, 3.71; N, 19.67.

General procedure for the Preparation of compounds 4a-d

4-Amino-2-chloro-6-substituted phenylpyrimidine-5-carbonitrile **2a-d** (0.01 mol) was refluxed with hydrazine hydrate (5 ml) for 3 h in the presence of absolute ethanol (20 ml), after that the mixture was allowed to cool, filtered, dried and recrystallized from ethanol.

4-Amino-2-hydrazino-6-phenylpyrimidine-5-carbonitrile 4a

Color: orange; yield: 64%; m.p: 285–287 °C; IR (KBr) cm^{-1}: 3444, 3323 and 3219 (NH, NH_2), 3037 (C-H aromatic), 2204 (C≡N), 1609 (C=C), 1565 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 4.27 (s, 2 H, NH_2 (D_2O exchangeable)), 6.78 (s, 2 H, NH_2, (D_2O exchangeable)), 7.47–7.59 (m, 5 H, ArH), 11.89 (s,1 H, HN (D_2O exchangeable)); Anal. Calcd for $C_{11}H_{10}N_6$ (226): C, 58.41; H, 4.42; N, 37.17. Found: C, 58.64; H, 4.31; N, 37.33.

4-Amino-6-(4-chlorophenyl)−2-hydrazinopyrimidine-5-carbonitrile 4b

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Color: reddish brown; yield: 58%; m.p: 280–282 °C; IR (KBr) cm^{-1}: 3446, 3335 and 3218 (NH, NH_2), 3015 (C-H aromatic), 2205 (C≡N), 1616 (C=C), 1650 (C=N), 782 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 4.37 (s, 2 H, NH_2, (D_2O exchangeable)), 6.85 (s, 2 H, NH_2 (D_2O exchangeable)), 7.55–7.67 (dd, 4 H, ArH, J = 40.8, 8.4 Hz), 11.93 (s,1 H, HN (D_2O exchangble)); Anal. Calcd for $C_{11}H_9N_6Cl$ (260.45): C, 50.68; H, 3.46; N, 32.25. Found: C, 50.52; H, 3.61; N, 32.09.

4-Amino-2-hydrazino-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile 4c

Color: yellow; yield: 66%; m.p: 289–291 °C; IR (KBr) cm^{-1}: 3479, 3426, 3367, 3218, 3153 (NH, NH_2), 2929 (C-H aliphatic), 2204 (C≡N), 1625 (C=C), 1580 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.82 (s, 3 H, OCH_3), 4.32 (s, 2 H, NH_2 (D_2O exchangble)), 6.66 (s, 2 H, NH_2 (D_2O exchangble)), 7.06–7.42 (dd, 4 H, ArH, J = 175.5, 8.5 Hz),11.92 (s, 1 H, HN (D_2O exchangble)); Anal. Calcd for $C_{12}H_{12}ON_6$ (256): C, 56.25; H, 4.69; N, 32.81. Found: C, 56.04; H, 4.87; N, 32.65.

4-Amino-2-hydrazino-6-(4-methyl phenyl)pyrimidine-5-carbontrile 4d

Color: pale brown; yield: 54%; m.p: 270–272 °C; IR (KBr) cm^{-1}: 3422, 3364, 3320, 3186 (NH, NH_2), 2921, 2851 (C-H aliphatic), 2204 (C≡N), 1624 (C=C), 1581 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.34 (s, 3 H, CH_3), 4.29 (s, 2 H, NH_2 (D_2O exchangeable)), 6.68 (s, 2 H, NH_2 (D_2O exchangeable)), 7.10–7.35 (dd, 4 H, ArH, J = 80, 8 Hz), 11.76 (s,1 H, HN (D_2O exchangeable)); Anal. Calcd for $C_{12}H_{12}N_6$ (240): C, 60.00; H, 5.00; N, 35.00. Found: C,59.91; H, 4.92; N, 35.12.

General procedure for the Preparation of compounds 5a-d

Compounds **2a-d** (0.01 mol) were refluxed with ethanol amine (5 ml) in absolute ethanol (20 ml) for 3 h. Then the reaction mixture was allowed to cool, filtered, and dried and the precipitate was recrystallized from ethanol to yield compounds **5a-d**.

4-Amino-2-((2-hydroxyethyl)amino)−6-phenylpyrimidine-5-carbonitrile 5a

Color: orange; yield: 59%; m.p: 210–212 °C; IR (KBr) cm^{-1}: 3518 (OH), 3443 (NH), 3322, 3217 (NH_2), 2924 (C-H aliphatic), 2204 (C≡N), 1609 (C=C), 1564 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.48–3.56 (t, 4 H, $2CH_2$), 4.75 (s, 1 H, OH (D_2O exchangeable)), 7.19 (s, 2 H, NH_2 (D_2O exchangeable)), 7.21–7.48 (m, 5 H, ArH), 7.69 (s, 1 H, NH (D_2O exchangeable)); Mass spectrum (m/z,%): 255 (M^+, 16), 189 (54), 170 (58), 75 (100), 77 (14); Anal. Calcd for $C_{13}H_{13}ON_5$ (255): C, 61.18; H, 5.10; N, 27.45. Found: C, 60.89; H, 5.15; N, 27.70.

4-Amino-2-((2-hydroxyethyl)amino)−6-(4-chlorophenyl)pyrimidine-5-carbonitrile 5b

Color: dark yellow; yield: 54%; m.p: 226–228 °C; IR (KBr) cm^{-1}: broad band at 3421, 3339, 3240 (OH, $NH_2$and NH), 2920, 2851 (C-H aliphatic), 2207 (C≡N), 1646 (C=C), 1584 (C=N), 775 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ ppm: 3.49–3.56 (t, 2 H, N-CH_2), 3.63–3.66 (t, 2 H, O-CH_2), 4.76 (s, 1 H, OH (D_2O exchangeable)), 7.27 (s, 2 H, NH_2 (D_2O exchangeable)), 7.44–7.62 (dd, 4 H, ArH, J = 45, 24 Hz), 7.64 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{13}H_{12}ON_5Cl$ (289.45): C, 53.89; H, 4.16; N, 24.18. Found: C, 53.96; H, 4.25; N, 24.06.

4-Amino-2-((2-hydroxyethyl)amino)−6-(4-methoxyphenyl)pyrimidine-5-carbonitrile 5c

Color: yellow; yield: 66%; m.p: 229–231 °C; IR (KBr) cm^{-1}: 3448, 3419, 3381, 3335 (OH, NH and NH_2), 2958, 2925, 2848 (C-H aliphatic), 2206 (C≡N), 1643 (C=C), 1583 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.44–3.48 (t,2 H, N-CH_2), 3.54–3.57 (t, 2 H, O-CH_2), 3.84 (s, 3 H, OCH_3), 4.82 (s, 1 H, OH (D_2O exchangeable)), 6.86 (s, 2 H, NH_2 (D_2O exchangeable)), 7.09–7.45 (dd, 4 H, ArH, J = 136.4, 8.4 Hz), 7.62 (s, 1 H, NH_2 (D_2O exchangeable)); Anal. Calcd for $C_{14}H_{15}O_2N_5$ (285): C, 58.94; H, 5.26; N, 24.56. Found: C, 58.78; H, 5.49; N, 24.71.

4-Amino-2-((2-hydroxyethyl)amino)−6-(4-methylphenyl)pyrimidine-5-carbonitrile 5d

Color: light brown; yield: 58%; m.p: 240–242 °C; IR (KBr) cm^{-1}: 3491, 3422, 3348, 3144 (OH, NH and NH_2), 2964, 2921, 2852 (C-H aliphatic), 2207 (C≡N), 1647 (C=C), 1623 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.39 (s, 3 H, CH_3), 3.44–3.49 (t, 2 H, N-CH_2), 3.54–3.61 (t, 2 H, O-CH_2), 4.66 (s, 1 H, OH (D_2O exchangeable)), 7.06 (s, 2 H, NH_2 (D_2O exchangeable)), 7.18–7.36 (dd, 4 H, ArH, J = 52, 40 Hz), 7.77 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{14}H_{15}ON_5$ (269): C, 58.94; H, 5.30; N, 24.55. Found: C, 58.80; H, 5.51; N, 24.63.

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General procedure for the Preparation of compounds 6a-d

A mixture of compounds **2a-d** (0.01 mol) with 40% methyl amine solution (5 ml) in the presence of absolute ethanol (20 ml) was heated under reflux for 3 h. The reaction mixture was allowed to cool, and the formed precipitate was subsequently filtered and recrystallized from ethanol to afford compounds **6a-d**.

4-Amino-2-(methylamino)−6-phenyl pyrimidine-5-carbonitrile 6a

Color: light brown; yield: 62%; m.p:175–177 °C; IR (KBr) cm^{-1}: 3488, 3346, 3229 (NH and NH_2), 2962, 2922 (C-H aliphatic), 2207 (C ≡ N), 1629 (C = C), 1563 (C = N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.89 (s, 3 H, CH_3), 7.39 (s, 2 H, NH_2 (D_2O exchangeable)), 7.42–7.54 (m, 5 H, ArH), 7.96 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{12}H_{11}N_5$ (225): C, 63.99; H, 4.88; N, 31.11. Found: C, 64.12; H, 5.11; N, 31.30.

4-Amino-6-(4-chlorophenyl)−2-methylaminopyrimidine-5-carbonitrile 6b

Color: light brown; yield: 67%; m.p:199–201 °C; IR (KBr) cm^{-1}: 3444, 3350, 3239 (NH, NH_2), 2925, 2891 (C-H aliphatic), 2204 (C ≡ N), 1640 (C = C), 1585 (C = N), 774 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.89 (s, 3 H, CH_3), 7.48 (s, 2 H, NH_2 (D_2O exchangeable)), 7.51–7.65 (dd, 4 H, ArH, J = 48, 8 Hz), 7.95 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{12}H_{10}N_5Cl$ (259.45): C, 55.50; H, 3.85; N, 26.98. Found: C, 55.39; H, 3.72; N, 26.83.

4-Amino-6-(4-methoxyphenyl)−2-methylaminopyrimidine-5-carbonitrile 6c

Color: light brown; yield: 71%; m.p: 206–208 °C; IR (KBr) cm^{-1}: 3469, 3379, 3365 (NH, NH_2), 2927, 2864 (C-H aliphatic), 2201 (C ≡ N), 1627 (C = C), 1557 (C = N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.89 (s, 3 H, CH_3), 3.85 (s, 3 H, OCH_3), 6.93 (s, 2 H, NH_2 (D_2O exchangeable)), 7.09–7.45 (dd, 4 H, ArH, J = 136, 8 Hz), 7.95 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{13}H_{13}ON_5$ (255): C, 61.18; H, 5.10; N, 27.45. Found: C, 61.30; H, 5.05;N, 27.33.

4-Amino-6-(4-methylphenyl)−2-methylaminopyrimidine-5-carbonitrile 6d

Color: brown; yield: 69%; m.p: 203–205 °C; IR (KBr) cm^{-1}: 3472, 3365, 3302 (NH, NH_2), 2919, 2850 (C-H aliphatic), 2204 (C ≡ N), 1637 (C = C), 1564 (C = N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.40 (s, 3 H, CH_3), 2.89 (s, 3 H, N-CH_3), 6.79 (s, 2 H, NH_2 (D_2O exchangeable)), 7.25–7.60 (dd, 4 H, ArH, J = 108, 32 Hz), 7.82 (s, 1 H, NH (D_2O exchangeable)); Anal. Calcd for $C_{13}H_{13}N_5$ (239): C, 65.27; H, 5.44; N, 29.29; Found: C, 65.36; H, 5.37; N,29.13.

General procedure for the preparation of compounds 7a-d

4-Amino-6-aryl-2-(dimethylamino)pyrimidine-5-carbonitrile **7a-d** were synthesized by refluxing of **2a-d** (0.01 mol) with a dimethyl amine solution of 40% (5 ml) in absolute ethanol (20 ml) for 3 h. The mixture was left to cool, then filtered, dried and recrystallized from ethanol.

4-Amino-2-(dimethylamino)−6-phenylpyrimidine-5-carbonitrile 7a

Color: brown; yield: 64%; m.p: 253–255 °C; IR (KBr) cm^{-1}: 3321, 3220 (NH_2), 3056 (C-H aromatic), 2927 (C-H aliphatic), 2205 (C ≡ N), 1625 (C = C), 1564 (C = N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.20 (s, 6 H, $2CH_3$), 7.06 (s, 2 H, NH_2 (D_2O exchangeable)), 7.36–7.62 (m, 5 H, ArH); Anal. Calcd for $C_{13}H_{13}N_5$ (239): C, 65.24; H, 5.44; N, 29.29. Found: C, 65.17; H, 5.56; N, 29.37.

4-Amino-6-(4-chlorophenyl)−2-(dimethylamino)pyrimidine-5-carbonitrile 7bColor: light brown; yield: 51%; m.p: 256–258 °C; IR (KBr) cm^{-1}: 3488, 3374 (NH_2), 2921 (C-H aliphatic), 2205 (C ≡ N), 1611 (C = C), 1579 (C = N), 775 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.24 (s, 6 H, $2CH_3$), 7.10 (s, 2 H, NH_2, (D_2O exchangeable)), 7.53–7.64 (dd, 4 H, ArH, J= 36, 8 Hz); ^{13}C-NMR (DMSO-d_6) δ/ppm: 39.32 ($2CH_3$),80.45 (C-CN), 116.68 (C ≡ N), 129.17 (2 C), 131.09 (2 C), 134.82, 135.24, 159.70, 160.39, 161.21 (aromatic carbons); Anal. Calcd for $C_{13}H_{12}N_5Cl$ (273.45): C, 57.04; H, 4.39; N, 25.59. Found: C, 57.19; H, 4.58; N, 25.36.

4-Amino-2-(dimethylamino)−6-(4-methoxyphenyl)pyrimidine-5-carbonitrile 7c

Color: reddish brown; yield: 66%; m.p: 279–281 °C; IR (KBr) cm^{-1}: 3410, 3350 (NH_2), 2927, 2877 (C-H aliphatic), 2203 (C ≡ N), 1635 (C = C), 1560 (C = N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.24 (s, 6 H, $2CH_3$), 3.85 (s, 3 H, OCH_3), 6.96 (s, 2 H, NH_2 (D_2O exchangeable)), 7.10–7.48 (dd, 4 H, ArH, J= 144, 8 Hz); ^{13}C-NMR (DMSO-d_6) δ/ppm:

39.25 ($2CH_3$), 55.75 (OCH_3), 80.54 (C-CN), 114.36 (2 C), 117.10 (CN), 127.84 (2 C), 130.89, 136.94, 160.89, 160.98, 162.03 (aromatic carbons); Anal. Calcd for $C_{14}H_{15}ON_5$ (269): C, 62.45; H, 5.58; N, 26.02. Found: C,62.25; H, 5.73; N, 25.92.

4-Amino-2-(dimethylamino)−6-(4-methylphenyl)pyrimidine-5-carbonitrile 7d

Color: brown; yield: 72%; m.p: 286–288 °C; IR (KBr) cm^{-1}: 3410, 3337 (NH_2), 3100 (C-H aromatic), 2964, 2932, 2840 (C-H aliphatic), 2214 (C≡N), 1646 (C=C), 1610 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.34 (s, 3 H, CH_3),3.25 (s, 6 H, $2CH_3$), 7.06 (s, 2 H, NH_2 (D_2O exchangeable)), 7.33–7.48 (dd, 4 H, ArH, J=43, 16 Hz); Anal. Calcdfor $C_{14}H_{15}N_5$ (253):C, 66.40; H, 5.93; N, 27.67. Found:C, 66.31; H, 6.10; N, 27.73.

General procedure for the preparation of compounds 8a-d

To obtain compounds **8a-d**, compounds **2a-d**, (0.01 mol) were added to (5 ml) ethylamine solution in (20 ml) absolute ethanol, refluxed for 3 h, and then left to cool at room temperature. The precipitate formed was collected by filtration, dried and recrystallized from ethanol.

4-Amino-2-(ethylamino)−6-phenylpyrimidine-5-carbonitrile 8a

Color: reddish brown; yield: 61%; m.p: 233–235 °C; IR (KBr) cm^{-1}: 3489, 3378, 3336 (NH, NH_2), 2974, 2930 (C-H aliphatic), 2204 (C≡N), 1622 (C=C), 1562 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 1.12–1.16 (t, 3 H, CH_3),3.42–3.50 (q, 2 H, CH_2), 6.88 (s,1 H, NH (D_2O exchangeable)), 7.29–7.61 (m, 7 H, NH_2 (D_2O exchangeable) and ArH); Mass spectrum (m/z, %): 239 (M^+, 39.50), 220 (83), 132 (100) and 129 (97); Anal. Calcd for $C_{13}H_{13}N_5$ (239): C, 65.27; H, 5.44; N, 29.29. Found: C, 65.38; H, 5.29; N, 29.32 .

4-Amino-6-(4-chlorophenyl)−2-(ethyamino)pyrimidine-5-cabonitrile 8b

Color: brown; yield: 63%; m.p: 257–259 °C; IR (KBr) cm^{-1}: 3485, 3332, 3225 (NH, NH_2), 2972, 2929 (C-H aliphatic), 2209 (C≡N), 1631 (C=C), 1578 (C=N), 780 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 1.12–1.15 (t, 3 H, CH_3), 3.41–3.46 (q, 2 H, CH_2), 7.35 (s,1 H, NH (D_2O exchangeable)), 7.51–7.64 (m, 6 H, NH_2, (D_2Oexchangeable) and ArH); Anal. Calcd for $C_{13}H_{12}N_5Cl$ (273.45): C, 57.04; H, 4.39; N, 25.59. Found: C, 56.91; H,4.57; N, 25.38.

4-Amino-2-(ethylamino)−6-(4-methoxyphenyl)pyrimidine-5-carbonitrile 8c

Color: dark brown; yield: 69%; m.p: 261–263 °C; IR (KBr) cm^{-1}: 3469, 3336, 3229 (NH, NH_2), 2963, 2932, 2871 and 2837 (C-H aliphatic), 2207 (C≡N), 1632 (C=C), 1583 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 1.12–1.15 (t, 3 H, CH_3), 3.41–3.46 (q, 2 H, CH_2), 3,85 (s, 3 H, OCH_3), 6.91 (s, 1 H, NH (D_2O exchangeable)), 7.08–7.45 (m, 6 H, NH_2 (D_2O exchangeable) and ArH); Anal. Calcd for $C_{14}H_{15}N_5O$ (269): C, 62.45; H, 5.57; N, 26.03. Found: C, 62.31; H, 5.77; N, 25.96.

4-Amino-2-(ethylamino)−6-(4-methylphenyl)pyrimidine-5-carbonitrile 8d

Color: dark brown; yield: 75%; m.p: 255–257 °C; IR (KBr) cm^{-1}: 3491, 3379, 3301 (NH, NH_2), 3069 (C-H aromatic), 2920, 2850 (C-H aliphatic), 2207 (C≡N), 1633 (C=C), 1550 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm:1.14–1.18 (t, 3 H, CH_3), 2.41 (s, 3 H, CH_3), 3.49–3.54 (q, 2 H, CH_2), 6.88 (s, 1 H, NH (D_2O exchangeable)), 7.07–7.24 (m, 6 H, NH_2 (D_2O exchangeable) and ArH); Anal. Calcd for $C_{14}H_{15}N_5$ (253): C, 66.40; H, 5.93; N,27.68. Found: C, 66.29; H, 6.08; N, 27.42.

General procedure for the preparation of compounds 9a-d

A mixture of **2a-d** (0.01 mol) with morpholine (5 ml) in absolute ethanol (20 ml) was refluxed for 3 h. The reaction mixture was allowed to cool and then filtered, dried and recrystallized from ethanol to afford compounds **9a-d**.

4-Amino-2-morpholino-6-phenylpyrimidine-5-carbonitrile 9a

Color: light brown; yield: 57%; m.p: 245–247 °C; IR (KBr) cm^{-1}: 3387, 3340 (NH_2), 2958, 2922, 2853 (C-H aliphatic), 2208 (C≡N), 1619 (C=C), 1558 (C=N), 1303 (C-O), 1270 (C-N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.67–3.69 (t, 4 H, $2CH_2N$), 3.73–3.75 (t, 4 H, $2CH_2O$), 7.44–7.53 (m, 7 H, NH_2 (D_2O exchangeable) and ArH); Anal. Calcd for $C_{15}H_{15}N_5O$ (281): C, 64.06; H, 5.34; N, 24.91. Found: C, 64.16; H, 5.50; N, 24.83.

4-Amino-6-(4-chlorophenyl)−2-morpholinopyrimidine-5-carbonitrile 9b

Color: brown; yield: 51%; m.p: 281–283 °C; IR (KBr) cm^{-1}: 3395, 3311 (NH_2), 2977, 2903, 2856 (C-H aliphatic), 2209

(C≡N), 1647 (C=C), 1575 (C=N), 1300 (C-O), 1270 (C-N), 782 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.70–3.74 (t, 4 H, 2CH_2N), 3.75–3.79 (t, 4 H, 2CH_2O), 7.09 (s, 2 H, NH_2 (D_2O exchangeable)), 7.55–7.65 (dd, 4 H, ArH, J=32.8, 8.4 Hz); ^{13}C-NMR (DMSO-d_6) δ/ppm: 48.15 (CH_2-N-CH_2), 66.39 (CH_2-O-CH_2), 81.93 (C-C-N), 116.37 (C≡N), 129.23 (2 C), 131.12 (2 C), 134.50, 135.43, 160.14, 161.09, 162.13 (aromtic carbons); Anal. Calcd for $C_{15}H_{14}N_5OCl$ (315.45): C, 57.06; H, 4.44; N, 22.19. Found: C, 57. 22; H, 4.57; N, 22.05.

4-Amino-6-(4-methoxyphenyl)−2-morpholinopyrimidine-5-carbonitrile 9c

Color: light brown; yield: 60%; m.p: 230–232 °C; IR (KBr) cm^{-1}: 3444, 3367 (NH_2), 2963, 2925, 2852 (C-H aliphatic), 2205 (C≡N), 1612 (C=C), 1560 (C=N), 1301 (C-O), 1253 (C-N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.68–3.70 (t, 4 H, 2CH_2N), 3.71–3.74 (t, 4 H, 2CH_2O), 3.89 (s, 3 H, OCH_3), 6.88 (s, 2 H, NH_2 (D_2O exchangeable)), 7.26–7.32 (dd, 4 H, ArH, J=16, 8 Hz); Anal. Calcd for $C_{16}H_{17}N_5O_2$ (311): C, 61.74; H, 5.47; N, 22.51. Found: C, 61.59; H, 5.61; N, 22.42.

4-Amino-6-(4-methylphenyl)−2-morpholinopyrimidine-5-carbonitrile 9d

Color: brown; yield: 67%; m.p: 266–268 °C; IR (KBr) cm^{-1}: 3485, 3374 (NH_2), 2920, 2851 (C-H aliphatic), 2201 (C≡N), 1621 (C=C), 1575 (C=N), 1318 (C-O), 1296 (C-N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.40 (s, 3 H, CH_3), 3.74–3.75 (t, 4 H, 2CH_2N), 3.78–3.79 (t, 4 H, 2CH_2O), 6.96 (s, 2 H, NH_2 (D_2O exchangeable)), 7.24–7.35 (dd, 4 H, ArH, J=36, 8 Hz); Anal. Calcd for $C_{16}H_{17}N_5O$ (295): C, 65.08; H, 5.76; N, 23.73. Found: C, 65.20; H, 5.91;N, 23.62.

General procedure for the preparation of compounds 10a-c

A mixture of **4a-c** (0.01 mol) and malononitrile (0.012 mol) was refluxed in absolute ethanol (20 ml) for 3 h. The mixture was allowed to cool, filtered, and dried, after which the precipitate was recrystallized from ethanol.

4-Amino-2-(3,5-diamino-1 H-pyrazol-1-yl)−6-phenylpyrimidine-5-carbonitrile 10a

Color: brown; yield: 52%; m.p: 281–283 °C; IR (KBr) cm^{-1}: 3475, 3424, 3362, 3221, 3161 (NH_2), 2206 (C≡N), 1624 (C=C), 1558 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 6.68 (s, 1 H, CH), 7.25–7.52 (m, 11 H, 3NH_2 (D_2O exchangeable) and ArH); Anal. Calcd for $C_{14}H_{12}N_8$ (292): C, 57.53; H, 4.11; N, 38.36. Found: C, 57.70; H, 4.02; N, 38.41.

4-Amino-6-(4-chlorophenyl)−2-(3,5-diamino-1 H-pyrazol-1-yl)pyrimidine-5-carbonitrile 10b

Color: light brown; yield: 63%; m.p: 277–279 °C; IR (KBr) cm^{-1}: 3430, 3342, 3235, 3201 (NH_2), 2208 (C≡N), 1630 (C=C), 1579 (C=N), 780 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 6.59 (s, 1 H, CH), 7.46–7.67 (m, 10 H, 3NH_2 (D_2O exchangeable) and ArH); Anal. Calcd for $C_{14}H_{11}N_8Cl$ (326.45): C, 51.46; H, 3.37; N, 34.31. Found: C,51.59; H, 3.20; N, 34.16.

4-Amino-2-(3,5-diamino-1 H-pyrazol-1-yl)−6-(4-methoxyphenyl)pyrimidine-5-carbonitrile 10c

Color: light brown; yield: 57%; m.p:263–265 °C; IR (KBr) cm^{-1}: 3449, 3420, 3382, 3355, 3220 (NH_2), 2919, 2850 (C-H aliphatic), 2208 (C≡N), 1623 (C=C), 1577 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 3.88 (s, 3 H, OCH_3), 6.51 (s, 1 H, CH), 7.49–7.97 (m, 10 H, 3NH_2 (D_2O exchangeable) and ArH); Anal. Calcd for $C_{15}H_{14}N_8O$ (322): C, 55.90; H, 4.35; N, 34.78. Found: C, 56.02; H, 4.23; N, 34.87.

General procedure for the preparation of compounds 11a-c

Compounds **4a-c** (0.01 mol) were refluxed with acetylacetone (0.012 mol) in the presence of absolute ethanol (20 ml) and two drops of conc. HCl for 3 h, and the reaction mixture was allowed to cool, filter, dry, and then recrystallized from ethanol.

4-Amino-2-(3,5-dimethyl-1 H-pyrazol-1-yl)−6-phenylpyrimidine-5-carbonitrile 11a

Color: brown; yield: 54%; m.p: 288–290 °C; IR (KBr) cm^{-1}: 3410, 3337 (NH_2), 2964, 2932, 2840 (C-H aliphatic), 2207 (C≡N), 1646 (C=C), 1610 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.41 (s, 3 H, CH_3), 3.18 (s, 3 H, CH_3), 6.38 (s, 1 H, CH), 7.32 (s, 2 H, NH_2 (D_2O exchangeable)), 7.54–7.72 (m, 5 H, ArH); Anal. Calcd for $C_{16}H_{14}N_6$ (290): C, 66.21; H, 4.83; N, 28.97. Found: C, 66.30; H, 4.71; N, 28.82.

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4-Amino-6-(4-chlorophenyl)−2-(3,5-dimethyl-1 H-pyrazol-1-yl)pyrimidine-5-carbonitrile 11b

Color: light brown; yield: 56%; m.p: 279–281 °C; IR (KBr) cm^{-1}: 3324, 3195 (NH_2), 2922, 2851 (C-H aliphatic), 2210 (C≡N), 1622 (C=C), 1592 (C=N), 720 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.34 (s, 3 H, CH_3), 3.13 (s, 3 H, CH_3), 6.30 (s, 1 H, CH), 7.11 (s, 2 H, NH_2 (D_2O exchangeable)), 7.25–7.34 (dd, 4 H, ArH, J = 28, 8 Hz); Anal. Calcd for $C_{16}H_{13}N_6Cl$ (324.45): C, 59.17; H, 4.01; N, 25.88. Found: C, 59.29; H, 3.97; N, 25.94.

4-Amino-6-(4-methoxyphenyl)−2-(3,5-dimethyl-1 H-pyrazol-1-yl)pyrimidine-5-carbonitrile 11c

Color: brown; yield: 62%; m.p: 259–261 °C; IR (KBr) cm^{-1}: 3429, 3338 (NH_2), 2997, 2954, 2933 (C-H aliphatic), 2209 (C≡N), 1629 (C=C), 1577 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.44 (s, 3 H, CH_3), 3.17 (s, 3 H, CH_3), 3.82 (s, 3 H, OCH_3), 6.34 (s, 1 H, CH), 7.31 (s, 2 H, NH_2 (D_2O exchangeable)), 7.52–7.69 (dd, 4 H, ArH, J = 60, 8 Hz); Anal. Calcd for $C_{17}H_{16}N_6O$ (320): C, 63.75; H, 5.00; N, 26.25. Found: C, 63.86; H, 4.90; N, 26.4

5. ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the synthesized compounds was tested against a series of two gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), and gram-negative bacteria (Escherichia coli). The antifungal activities of the compounds were tested against two fungi (Candida albicans and Aspergillus flavus).

Method Each of the compounds was dissolved in DMSO, and a solution at a concentration of 1 mg/ml was prepared. Separately paper discs of Whatman filter paper were prepared with standard size (5 cm) were cut and sterilized in autoclave. The paper discs, soaked in the desired concentration of the compound solution, were placed aseptically in Petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seed- ed with Staphylococcus aureus, Bacillus subtilis, E. coli, Candida albicans and Aspergillus flavus.The Petri dishes were incubated at 36 °C, and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic (ampicillin) and antifungal

agent (clotrimazole) was also recorded using the same procedure described above, at the same concentration and in the same solvents[59,60]. The % activity index for the complex was calculated using the following formula.

$$\%\ Activity\ Index = \frac{\textbf{Zone of inhibition by test compound (diametre)}}{\textbf{Zone of inhibition by standard (diametre)}} \times 100.$$

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory properties of the newly synthesized compounds were evaluated by assessing their anti-hemolytic activities (or human R.B.C. membrane stabilization) through the maintenance of the stability of the human red blood cell membrane. Blood samples (which was collected from healthy human volunteers who had not taken any NSAIDS for the two weeks prior to the experiment) were mixed with an equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.4% NaCl) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension. One milliliter of each synthesized compound at a concentration of 50 µg/mL was dissolved in phosphate buffer, and 2 mL of hyposaline along with 0.5 mL of human red blood cell membrane stabilisation (HRBC) suspension were added. The mixture was incubated at 37 °C for 30 min and then centrifuged at 3000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm[61]. Aspirin or acetylsalicylic acid (50 µg/ mL, Arab Drug Co., Cairo, A.R.E., Batch No. 2050089) was used as a reference anti-inflammatory drug, and phosphate buffer solution was used as the control. The percentage of anti-hemolytic properties was calculated using the following equation: % = 100 × (1 − A2/A1), where A1 = the optical density of the control sample, and A2 = the optical density of the compound sample. All the synthesized compounds were tested in triplicate, and the results are presented as means ± standard deviations.

ANTIOXIDANT ACTIVITY

The antioxidant activity of the tested compounds was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with L-ascorbic acid as a reference. Each tested sample and L-ascorbic acid (50 µg) were dissolved in 1 mL of dimethyl sulfoxide (DMSO). The dissolved sample (250 ml) was added to 1 mL of DPPH/DMSO solution (6 µg/50 ml) and the total volume was adjusted to 3 mL with DMSO. An equal amount of DMSO was used as a control. After good shaking, the mixture was incubated for 30 min in the dark at room temperature. The absorbance was measured via a spectrophotometer at 517 nm, and the DPPH radical scavenging percentage was calculated according to the equation: 1- (A sample/A control) × 100[62]. All the synthesized compounds were tested in triplicate, and the results are presented as means ± standard deviations.

6. CONCLUSION

Novel pyrimidine derivatives were synthesized from 6-amino-4-aryl-2-oxo-pyrimidine- 5-carbonitrile **1a-d**, where pyrimidines **1a-d** reacted with $POCl_3$ to yield 2-chloropyrimidine derivatives **2a-d**. The reactions of compounds **2a-d** with acetic acid and acetic anhydride in the presence of concentrated H_2SO_4, produced **3a-d**, while their reactions with hydrazine hydrate in ethanol resulted in the formation of 2-hydrazinopyrimidines **4a-d**. Moreover, the reactions of the 2-chloro derivatives **2a-d** with different alkyl amines produced novel pyrimidines **5a-d**, **6a-d**, **7a-d**, **8a-d** and **9a-d** corresponding to the respective amines. The reactions of compounds **4a-c** with malononitrile yielded 2-pyrazolyl pyrimidines **10a-c**. On the other hand, 2-hydrazinopyrimidines **4a-c** reacted with acetyl acetone to give 2-pyrazolyl pyrimidines **11a-d**. The newly synthesized compounds were tested for their cytotoxic, antioxidant, antimicrobial and anti-inflammatory properties. The results indicated that compounds **3b**, **10b** and **10c** exhibited excellent cytotoxic activities against the three tested cancer cell lines while demonstrating a safe profile on normal cells. Furthermore, compounds **4b**, **10c**, **11a-c** showed potent anti-inflammatory and antioxidant properties, whereas compounds **3a**, **3b**, **3d**, **4a-d**, **9c** and **10b** proved to be excellent antimicrobial agents. Based on these results, compounds **3b**, **10b** and **10c** are promising candidates for antitumor agents against several types of cancer. Further molecular and in vivo studies may be necessary to validate their efficacy as new anticancer drugs.

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