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Research Article

Synthesis of N,1-disubstituted-6-[(phenylamino)methyl]-1H-pyrazolo[3,4-d]pyrimidin-4-amine derivatives as biologically active agents

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Abstract

Pyrazolo [3,4-*d*]pyrimidin-4-amine are found most active molecules are synthesized by propanedinitrile, diethoxymethoxyethane and acetic anhydride and boiled at 180 degrees & yielded good products Ethoxy methylidene propanedinitrile which was then boiled and reacted with phenyl hydrazine and yielded 5-amino-1-phenyl-4,5-dihydro-1*H*-pyrazole-4-carbonitrile and good percentile which then reacted with concentrated sulphuric acid and yielded 5-amino-1-phenyl-4,5-dihydro-1*H*-pyrazole-4-carboxamide and cyclysed with ester methyl chloro acetate and polymerised the pyrimidine ring the keto functional group on the molecules further chlorinated with phosphorous oxy chloride and yielded with good results 6-(chloromethyl)-1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4 -*d*]pyrimidin-4-one which further reacted with amines are substituted chlorines with amines groups. The antioxidant activity of the molecules was determined by free radical scavenging assay by *in vitro* method called DPPH assay. All the compounds (A1-A10) were found active in SOD assay procedure. *In vivo* anti oxidant activity evaluation of antioxidant activity using azathioprine induced oxidative stress in rats, superoxide dismutase**:** Superoxide dismutase is class of enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defence in nearly all cells exposed to oxygen. Superoxide dismutase activity was estimated in tissue homogenate with help of pure bovine superoxide dismutase standard and compounds A1was highly effective with anti oxidant activity.

Keywords: *Pyrazolo pyrimidine, Azathioprine, Ethoxy methylidene propanedinitrile, ethoxymethoxyethane and anti oxidant activity.*

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Introduction

Pyrazolo pyrimidine derivatives have attracted the attention of numerous researchers over many years due to their important biological activities. The structural similarity of pyrazolo[3,4-*d*]pyrimidines with purines(A. Bendich et.al., 1954) have made them a prime target for scientific research and in this context several reports dealing with the synthesis of these fused heterocyclic compounds have appeared in the literature(B.S. Holla et.al., 2006) An array of biological activities such as antibacterial, antifungal(H. Liu.et.al 2007) antiphlogistic(J.H. Chern. Et.al., 2004), antitumor(J.M Quintela.et.al., 2003), and herbicidal has been reported to be shown by various pyrazolopyrimidines. It has been proved that these heterocyclic compounds are effective as inhibitors of inflammatory mediators in intact cells (M. A El-Sherbeny 2000), M. tuberculosis(M. Bakavoli.et.al., 2010) and human enterovirus(M. Ghorab.et.al., 2010). They also show inhibitory activity towards both tubulin polymerization, cyclin-dependent kinase(N.D. Adams.et.al., 2007) and enzymatic assays on Src and Abl tyrosine kinases(O. Moukha-Chafiq.et.al., 2002). Prompted by these claims and in continuing our synthetic studies on bioactive heterocycles(V. Krystof.et.al., 2006), we have now synthesized a new series of some novel N,1-disubstituted-6-[(phenylamino)methyl]-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-amine derivative and their anti oxidant activity.

Materials & Methods

Synthesis of Ethoxy methylidene propanedinitrile:

Propanedinitrile (4.1g, 60mmol), acetic anhydride (20.0 ml, 20mmol) and diethoxymethoxyethane (11.2ml, 60mmol) were stirred in a round bottom flask and then refluxed for a duration of 12 h at 110oC. The solvent acetic anhydride was removed from the reaction medium under reduced pressure by distillation the residue was added to crushed ice and washed with cool water thus obtained crystals were filtered and dried.

Synthesis of 5-amino-1-phenyl-4,5-dihydro-1H-pyrazole-4-carbonitrile:

Ethoxy methylidene propanedinitrile (4.0 g, 40mmol),is added to phenyl hydrazine (3.0 ml, 40mmol) and dissolved in solvent ethanol (20.0 ml) in a neat freshly dried round bottom flask and refluxed for 2h at 800C. The excess solvent ethanol was removed from the reaction medium by distillation then the resultant crude was transferred to beaker of crushed ice. Then obtained precipitate was washed with cool water, then filtered and dried.

Synthesis of 5-amino-1-phenyl-4,5-dihydro-1*H*-pyrazole-4-carboxamide :

A concentrated ice cooled sulphuric acid at 0^{o}C **(30.0 ml)** in a large beaker, to w of 5-amino-1-phenyl-4,5-dihydro-1*H*-pyrazole-4-carboxamide **(**2**) (6.5g, 40mmol)** was added drop by drop with vigorous stirring for 3 **h.** The mixture was then neutralized with aqueous sodium hydroxide solution, and then the P^{H} **7** was obtained, under ice cold condition. The product was crystallized in ice cold condition was filtered and dried.

Synthesis of 6-(chloromethyl)-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4 -d]pyrimidin-4-one :

A basic solvent ethanol was mixed with sodium metal turnings and sodium ethoxide was prepared to a quantity of 150.0 ml, under anhydrous condition, 5-amino-1-phenyl-1-pyrazole-4-carboxamide (3.4 g, 30 mmol) and then ester methylchloroacetate (16 ml, 170.3 mmol) is added drop by drop for 1 hour. The resultant reaction mixture was transferred into container of cool water. Conc. HCL was added slowly until precipitate was formed. The resultant precipitate was filtered and dried.

Synthesis of COMPOUND A1:

Dimethly sulphoxide (150.0 ml). was taken in RBF boiled at 180oC and then slowly to the solvent 6-(chloromethyl)-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4 -d]pyrimidin-4-one (3.4 g, 20 mmol) was added then methyl iodide was added and refluxed for 12 hours. The reaction mixture was poured into beaker, added Conc. HCL drop wise. The resulting precipitate was filtered off, air dried and crystallized from chloroform.

Synthesis of COMPOUND A2:

6-(chloromethyl)-5-methyl-1-phenyl-1,5-dihydro-4H-pyrazolo [3,4-d]pyrimidin-4-one (0.5 g, 1.72 mmol) and 20.0 ml of phosphorus oxy chloride in a round bottom flask and boiled up to 1100C for 14 h under anhydrous conditions. The excess phosphorus oxy chloride was distilled off from the reaction medium under reduced pressure. The mixture was added to crush ice. It was neutralized with dilute sodium bicarbonate. The resultant precipitate obtained was filtered off, air dried.

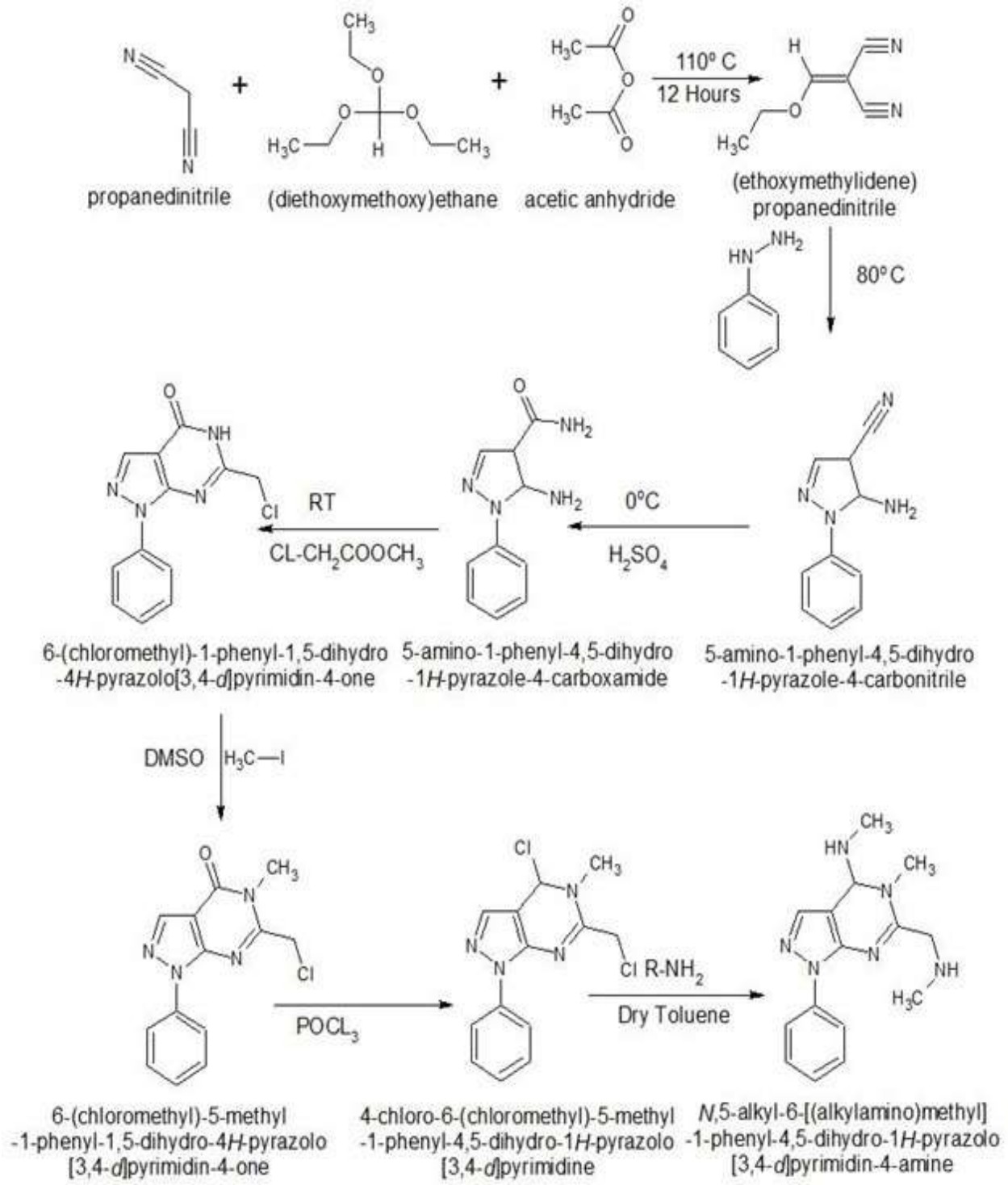

Fig. 1 Schematic representation of synthetic reactions

Synthesis of COMPOUND A3:

A solution of 4-chloro-6-(chloromethyl)-5-methyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidine, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then aniline (4 ml, 40 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

Synthesis of COMPOUND A4:

A solution of compound A3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then ethanamine (4 ml, 50 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

Synthesis of COMPOUND A5:

A solution of compound A3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then propan-1-amine (4 ml, 50 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

Synthesis of COMPOUND A6:

A solution of compound A3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then propan-2-amine (4 ml, 50 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

Synthesis of COMPOUND A7:

A solution of compound A3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then morpholine (3.4 ml, 50 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

Synthesis of COMPOUND A8:

A solution of compound A3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then N-ethylethan amine (4.5 ml, 50 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

Synthesis of COMPOUND A9:

A solution of compound A3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then butan-1-amine (4.5 ml, 50 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

Synthesis of COMPOUND A10:

A solution of compound A3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then 2-methylpropan-1-amine (5.2 ml, 50 mmol) is added in a drop by drop manner at room temperature and stirred at room temperature for 24h. The excess toluene was removed from the reaction medium by distillation. The mixture was transferred to crushed ice. The resultant precipitate obtained was filtered and dried.

In Vitro Anti Oxidant Activity

DIPHENYLPICRYLHYDRAZYL METHOD (DPPH):

The antioxidant activity of the molecules was determined by free radical scavenging assay by in vitro method called DPPH assay.

Materials:

α, α-diphenyl-β-picrylhydrazyl , Ethanol, Butylated hydroxy anisole, tris HCL buffer, distilled water, LABINDIA UV-Visible Spectrophotometer, test tubes, thermometer and incubator.

Preparation of drug concentrations:

All the molecules were prepared in different concentrations such as (50, 100, 150 and 200) µL with solvent ethanol.

Preparation of samples:

All the above concentrations were added 0.8ml of tris HCL buffer and then pH was then adjusted 7.4, then reagent DPPH (500mM with Ethanol) of 1ml was added to all the dilutions. The samples was shaken vigorously and then incubated at room temperature for duration of 30mins.

Blank Preparation:

Ethanolic solution of the drug sample (0.2ml) was served as the blank reagent.

Control Preparation:

Ethanolic solution of the DPPH (1m, 500mM) sample was served as the standard reagent.

Standard Preparation:

Butylated hydroxy anisole (BHA) is used as standard drug for the comparative study as of the above dilutions were prepared.

Assay Procedure:

Assay was performed at 517nm on (LABINDIA) UV-Visible Spectrophotometer and observation were recorded and calculated as per the formula given below.

$$\% \text{ DPPH radical scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample}}{(\text{Absorbance of control}) \times 100} \times 100$$

Decreased absorbance of the reaction mixture indicates stronger DPPH radical scavenging activity.

In Vivo Anti Oxidant Activity

Animals for Experiment:

Albino rats weighing 140-160g were utilized in the study. They are procured and stored in polypropylene cages and maintained at room temperature 23^{o}C ± 2^{o}C and relative 50% humid climate conditions are maintained. They are stored at 12h day light and 12h dark light for acclimatization for the experimental study. They are also provided with rodent pellet diet food and water were provided *ad libitum.*

Induction Procedure for rats:

Oxidative stress:

Azathioprine 3g/ml solution was feeded through oral route and through retro-orbital plexus root samples were collected at regular intervals and renal parameters like Creatinine and urea was estimated for induced oxidative stress on the animal.

Animal group alignment for experimental study:

Total animals were divided in to five groups with each group containing six rats.

Control Group: This group of rats is orally administered with normal saline for 21 days.

Blank Group: This group of rats is orally administered with Azathioprine 20mg/kg for 21 days. Dose I**:** This group of rats is orally administered with Azathioprine 20mg/kg and drug of compounds A1, of 100mg/kg for 21 days. Dose I**:** This group of rats is orally administered with Azathioprine 20mg/kg and drug of compounds B5 of 100mg/kg for 21 days. Dose I**:** This group of rats is orally administered with Azathioprine 20mg/kg and drug of compounds C10 of 100mg/kg for 21 days. Dose II**:** This group of rats is orally administered with Azathioprine 20mg/kg and drug of compounds A1 of 200mg/kg for 21 days. Dose II**:** This group of rats is

orally administered with Azathioprine 20mg/kg and drug of compounds B5 of 200mg/kg for 21 days. Dose II**:** This group of rats is orally administered with Azathioprine 20mg/kg and drug of compounds C10 of 200mg/kg for 21 days. Standard Group**:** This group of rats is orally administered with Azathioprine 20mg/kg and treated with ascorbic acid 10mg/kg for 21 days.

Sample Collection from Blood & Organs: After 24 hrs of 21days treatment blood samples were collected from puncture of retro plexus and centrifuged at 3000 (RPM) revolution per minute for a duration of 15mins. Serum was extracted and stored at -20^{o}C and utilized for the estimation of creatinine and urea. Then the rats were killed with anesthetic effect and with a midline incision and open up of the abdominal cavity then accessed for liver and kidney. Then washed with saline and fixed quickly with formaldehyde solution. The left over kidney and liver were homogenized in 0.25M sucrose solution and centrifuged at 5000rpm for duration of 5 mins. The supernatant layer obtained was stored at 20^{o}C for estimation of superoxide dismutase with in a period of 48 hours by UV Spectrophotometer.

Superoxide Dismutase (SOD) Estimation: SOD are the enzymes play major role in the human body by catalyzing the dismutaton of superoxide in to oxygen and hydrogen peroxide. They are an important antioxidant defense in all cells exposed to oxygen.

Reagent preparation SOD estimation, 0.01M phosphate buffer with pH 7.5 s prepared.
Potassium phosphate buffer preparation:
1.741gms of K_2HPO_4 is dissolved in 1000ml of distilled water and 680.45mg and dissolved in 50ml individually, then the pH of K_2HPO_4 is observed. To K_2HPO_4 add liquid of K_2HPO_4 till 7.5 pH shall be attained and measured by a pH meter. Riboflavin Solution Riboflavin (5mg) was weighed, and dissolved in 1lit of potassium phosphate buffer, to attain concentration of $1.3*10^{-5}$ M.
Preparation of O-dianisidine solution:
O-dianisidine solution was formulated by adding 122mg was dissolved 50ml of ethanol.
Extraction procedure:
3ml of packed blood cells were lysed by the addition of equal volume of cold deionized water. Hemoglobin was then precipitated by the addition of chloroform: ethanol (1.5:1). This was diluted with 500µl of water and centrifuged for 15 minutes at 3000 rpm. The supernatant containing SOD was taken for the measurement of its activity.
Assay procedure:
0.88ml of riboflavin solution (1.3 × 10–5 M in 0.01M potassium phosphate buffer, pH 7.5) was added to 60µl of O-dianisidine solution (10-2 M in ethanol) and to this 100µl of clear separated SOD was added and optical density was measured at 460nm. Then the cuvette containing reaction mixture was transferred to the illuminating box, illuminated for 4min., and optical density was re-measured against blank containing ethanol in place of enzyme. The change in the optical density was determined. The SOD content was determined from the standard graph prepared using pure bovine SOD. Statistical analysis: All the values were expressed as mean

±standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance (ANOVA) followed by dunnett's test. P <0.05 will be considered as statistically significant.

Results

Table No 1 Physical Properties of the synthesized Molecules:

Compound ID	IUPAC Name	Mol Formula	Mol Weight	Mol Composition	Melting Point	Rf Value
A1	**6-(chloromethyl)-5-methyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo [3,4-*d*] pyrimidin-4-one.**	$C_{13}H_{11}ClN_4O$	274.67	C(56.84%) H(4.04%) Cl(12.91%) N(20.40%) O(5.82%)	201	0.46
A2	**4-chloro-6-(chloromethyl)-5-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine**	$C_{13}H_{12}Cl_2N_4$	295.16	C(52.90%) H(4.10%) Cl(24.02%) N(18.98%)	210	0.72
A3	**6-(anilinomethyl)-*N*-phenyl-5-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-amine**	$C_{25}H_{24}N_6$	408.49	C(73.51%) H(5.92%) N(20.57%)	265	0.42
A4	***N*-ethyl-6-[(ethylamino)methyl]-5-methyl-1-phenyl-4,5- dihydro-1*H*-pyra zolo[3,4-*d*]pyrimidin-4-amine**	$C_{17}H_{24}N_6$	312.41	C(65.36%) H(7.74%) N(26.90%)	255	0.72
A5	***N*-propyl-6-[(propylamino) methyl]-5-methyl-1-phenyl-4,5-dihydro-1*H*-pyra zolo[3,4-*d*]pyrimidin-4-amine**	$C_{19}H_{28}N_6$	340.46	C(67.03%) H(8.29%) N(24.68%)	305	0.46
A6	***N*-propan-2-yl-6-[(propan-2-ylamino)methyl]-5-methyl-1-phenyl-4,5-di hydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine**	$C_{19}H_{28}N_6$	340.46	C(67.03%) H(8.29%) N(24.68%)	305	0.46
A7	**6-(morpholin-4-ylmethyl)-4-(morpholino-4-yl)-5-methyl-1-phenyl-1*H*-pyra zolo[3,4-*d*]pyrimidine**	$C_{21}H_{28}N_6O_2$	396.48	C(63.62%) H(7.12%) N(21.20%) O(8.07%)	320	0.72
A8	**6-[(diethylamio)methy]-N,N-diethyl-5-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine**	$C_{21}H_{32}N_6$	368.51	C(68.44%) H(8.75%) N(22.80%)	285	0.66
A9	**N-butyl-6-[(butylamio)methyl]-5-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo [3,4-*d*]pyrimidine**	$C_{21}H_{32}N_6$	368.51	C(68.44%) H(8.75%) N(22.80%)	265	0.74
A10	**1-phenyl-5-methyl-N-(2-methyl propy)l-6-{[(2-methyl pro pyl) amino] methyl}-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine**	$C_{21}H_{32}N_6$	368.51	C(68.44%) H(8.75%) N(22.80%)	285	0.66

Table No 2 Spectral Properties of the synthesized Molecules:

Compound ID	IR Spectra	NMR Spectra	Mass Spectra
A1	**IR (Cm^{-1}) (KBr):** 3442.7 (-NH, 2° amide); 2971.9 (-CH_2); 1693.4 (-C=O); 1594.1 (C=C, aromatic); 779.1 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):**δ 4.63 (s, 2H, -CH_2 -Cl), 7.41-7.44 (t, *J*= 7.4 Hz, 1H, H-4' Ph), 7.56-7.60 (t, *J*= 7.8 Hz, 2H,H-2 & 6' Ph), 8.03-8.05 (d, *J*= 8.0 Hz, 2H, H-3' & 5' Ph), 8.35 (s, 1H, H-3);	**Mass** m/z :275 (M+1), 273 (M-1);
A2	**IR (Cm^{-1}) (KBr):** 3442.7 (-NH, 2° amide); 2971.9 (-CH_2); 1693.4 (-C=O); 1594.1 (C=C, aromatic); 779.1 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):**δ 4.63 (s, 2H, -CH_2 -Cl), 7.41-7.44 (t, *J*= 7.4 Hz, 1H, H-4' Ph), 7.56-7.60 (t, *J*= 7.8 Hz, 2H,H-2 & 6' Ph), 8.03-8.05 (d, *J*= 8.0 Hz, 2H, H-3' & 5' Ph), 8.35 (s, 1H, H-3);	**Mass** m/z :296 (M+1), 294 (M-1);
A3	**IR (Cm^{-1}) (KBr):** 3397.2 (-NH); 2926.1 (-CH_2); 1575.7 (C=C, aromatic); 787.1 (-CH oop).	**^{1}H NMR (400 MHz, DMSO -d_6):** δ 4.47 (s, 2H, -CH_2-NH-C_6H_5), 6.58-6.62 (t, *J* = 7.0 Hz, 1H, H-4''' Ph), 6.76-6.62 (d, *J* = 7.6 Hz, 2H, H-2''' & 6''' Ph), 7.10-7.14 (t, *J* = 7.2 Hz, 3H, H-3''',5''' & 4'' Ph), 7.34-7.38 (t, *J* = 6.8 Hz, 3H, H-2'',6'' & 4' Ph), 7.50-7.54 (t, *J* = 7.8 Hz, 2H, H-3'' & 5'' Ph), 7.83-7.85 (d, *J* = 7.6 Hz, 2H, H-2' & 6' Ph), 8.17-8.19 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.51 (s, 1H, H-3);	**Mass** m/z :409 (M+1), 407 (M-1);
A4	**IR (Cm^{-1}) (KBr):** 3441.5 (-NH); 2966.3, 2924.3 (-CH_2); 1631.4 (C=C, aromatic); 758.9 (-CH oop)	**^{1}H NMR (400 MHz, DMSO -d_6):** δ 0.69-0.73 (t, *J* = 7.4 Hz, 3H, -CH_2-NH-CH_2-CH_3), 1.22-1.25 (t, *J* = 7.2 Hz, 3H, -NH-CH_2-CH_3), 2.67-2.73 (pentet, *J* = 6.8 Hz, 2H, -CH_2-NH-CH_2-CH_3), 3.52-3.59 (pentet, *J* = 6.8 Hz, 2H, -CH_2-NH-CH_2-CH_3), 3.47-3.52 (q, *J* = 6.6 Hz, 2H, -NH-CH_2-CH_3), 3.96-4.05 (q, *J* = 6.2 Hz, 2H, -CH_2-NH-CH_2 -CH_3), 4.47 (s, 2H, -CH_2-NH-CH_2-CH_3), 7.30-7.34 (t, *J* = 7.4 Hz, 1H, H-4' Ph), 7.52-7.56 (t, *J* = 7.8 Hz, 2H, H-2' & 6' Ph), 8.20-8.22 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.31 (s, 1H, H-3);	**Mass** m/z :313 (M+1), 311 (M-1);
A5	**IR (Cm^{-1}) (KBr):** 3449.1 (-NH); 2959.6, 2924.7 (-CH_2); 1592.4 (C=C, aromatic); 758.4 (-CH oop).	**^{1}H NMR (400 MHz, DMSO -d_6):** δ0.48-0.51 (t, *J* = 4.4 Hz, 3H, -NH-CH_2-CH_2-CH_3), 0.94-0.98 (t, *J* = 7.4 Hz, 3H, -CH_2-NH-CH_2-CH_2-CH_3), 1.60-1.69 (sextet, *J* = 7.2 Hz, 2H, -NH-CH_2-CH_2-CH_3), 1.89-1.99 (pentet, *J* = 7.8 Hz, 2H, -CH_2-NH-CH_2-CH_2-CH_3), 3.47-3.52 (q, *J* = 6.6 Hz, 2H, -NH-CH_2-CH_2-CH_3), 3.96-4.05 (q, *J* = 6.2 Hz, 2H, -CH_2-NH-CH_2-CH_2-CH_3), 4.48 (s, 2H, -CH_2-NH-CH_2-CH_2-CH_3), 7.30-7.34 (t, *J* = 7.4 Hz, 1H, H-4' Ph), 7.52-7.56 (t, *J* = 7.8 Hz, 2H, H-2' & 6' Ph), 8.20-8.22 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.31 (s, 1H, H-3);	**Mass** m/z :341 (M+1), 339 (M-1);
A6	**IR (Cm^{-1}) (KBr):** 3447.8 (-NH); 2961.4, 2925.0 (-CH_2); 1593.5 (C=C, aromatic); 759.2 (-CH oop).	**^{1}H NMR (400 MHz, DMSO -d_6):**δ 1.20-1.28 (d, *J* = 6.8 Hz, 6H, -NH-CH-$(CH_3)_2$), 1.48-1.50 (d, *J* = 6.4 Hz, 6H, CH_2-NH-CH-$(CH_3)_2$), 3.04-3.11 (q, *J* = 8.0 Hz, 1H, CH_2-NH-CH-$(CH_3)_2$), 4.43-4.47 (q, *J* = 6.7 Hz, 1H, -NH-CH-$(CH_3)_2$), 4.62 (s, 2H, -CH_2-NH- CH-$(CH_3)_2$), 7.32-7.35 (t, *J* = 7.4 Hz, 1H, H-4' Ph), 7.52-7.56 (t, *J* = 7.8 Hz, 2H, H-2' & 6' Ph), 8.21-8.24 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.37 (s, 1H, H-3);	**Mass** m/z :341 (M+1), 339 (M-1);
A7	**IR (Cm^{-1}) (KBr):** 2966.3, 2924.3 (-CH_2); 1631.4 (C=C, aromatic); 758.9 (-CH oop).	**^{1}H NMR (400 MHz, DMSO -d_6):**δ 2.32-2.34 (t, *J* = 5.2 Hz, 4H, H-2''' & 6''' morph), 3.53-3.55 (t, *J* = 4.8 Hz, 4H, H-3''' & 5''' morph), 3.77-3.79 (t, *J* = 4.8 Hz, 4H, H-2'' & 6'' morph), 3.96-3.98 (t, *J* = 4.8 Hz, 4H, H-3'' & 5'' morph), 4.47 (s, 2H, -CH_2-), 7.35-7.39 (t, *J* = 7.4 Hz, 1H, H-4' Ph), 7.54-7.58 (t, *J* = 7.6 Hz, 2H, H-2' & 6' Ph), 8.17-8.19 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.41 (s, 1H, H-3); ' Ph), 8.37 (s, 1H, H-3);	**Mass** m/z :397 (M+1), 395 (M-1);
A8	**IR (Cm^{-1}) (KBr):** 3447.8 (-NH); 2961.4, 2925.0 (-CH_2); 1593.5 (C=C, aromatic); 759.2 (-CH oop).	**^{1}H NMR (400 MHz, DMSO -d_6):**δ 2.32-2.34 (t, *J* = 5.2 Hz, 4H, H-2''' & 6'''), 3.53-3.55 (t, *J* = 4.8 Hz, 4H, H-3''' & 5'''), 3.77-3.79 (t, *J* = 4.8 Hz, 4H, H-2'' & 6''), 3.96-3.98 (t, *J* = 4.8 Hz, 4H, H-3'' & 5''), 4.47 (s, 2H, -CH_2-), 7.35-7.39 (t, *J* = 7.4 Hz, 1H, H-4' Ph), 7.54-7.58 (t, *J* = 7.6 Hz, 2H, H-2' & 6' Ph), 8.17-8.19 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.41 (s, 1H, H-3);	**Mass** m/z :369(M+1), 367 (M-1);
A9	**IR (Cm^{-1}) (KBr):** 3447.8 (-NH); 2961.4, 2925.0 (-CH_2); 1593.5 (C=C, aromatic); 759.2 (-CH oop)	**^{1}H NMR (400 MHz, DMSO -d_6):**δ 2.32-2.34 (t, *J* = 5.2 Hz, 4H, H-2''' & 6'''), 3.53-3.55 (t, *J* = 4.8 Hz, 4H, H-3''' & 5'''), 3.77-3.79 (t, *J* = 4.8 Hz, 4H, H-2'' & 6''), 3.96-3.98 (t, *J* = 4.8 Hz, 4H, H-3'' & 5''), 4.47 (s, 2H, -CH_2-), 7.35-7.39 (t, *J* = 7.4 Hz, 1H, H-4' Ph), 7.54-7.58 (t, *J* = 7.6 Hz, 2H, H-2' & 6' Ph), 8.17-8.19 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.41 (s, 1H, H-3);	**Mass** m/z :369(M+1), 367(M-1);
A10	**IR (Cm^{-1}) (KBr):** 3447.8 (-NH); 2961.4, 2925.0 (-CH_2); 1593.5 (C=C, aromatic); 759.2 (-CH oop)	**^{1}H NMR (400 MHz, DMSO -d_6):**δ 2.32-2.34 (t, *J* = 5.2 Hz, 4H, H-2''' & 6'''), 3.53-3.55 (t, *J* = 4.8 Hz, 4H, H-3''' & 5'''), 3.77-3.79 (t, *J* = 4.8 Hz, 4H, H-2'' & 6''), 3.96-3.98 (t, *J* = 4.8 Hz, 4H, H-3'' & 5''), 4.47 (s, 2H, -CH_2-), 7.35-7.39 (t, *J* = 7.4 Hz, 1H, H-4' Ph), 7.54-7.58 (t, *J* = 7.6 Hz, 2H, H-2' & 6' Ph), 8.17-8.19 (d, *J* = 8.0 Hz, 2H, H-3' & 5' Ph), 8.41 (s, 1H, H-3);	**Mass** m/z :369(M+1), 367(M-1);

Anti Oxidant Activity

Table 3

In vitro diphenylpicrylhydrazyl assay results

S.No	Sample Code	50µl	100 µl	150 µl	200 µl
1	A1	1.13	1.032	0.988	0.761
2	A2	1.12	1.034	0.981	0.769
3	A3	1.11	1.036	0.989	0.762
4	A4	1.13	1.038	0.982	0.768
5	A5	1.12	1.04	0.988	0.769
6	A6	1.14	1.038	0.983	0.763
7	A7	1.15	1.034	0.987	0.767
8	A8	1.1	1.032	0.984	0.764
9	A9	1.14	1.03	0.986	0.766
10	A10	1.16	1.035	0.985	0.765
31	BHA	1.1	0.081	0.539	0.384

In Vivo Anti Oxidant Activity

EVALUATION OF ANTIOXIDANT ACTIVITY USING AZATHIOPRINE INDUCED OXIDATIVE STRESS IN RATS, SUPEROXIDE DISMUTASE: Superoxide dismutase is class of enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defence in nearly all cells exposed to oxygen. Superoxide dismutase activity was estimated in tissue homogenate with help of pure bovine superoxide dismutase standard. The values were shown in below table, and figure.

Table 2:

Standard graph values of superoxide dismutase

SOD(µU)	Absorbance
1000	0.015
3000	0.017
10000	0.039
30000	0.062
100000	0.16

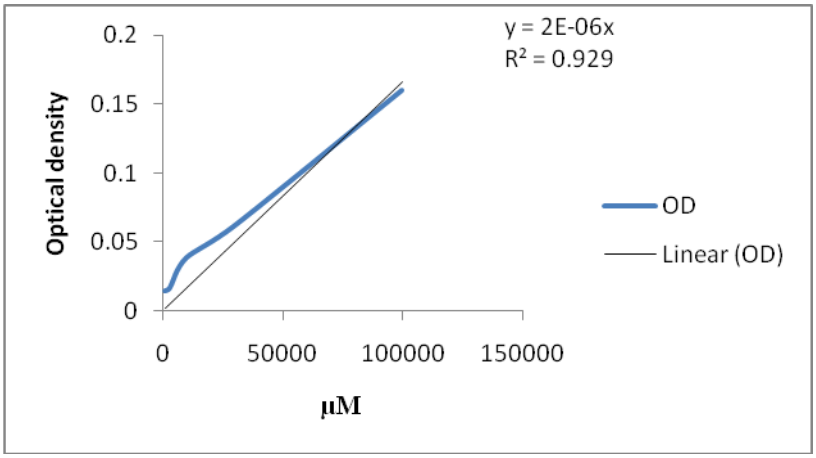

Figure 2: Standard graph of superoxide dismutase

Table 3:

Superoxide dismutase levels in kidney tissue homogenate

Group	SOD(U/mg) in kidney
Normal group	98.6±0.95
Toxic control (20mg/kg)	11.3±0.71
A1 low dose(100mg/kg)	38.07±0.52**
A1high dose (300mg/kg)	56.46±1.08***
Standard ascorbic acid(10mg/kg)	80.2±0.84***

All the values are expressed as mean ±SD (n=6); ** indicates p<0.001, *** indicates p<0.0001 vs toxic control.

Conclusion:

N,1-disubstituted-6-[(phenylamino)methyl]-1H-pyrazolo[3,4-d]pyrimidin-4-amine were treated with different amines under mild conditions to give corresponding amino derivatives (A1-A10), the title compounds in good yields. An optimized method with prominent conditions for the schematic synthesis of the compounds listed as per the title. Synthesized compounds structures were confirmed with spectroscopic data such as mass spectrum, NMR spectrum and Infra red spectrum. Synthesized compound physical properties such as Molecular formula, Molecular weight were developed and recorded. The title compounds were screened for antioxidant activity against antioxidant activity using azathioprine induced oxidative stress in rats All compounds (A**1-A10)** showed similar antioxidant activity of compounds (A1, B5, C10) were more potent when compared to the rest of the compounds synthesized. This acts as a lead for further optimization.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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