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Research Article

Anti cancer activity of novel series of N-substituted-5,6-dimethyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]- pyrimidine derivatives.

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Abstract

N-substituted-1-phenyl-5,6-dimethyl-1,5-dihydro-4*H*-pyrazolo [4,3-d]pyrimidine has been synthesized by 1-phenyl-5-amino-4,5-dihydro-1*H*-pyrazole-4-carbox-amide derivatives as novel series were reacted with ester ethylacetate and aromtised as the pyrimidine ring the carbonyl functional group of the molecules were further halogenated by chlorination with phosphorous oxy chloride and yielded 1-phenyl-4-chloro-5,6-dimethyl-4,5-dihydro-1*H*-pyrazolo[4,3-d]pyrimidine: which further aminated with alkyl and aromatic amines are substituted chlorines. Compounds prepared were diluted to a concentration 50µL and other dilutions were prepared in 96well plates in triplicate and final volume made up to 100µL using DMSO solvent. 50-100µL of tris-base solution with pH10.5 were added to all wells and shaked in an equipment for shaking called orbital shaker and incubated for a duration of 10 min to stabilise the dye bounded to a protein. Absorbance has been measured at 510nm at a micro plate to obtain observations. Cell % growth = Absorbance sample/ Absorbance negative control X 100 % Growth inhibition = 100 - % Cell growth.

Keywords: *Pyrazolo pyrimidine, doxurubicin, Ethoxy methylidene propanedinitrile, ethoxymethoxyethane and anti cancer activity.*

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Introduction

Spiro cyclic systems containing one quaternary carbon center and two fused rings arc structurally interesting. Spiro compounds were naturally occurring substances isolated from various sources (Conroy, H.et.al., 1959), Compounds with spiro moiety exhibit highly pronounced biological and

pharmacological properties(Cui, C. B.et.al., 1996). The rigidity of spiro compounds affords them high thermal stability, and exhibits various photochemical properties(Cui, C. B.et.al., 1996). The construction of ring structures from o-aminocarboxamide as a starting material has wide applicability for the annulation of heterocyclic systems. Pyrazolo[4',3';5,6] pyrido [2,3-t]pyrimidines have wide applications as colorants(Cui, C. B.et.al., 1997), heat and moisture resistant and thermal transfer printing agents(Daly, J. W.et.al., 1870), as well as photographic couplers(Okabe, K. J.et.al 1967). They also display wide range of pharmacological activities such as anticonvulsants(Sakabe, N.et.al.,1967), anti-malarial agents8, anti-inflammatories and central nervous system depressants(James, D.et.al., 1991), Moreover, these types of compounds are inhibitors of cyclic guanosine-3',5'-monophophate phosphodiesterase {cGMP PDE), and are thus agents against erectile dysfunction(Kobayashi, J.et.al., 1991), They also act as antiproliferative agents (Dolle, R. E.et.al., 2001), The multicomponent reaction of 5-aminopyrazole 1 with barbituric acids 2 and aromatic aldehyde 3 under conventional heating, microwave irradiation, or ultrasonic irradiation at various temperatures leads to pyrazolo[4',3':5,6] pyrido [2,3-f]pyrimidines 4 and 5 in high yields. While four component cyclocondensation 5-aminopyrazoles with barbituric acids and aromatic aldehydes in DMF under sonication at room temperature for three hours yielded spiro compounds 4,6-diaryl-1,4,6,7-tetrahydro-277-spiro[pyrazolo[3,4-]pyridine-5,5'- pyrimidine(Gonzalez-Vera. Et.al., 2005).

Experimental Methodology

Synthesis of COMPOUND C1:

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 ethyl acetate (17 ml, 200 mmol) is added drop by drop for 1 hour and refluxed for 14 hours. 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 C2:

Dimethly sulphoxide (150.0 ml). was taken in RBF boiled at 180oC and then slowly to the solvent 6-methyl-1-phenyl-1,5-dihydro-4H-pyrazolo[4,3-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.

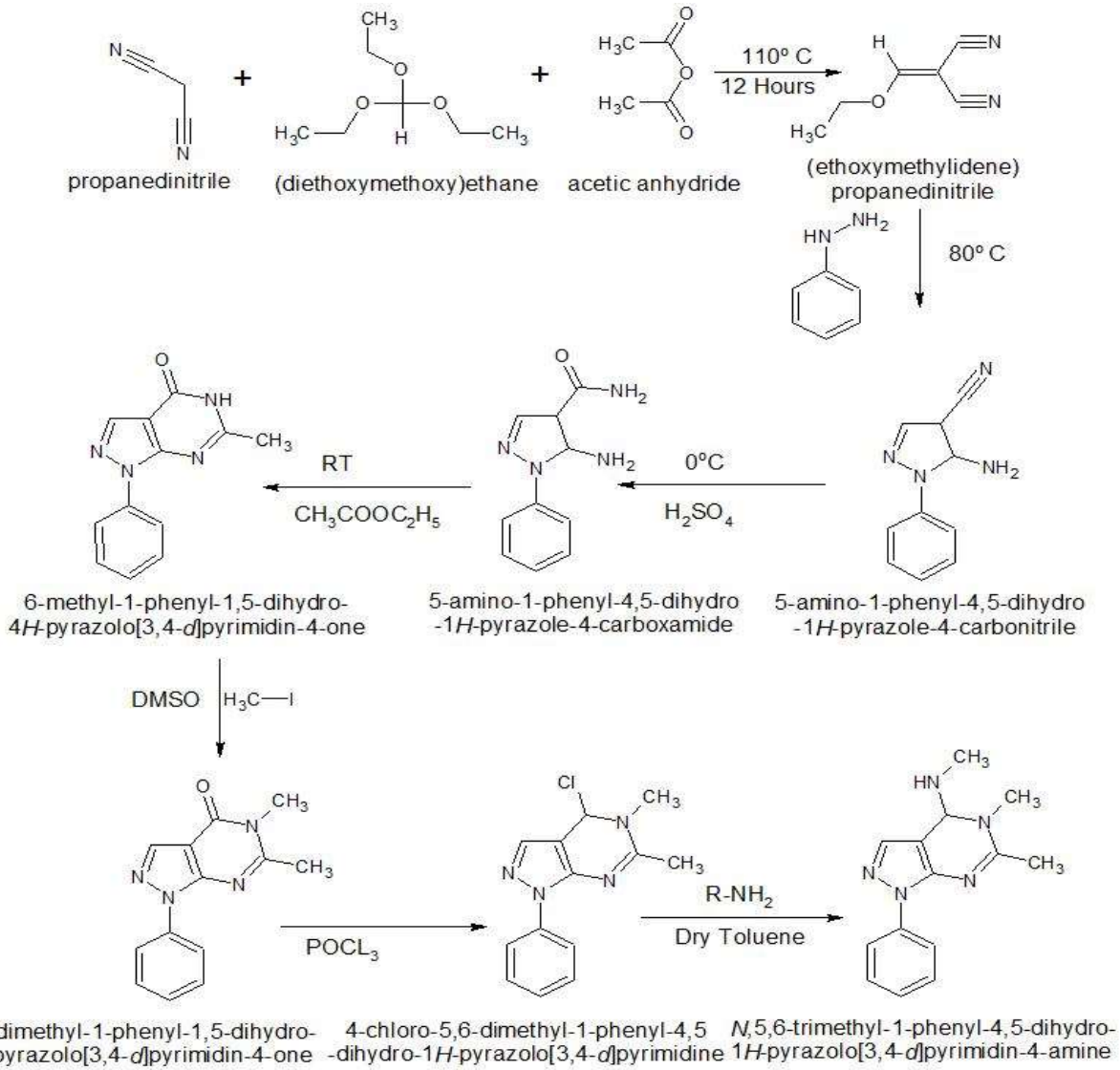

Fig. 1 Schematic representation of synthetic reactions

Synthesis of COMPOUND C3:

5,6-methyl-1-phenyl-1,5-dihydro-4H-pyrazolo[4,3-d]pyrimidin-4-one (0.6 g, 1.82 mmol) and 20.0 ml of phosphorusoxychloride 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.

Synthesis of COMPOUND C4:

A solution of compound C3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then propan-1-amine (6.5 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 C5:

A solution of compound C3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then furan-2-amine (6.7 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 C6:

A solution of compound C3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then butan-1-amine (6.5 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 C7:

A solution of compound C3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then propan-2-amine (6.5 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 C8:

A solution of compound C3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then morpholine (7.5 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 C9:

A solution of compound C3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then ethanamine (6.5 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 NCOMPOUND C10:

A solution of compound C3, (500mg, 1.80 mmol) in freshly dried toluene (5.0 ml), then methaamine (4.5 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.

Anti Cancer Activity

Materials and Methods:

Polysterene tissue-culture plates of 96, 384 flat bottomed wells, PCR plate of 96well, falcon tubes of 15ml, eppendorf tubes of 1.5ml, tissue culture plates of 100mm, pipette tips,1250µL and

125µL matrix pipette, skin cancer cell line, sterile and on sterile reservoir, culture medium, PBS phosphate buffer saline. Serum free medium, fatal bovine serum, trypsin solution, trichloro acetic acid, sulforhodamine B sodium salt in 1% v/v acetic acid, tryptan blue, 1% acetic acid,unbuffered tris base solution, Lipofectamine RNA imax, mi RNA precursor molecules – negative control and mirvana miRNA mimics.Sulforhodamine B assay Testing method.

Sample dilution preparation:

Synthetic compounds prepared were diluted to a concentration 50µL and other dilutions were prepared in 96well plates in triplicate and final volume made up to 100µL using DMSO solvent.

Table No 1 List of dilutions of synthesized compounds

S.NO	SAMPLE ID	DILUTION-1	DILUTION-2	DILUTION-3	DILUTION-4
1	C1	10^{-7}	10^{-6}	10^{-5}	10^{-4}
2	C2	10^{-7}	10^{-6}	10^{-5}	10^{-4}
3	C3	10^{-7}	10^{-6}	10^{-5}	10^{-4}
4	C4	10^{-7}	10^{-6}	10^{-5}	10^{-4}
5	C5	10^{-7}	10^{-6}	10^{-5}	10^{-4}
6	C6	10^{-7}	10^{-6}	10^{-5}	10^{-4}
7	C7	10^{-7}	10^{-6}	10^{-5}	10^{-4}
8	C8	10^{-7}	10^{-6}	10^{-5}	10^{-4}
9	C9	10^{-7}	10^{-6}	10^{-5}	10^{-4}
10	C10	10^{-7}	10^{-6}	10^{-5}	10^{-4}
11	DOXRUBICIN	10^{-7}	10^{-6}	10^{-5}	10^{-4}

Cell line preparation:

Trypnization: Monolayers of cell lines are washed with sterilized PBS phosphate buffer saline. The buffers from the cells are removed by adding 1ml of 0.25%w/v and evenly cover the cell lines. Then incubated at 37ºC for duration of 5 min until cell dissociate from the surface. Then trypsin in the cultures are inactivated by adding 10 volumes of culture medium with FBS fetal bovine serum was mixed until single homogenized medium formed in the cell suspension and then transferred to falcon tubes.

Cell concentration determination:

Falcon tubes are filled with a single cell suspension with 0.4% tryptan blue solution of a ratio 1:1 then suspension was measured for concentration of cells by hematocytometer chamber and measured under microscope and observed for cell viability for prior cell seeding and observed for good cell health. Then the concentration of the cell is adjusted to 10% with FBS growth medium in 96 well a cell density of 50µL. Then the suspension was in a sterile reagent container for operating with multi channel pipette.

Drug exposure to cell lines:

50µL of sample for exposure were transferred into 96 well cell plate and added to each cell. Suspension cell of 50µL is added to each well. Even distribution in the each cell was observed from the bottom cell plate is underwent for short spin of 20 sec for even mixup. Standard drug doxorubicin was incubated in three well as same as the above procedure.

Blank solution preparation:

Three wells in the 96 well plates were filled with DMSO and cell suspension to estimated untreated vehicle control for background subtraction.

Incubation: All the plates were incubated at 37^{o}Cwith humidified incubator in 5% CO until plates verified and observations recorded.

Fixation and staining of cells:

All the treated cell lines were added with 25µL of ice cold trichloroacetic acid of 50% and then supernatant medium and cell plates were incubated at 4oC for duration of one hour. Four times plates washed by submerging the plate ina tub of slow running water. Excess water is removed by gentle tapping into a paper towel and air dried at room temperature. 50µl of 0.04% SRB solution is added for each well and at room temperatures were incubated for duration of one hour then quickly rinsed the plates and washed multiple times with 1% acetic acid and then removed unbound dye. Then air dried at room temperature and observed for bubbles and removed.

Measurement of absorbance:

50-100µL of tris-base solution with pH10.5 was added to all wells and shaked in an orbital shaker and incubated for 10 min to stabilise the protein bounded dye. Absorbance then measured at 510nm on a micro plate reader.

Calculations: Absorbance of background was removed for all the cell wells. Cell % growth = Absorbance sample/ Absorbance negative control X 100 % Growth inhibition = 100 - % Cell growth. Growth inhibition was calculated of 50% was calculated as GI_{50}. TGI: Drug concentration resulting in total growth inhibition (TGI).LC_{50}: Concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment (concentration of drug causing lethality to 50 % of the cells as compared to that at the beginning) indicating a net loss of cells following treatment.

Results

Table No 2 Physical Properties of the synthesized Molecules:

Compound ID	IUPAC Name	Mol Formula	Mol Weight	Mol Composition	Melting Point	Rf Value
C1	6-methyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[4,3-d] pyrimidin-4-one	$C_{12}H_{10}N_4O$	226.2	C(63.71%) H(4.46%) N(24.76%) O(7.07%)	298	0.72
C2	5,6-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo [4,3-d]pyrimidin-4-one.	$C_{13}H_{12}N_4O$	240.3	C(64.99%) H(5.03%) N(23.32%) O(6.66%)	312	0.66
C3	4-chloro-5,6-dimethyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo [4,3-d]pyrimidine	$C_{13}H_{13}ClN_4$	260.7	C(59.89%) H(5.03%) Cl(13.60%) N(21.49%)	258	0.42
C4	5,6-dimethyl-1-phenyl-*N*-propyl-4,5-dihydro-1*H*-pyrazolo [4,3-d]pyrimidin-4-amine.	$C_{16}H_{21}N_5$	283.4	C(67.82%) H(7.47%) N(24.71%)	258	0.72
C5	*N*-(furan-2-yl)-5,6-dimethyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[4,3-d]pyri midin-4-amine.	$C_{17}H_{17}N_5O$	307.3	C(66.43%) H(5.58%) N(22.79%) O(5.21%)	298	0.46
C6	*N*-butyl-5,6-dimethyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo [4,3-d]pyrimidin-4-amine.	$C_{17}H_{23}N_5$	297.4	C(68.66%) H(7.80%) N(23.55%)	288	0.72
C7	5,6-dimethyl-1-phenyl-*N*-(propan-2-yl)-4,5-dihydro-1*H*-pyrazolo[4,3-d]pyrimidin-4-amine.	$C_{16}H_{21}N_5$	283.4	C(67.82%) H(7.47%) N(24.71%)	320	0.66
C8	5,6-dimethyl-4-(morpholin-4-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[4,3-d]pyrimidine.	$C_{17}H_{21}N_5O$	311.4	C(65.57%) H(6.80%) N(22.49%) O(5.14%)	295	0.72
C9	*N*-ethyl-5,6-dimethyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo [4,3-d]pyrimidin-4-amine.	$C_{15}H_{19}N_5$	269.3	C(66.89%) H(7.11%) N(26.00%)	275	0.42
C10	*N*,5,6-trimethyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo [4,3-d]pyrimidin-4-amine.	$C_{14}H_{17}N_5$	255.3	C(65.86%) H(6.71%) N(27.43%)	268	0.72

Table No 3 Spectral Properties of the synthesized Molecules:

Compound ID	IR Spectra	NMR Spectra	Mass Spectra
C1	**IR (Cm^{-1}) (KBr):** 3151.4 (-NH, 2° amide); 2923.9 (-CH_3); 3013.5(=C-H, aromatic); 1743.1 (-C=O); 1501.9 (C=C, aromatic); 780.5 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):** □ 2.42 (s, 3H, -C<u>H</u>$_3$), 7.37-7.41 (t, *J* = 7.4 Hz, 1H, H-4' ph), 7.54-7.58 (t, *J* = 8.0 Hz, 2H, H-2' & 6' ph), 8.04-8.06 (d, *J* = 8.0 Hz, 2H, H-3' & 5' ph), 8.25 (s, 1H, H-3).	**Mass** m/z :227(M+1), 225(M-1);
C2	**IR (Cm^{-1}, KBr):** 3161.5 (-NH, 2° amide); 3037.0(=C-H, aromatic); 1595.6 (-C=O); 1517.0 (C=C, aromatic); 782.5 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):** □ 7.38-7.42 (t,*J*= 7.4 Hz, 1H, H-4' Ph), 7.4-7.58 (t,*J*= 7.8 Hz, 2H, H-3' & 5' Ph),8.05-8.07 (d,*J*= 8.0 Hz, 2H, H-2' & 6' Ph), 8.20 (s, 1H, H-3), 8.30 (s, 1H, H-6), 8.48 (s, 1H, -N<u>H</u>).	**Mass m/z** :241(M+1), 239(M-1);
C3	**IR (Cm^{-1}, KBr):** 3161.5 (-NH, 2° amide); 3037.0(=C-H, aromatic); 1595.6 (-C=O); 1517.0 (C=C, aromatic); 782.5 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):** □ 7.38-7.42 (t,*J*= 7.4 Hz, 1H, H-4' Ph), 7.4-7.58 (t,*J*= 7.8 Hz, 2H, H-3' & 5' Ph),8.05-8.07 (d,*J*= 8.0 Hz, 2H, H-2' & 6' Ph), 8.20 (s, 1H, H-3), 8.30 (s, 1H, H-6), 8.48 (s, 1H, -N<u>H</u>).	**Mass** m/z :261(M+1), 259(M-1);
C4	**IR (Cm^{-1}) (KBr):** 3447.9 (-NH); 2955.7, 2922.8 (-CH_3); 1606.2 (C=C, aromatic); 784.9 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):** □ 0.94-0.98 (t, *J* = 7.4 Hz, 3H,-CH_2-CH_2-C<u>H</u>$_3$), 1.60-1.69 (sextet, *J* = 7.2 Hz, 2H, -CH_2-C<u>H</u>$_2$-CH_3), 2.48 (s, 3H, -C<u>H</u>$_3$), 3.46-3.51 (q, *J* = 6.5 Hz, 2H,–C<u>H</u>$_2$-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 (dd, *J* = 8.0 Hz, 2H,H-3' & 5' ph), 8.33 (s, 1H, H-3).	**Mass** m/z :284(M+1), 282(M-1);
C5	**IR (Cm^{-1}) (KBr):** 3451.14 (-NH); 3071.76 (=C-H, aromatic); 2911.84 (-CH_2); 1591.64 (C=C, aromatic); 739.76 (-CH oop).	**^{1}H NMR (400 MHz, $CDCl_3$):** □4.86-4.87 (d, *J* = 5.2 Hz, 2H, -NH-C<u>H</u>$_2$-), 5.85 (s, 1H, N<u>H</u>), 6.34-6.36 (d, *J* = 3.2 Hz, 2H, H-2''&6'' ph), 7.30-7.34 (t, *J* = 7.4 Hz, 1H, H-4'' ph), 7.40 (m, 1H, H-4'''), 7.48-7.52 (d, *J* = 8.0 Hz, 2H, H-3''& 5''), 8.04 (s, 1H, H-3), 8.13-8.15 (d, *J* = 8.0 Hz, 2H, H-3'&5' ph), 8.51 (s, 1H, H-6).	**Mass** m/z :308 (M+1), 306(M-1);
C6	**IR (Cm^{-1}) (KBr):** 3446.21 (-NH); 3077.47 (=C-H, aromatic); 2931.03 (-CH_2); 1591.60 (C=C, aromatic); 750.70 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):** □ 0.97-1.01 (t, *J* = 7.4 Hz, 3H,-CH_2-CH_2-CH_2-C<u>H</u>$_3$), 1.40-1.49 (sextet, *J* = 7.3 Hz, 2H,-CH_2-CH_2-C<u>H</u>$_2$-CH_3), 1.63-1.71 (pentet, *J* = 7.2 Hz, 2H,-CH_2-C<u>H</u>$_2$-CH_2-CH_3), 3.56-3.61 (q, *J* = 6.5 Hz, 2H,–C<u>H</u>$_2$-CH_2-CH_2-CH_3), 7.37-7.41 (t, *J* = 7.4 Hz, 1H, H-4' ph), 7.57-7.61 (d, *J* = 7.8 Hz, 2H,H-2' & 6' ph), 8.24-8.26 (d, *J* = 7.6Hz, 2H,H-3' & 5' ph), 8.42 (s, 1H, H-3), 8.44 (s, 1H, H-6).	**Mass** m/z :298 (M+1), 296(M-1);
C7	**IR (Cm^{-1}) (KBr):** 3446.29 (-NH); 3087.90 (=C-H, aromatic); 2970.40 (-CH); 1585.79 (C=C, aromatic); 747.31 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):** □ 1.26-1.28 (d, *J* = 6.8 Hz, 6H,-CH-[C<u>H</u>$_3$]$_2$), 4.43-4.47 (heptet, *J* = 5.2 Hz, 1H, -C<u>H</u>-[CH_3]$_2$), 7.32-7.35 (t, *J* = 7.4 Hz, 1H, H-4' ph), 7.52-7.56 (d, *J* = 8.0 Hz, 2H,H-2' & 6' ph), 8.21-8.23 (d, *J* = 8.0 Hz, 2H,H-3' & 5' ph), 8.37 (s,1H, H-3), 8.4 (s,1H, H-6).	**Mass** m/z :284 (M+1), 282(M-1);
C8	**IR (Cm^{-1}) (KBr):** 3018.2 (=C-H, aromatic); 2917.65 (-CH_2); 1570.75 (C=C, aromatic); 771.58 (-CH oop).	**^{1}H NMR (400 MHz, DMSO-d_6):** □ 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), 7.35-7.39 (t, *J* = 7.4 Hz, 1H, H-4' ph), 7.54-7.58 (d, *J* = 7.6 Hz, 2H, H-2'& 6' ph), 8.17-8.19 (d, *J* = 8.4 Hz, 2H, H-3' & 5' ph), 8.41 (s, 1H, H-3), 8.60 (s, 1H, H-6).	**Mass** m/z :312 (M+1), 310(M-1);
C9	**3 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):** □ 0.69-0.73 (t, *J* = 7.4 Hz, 3H, -CH_2-NH-CH_2-C<u>H</u>$_3$), 1.22-1.25 (t, *J* = 7.2 Hz, 3H, -NH-CH_2-C<u>H</u>$_3$), 2.67-2.73 (pentet, *J* = 6.8 Hz, 2H, -CH_2-NH-C<u>H</u>$_2$-CH_3), 3.52-3.59 (pentet, *J* = 6.8 Hz, 2H, -CH_2-NH-C<u>H</u>$_2$-CH_3), 3.47-3.52 (q, *J* = 6.6 Hz, 2H, -NH-C<u>H</u>$_2$-CH_3), 3.96-4.05 (q, *J* = 6.2 Hz, 2H, -CH_2-NH-C<u>H</u>$_2$ -CH_3), 4.47 (s, 2H, -C<u>H</u>$_2$-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 :270 (M+1), 268(M-1);
C10	**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):** □ 0.69-0.73 (t, *J* = 7.4 Hz, 3H, -CH_2-NH-CH_2-C<u>H</u>$_3$), 1.22-1.25 (t, *J* = 7.2 Hz, 3H, -NH-CH_2-C<u>H</u>$_3$), 2.67-2.73 (pentet, *J* = 6.8 Hz, 2H, -CH_2-NH-C<u>H</u>$_2$-CH_3), 3.52-3.59 (pentet, *J* = 6.8 Hz, 2H, -CH_2-NH-C<u>H</u>$_2$-CH_3), 3.47-3.52 (q, *J* = 6.6 Hz, 2H, -NH-C<u>H</u>$_2$-CH_3), 3.96-4.05 (q, *J* = 6.2 Hz, 2H, -CH_2-NH-C<u>H</u>$_2$ -CH_3), 4.47 (s, 2H, -C<u>H</u>$_2$-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 :256 (M+1), 254(M-1);

Table No 4 Anti cancer activity results

	Average Values			
Sample Code	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
C1	100.0	96.5	86.3	-61.0
C2	101.0	85.5	65.9	30.9
C3	100.0	96.4	83.3	40.0
C4	100.0	94.7	84.7	-34.7
C5	100.0	96.5	86.3	-61.0
C6	100.0	100.0	100.0	13.5
C7	100.0	100.0	99.8	43.9
C8	100.0	100.0	100.0	61.5
C9	100.0	100.0	100.0	-30.0
C10	100.0	92.5	77.6	6.9
ADR	-24.0	-62.5	-64.2	-70.7

Table. 5

Anti cancer activity on skin cell line at different levels

G361	Molar drug concentration		
	LC 50	TGI	GI50
C1	>10-4	3.2*10-5	2.02*10-6
C2	>10-4	>10-4	3.2*10-5
C3	>10-4	>10-4	2.6*10-6
C4	>10-4	>10-4	2.3*10-6
C5	>10-4	>10-4	3.2*10-5
C6	>10-4	3.2*10-5	2.02*10-6
C7	>10-4	>10-4	2.8*10-6
C8	>10-4	>10-4	3.8*10-5
C9	>10-4	3.7*10-5	2.2*10-6
C10	>10-4	3.2*10-5	2.04*10-6
ADR	1.7*10-7	<10-7	<10-7

Conclusion

N-substituted-5,6-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[4,3-d]pyrimidine 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 anticancer activity against human sin cancer cell lines with doxirubicin as standard comparative drug. All compounds (C1-C10) showed similar antioxidant activity of compounds (C1, C3, C4, C6, C7, C9 and 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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