ISSN 2515-8260 Volume 07, Issue 11, 2020

Antioxidant Activity Of Pyrazolopyrimidine Derivatives

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Abstract:
A novel series of substituted pyrazolopyrimidine derivatives have been synthesized from substituted hydrazine derivatives and ethoxy (methylene) malanonitrile gives an intermediate (5-amino-1- substituted phenyl pyrazole-4-carbonitrile) (1) which on further react with formic acid, acetic acid/HCl and formamide gives series of (1-substitutedphenyl)1H pyrazole [3,4-d] pyrimidine-4(5H)-one (2). The synthesized compounds were characterized on the basis of IR, 1H NMR and Mass spectral data. Furthermore, these newly synthesized compounds were screened for their antioxidant activity by DPPH free radical scavenging method. Unfortunately none of the compound shows antioxidant activity.

Keywords: Pyrazolopyrimidine, Antioxidants activity, Ascorbic acid.

1. INTRODUCTION

Free radicals originate from a large variety of normal and pathological metabolic transformations, from host-defense against undesirable invasion (chemical or biological), and from host-response to a disturbance of the tissues integrity (due to trauma, cellular damage, etc.). The balance between formation and elimination of free radicals determines the overall stability of a living body. Free-radical chain reactions in the body are initiated mostly by Reactive Species [RS - molecules, ions, free-radicals; Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS)] possessing oxygen or nitrogen atom with an unpaired electron. If more RS are formed than needed for the normal redox-signaling and self-defense of the host, oxidative stress (OS) occurs leading to an oxidative cellular damage, even to cellular death. Free-radicals induce the cell damage by altering the biological activities of lipids, proteins, DNA and carbohydrates.

A great deal of progress has been made in recent years in relating ageing to oxidation in biological cells. The ROS are basically involved in detoxification of invading organisms and chemicals, but stray ROS also initiate lipid peroxidation in healthy cells. Lipid peroxidation initiated by oxygen radicals eventually results in membrane degradation and cell death[1] leading to diverse pathologies, such as Alzheimer's disease, atherosclerosis, diabetes, Parkinson's disease, etc.[2]Thus, reduction of the rate of these life-limiting metabolic processes by use of chemicals has been a subject of current research.[3-5]The negative effects of the RS may be diminished by limiting their formation (control over metabolic transformations and enzymes producing RS) through radical neutralization processes, for example by (a) recombination of the RS already formed (e.g. “radical scavenging”), and (b)

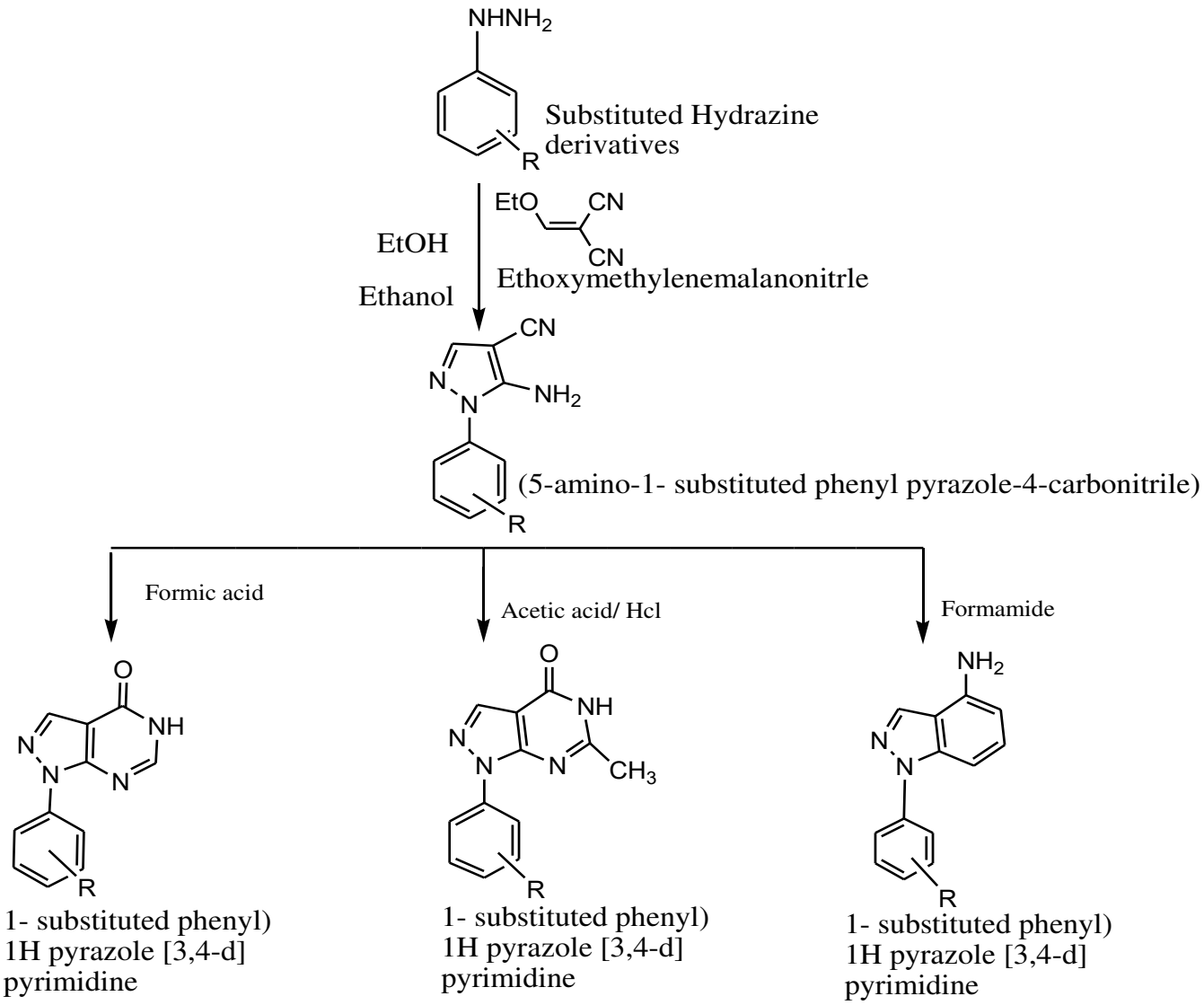
altering the RS effects. Inhibitors of the RS synthesis [NADPH- oxidases, xanthine oxidase (XO) inhibitors, leukocytes' antibodies, etc.] may also be administered.

Antioxidants are the substances, which when present at low concentrations compared to those of an oxidisable substrate, significantly delay or prevent oxidation of that substrate. The key role of antioxidants is to intercept and react with free radicals so that cascade effect of ROS propagation is prevented by readily donating its proton to the ROS. Basically, the antioxidant property of a compound is attributed to its ability of (*a*) oxygen radical scavenging, (*b*) inhibiting cellular microsomal P-450-linked mixed function oxidation (MFO) reaction, and (*c*) suppressing the formation of ROS. Thus, antioxidants are considered as remedies to overcome the lethal action of oxygen free radicals. Polyphenolic compounds have drawn greater attention compared to any other class of natural products for their significant biological functions as antioxidants and anticarcinogens or antimutagens, which have led to their recognition as potential neutraceuticals. Among them, phenolics that include coumarins, xanthones, flavones, etc. have attracted considerable attention. Pyrazolopyrimidine derivativeshave received a great deal of attention due to theirpharmacological activity[6], such as allopurinol [7], whichis still the drug of choice for the treatment ofhyperurecemia and gouty arthritis. [8]Pyrazolopyrimidine are purine analogues and as suchthey have useful properties as antimetabolites in purinebiochemical reaction. [9] Moreover, these compoundsalso display marked antitumor and antileukemicactivity. [10] Pyrazolopyrimidine derivatives havedemonstrated promising antimicrobial activity againstGram-positive bacteria. [11] Synthesis of such biologicallyimportant compounds assumes great importance.Recently, some new methods such as microwaveirradiation [12], supported solid catalyst [13], solid statereactions etc. [14] have been applied to facilitate thisreaction.

2. MATERIALS AND METHODS:

The melting point of products were determined by open capillaries method and are uncorrected. IR Spectra (KBr) were recorded on FTIR Spectrophotometer (Shimadzu FTIR 84005, 4000-400cm^{-1}). The electrospray mass spectra were recorded on a THERMO Finnigan LCQ Advantage max ion trap mass spectrometer. The 10 µl samples (dissolve in solvent such as methanol/ acetonitrile/ water) were introduced into the ESI source through Finnigen survey or autosampler. The mobile phase 90: 10 MeOH/ ACN : H_2O flowed at the rate of 250 µl/ min by MS pump. Ion spray voltage was set at 5.3 KV and capillary voltage 34 V. ^{1}H NMR were recorded on a Bruker DRX-300 MHz spectrometer in $CDCl_3$ using TMS as an internal standard, with ^{1}H resonance frequency of 300 MHz Chemical shift values are expressed in δ ppm.

ISSN 2515-8260 Volume 07, Issue 11, 2020



Scheme

R= H, *p*-nitro 2,4-di nitro, 2,4-di amino, *p*-amino, 3,5-di chloro

General procedures for the preparation of the compounds:

2.1. Synthesis of substituted 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile:

In reaction substituted hydrazine derivatives (1ml) in 20 ml anhydrous ethanol, ethoxymethylenemalanonitrile (1ml) was added and the reaction mixture were refluxed for 2-3 hrs, respectively. Anhydrous condition have to be maintained by using calcium chloride guard was filtered off, (compound 1) dried and recrystallized from ethanol.

General procedure for the synthesis of the compounds PPA-PPC

A Formic acid: Compound 1 (0.01 mol) was refluxed in formic acid (30 mL, 85%) for 5 h. The reaction mixture was cooled and poured into water. The formed solid compound PPA was filtered off, dried, and recrystallized from dioxane.

B Acetic acid/ HCl: Compound 1 (0.01 mol) was refluxed in a mixture of hydrochloric acid (3 mL) and acetic acid (9 mL) for 3 h. The reaction mixture was cooled, poured into water and the solid compound PPB formed was filtered off, dried and recrystallized from dioxane.

C Formamide: Compound 1 (0.01 mol) was refluxed in formamide (20 mL) for 3 h. The reaction mixture was cooled and poured into water. The solid compound PPC formed was filtered off, dried and recrystallized from dioxane.

Synthesis of 1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-1A:
Yield**:** 60%, mp 178-180°C, **IR (KBr, c^{m-1}):** 3540.60 (N-H secondary amine), 3346.21 (C-H Ar str), 2223.77 (C=N Ar),1647.10 (C=O str), 1598 (C=C str).**^{1}H NMR**: **(CDC_{l3}, δ, ppm):** 7.4(s, 1H, CH of pyrazole), 7.3-7.5 (m, 5H, Ar-H), 8.0 (s, 1H, NH), 8.7 (s, 1H, CH of pyrimidine).**MS *(m/z)*:** (M^+= 212); 125, 196, 145.

3.2. Synthesis of 6-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-1B:
Yield:50%, 156-158 °C, **IR (KBr, cm^{-1})**: 3460.60 (N-H secondary amine), 3396.41 (C-H Ar str), 2223.77 (C=N Ar)1647.10 (C=O str), 1598 (C=C str), **^{1}H NMR**: **($CDCl_3$, δ, ppm):**7.5 (s, 1H, CH of Pyrazole), 7.2-7.6 (m, 5H, Ar-H), 8.4 (s, 1H, NH of pyrimidine), 1.6 (s, 3H, CH of pyrimidine) .**MS (*m/z*)** : (M^+= 223); 200, 160, 186.

3.3.Synthesis of 1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine PP-1C:
Yield: 57.40 %, 200- 202 °C, **IR (KBr, cm^{-1}):** 3375.20 (N-H Ar), 3062 (C-H Ar), 2341 (C=N Ar), 1504.37 (C=C Ar str), 1305.72 (NH_2 str). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (d, 1H, CH of pyrazole), 7.0- 7.3 (m, 5H, Ar- H), 8.4 (s, 2H, Ar- C-NH_2), 8.9 (s, 1H, CH of pyrimidine). **MS (*m/z*)** : (M^+= 211), 105, 118, 134.

3.4.Synthesis of 1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-2A:
Yield: 90 % , 210-212 °C, **IR (KBr, cm^{-1}):** 3200 (NH str), 3232 (C-H str), 1616 (C=O str), 1506 (C=N str), 1458 (Ar- NO_2), 1496 (C=C Ar str).**^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.5 (s, 1H, CH of pyrazole), 7.5-8.2 (m, 5H, Ar-H), 8.0 (s, 2H, Ar C-NH_2) 8.9 (s, 1H, CH of pyrimidine). **MS (*m/z*)** : (M^+ = 257), 230, 130.

3.5. Synthesis of 6-methyl-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one
PP-2B: Yield: 81.08 % , 214-216 °C **IR (KBr, cm^{-1})**: 3500 (NH str), 3082 (C-H str), 1616 (C=O str), 1596 (C=N str), 1548 (Ar- NO_2), 1506 (C=C Ar str).**^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.6 (s, 1H, CH of pyrazole), 6.6-7.3 (m, 4H, Ar- H), 8.8 (s, 1H, NH pyrimidine), 1.5 (s, 3H, CH_3 of pyrimidine). **MS (*m/z*)** : (M^+= 263), 249, 271.

3.6. Synthesis of 1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine PP-2C: Yield: 81.01 % , 198-200 °C, **IR (KBr, cm^{-1})**: 3570 (NH str), 3152 (C-H str), 1606 (C=O str), 1545 (C=N str), 1475 (Ar- NO_2), 1578.02 (C=C Ar str). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.72 (s, 1H, CH of pyrazole), 7.5-8.2 (m, 4H, Ar-H), 8.5 (s, 2H, Ar C-NH_2), 8.4 (s, 1H, CH of pyrimidine). **MS (*m/z*)** : (M^+= 256), 240, 229.

3.7. Synthesis of 1-(2,4-dinitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-3A: Yield: 53 % , 222-224 °C, **IR (KBr, cm^{-1})**; 3456.87 (C-H Ar), 2459 (NH str),1582 CN str, 751(*p*- nitro), 736 (*o*- nitro Ar substitution). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.9 (s, 1H, CH of pyrazole), 7.9 (d, 2H, Ar-H), 8.1 (s, 1H, Ar-H) 8.0 (m, 1H, NH of pyrimidine), 8.7 (s, 1H, CH of pyrimidine).**MS (*m/z*)** : (M^+= 319.1).

3.8. Synthesis of 6-methyl-1-(2,4-dinitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-3B: Yield: 81.00 % , 206-208 °C, **IR (KBr, cm^{-1})**; 3386.77 (C-H Ar), 2358 (NH str), 1591 CN str, 781(*p*- nitro), 744 (*o*- nitro Ar substitution). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.3 (s, 1H, CH of pyrazole), 7.6 (m, 2H, Ar-H), 8.4 (s, 1H, Ar-H) 8.6 (s, 1H, NH pyrimidine), 1.2 (s, 3H, CH_3 of pyrimidine).**MS (*m/z*)** : (M^+= 308), 140, 185, 277, 184.

3.9. Synthesis of 1-(2,4-dinitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine PP-3C: Yield: 64%, 196-198 °C, **IR (KBr, cm^{-1})**: 3095.54 (CH str), 2360 (NH str), 1593 (C=C

str), 835(*p*- nitro), 709 (*o*- nitro Ar substitution).**^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (s, 1H, CH), 8.8 (s, 2H, Ar-H), 9.1 (d, 1H, Ar-H), 4.0 (s, 2H, ArC-NH_2), 8.3 (s, 1H, NH pyrimidine). **MS (*m/z*)** : (M^+= 301), 242, 167, 134

4.10 Synthesis of 1-(2,4-diaminophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-4A: Yield: 75.90%, 224-226 °C, **IR (KBr, cm^{-1})**: 3095.54 (CH str), 2360 (NH str), 1593 (C=C str), 3165.3 (-NH, 2° amide), 3018 (= C-H, aromatic), 1733.6 (- C=O). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (s, 1H, CH of pyrazole), 5.5 (d, 2H, Ar-H), 5.9 (s, 1H, Ar-H) 4.0 (s, 4H, ArC-NH_2), 8.3 (s, 1H, NH of pyrimidine).**MS (*m/z*)** : (M^+= 242); 160, 147, 201**.**

4.11 Synthesis of 1-(2, 4- diaminophenyl)-6-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-4B: Yield: 61%, 222-224°C, **IR (KBr, cm^{-1})**: 3127.3(-NH, 2° amide), 3014 (=C-H aromatic), 2929 (-CH_2-), 1684.9 (- C=O), **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.6 (s, 1H, CH), 6.6 (d, 2H, Ar-H), 7.1 (s, 1H, Ar-H), 4.1 (s, 4H, Ar C-NH_2), 8.5 (s, 1H, NH pyrimidine), 1.6 (s, 3H, CH_3 of pyrimidine) **MS (*m/z*)** : (M^+=256), 241, 228, 172, 188.

4.12 Synthesis of 4-(4-amino-1H-pyrazolo[3,4-d]pyrimidin-1yl) benzene-1,3-diamine PP-4C: Yield: 55%, 214-216 °C, **IR (KBr, cm^{-1})**: 3205 (NH_2), 3075.1 (=C-H aromatic), 1509.2 (C=C aromatic). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (d, 1H, CH of pyrazole), 5.5 (d, 2H, Ar-H), 5.8 (s, 1H, Ar-H), 4.0 (m, 4H, ArC-NH_2), 4.6 (m, 2H, Ar C-NH_2), 8.3 (s, 1H, CH pyrimidine). **MS (*m/z*)** : (M^+=241), 134, 107,148.

4.13 Synthesis of 1-(3,5-dichlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-5A: Yield: 66%, 228-230 °C, **IR(KBr,cm^{-1})**: 3125.54 (CH str), 2460 (NH str), 1453 (C=C str), 3255.3 (-NH, 2° amide), 3108 (= C-H, aromatic), 1653.6 (- C=O).**^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.6 (d, 1H, CH of pyrazole), 6.8-7.4 (m, 4H, Ar-H), 3.25 (s, 2H, ArC-NH_2), 8.0 (s, 1H, NH pyrimidine), 8.7 (s, 1H, CH pyrimidine). **MS (*m/z*)** : (M^+= 231), 124, 214, 122.

4.14 Synthesis of 1-(4-aminophenyl)-6-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-5B: Yield: 59%, 218-220 °C, **IR(KBr,cm^{-1})**: 3235.2 (-NH, 2° amide), 3120.12(=C-H aromatic), 2892 (-CH_2-), 1574.9 (- C=O). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (d, 1H, CH of pyrazole), 6.5-7.0 (m, 4H, Ar-H), 3.95 (s, 2H, ArC-NH_2), 8.6 (s, 1H, NH pyrimidine), 1.6 (s, 3H, CH_3 of pyrimidine). **MS (*m/z*)** : (M^+=241), 149, 145, 161.

4.15 Synthesis of 1-(4-aminophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine PP-5C: Yield : 65%, 188-190 °C, **IR(KBr,cm^{-1})**: 3225 (NH_2), 3125.1 (=C-H aromatic), 1485.2 (C=C aromatic). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (d, 1H, CH of pyrazole), 6.5-7.0 (m, 4H, Ar-H), 8.3 (d, 1H, CH of pyrimidine), 3.95 (s, 2H, ArC-NH_2), 3.86 (s, 2H, ArC-NH_2 of pyrimidine). **MS (*m/z*)** : (M^+= 226), 134, 172.

4.16 Synthesis of 1-(3,5-dichlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP-6A: Yield : 58.13%, 212-214 °C, **IR(KBr,cm^{-1})**: 3102.54 (CH str), 2365 (NH str), 1353 (C=C str), 3355.3 (-NH, 2° amide), 3208 (= C-H, aromatic), 1553.6 (- C=O).**^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (d, 1H, CH of pyrazole), 6.6-7.7 (m, 4H, Ar-H), 8.5 (s, 1H, NH pyrimidine), 8.9 (s, 1H, CH pyrimidine). **MS (*m/z*)** : (M^+= 279), 144, 208, 121, 209.

4.17 Synthesis of 1-(3,5-dichlorophenyl)-6-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one PP- 6B: Yield: 65.66%, 224-226 °C, **IR (KBr, cm^{-1})**: 3335.2 (NH_2), 3025.1 (=C-H aromatic), 1585.2 (C=C aromatic). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.7 (d, 1H, CH), 7.2-7.3 (m, 3H, Ar-H), 8.7 (s, 1H, NH of pyrimidine), 1.8 (s, 3H, CH_3 of pyrimidine). **MS (*m/z*)** : (M^+=295), 149, 144, 197.

4.18 Synthesis of 1-(3,5-dichlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine PP-6C: : Yield: 75.53%, 218-220 °C, **IR (KBr,cm^{-1})**: 3225 (NH_2), 3125.1 (=C-H aromatic), 1485.2 (C=C aromatic). ^{1}H NMR: **($CDCl_3$, δ, ppm):** 7.8 (d, 1H, CH of pyrazole), 6.5-7.1 (m, 3H, Ar-H), 3.2 (s, 2H, Ar C-NH_2), 8.9 (s, 1H, CH pyrimidine). **MS (*m/z*)** : (M^+= 270), 199, 262, 140, 134.

ISSN 2515-8260 Volume 07, Issue 11, 2020

"Table 2: Physicochemical data for the newly prepared compounds"

S. No.	Compd no.	R	Molecular Formula	M.W	R_f* value	(%) Yield	m.p. (°C)
1	PP-1A	H	$C_{10}H_9N_3O$	212	0.72	60.00	178-180
2	PP-1B	H	$C_{13}H_{11}N_3O$	223	0.87	50.00	156-158
3	PP-1C	H	$C_{11}H_9N_5$	211	0.81	57.40	200-202
4	PP-2A	*p*-nitro	$C_{11}H_7N_5O_3$	257	0.86	90.09	210-212
5	PP-2B	*p*-nitro	$C_{12}H_9N_5O_3$	263	0.71	81.80	214-216
6	PP-2C	*p*-nitro	$C_{11}H_8N_6O_2$	256	0.69	81.10	198-200
7	PP-3A	2,4-di nitro	$C_{11}H_6N_6O_5$	319	0.76	54.00	222-224
8	PP-3B	2,4-di nitro	$C_{12}H_8N_6O_5$	308	0.78	81.00	206-208
9	PP-3C	2,4-di nitro	$C_{11}H_7N_7O_4$	301	0.70	64.00	196-198
10	PP-4A	2,4-di amino	$C_{11}H_{10}N_6O$	242	0.82	75.90	224-226
11	PP-4B	2,4-di amino	$C_{12}H_{12}N_6O$	256	0.75	61.00	222-224
12	PP-4C	2,4-di amino	$C_{11}H_{11}N_7$	241	0.69	55.00	214-216
13	PP-5A	*p*-amino	$C_{11}H_9N_5O$	231	0.81	66.00	228-230
14	PP-5B	*p*-amino	$C_{12}H_{11}N_5O$	241	0.76	59.00	218-220
15	PP-5C	*p*-amino	$C_{11}H_{10}N_6$	226	0.65	65.00	188-190
16	PP-6A	3,5-di chloro	$C_{11}H_6Cl_2N_4O$	281	0.75	58.13	212-214
17	PP-6B	3,5-di chloro	$C_{14}H_9Cl_2N_2O$	292	0.68	65.66	224-226
18	PP-6C	3,5-di chloro	$C_{13}H_8Cl_2N_3$	276	0.67	75.53	218-220

*** Solvent system: Petroleum ether: ethyl acetate (7:3)**

[15, 16]

Antioxidant Studies

Free radical scavenging activity by dpph assay method:

Procedure:

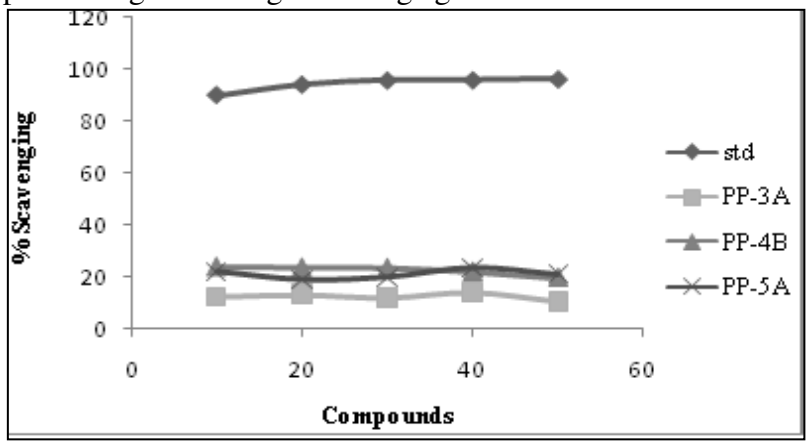
Free radical scavenging activity of the test compounds were determined by DPPH assay method and compared with of Ascorbic acid as standard. Drug stock solutions (1µg/ml) were diluted to final concentrations of 10, 20, 30, 40, and 50µg/ml in methanol. One ml of 0.3 mM (1.2 gm in 100 ml) DPPH methanol solution was added to 2.5 ml of drug solution of different concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 517 nm and converted to percentage inhibition It was calculated by the following formulae:

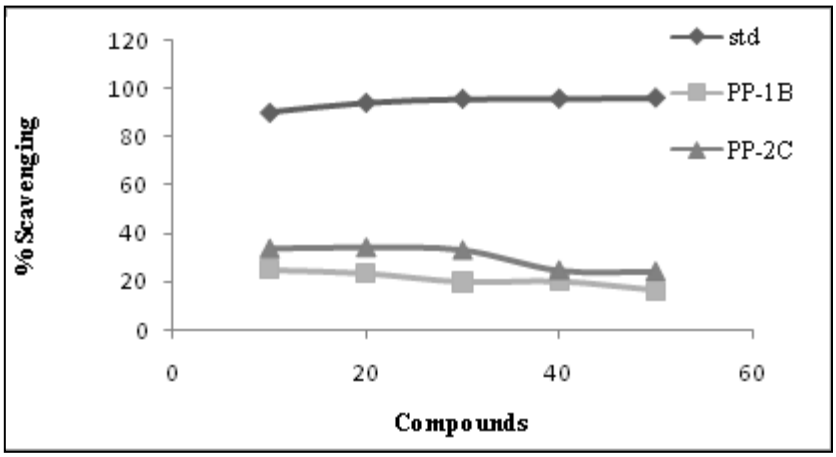
$$\% \text{ Inhibition} = \frac{\text{Abs of control-Abs of test/standard}}{\text{Abs. of control}} \times 100$$

Methanol (1ml) and drug solution (2.5 ml) was used as a blank. DPPH solution (1ml, 0.3 mM) and methanol (2.5 ml) was used as a control. Ascorbic acid was the standard solution.

ISSN 2515-8260 Volume 07, Issue 11, 2020

"Table 2: Graph showing Percentage Scavenging at different concentration of compounds"





3. RESULT AND DISCUSSION

Antioxidants activity was performed by free radical scavenging activity by DPPH assay method. The DPPH antioxidant assay measure the hydrogen donating capacity of the molecules in the sample. When the free radical DPPH is reduced by the sample, its colour changes from violet to yellow. The absorbance decline is measured and the antioxidant capacity can be determined To evaluate antioxidant activity changes in absorbance of DPPH were measured at 517nm, standard drug Ascorbic acid and control methanol and DPPH were used at a concentrations of 10, 20, 30, 40 and 50 µg/ml. None of the synthesized compounds shows antioxidant activity.

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