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RESEARCH ARTICLE

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A Path for Horizing Your Innovative Work

SYNTHESIS AND PHARMACOLOGICAL SCREENING OF PYRAZOLOPYRIDINE COMPOUNDS AS ANXIOLYTICS

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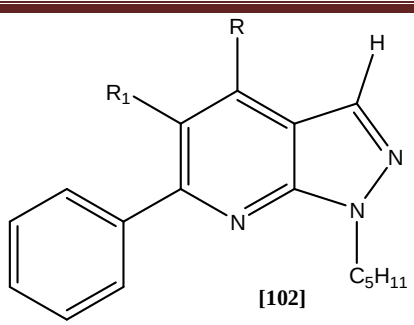
Abstract: Amine & chloro derivatives of Ethyl 4-substituted-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate & carboxylic acid were synthesized using liquid ammonia & phosphorous oxychloride from 1-pentyl-5 amino pyrazole respectively. 3 amino-2 substituted-4,7-dihydro-2H-Pyrazolo[4,3-c]pyridine-4,6-diol were synthesized from 3-cyanometyl-4-cyano-5-amino-1-substituted pyrazole using active methylene compound, Malononitrile & substituted hydrazine. Pyrazolo[3,4-b]pyridinone was synthesized from 3-amino-1-phenyl pyrazolone & acetylacetone. Completion of reactions was checked by TLC using hexane:ethyl acetate as mobile phase. Reagents like Diethyl Benzoyl malonate & β-Cyano Ethylhydrazine were also synthesized. All the synthesized compounds were characterized by IR, Mass & NMR analytically & by melting point & TLC physically. The synthesized compounds were tested using Elevated Plus Maze model.

Keywords: Anxiety, Pyrazolo[3,4-b]pyridine, Pyrazolo[4,3-c]pyridine, Pyrazolo[3,4-b]pyridinone, Diethyl benzoyl malonate, β-cyanoethyl hydrazine

INTRODUCTION

Fused pyridines continue to attract considerable attention of researchers in different countries because of their great practical usefulness, primarily, due to a very wide spectrum of their biological activities[1]. Along with some other pyridine systems containing an annelated five membered heteroaromatic ring, pyrazolopyridines are isosters of bioactive indoles or indazoles. Pyrazolopyridines are allosteric modulators of benzodiazepine receptors. They are a class of non-sedating anxiolytics, agreeably more potent than benzodiazepines. The pyrazolo[3,4-b]pyridine moieties represent important building blocks in both natural & synthetic bioactive compounds[1]. They show anxiolytic activity along with Xanthine oxidase inhibitors, cholesterol formation-inhibitor, & Anti-Alzheimer[4]. They are also used in gastrointestinal diseases anorexia nervosa, drug and alcohol withdrawal symptoms, drug addiction, and infertility activities. They also act as potent and selective inhibitors of A1 adenosine receptors, phosphodiesterase (PDE4) inhibitors in immune and inflammatory cells, glycogen synthase kinase-3(GSK-3) inhibitors, kinase inhibitors of p38 as anti-inflammatory[2].

Recent research efforts in the anxiolytic area have been aimed at discovering anxioselective agent, i.e., compounds which at therapeutically effective doses show a reduced propensity to cause one or more of the unwanted ancillary activities associated with BZ usage[3]. Bare et al. reported the synthesis of pyrazolopyridine esters and amides which gave compounds with potent anxiolytic action.Pyrazolo[3,4-b]pyridines have been prepared generally by cyclization reactions starting from different heterocyclic reagents[7]. D.B. Kendre & co-workers reported the synthesis of novel pyrazolo[3,4-*b*]pyridines has been achieved successfully by sequence of *Gould - Jacobs* reaction between 5-aminopyrazole and diethylethoxymethylenemalonate in good yield[9]. So based on the earlier reports in order to find out more potent and safe anxiolytic, it was thought of interest to carryout synthesis of substituted pyrazolopyridines. This prompted us to synthesize some more derivatives in the series to carryout systematic SAR study by changing various substituents on pyridine ring R & R_1



[102]

R=NH_2, Cl
R_2 = COOH, COOEt

To check the biological versatility of the pyrazolopyridine compounds, apart from pyrazolo[3,4-b]pyridines, pyrazolo[4,3-c]pyridine & pyrazolo[3,4-b]pyridinone was synthesised.

MATERIALS & METHODS

Apparatus

All the Chemicals and Solvents were obtained from E-Merck, India (AR grade) and were used further purification. The melting point of the synthesized compounds were determined in open capillary using VEEGO MELTING POINT APPARATUS model VMP-D and recorded in Celsius without correction. Purity of the compound was verified by precoated TLC plates (E- Merck Kieselgel 60 F_{254}). The Infrared spectra for the synthesized compounds were recorded using SHIMADZU-FTIR 8400S spectrometer using KBr as a back ground. ^{1}H NMR spectra of the synthesized compounds were taken using BRUKER Advance-II 400 NMR spectrometer using Tetramethyl silane as an internal standard. ^{1}H NMR spectra were recorded with $CDCl_3$ as a solvent & the chemical shift data were expressed as delta values related to TMS. Mass spectra of the synthesized compounds were taken using 2010EV LCMS SHIMADZU instrument at 70 eV.

Reagents and Materials

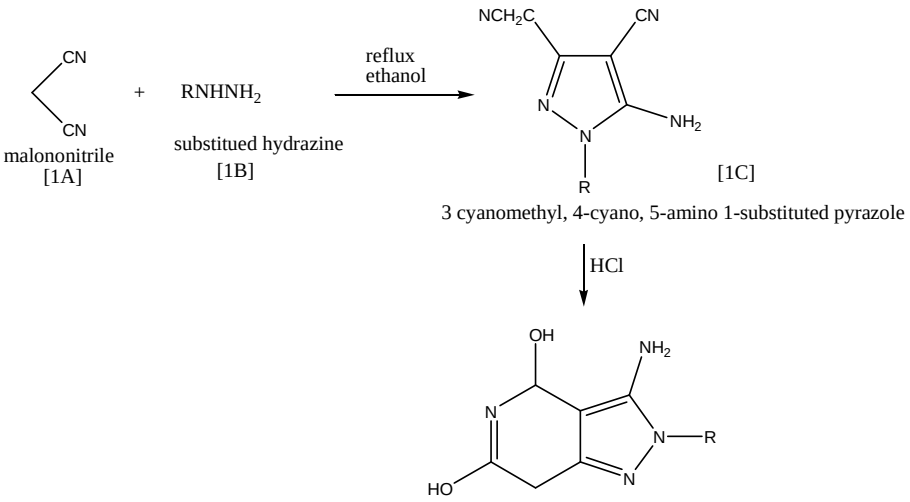
Malononitrile, Methyl hydrazine, Phenyl hydrazine, Conc. HCl, Ethyl cyanoacetate, Valeraldehyde, Acrylonitrile, Hydrazine hydrate, Sodium metal, Magnesium metal turnings, Diethyl malonate, Ethyl chloro carbonate, Benzoic acid, Acetyl acetone, Phosphorous oxychloride, NaOH, Ammonia solution, Diphenyl ether, Sodium sulphate, Magnesium Chloride, Calcium chloride, Glacial acetic acid, Conc. Sulphuric acid, iodine.

Solvents used: Ethanol, Methanol, Ethyl acetate, Butanol, Toluene, Hexane, Acetone.

EXPERIMENTAL:

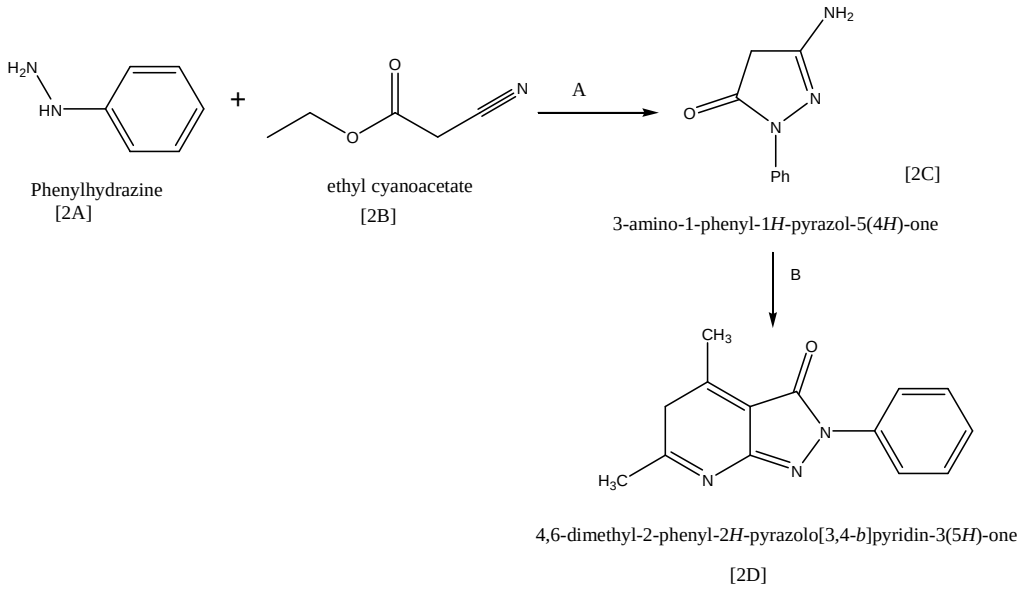
SYNTHETIC APPROACH:

Scheme 1: Synthesis of substituted Pyrazolo[4,3-c]pyridine[10]:



R= Methyl hydrazine, Hydrazine hydrate

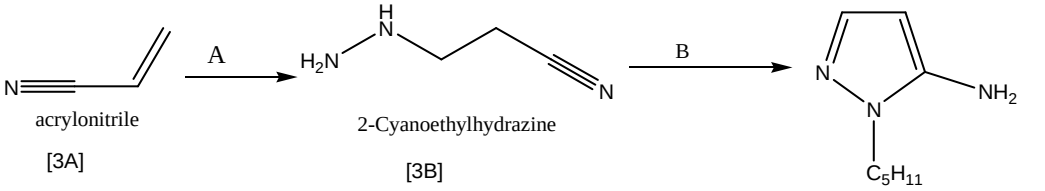
Scheme 2: Synthesis of 4,6-dimethyl, 2-phenyl, 2H-pyrazolo[3,4-b]pyridine-3(5)-one[11]:



A=NaOEt, EtOH,aq.HOAc
B=acetyl acetone, glacial acetic acid

Scheme 3: Synthesis of Pyrazolo[3,4-b]pyridine:

Step-1: synthesis of 1-pentyl, 5-amino pyrazole[7]:



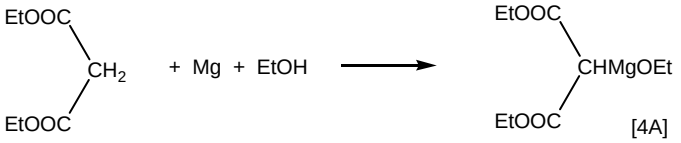
acrylonitrile [3A]

2-Cyanoethylhydrazine [3B]

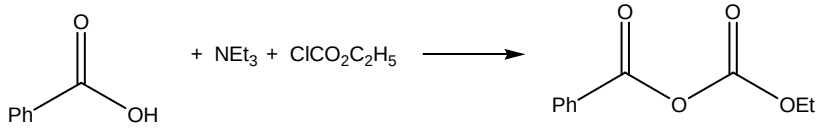
1-pentyl-1*H*-pyrazol-5-amine [3C]

A= Hydrazine hydrate
B= toluene, sodium butoxide

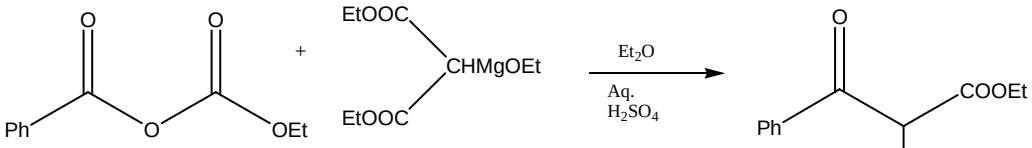
Step-2: synthesis of diethyl benzoyl malonate[13]:



[4A]



[4B]



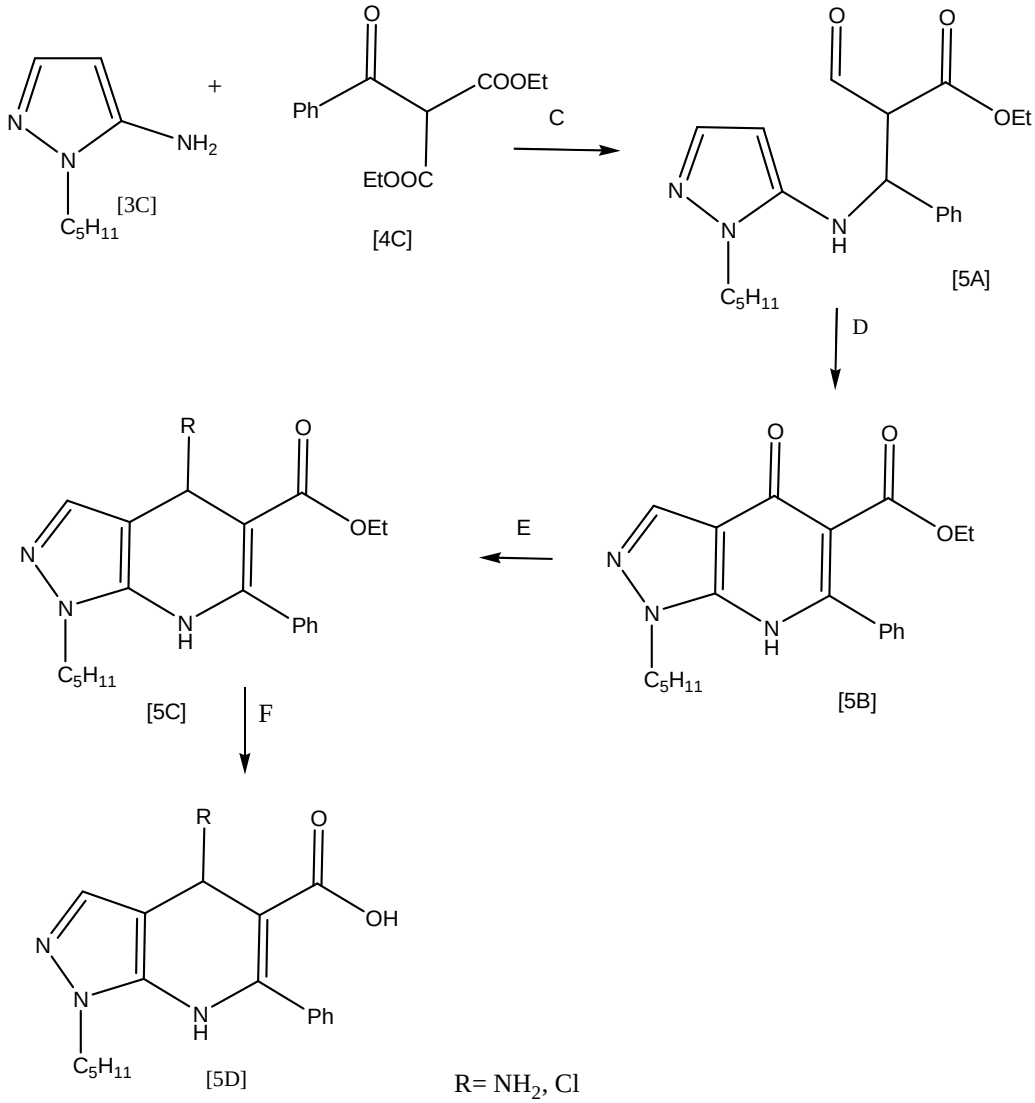
Ethoxymagnesium malonic ester

mixed Benzoic- carbonic anhydride

Diethyl benzoyl malonate

[4C]

Synthesis of ethyl 4-substituted-1-pentyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate(5D)[7]



C= ethanol, reflux

D= diphenyl ether, reflux

E= phosphorous oxychloride, liquid ammonia,reflux

F= NaOH, HCl

SCHEME-1: Synthesis of 2-Substitued, 3-Amino, 4-dihydroxypyrazolo[4,3-c]pyridine:[1D]

Step 1: Synthesis of 1 substituted- 3 cyanomethyl- 4 cyano- 5 aminopyrazole**:** [1C]

- To a boiling solution of 0.5 mole of malononitrile in 90 ml of ethanol was added a solution of substituted hydrazine in 15 ml of ethanol at such a rate that the mixture continued to boil without any external boiling.
- After addition of all the methyl hydrazine solution, the mixture was allowed to reflux for 30 min.,and cooled at refrigerating temperature to give 12.0 gm of crude, white needle shape crystals.
- Second crop of crystals was obtained on cooling the filterate. Recyrstallisation with water gave white crystals.

1-phenyl- 3 cyanomethyl- 4 cyano- 5 aminopyrazole:[1C-A]

Yield: 69%, Molecular formula: $C_{11}H_{11}N_5$, Molecular weight: 213 gm/mole, Melting point:208-210^{0}C, Solvent system:Hexane : Ethyl acetate (9:1), Rf value: 0.53

1-methyl- 3 cyanomethyl- 4 cyano- 5 aminopyrazole:[1C-B]

Yield: 65%, Molecular formula: $C_7H_7N_5$, Molecular weight: 161 gm/mole, Melting point:178-179^{0}C, Solvent system:Hexane : Ethyl acetate (9:1), Rf value: 0.56

3 cyanomethyl- 4 cyano- 5 aminopyrazole:[1C-C]

Yield: 70% , Molecular formula : $C_6H_5N_5$, Molecular weight: 147 gm/mole, Melting point:198-200^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.5 cm

Step-2: Synthesis of 2-Substitued, 3-Amino, 4-dihydroxypyrazolo[4,3-c]pyridine:[1D]

- A suspension of 1.5 gm of 1-substituted 3 cyanomethyl 4-cyano- 5 aminopyrazole in 15 ml HCl was heated till boiling to give a clear solution.
- Additional 10 ml cold water was added followed by slow cooling, resulting into the separation of yellow crystals which were collected by filteration & washed with water.
- Recrystallisation with water yielded light yellow needles.

2-phenyl, 3-Amino, 4-dihydroxypyrazolo[4,3-c]pyridine:

Yield:38%, Molecular formula: $C_{12}H_{13}N_4O_2$, Molecular weight: 245 gm/mole, Melting point:2274-276^{0}C, Solvent system: Hexane : Ethyl acetate

(9:1), Rf value: 0.38 cm, IR frequency(cm^{-1}), 1505.49 (C=C), 1301.03 (C-N), Mass (m/z): 168 (M+), 167.9 (M-1), ^{1}HNMR-dmso

(ppm): 2.12-2.55 δ (d, 1H, OH),3.30 δ (d, 2H, N-CH_3),4.30 δ (s, 2H, NH_2).

2-methyl, 3-Amino, 4-dihydroxypyrazolo[4,3-c]pyridine:

Yield:35%, Molecular formula: $C_6H_8N_4O_2$, Molecular weight:168 gm/mole, Melting point :288-290^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.32 cm, IR frequency (cm^{-1}), 1264.38(C-N)3170(OH), Mass (m/z): 183.1 (M+1) 179.9 (M-1), ^{1}HNMR-dmso(ppm): 2.55 δ (d, 1H, OH),4.50 δ (s, 2H, NH_2).

3-Amino, 4-dihydroxypyrazolo[4,3-c]pyridine:

Yield: 40% ,Molecular formula: $C_7H_{10}N_4O_2$, Molecular weight: 182 gm/mole, Melting point: 320-22^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.35 cm

SCHEME 2: Synthesis of 4,6-dimethyl, 2-substituted, 2H-pyrazolo[3,4-b]pyridine-3(5)-one[12]:

Step 1: Synthesis of 3-amino, 1-substituted, 5-pyrazolone:

- Sodium ethoxide is prepared from 46 g. (2 gram atoms) of sodium and 800 ml. of absolute ethanol in a 2-l. three-necked flask equipped with stirrer and a reflux condenser.
- To the hot solution is added 113 g. (106 ml., 1 mole) of ethyl cyanoacetate followed by 108 g. (98 ml., 1 mole) of substituted hydrazine, and the mixture is stirred and heated in an oil bath at 120° for 16 hours.
- Then most of the ethanol is removed under reduced pressure and the residue is dissolved in 1 l. of water; the mixture is warmed to about 50° and stirred to facilitate solution. After being cooled to room temperature, the solution is extracted with three 100-ml. portions of ether.
- The aqueous phase is acidified by the addition of 100 ml. of glacial acetic acid, cooled in ice, and filtered.
- The crude product is washed on the filter with 100 ml. of 95% ethanol; it is then transferred to a flask and boiled with 500 ml. of the same solvent, and this mixture is cooled and filtered.
- The solid is washed with ethanol and dried. The tan crystalline 1-phenyl-3-amino-5-pyrazolone, melting with decomposition at 216–218°, weighs 76–82 g.(43–47%)

3-amino, 1-phenyl, 5-pyrazolone

Yield: 56%, Molecular formula:$C_9H_9N_3O$, Molecular weight:175 gm/mole, Melting point: 176-178^{0}C, Solvent system:

Hexane : Ethyl acetate (9:1), Rf value: 0.38

3-amino, 1-methyl, 5-pyrazolone:

Yield: 53%, Molecular formula:$C_4H_7N_3O$, Molecular weight:113 gm/mole, Melting point: 166-168^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.42

3-amino, 1-ethyl, 5-pyrazolone:

Yield: 50%, Molecular formula:$C_5H_9N_3O$, Molecular weight:127 gm/mole, Melting point: 158-160^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.42

3-amino, 5-pyrazolone:

Yield: 48%, Molecular formula:$C_3H_5N_3O$, Molecular weight: 99 gm/mole, Melting point: 148-150^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.42

Step 2: Synthesis of 4,6-dimethyl, 2-phenyl, 2H-pyrazolo[3,4-b]pyridine-3(5)-one

- 8.75 gms of 3-amino, 1-substituted, 5-pyrazolone, 10 ml of glacial acetic acid & 5.5 ml acetyl acetone were kept on steam bath for 16 hrs.
- After addition of 25 ml of water, the reaction product crystallized on cooling. It was filtered, washed with water & recrystallised with dilute alcohol.
- The orange crystals melted with decomposition at 196-197^{0}C

4,6-dimethyl, 2-phenyl, 2H-pyrazolo[3,4-b]pyridine-3(5)-one:

Yield: 58%, Molecular formula: $C_{14}H_{13}N_3O$, Molecular weight: 239, Melting point: 224-226^{0}C, Solvent system: Hexane: Ethyl acetate (9:1), Rf value: 0.5, IR frequency:1625(C=O), 1339.61 (C-N), Mass m/z (ppm): 239.8 (M^+)

4,6-dimethyl, 2-ethyl, 2H-pyrazolo[3,4-b]pyridine-3(5)-one:

Yield: 40%, Molecular formula: $C_{12}H_{11}N_3O$, Molecular weight: 192, Melting point: 236-238^{0}C, Solvent system: Hexane: Ethyl acetate (9:1), Rf value: 0.52

4,6-dimethyl, 2-methyl, 2H-pyrazolo[3,4-b]pyridine-3(5)-one:

Yield: 45%, Molecular formula: $C_{11}H_{12}N_3O$, Molecular weight: 178, Melting point: 192-194-226^{0}C, Solvent system: Hexane: Ethyl acetate (9:1), Rf value: 0.54

4,6-dimethyl, 2H-pyrazolo[3,4-b]pyridine-3(5)-one:

Yield: 56%, Molecular formula: $C_8H_9N_3O$, Molecular weight: 165, Melting point: 1680-17^{0}C, Solvent system: Hexane: Ethyl acetate (9:1), Rf value: 0.55

SCHEME 3: Synthesis of ethyl 4-substituted-1-pentyl-6-phenyl-4,7-

dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate (5D):

Step 1: Synthesis of β-cyanoethylhydrazine

- To a 500ml two-necked flask fitted with a thermometer and a pressure-equalizing funnel are added a large magnetic stirring bar and 4.17 g. (0.60 moles of $N_2H_4 \cdot H_2O$) of 72% aqueous hydrazine hydrate.
- Acrylonitrile (3.18 g., 0.60 moles) is gradually added with stirring during 2 hours. The internal temperature is kept at 30–35° by occasional cooling of the flask.
- The funnel is replaced by a distillation condenser. Removal of water by distillation at 40 mm. at a bath temperature of 45–50° gives 49–51gm of β-cyanoethylhydrazine as yellow oil. This product can be purified by distillation.

Yield: 82%, Molecular formula: $C_3H_7N_3$, Molecular weight: 85.11, Boiling point: 76–79°,Solvent system: Hexane:ethyl acetate (9:1), Rf value: 0.63

Step 2: Synthesis of 1-pentyl, 5-amino pyrazole:

- Into a 150ml beaker, stirred solution of 0.0473 mol β-cyanoethylhydrazine in 40 ml toluene was added dropwise to a solution of 0.0497 mol of valeraldehyde in 10 ml toluene.
- After stirring at 3 hr at room temp., the reaction mixture was concentrated to give 8. 5 gm crude intermediate hydrazone as amber oil.
- This oil was then added to a solution of sodium butoxide [prepared by reacting 0.2 gm sodium in 45 ml of butanol] & refluxed for 5 hrs.
- The resulting cooled solution was concentrated to give 10.5 gm of dark, viscous oil, which was then distilled to give light yellow oil

1-pentyl, 5-amino pyrazole:

Yield: 40%, Molecular formula: $C_8H_{15}N_3$, Molecular weight: 153.11, Boiling point:114^0C, Solvent system: Hexane : ethyl acetate (9:1), Rf value: 0.54

1-butyl, 5-amino pyrazole:

Yield: 43%, molecular formula: $C_7H_{13}N_3$, Molecular weight: 139.12, Melting point:144-46^0C, Solvent system: Hexane : ethyl acetate (9:1), Rf value: 0.38

1-ethyl, 5-amino pyrazole:

Yield: 35%, Molecular formula: $C_5H_9N_3$, Molecular weight: 111.15, melting point:136-38^0C, Solvent system: Hexane : ethyl acetate (9:1), Rf value: 0.52

1-phenyl, 5-amino pyrazole:

Yield: 40%, Molecular formula: $C_9H_9N_3$, Molecular weight: 159.1, Melting point: 154-56^0C, Solvent system: Hexane : ethyl

acetate (9:1), Rf value: 0.62

1-methyl, 5-amio pyrazole:

Yield: 40%, Molecular formula: $C_4H_7N_3$, Molecular weight: 97.12, Melting point: 128-30^{0}C, Solvent system: Hexane : ethyl acetate (9:1), Rf value: 0.44

Step 3: Synthesis of diethyl benzoyl malonate:

A. *Synthesis of Ethoxymagnesiummalonic ester*:

- In a 250-ml. three-necked flask equipped with a dropping funnel and an efficient reflux condenser fitted with a calcium chloride drying tube are placed 5.0 g. (0.2 g. atom) of magnesium turnings (Grignard), 5 ml. of absolute alcohol, 0.2 ml. of carbon tetrachloride, and 6 ml. of a mixture of 32.0 g. (30.2 ml., 0.2 mole) of diethyl malonate and 16 ml. of absolute alcohol.
- The reaction will proceed in a few minutes and may require occasional cooling before the addition of the remainder of the diethyl malonate solution.
- The addition should be controlled so that the reaction goes at a fairly vigorous rate. When the reaction mixture has cooled to room temperature, 60 ml. of ether dried over sodium wire is cautiously added.
- When the reaction again appears to subside, gentle heating by means of a steam bath is begun and continued until nearly all the magnesium has disappeared.
- The alcohol and ether are removed by distillation, first at atmospheric pressure and then at reduced pressure secured with a water pump. To the partially crystalline product is added 60 ml. of dry benzene, and the solvent is again removed by distillation at atmospheric and then reduced pressure.
- The residue is dissolved in 60 ml. of dry ether to await the completion of the mixed carbonic-carboxylic anhydride preparation.

B. *Mixed benzoic-carbonic anhydride:*

- In a 500-ml. three-necked flask, equipped with a low-temperature thermometer, an efficient sealed stirrer, and an adaptive joint carrying a drying tube and a dropping funnel, is placed a solution of 24.4 g. (0.2 mole) of benzoic acid and 20.2 g. (0.2 mole) of triethylamine in 200 ml. of dry toluene.
- The solution is cooled below 0° by means of an ice-salt mixture, and 21.7 g. (0.2 mole) of ethyl chlorocarbonate is added at such a rate that the temperature does not rise above 0° (approximate time for addition is 25–30 minutes).

- Triethylamine hydrochloride precipitates both during the addition and while the mixture is stirred for 15–25 minutes thereafter.

C. *Diethyl benzoylmalonate*:

- The dropping funnel used for the chlorocarbonate addition is replaced by another into which the ethereal solution of the ethoxymagnesium compound has been transferred.
- Approximately 30 ml. of dry ether is used to rinse the flask, and this is also added to the dropping funnel. The ether solution is added to the mixed anhydride with stirring, as the temperature is held at -5° to 0°.
- After the mixture has been allowed to stand overnight and to come to room temperature during this time, it is treated cautiously with 400 ml. of 5% sulfuric acid; then the aqueous solution is separated and extracted once with ether.
- The two organic layers are combined, washed once with dilute sulfuric acid and then with a concentrated sodium bicarbonate solution until no further benzoic acid is obtained from acidification of the bicarbonate extracts.
- The organic layer is washed with water and dried over anhydrous sodium sulfate. After removal of the sodium sulfate by filtration, the solvent is removed at water-pump pressure from a water bath held at about 50°.
- The resulting product is purified by distillation, and the fraction boiling at 144–149°/0.8 mm. is collected. The yield is 35.8–39.4 g. (68–75%)

Yield: 42%, Molecular formula: $C_{14}H_{16}O_5$, Molecular weight: 264.2, Boiling point: 144-149^{0}C, Solvent system:Hexane : ethyl acetate (9:1), Rf value: 0.47

Step 4: Synthesis of ethyl 2-formyl-3-(1-pentyl-1H-pyrazol-5-ylamino)-3-phenylpropanoate:

- A solution of 1-pentyl, 5-Amino pyrazole (10ml), 10 mmol & diethyl benzoyl malonate, 10 mmol in absolute ethanol, 20 ml was refluxed for 12-15 hr.
- The solid formed on cooling was filtered by suction, washed with ethanol and dried to afford solid in 90% yield.

Yield: 90%, Molecular formula: $C_{20}H_{27}N_3O_3$, Molecular weight:: 358 , Melting point: 146-148^{0}C, , Solvent system: Hexane : ethyl acetate (9:1), Rf

value: 0.4

Step 5: Synthesis of ethyl 4-oxo-1-pentyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate (5B):

- A solution of 5A, 10 mmol in diphenyl ether, 20 ml was heated at 230-240^{0}C for 1hr.
- Then the solution was allowed to cool to room temperature & the reaction mixture was poured into diethyl ether (50ml) & stirred for an hour.
- The solid that separated out was filtered and washed with ether (250 ml), dried to give 5B in 70% yield.

Yield: 70%, Molecular formula: $C_{20}H_{23}N_3O_3$, Molecular weight:: 353.41 , Melting point: 116-118^{0}C, Solvent system: Hexane : ethyl acetate (9:1)

Step 6: Synthesis of ethyl 4-chloro-1-pentyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate (5C):

- A solution of 5B, 10 mmol in phosphorous oxychloride, 20 ml, was refluxed at 110-130^{0}C for 5-8 hr.
- Completion of reaction was checked by TLC.
- Then the solution was allowed to cool to room temperature & was dropwise poured to crushed ice with constant stirring.
- The obtained solid was washed with cold water, dried to give 5C

Yield: 65%, Molecular formula: $C_{20}H_{24}ClN_3O_2$, Molecular weight: 371, Melting point: 168-170^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.48

Step 7: Synthesis of ethyl 4-amino-1-pentyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate (5D):

- A solution of 4.02 g (4.5 mmol) of 5C in 10 mL of toluene was prepared in a 50 ml beaker.
- This was added to an azeotropic solution of toluene & ammonia dropwise at 0-5^{0}C with stirring. Maintain the reaction upto 5^{0}C through ice-salt mixture.
- The reaction mixture was controlled by TLC
- The residue was distilled off, to give a viscous liquid which cooled to give brown solid. Recrystallization of this solid from hexane gave 11.23 g (85%) of amino ester 5D as white crystals.

Ethyl 4-amino-1-pentyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate:

Yield: 85%, Molecular formula: $C_{20}H_{26}N_4O_2$, Molecular weight: 352, Melting point:188-190^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.42, IR frequency(cm^{-1}) : 3086.21 (C-H), 1652.09 (C=N), 1304.89(C-N), Mass (m/z): 353.9 (M+1), 351.7(M-1), ^{1}H-NMR-dmso(ppm): 1.98δ (s, 3H, CH_3), 7.92-7.95 δ (d, 2H,-C_6H_5), 3.70 δ (s, 2H, CH_2), 4.21-4.28(m, 5H,-COOEt), 4.35 δ (s, -NH_2), 0.9-1.8 δ (m, 11H, C_5H_{11}).

Ethyl 4-chloro-1-pentyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate:

Yield: 80%, Molecular formula: $C_{20}H_{22}ClN_3O_2$, Molecular weight: 371, Melting point: 168-170^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.48, IR frequency(cm^{-1}) : 1051.24(C-Cl), Mass (m/z): 372.9 (M+2), 370.8 (M-1), ^{1}H-NMR-dmso(ppm): 1.98δ (s, 3H, CH_3), 7.92-7.95 δ (d, 2H,-C_6H_5), 3.70 δ (s, 2H, CH_2), 8.21-8.28(m, 5H,-COOH), 4.35 δ (s, -NH_2), 0.9-1.8 δ (m, 11H, C_5H_{11}).

Step 8: Synthesis of 4-amino-1-pentyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid (5E):

- A solution of **5C** (10 mmol) in ethanol (25 mL) and 10 % aq. NaOH (2 mL) was heated at reflux temperature for 7-8 h.
- Then the ethanol was removed under reduced pressure and the residue dissolved in water (50 mL).
- The mixture was acidified with conc. HCl and the resulting precipitate collected by suction filtration and washed with water, dried to afford **5E** in 70 % yield.

4-amine-1-pentyl-6-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid:

Yield: 80%, Molecular formula: $C_{18}H_{20}N_4O_2$, Molecular weight: 324, Melting point: 176-178^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value: 0.39, IR frequency(cm^{-1}) : : C=N (1652), C-N (1303.92), C=C (1596.15) , Mass (m/z): 324.9 (M+), ^{1}H-NMR-dmso(ppm): 1.93δ (s, 3H, CH_3), 7.96-7.97 δ (d, 2H, C_6H_5), 3.68 δ (s, 2H, CH_2), 0.85-1.25 δ (m, 11H, C_5H_{11}), 4.33 δ (s, -NH_2).

4-chloro-1-pentyl-6-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid:

Yield: 75%, Molecular formula: $C_{18}H_{18}ClN_3O_2$, Molecular weight: 343.5, Melting point: 192-194^{0}C, Solvent system: Hexane : Ethyl acetate (9:1), Rf value:

0.47, IR frequency(cm^{-1}) : 1055.10 (C-Cl), Mass (m/z): 344.9 (M+2), ^{1}H-NMR-dmso(ppm): 1.93δ (s, 3H, CH_3), 7.96-7.97 δ (d, 2H, C_6H_5), 3.68 δ (s, 2H, CH_2), 0.85-1.25 δ (m, 11H, C_5H_{11}), 4.33 δ (s, -NH_2).

PHARMACOLOGICAL SCREENING:

The Elevated plus maze test[14]:

Method : The elevated plus maze test
Animals used : Rat
Number of animals use : 39 for each compound as one group
Dose of test compound : 1 mg/kg
Dose of standard drug : Diazepam (1 mg/kg)
Route of administration : Oral or intraperitonial

- The apparatus was made of Plexiglas and consisted of two open arms (50 cm x 10 cm) and two enclosed arms (50 cm x 10 cm x 40 cm).
- The arms extended from a central platform (10 x 10 cm2) forming a plus-sign with like arms opposite each other.[82]
- The maze was elevated 50 cm from the floor. Rats weighing 150-200 g were divided into ten groups (n=3).
- Control group was given distilled water (10 ml/kg i.p.), standard group was administered the diazepam (1 mg/kg i.p.) & the test group received the synthesized compounds.[83]
- Thirty minutes after *i.p* injection with diazepam and 1 h after oral treatment with the extract and vehicle, rats were placed individually in succession in the central platform of the maze for 5 minutes and their behaviour was observed for atleast 5 min.
- Behavioural parameters like number of entries into and time spent in each arm i.e. closed and open arms were observed[15].
- Entry into an arm was defined as the animal placing all four paws into the arm. Protected head dipping was defined as the mouse stretching to dip its head into the open space and observing the environment with the body remaining in a closed arm or the central platform while in nonprotected head dipping, the mouse dips its head into the open space and observing the environment with the body being in an open arm.
- Protected stretch attend postures were defined as the mouse stretching forward and retracting without moving forward its feet whilst in the closed arm or central platform of the maze

whereas unprotected stretch-attend postures were defined as the rat exhibiting this behaviour whilst in the open arms[16].

Figure 1 : pictoral view of elevated plus maze

Dose Calculation:

Table 1

For control:

Sr. no	Body weight(gm)
1	200
2	230
3	240

Table 2

For standard:

Sr.	Body	Dose (mg)	Volume of (0.5 ml/100gm) drug to be

no	weight(gm)		administered from stock solution (2.5 mg/ml)
1	250	0.25	0.10
2	190	0.19	0.07
3	260	0.26	0.10

Table 3

For test compounds:

Sr. no	Group code	Compound code	Body weight (gm)	Dose (mg)	Volume of (0.5 ml/100gm) drug to be administered from stock solution (2.5 mg/ml)
1	A	SKSK061201	220	0.22	0.08
			200	0.2	0.08
			260	0.26	0.10
2	B	SKSK061202	250	0.25	0.1
			220	0.22	0.08
			150	0.15	0.06
3	C	SKSK061203	230	0.23	0.09
			260	0.26	0.10
			210	0.21	0.08
4	D	SKSK061204	190	0.19	0.07
			220	0.22	0.088
			200	0.20	0.08
5	E	SKSK061205	240	0.24	0.09
			230	0.23	0.09
			260	0.26	0.10
6	F	SKSK061206	250	0.25	0.10
			270	0.27	0.10
			230	0.23	0.09
7	G	SKSK061207	250	0.25	0.10
			270	0.27	0.10

	250	0.25	0.10

Observation:

Table 4

For control:

Sr. no	No of entries		Time spent(s)		Average time (s)	
	Open	**Close**	**Open**	**Close**	**Open**	**Close**
1	0	1	0	300	0	300
2	0	1	0	300	0	300
3	2	2	35	285	17	142.5

Table 5

For standard:

Sr. no	No of entries		Time spent(s)		Average time (s)	
	Open	**Close**	**Open**	**Close**	**Open**	**Close**
1	5	2	270	32	54	16
2	4	2	276	25	69	14.5
3	5	1	285	15	57	20

Table 6

For test compounds

Sr. no	Group code	Compound code	Animal no.	No of entries		Time spent (sec)		Average time (sec)	
				Open	**Close**	**Open**	**Close**	**Open**	**Close**
1	A	SKSK061201	1	4	3	260	45	65	15
			2	5	1	272	32	54.4	32
			3	6	2	35	285	17	142.5

2	B	SKSK061202	1	3	2	265	37	88.3	18
			2	5	2	271	32	54.2	16
			3	4	1	269	40	67.2	21.5
3	C	SKSK061203	1	6	2	264	36	44	18
			2	4	1	270	34	67.5	34
			3	2	3	280	22	40	7.3
4	D	SKSK061204	1	6	3	240	65	40	21.6
			2	5	2	230	72	46	36
			3	3	1	253	49	84.3	49
5	E	SKSK061205	1	3	2	230	73	76.6	36.5
			2	3	3	244	6	81.3	22.3
			3	3	2	271	32	90.3	16
6	F	SKSK061206	1	4	3	220	79	55	26.3
			2	3	1	210	92	70	92
			3	4	2	200	103	50	51.2
7	G	SKSK061207	1	5	3	254	39	6.51	13
			2	6	3	274	27	45.6	9
			3	4	1	267	36	66.7	36

BIOSTATISTICAL ANALYSIS OF ANXIOLYTIC ACTIVITY:

- In all experiments, a sample size of three (n=3) was utilized. All data are presented as mean ± SEM.
- To compare differences between groups, one-way ANOVA was performed with *post* test.
- Graphpad Instat3 for Windows 7 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis.

Table 7

Results for No. of open entries

Compound code	No. of samples	Mean	Median	SEM	SD
SKSK061201	3	5	5	0.57	1
SKSK061202	3	4	4	0.57	1

SKSK061203	3	4	4	1.15	2
SKSK061204	3	4.6	5	0.88	2.5
SKSK061205	3	3	3	0	0
SKSK061206	3	3.6	4	0.33	0.57
SKSK061207	3	5	5	0.57	1

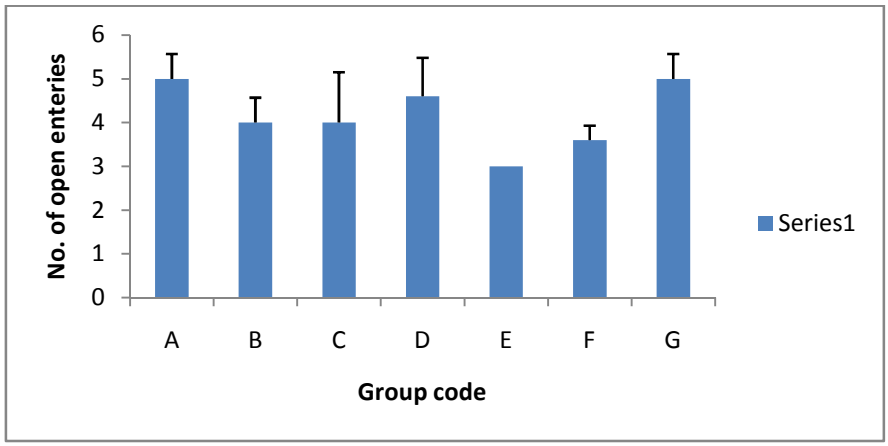


Figure 2: mean ± SEM for the no. of entries in open arm

Table 8

Results of No. of close entries

Compound code	No. of samples	Mean	Median	SEM	SD
SKSK061201	3	2	2	0.57	1
SKSK061202	3	1.6	2	0.33	0.5
SKSK061203	3	2	2	0.57	1
SKSK061204	3	2	2	0.57	1
SKSK061205	3	2.3	2	0.33	0.5
SKSK061206	3	2	2	0.57	1
SKSK061207	3	2.3	3	0.66	1.15

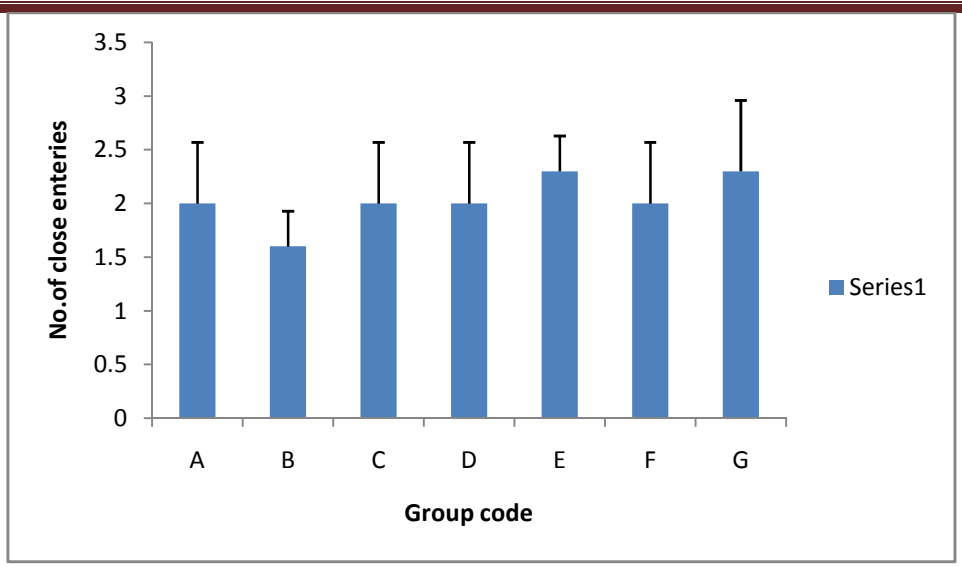


Figure 3: mean ± SEM for the no. of entries in close arm

Table 9

Results of average time of open entries:

Compound code	No. of samples	Mean	Median	SEM	SD
SKSK061201	3	45.46	54.400	14.56	25.21
SKSK061202	3	69.9	67.200	9.93	17.21
SKSK061203	3	50.5	44.000	8.58	14.85
SKSK061204	3	56.76	46.000	13.87	24.03
SKSK061205	3	82.73	81.300	4.02	6.96
SKSK061206	3	58.33	55.000	6.01	10.41
SKSK061207	3	39.6	45.600	17.63	30.54

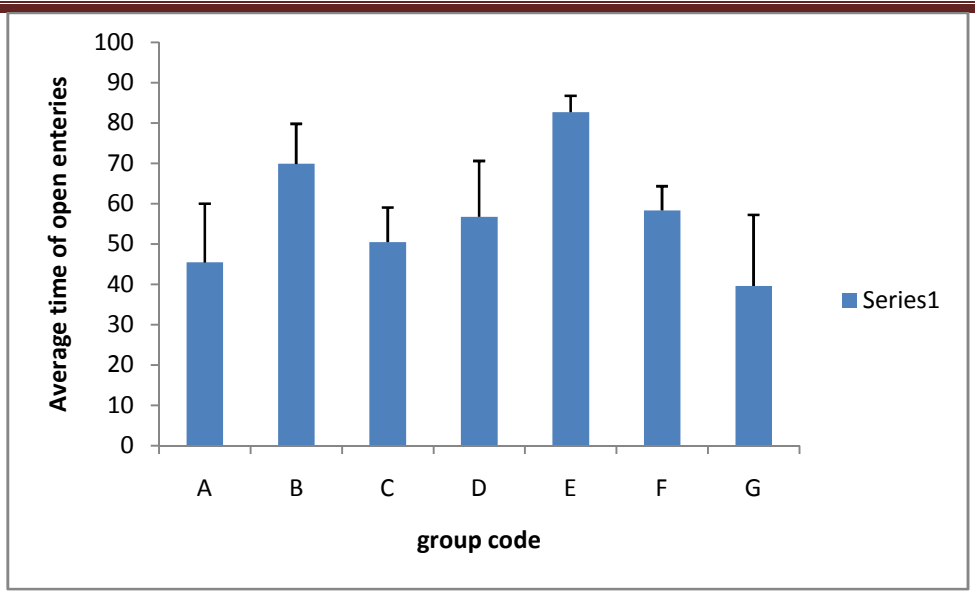


Figure 4: mean ± SEM for the average time spent in open arm

Table 10

Results for average time of close entries

Compound code	No. of samples	Mean	Median	SEM	SD
SKSK061201	3	63.167	32.000	39.97	69.228
SKSK061202	3	18.500	18.000	1.607	2.784
SKSK061203	3	19.767	18.000	7.76	13.437
SKSK061204	3	35.533	36.000	7.913	13.7
SKSK061205	3	24.933	22.300	6.06	10.5
SKSK061206	3	56.500	51.200	19.15	33.17
SKSK061207	3	19.33	13.000	8.4	14.57

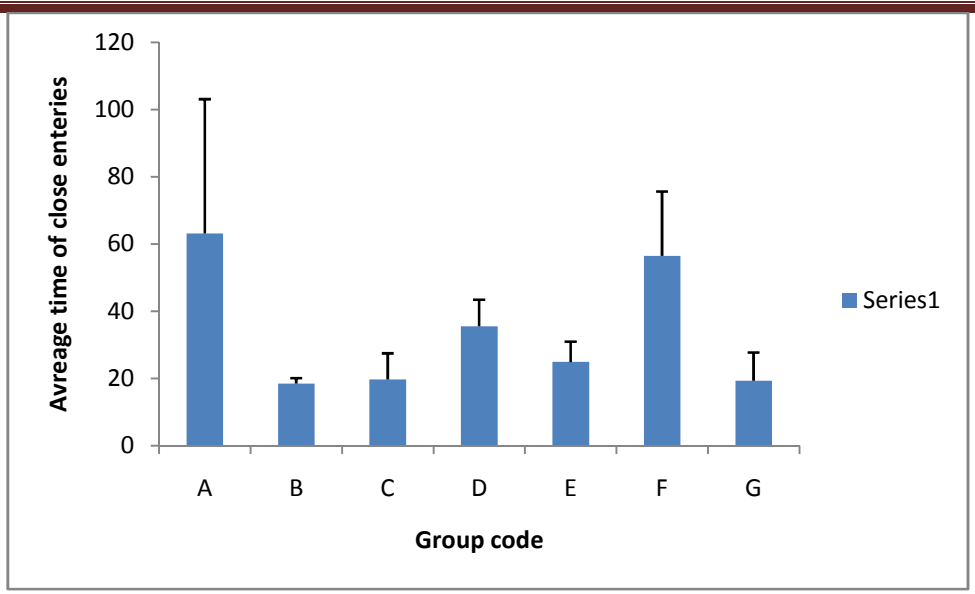
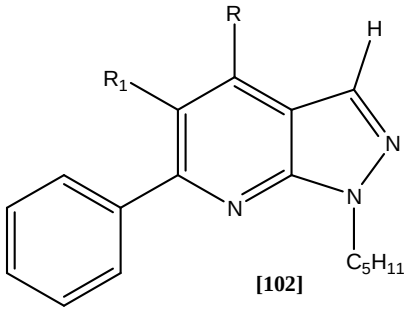


Figure 5: mean ± SEM for the no. of entries in open arm

RESULTS & DISCUSSION

- Pyrazolopyridines are allosteric modulators of benzodiazepine receptors. They are a class of non-sedating Anxiolytics, agreeably more potent than benzodiazepines.
- The pyrazolo[3,4- b]pyridine moieties represent important building blocks in both natural and synthetic bioactive compounds.

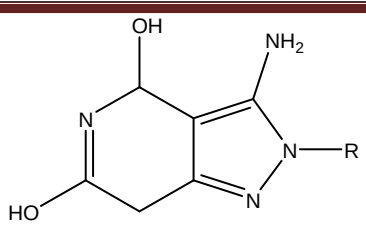


[102]

$R=NH_2$, Cl
R_2 = COOH, COOEt

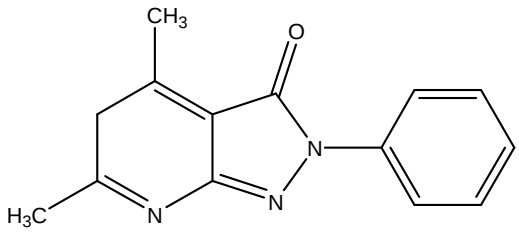
- Optimal *in vitro* and *in vivo* activity should be obtained with linear four or five carbon units at 1st position in [102]. As the length of the 1-alkyl substituent progressed past six carbon units, biological activity rapidly diminished.
- Branching at the 2nd position and di substitution at the 4th position leads to marked decreases in activity.

- Spectral evidence indicated that the carbonyl group of the ester participated in an intramolecular hydrogen bond with the 4-amino group. This interaction would be expected to cause the ester carbonyl to be coplanar (or nearly so) with the pyrazolopyridine ring system.
- The intramolecular hydrogen bond and coplanar arrangement of the ester carbonyl was presumed to be necessary for BZ receptor interaction; consequently, any modification which perturbed this active conformation would be detrimental to the biological properties of the molecule.
- The p-substituent must be a primary amino group to obtain potent displacing activity in the Flunitrazepam binding assay.
- The 5-carboxylic acid ester grouping is necessary for good biological activity. The primary 4-amino group is necessary for potent interaction with the BZ receptor
- If the ester grouping could be made more resistant to metabolism, a long-acting, more potent compound would be obtained.
- A single methyl group branch at the 3-position of this linear chain appears to be beneficial for interacting with the receptor.
- Keeping in view, the aforesaid details, we presumed to have good anxiolytic activity of the synthesised compounds, but the results were not observed as per our presumption.
- From the results of elevated plus maze model, we concluded that compounds SKSK061201, SKSK061203, SKSK061204 & SKSK061207 showed more number of entries in open arm, but as per one-way ANOVA analysis, as the data's were not significant, the compounds failed to show anxiolytic activity.
- Moreover, to prove the versatility of pyrazolopyridines, pyrazolo[4,3-c]pyridine & pyrazolo[3,4-b]pyridinones were also tested for the anxiolytic activity, but unfortunately negative results were observed.
- Pyrazolo[4,3-c]pyridine [103], pyrazolo[3,4-b]pyridinone [104] & pyrazolo[3,4-b]pyridine [102] compounds were synthesized from substituted amino pyrazoles.



3-amino-2-substituted-4,7-dihydro-2*H*-pyrazolo[4,3-*c*]pyridine-4,6-diol

[103] R= CH_3, H



4,6-dimethyl-2-phenyl-2*H*-pyrazolo[3,4-*b*]pyridin-3(5*H*)-one

[104]

- The chemical structure of compound was characterized by IR, MASS, NMR spectral data. The entire synthesized compound was screened for their anxiolytic activity

Conclusion

- ❑ Pyrazolopyridines are allosteric modulators of benzodiazepine receptors.They are a class of non-sedating anxiolytics, agreeably more potent than benzodiazepines.
- ❑ The pyrazolopyridine moieties represent important building blocks in both natural and synthetic bioactive compounds.
- ❑ Pyrazolo[4,3-c]pyridine, pyrazolo[3,4-b]pyridinone & pyrazolo[3,4-b]pyridine compounds were synthesized from substituted Amino Pyrazoles.
- ❑ Reagents such as β-cyanoethylhydrazine & diethyl benzoyl malonate were also synthesized.
- ❑ Anxiolytic activity was tested using Elevated plus maze model. The results were analysed through one-way ANOVA, using *post* test.
- ❑ From the results of elevated plus maze model, we concluded that compounds SKSK061201, SKSK061203, SKSK061204 & SKSK061207 showed more number of entries in open arm, but as per one-way ANOVA analysis, as the data's were

not significant, the compounds failed to show anxiolytic activity.

- Ethyl chloro carbonate was purchased from National chemicals, Vadodra.
- Magnesium turnings & ethyl cyanoacetate were purchased from Chemdyes Corporation, Rajkot.
- All the other requirements & chemicals were provided by Sat Kaival college of pharmacy.

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