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SYNTHESIS AND ANTIOXIDANT ACTIVITY OF NOVEL PYRIMIDINE DERIVATIVES

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Abstract
A new group of chalcones **(2a-h)** are synthesized by reacting substituted aromatic aldehydes derivatives and 4-nitro acetophenone in ethanol medium in presence of base. The chalcones **(3a-h)** underwent cyclization in the presence of guanidine carbonate to yield the title compounds substituted pyrimidine derivatives **(3a-h).**All the newly synthesized compounds were characterized on the basis of IR, ^{1}H-NMR, and Mass spectral data. The final compounds were evaluated for their *In-Vitro* antioxidant activity by DPPH assay, Nitric oxide radical scavenging assay and super oxide scavenging assay. Some of the tested compounds **3b, 3d, 3e, 3g, 3h** showed good antioxidant activity, when compared to standard ascorbic acid.

Key words: Chalcones, guanidine carbonate, Pyrimidine, Antioxidant activity, nitric oxide.

Introduction

More than half of the research is done in the field of organic chemistry is mainly focused on heterocyclic compounds [I]. Significance of heterocyclic compounds in nature can be understood by exploring the chemistry of products such as vitamins, antibiotics and hormones [II]. Many active constituents have been isolated from plant origin and subjected to optimization to develop active pharmaceutical ingredients [III].

Almost all synthetic compounds such as diazepam, metronidazole, captopril and chlorpromazine belong to Heterocycles class of drugs [IV]. Nitrogen atom has gained a very important place in the family of heterocyclic moieties and it has played a vital role in development of new drugs in pharmaceutical industry [V]. A variety of heterocyclic compounds such as azine or pyridine, diazine, pyridine and pyrimidine have been developed using nitrogen. Pyrimidine is a six membered ring falling under the classification of heterocyclic compounds containing nitrogen atoms. Pyrimidine contains two nitrogen atoms at positions 1 and 3 of the six-membered ring. Basicity of pyrimidine is comparatively weaker than pyridine and it is soluble in water [VI].

Study indicated that drugs possessing pyrimidine nucleus showed a broad range of pharmacological activity like 5-flourouracil is used as anticancer; Idoxuridine and

Triflouridine is used in treatment of various viral infections as antiviral drug; Zidovudine is used for treatment and prevention of AIDS/HIV ; Trimethoprim and Sulphamethiazine act as antibacterial drugs; Prazosin and Minoxidil as antihypertensive; Phenobarbitone as anticonvulsant and sedative hypnotic agents; Propylthiouracil is used to treat hyperthyroidism; Thinozylamine as H_1 – anti-histaminics [VII].

Pyrimidines have been extensively studied in search of drugs showing better pharmacological activities and it was found that a broad variety of effects are shown by the moieties containing pyrimidine as primary nucleus. In addition to this, various derivatives of pyrimidines have been found to possess antifungal[VIII-1X], antibacterial[X-X1], antileishmanial [XII], antihistaminic [XIII], anti-inflammatory [XIV], antihypertensive[XV], activities.

In the view of the above mentioned findings, it was contemplated was to synthesize a novel series of substituted pyrimidine derivatives and to screen for their antioxidant activity.

Materials and methods

Laboratory grade reagents and chemicals are purchased from Hi-Media. The purity and reaction monitoring was checked by thin layer chromatography (TLC) using silica gel coated plates (Merck, Silica gel 60 F254). FT-IR spectra are recorded by using Bruker's Alpha-FT-IR spectrometer using ATR technique in the range of 4000-600 cm^{-1}. ^{1}H-NMR spectra are recorded in $CDCl_3$and DMSO on Avance-II spectrometer by Bruker with 400 MHz magnetic capacity.TMS (δ=0) is used as internal standard and Chemical shift values were reported in ppm. ESI technique is used to analyse the compounds for their Mass spectra. Melting points of the compounds was determined by open capillary method on Equiptronics EQ 730 digital melting point apparatus and are uncorrected. Ethyl acetate: acetone in various proportions was used a mobile phase.

General procedure for synthesis of Pyrimidine derivatives (3a-h)

Chalcones **(2a-h)** (0.01M)were dissolved in 25ml of ethanol and 5-7 drops of concentrated HCl was added to the above solution. Guanidine carbonate (0.01M) was added and stirred the contents for ten minutes and the reaction mixture was refluxed 10-12 hours. The reaction contents were stirred vigorously after pouring into ice with water. The precipitated compound was filtered and recrystallized using ethanol. The physical data of Pyrimidine derivatives **(3a-h)** is given in table 1.

3a: 4-(4-nitrophenyl)-6-phenylpyrimidin-2-amine: IR (KBr, *v,* cm^{-1}): 3545(NH), 3062(C-H), 1594(C=N), 1518 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 5.09 (s, NH_2, 2H), 6.91-7.69 (m, Ar-H, 9H). MS (m/z): 292 (M+).

3b: 4-(4-bromophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine: IR (KBr, *v,*cm^{-1}): 3565(NH), 3013(C-H), 1587(C=N), 1537(C=C), 749(C-Br). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 5.11 (s, NH_2, 2H), 6.89-8.04 (m, Ar-H, 8H) MS (m/z): 371 (M+).

3c: 4-(2-amino-6-(4-nitrophenyl)pyrimidin-4-yl)phenol: IR (KBr, *v,* cm^{-1}): 3565 (NH), 3340(OH), 3067(C-H), 1594C=N), 1516 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 5.45 (s, NH_2, 2H), 7.11-9.60, 12.10 (s, OH, 1H). (m, Ar-H, 8H) MS (m/z): 308(M+).

3d: 4-(4-nitrophenyl)-6-p-tolylpyrimidin-2-amine-: IR (KBr, *v,* cm^{-1}): 3613(NH), 3024(C-H), 1630(C=N), 1520(C=C).^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 2.28 (s, CH_3, 3H), 6.99(s, NH_2, 2H), 7.01-8.33 (m, Ar-H, 8H) MS (m/z): 306 (M+).

3e: 4-(4-fluorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine: IR (KBr, *v,* cm^{-1}): 3546(NH), 3039(C-H), 1600(C=N), 1509 (C=C), 700 (C-F). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 6.43 (s, NH_2, 2H), 7.44-8.29 (m, Ar-H, 9H) MS (m/z): 310 (M+).

3f: 4-(4-chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine: IR (KBr, *v,*cm^{-1}): 3545(NH), 3075(C-H), 1597(C=N), 1506(C=C), 687 (C-Cl). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 6.44 (s, NH_2, 2H), 6.76-8.19 (m, Ar-H, 8H) MS (m/z): 326(M+).

3g: 4,6-bis(4-nitrophenyl)pyrimidin-2-amine-: IR (KBr, *v,* cm^{-1}): 3584(NH), 3062(C-H), 1603(C=N), 1541(C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 5.60 (s, NH_2, 2H), 6.58-8.45 (m, Ar-H, 8H) MS (m/z): 337(M+).

3h:4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-amine: IR (KBr, *v,* cm^{-1}): 3613(NH), 3023(C-H), 1627(C=N), 1558 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 3.48 (s, OCH_3, 3H), 6.43 (s, NH_2, 2H), 6.89-8.15 (m, Ar-H, 8H) MS (m/z): 322 (M+).

Antioxidant activity

α, α-Diphenyl-β -picryl-hydrazyl radical scavenging (DPPH) assay [XVI]

The potential of the synthesized compounds **(3a-h)** to scavenge free radical as a measure of anti-oxidant activity was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. 0.2mM Methanolic solution of DPPH was preparedand and 100µl of sample in various concentrations(10 -50µg/ml) was subjected to reaction with 100 µl of DPPH solution. The mixture was subjected to incubation for 30 minutes and the optical density was measured at 517nm. Ascorbic acid is used as reference standard. The experiment was conducted in triplicates. The DPPH antioxidant data is given in table-2. The % inhibition is calculated by using the formula:

% Inhibition = $(A_0 – A_1 / A_0) \times 100$ where; A_0 = absorbance of control; A1= absorbance of treated sample.

Superoxide radical scavenging assay [XVI]

All the reagents used in this estimation are prepared in 100 mM phosphate buffer, pH 7.4. 1 ml of NADH (468µM) solution is treated with 1 ml of Nitro blue tetrazolium (156NBT) and 0.1 ml of solution of sample in suitable solvent is added. The reaction is initiated by adding 100 µl of Phenazinemethosulphate solution (60µM PMS) in) to the mixture. Absorbance is measured at 560nm after incubating the mixture at 25°C for 5 minutes. The percentage inhibition is calculated based on the above formula and the results are tabulated in table-3.

Nitric oxide scavenging radical assay [XVI]

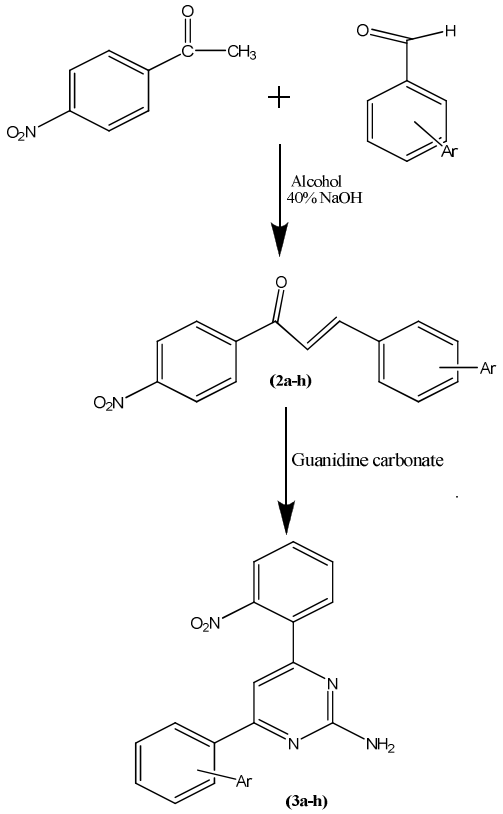
At physiological pH, Nitric oxide radicals are generated from sodium nitroprusside source. All the reagents used in this estimation are prepared in 100 mM phosphate buffer, pH 7.4.1ml of sample (10 -50µg/ml) is mixed with Sodium nitroprusside (1ml of 10mM). The solution mixture is subjected to incubation for 150 minutes at 25°C. 1 ml of the incubated solution is treated with 1ml of Griess's reagent (2% ortho phosphoric acid,1% sulphanilamide and 0.1% NEDA) is added. Absorbance of the intensity of purple colour is proportional to amount of free radical which is measured at 546 nm and the formula used in previous procedure is used to calculate the % inhibition considering optical density of sample and control. Ascorbic acid was used as standard drug material. The antioxidant data by nitric oxide method is given in table-4.

Results and Discussion

The route of synthesis is outlined in **Scheme-01.** Advanced spectroscopic techniques (FT-IR, NMR, Mass) and different physicochemical properties (TLC, mp) were utilized to characterize the newly synthesized compounds. TLC technique was employed to assess the purity of compounds. The key intermediate chalcone derivatives were prepared by the well known Claisen-Schmidth condensation reaction. Compounds **(3a-h)** were prepared by reacting chalcone analogues with guanidine carbonate in the presence of a few drops of HCl. The practical yield of the compounds was good. The IR spectral analysis of derivatives **(3a-h)** showed strong characteristic stretching bands at frequencies within 3550-3650 cm^{-1} for NH group. C=N stretching peak was observed in the range of 1630-1587 cm^{-1}. Compounds **(3a-h)** showed multiplets at δ 6.58-9.60 in ^{1}H-NMR spectra corresponding to aromatic

protons present in the final compound. A singlet peak in the range of δ 5.09-6.99 is due to the amino group which corresponds for two protons. Formation of pyrimidines was confirmed by characteristic peaks from Mass spectroscopy. Significantly stable ion peak consistent with molecular formula was observed in the Mass spectra of the compounds.

Scheme-01



Conclusion

In conclusion, pyrimidine derivatives were synthesized by cyclization of guanidine carbonate with chalcones The antioxidant study indicated the capacity of the compounds to scavenge free radicals and the results showed that many of the compounds exhibited moderate inhibitory potential in all the three antioxidant models. Among all the synthesized pyrimidines, compounds with electron withdrawing groups showed better activity and were found to be more potent in comparison to the other derivatives.

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Table-1: Physical data of pyrimidine derivatives (3a-h)

Comp	Ar-CHO	Mol. Weight	Mol. Formula	MP (^{0}C)	Yield (%)
3a	C_6H_5	292.29	$C_{16}H_{12}N_4O_2$	109-11	62
3b	4-Br	371.18	$C_{16}H_{11}BrN_4O_2$	158-60	70
3c	4-OH	308.29	$C_{16}H_{12}N_4O_3$	164-66	64
3d	4-CH_3	306.32	$C_{17}H_{14}N_4O_2$	126-28	74
3e	4-F	310.28	$C_{16}H_{11}FN_4O_2$	137-39	69
3f	4-Cl	326.73	$C_{16}H_{11}ClN_4O_2$	179-81	68
3g	4-NO_2	337.28	$C_{16}H_{11}N_5O_4$	146-48	65
3h	4-OCH_3	322.32	$C_{17}H_{14}N_4O_3$	151-53	60

Table-2: IC_{50} values of pyrimidine derivatives (3a-h)

Comp	DPPH	Nitric oxide	Superoxide
3a	38.14	134.22	33.9
3b	93.2	129.88	**25.06**
3c	128.66	33.98	74.45
3d	**28.3**	53.49	45.8
3e	62.33	**22.14**	70.83
3f	43.92	33.6	57.39
3g	211.73	31.17	**21.8**
3h	80.36	**16.26**	28.25
STD	23.12	26.85	24.99

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