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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT STUDY OF 6-METHYL-2*H*-PYRAZOLO[3,4-*d*]PYRIMIDINE-3,4-DIAMINE AND ITS DERIVATIVES.

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Abstract

A series of novel 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5a-j) have been designed and synthesized by condensation of 4-amino-2-methyl-6-(methylthio) pyrimidine-5-carbonitrile (3) with different derivatives of hydrazine (4a-j) by using anhydrous potassium carbonate as catalyst and solvent DMF. Compound (3) was prepared by reaction of acetamidine hydrochloride (1) and bis(methylthio)methylene malononitrile (2) with same reaction condition which is used for title compounds. The newly synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral analysis. Furthermore, these synthesized compounds were tested for antioxidant activity. The result of antioxidant activity reveals that most of the compounds shows potent activity. This procedure offers the advantages of mild reaction condition, easy work up, short reaction time and high yield.

Introduction:-

In last few decades, synthetic chemistry has been recognised to rich source of bioactive metabolites with varied biological and pharmacological activities. Currently free radicals and reactive oxygen species (ROS) play an important role in degenerative or pathological processes leading to many health disorders including inflammatory and cancer diseases [1]. The harmful effect of free radicals can however, be blocked by synthetic antioxidants such as butylated hydroxyanisole (BHA), ter-butylhydroquinone (TBHQ) and propyl gallate (PG) [2]. However, due to their adverse side effect search for more effective antioxidant has become crucial.

Pyrazolopyrimidines and its derivatives constitute a rich source of a wide variety of structurally unusual metabolites and seems to be an endless source of new chemicals constituents. After lot of literature survey we came to know that pyrazolopyrimidines and related fused heterocycles attracted organic chemist very much due to their broad spectrum biological and chemotherapeutic importance. They are known to exhibit widespread pharmacological importance [3-4] and were reported to posses antibacterial [5], antifungal [6], anti-inflammatory [7], antiviral [8], analgesic [9], antitumor [10], CNS-depressant [11-12], neuroleptic [13], tuberculostatic [14], protein kinase inhibitors [15], HIV reverse transcriptase inhibitors [16], non-narcotic analgesic activities [17]. More over pyrazolo[3,4-d]pyrimidines identifies as general class of adenosine receptors [18-20]. In last decades many synthetic methods have been reported for the synthesis of pyrazolopyrimidines and its derivatives [21-23]. Recently our research group have reported synthesis and antimicrobial activity of fused benzo[4,5]thiazolo[3,2-a]pyrazolo[3,4-*d*]pyrimidine derivatives [24]. In the literature we have found that, the replacement of 1H of pyrazole of pyrazolo[3,4-*d*]pyrimidine ring system by some other bioactive moiety drastically alters its pharmacological properties, keeping

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this in mind in present investigation we aimed to synthesize novel pyrazolo[3,4-*d*]pyrimidine derivatives with evaluation of their antioxidant activity.

Experimental:-

All compounds were purchased from SD-Fine, Spectrochem and Avra chemical companies and used without any additional purification. Melting points of synthesized compounds were determined by Electrothermal IA 9000 SERIES digital melting point apparatus and were uncorrected. Purity of all the products were routinely checked by thin layer chromatography (TLC) on pre-coated sheets of silica gel-C plates of 0.25 mm thickness. FT-IR spectra were recorded in Nujol or as KBr pallets on infrared spectrophotometer. Brukner advance spectrophotometer 400 MHz was used to record ^{1}H-NMR and ^{13}C-NMR spectra using tetramethylsilane (TMS) as internal standard, Mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 eV.

General procedure:-

Synthesis of 4-amino-2-methyl-6-(methylthio) pyrimidine-5-carbonitrile (3):-

A mixture of acetamidine hydrochloride (1) (0.01mol) and bis(methylthio)methylene malononitrile (2) (0.01mol) in 10 ml of DMF and anhydrous potassium carbonate (10mg) was refluxed for 6 hours. The reaction progress was monitored by thin layer chromatography (TLC) by using ethyl acetate:hexane (3:7) as irrigant. After completion of reaction, the reaction mixture was allow to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystallized from ethanol to give pure compound (3).

Synthesis of 6-methyl-2*H*-pyrazolo [3,4-*d*]pyrimidine-3,4-diamines (5a-j):-

As per scheme-2, a mixture of 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3) (0.001mol) and various derivatives of hydrazines (4a-j) (0.001mol) were independently refluxed in 10 ml of DMF and anhydrous K_2CO_3 (10mg) for 5-6 hours. After completion of reaction, the reaction mixture was allow to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystallized from ethanol to give pure compound (5a-j).

Table 1:- Physicochemical data.

Com.No.	MolecularFormula	Mol. Wt.	Colour	Yield %	M.P °C
3	$C_7H_8N_4S$	180	Gray	72.17	135-36
5a	$C_6H_8N_6$	164	Brown	68.05	194-95
5b	$C_7H_9N_7O$	207	Brown	59.42	162-64
5c	$C_7H_9N_7S$	223	Brown	66.19	169-71
5d	$C_{12}H_{12}N_6$	240	Gray	75.88	145-47
5e	$C_{13}H_{14}N_6$	254	Brown	72.36	170-72
5f	$C_{12}H_{10}N_8O_4$	330	Yellow	62.33	236-38
5g	$C_{13}H_{11}N_7S$	297	Brown	70.08	188-90
5h	$C_{14}H_{13}N_7S$	311	Yellow	64.47	215-17
5i	$C_{14}H_{13}N_7OS$	327	Brown	73.00	198-99
5j	$C_{15}H_{15}N_7S$	325	Brown	65.92	204-06

Spectral analysis:-

4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3):-

IR (KBr/cm^{-1}) 2206 (CN), 3359 (NH_2): ^{1}H-NMR (400 MHz,DMSO-d_6): δ 2.38 (s, 3H, CH_3), 2.53 (s, 3H, SCH_3), 7.5 (s, 2H, NH_2): ^{13}C-NMR (DMSO-d_6): δ 11.84 (SCH_3), 25.98(CH_3), 81.79 (C-CN), 114.54 (CN), 162.59 (C=N), 168.04 (C=N), 172.10 (C-SCH_3) EI-MS(m/z: RA%): 180 (M^+).

6-methyl-2-phenyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5d):-

IR (KBr/cm^{-1}) 3354 (NH_2): ^{1}H-NMR (400 MHz,DMSO-d_6): δ 2.36 (s, 3H, CH_3), 6.68 (s, 2H, NH_2), 6.94 (s, 2H, NH_2), 7.12-7.34 (m, 5H, Ar-H): EI-MS(m/z: RA%): 240 (M^+).

6-methyl-2-(2,4-di-nitrophenyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5f):-

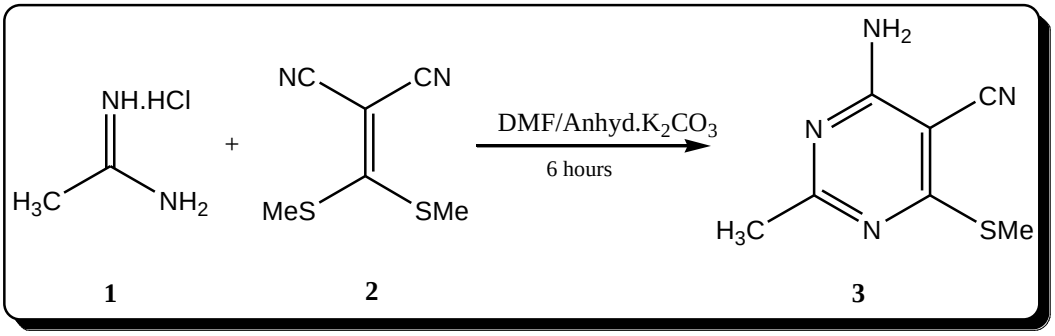
IR (KBr/cm^{-1}) 3295 (NH_2): ^{1}H-NMR (400 MHz,DMSO-d_6): δ 2.62 (s, 3H, CH_3), 6.58 (s, 2H, NH_2), 6.80 (s, 2H, NH_2), 7.53-7.84 (m, 3H, Ar-H): EI-MS (m/z: RA%): 330 (M^+).

6-methyl-2-(2-benzothiazolyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5g):-
IR (KBr/cm^{-1}) 3278 (NH_2): ^{1}H-NMR (400 MHz,DMSO-d_6): δ 2.53 (s, 3H, CH_3), 6.31 (s, 2H, NH_2), 6.83 (s, 2H, NH_2), 7.4-7.5 (m, 4H, Ar-H): EI-MS (m/z: RA%): 297 (M^+).

6-methyl-2-(6-methoxy-2-benzothiazolyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5i):-
IR (KBr/cm^{-1}) 3244 (NH_2): ^{1}H-NMR (400 MHz,DMSO-d_6): δ 2.49 (s, 3H, CH_3), 3.33 (s, 3H, OCH_3), 6.73 (s, 2H, NH_2), 7.30 (s, 2H, NH_2),7.4-7.5 (m, 3H, Ar-H): EI-MS (m/z: RA%): 327 (M^+).

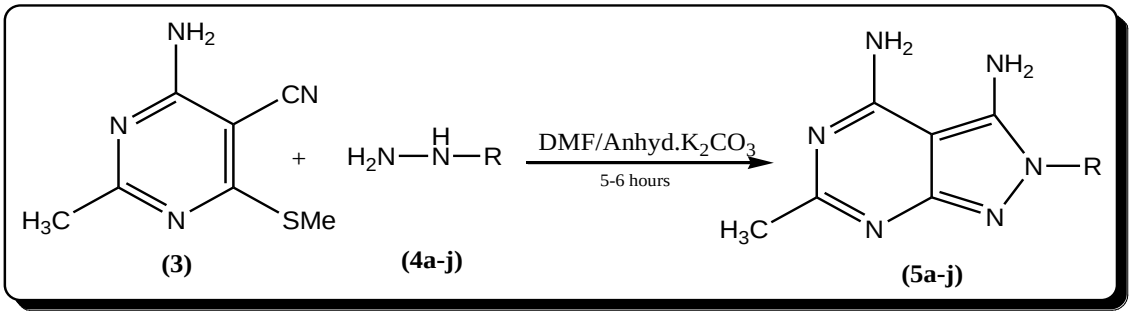
Result and Disscussion:-

During the course of our ongoing interest to the synthesis of various heterocyclic compounds using 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3), we observed that compound (3) is key intermediate for the synthesis of pyrazolopyrimidines. Thus in present view we have synthesized a series of 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5a-j). The key intermediate (3) was prepared by condensation of acetamidine hydrochloride (1) and bis(methylthio)methylene malononitrile (2) in DMF and catalytic amount of anhydrous K_2CO_3 to afford (3) **Scheme-1**.



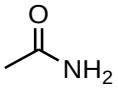
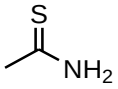
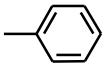
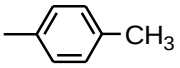
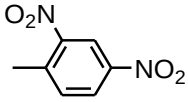
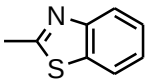
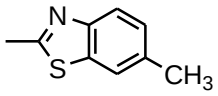
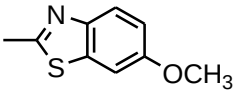
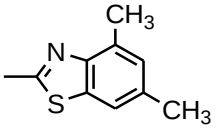
Scheme 1:- Formation of 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3).

The compound (3) posseses replaceable active thiomethyl group at 6-position and electron withdrawing nature of cyano group at 5-position. Due to presence of thiomethyl group and cyano group on compound (3) which has susceptibility for nucleophilic substitution-cyclization. When compound (3) was condensed independently with various hydrazine derivatives (4a-j) under similar experimental condition to afford 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5a-j) **Scheme-2.**



Scheme 2:- Formation of 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine derivatives (5a-j).

Table 2:- Compound numbers and various substituent's.

Comp. No.	R	Comp. No.	R
5a		**5b**	
5c		**5d**	
5e		**5f**	
5g		**5h**	
5i		**5j**	

The final compounds (5a-j) were characterized on the basis of physical and spectral (IR, ^{1}H-NMR and MS) data. Spectral analysis of these compounds are in agreement of the proposed structures.

The result of DPPH radical scavenging activity of newly synthesized 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines derivatives (5a-j) are summarized in table-3. DPPH is relatively stable nitrogen centred free radical that easily accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl hydrazyl, a stable free radical to decolourise in the presence of antioxidants. When DPPH accepts an electron from antioxidant compound, the DPPH is decolourise which can be quantitatively measured from change in absorbance. Such reactivity has been widely used to test the ability of compounds to act as free radical scavengers. The overall DPPH radical scavenging activity of tested 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines derivatives (5a-j) were in a range of 7.24-25.08 % as compared to the standard ascorbic acid (90.15%). The highest DPPH radical scavenging activity was exhibited by **5f** whereas **5d** demonstrate lowest activity. From the result of present work, it can be concluded that 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines derivatives are essential to boost the antioxidant activity.

Antioxidant activity:-

DPPH radical scavenging assay:-

The DPPH radical scavenging assay has been used for preliminary screening of the sample for antioxidant activity. The proton radical scavenging action is known as an important mechanism of antioxidants. The odd electron in DPPH radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from free radicals scavenging antioxidants to form reduced DPPH:H. 1ml (1 mM) of test sample was added in to equal quantity of 0.1 mM solution of DPPH in ethanol. After 10 minutes of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid was taken as standard reference. The result of DPPH reduction is summarized in table-3.

Table 3:- Antioxidant activity of selected compounds.

Sr. No.	Compounds	Antioxidant activity
		DPPH radical scavenging activity (%)
1	5a	24.23±0.09
2	5c	08.11±0.42
3	5d	07.24±0.20
4	5f	25.08±0.12
5	5i	25.04±0.51
6	Ascorbic acid	90.15±0.53

Conclusion:-

In summary, with the aim of have a good contribution in innovation of heterocyclic chemistry, we have demonstrated the preparation, characterization and antioxidant activity of novel heterocyclic compounds such as 6-methyl-2*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamines (5a-j) were obtained by simple route with good product yield. This protocol includes some important advantages such as mild reaction condition, easy work-up, product purity and short reaction time.

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