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Synthesis and Biological Activities of (4Z)-2-(1H-benzimidazol-2-ylmethyl)-4-arylidene-5-methyl-2,4-dihydro-3H-pyrazol-3-one Compounds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Pyrazoles are reported to be well known pharmacophores. This has motivated the synthesize some of the pyrazole derivatives by using hydrazine hydrate as well as adding benzimidazole in pyrazoles. A series of (4Z)-2-(1H-benzimidazol-2-ylmethyl)-4-arylidene-5-methyl-2,4-dihydro-3H-pyrazol-3-ones (IIIa3 to IIIe3) was synthesized by conventional method by refluxing compounds (IIIa2-IIIe2) with O-Phenylene diamine in absolute ethanol. A series of compounds (IIIa2-IIIe2) was prepared by reacting compounds (IIIa1-IIIe1) with chloroacetic acid. A series of (4E)-4-arylidene-5-methyl-2,4–dihydro-3H-pyrazol-3-ones (IIIa1-IIIe1) was prepared by the reaction between 5-methyl-2,4-dihydro- 3H-pyrazol-3-one (II) and different aldehydes in presence of Sodium acetate. All of the compounds were synthesised with high yields (58-80%) and identified using IR, 1H NMR spectrum data, as well as C, H, and N elemental analyses. At varying MIC values, all of the produced compounds showed antibacterial and antifungal activity, as well as analgesic and anti-inflammatory properties. Anticonvulsant, CNS depressive, ulcerogenic, and anthelmintic properties are thought to be exhibited by the produced compounds.

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Keywords: Pyrazole; benzimidazole; antibacterial; antifungal; analgesic; anti-inflammatory.

1. INTRODUCTION

Pyrazoles and Benzimidazoles are reported with the wide range of biological activities. Many attempts were made by researchers to find out an able potent pyrazole and benzimidazole derivatives and also combining both heterocyclic ring to enhance the biological activity [1-4]. Antiviral, antimicrobial properties possessing compounds is the need today as we are facing Covid-19 pandemic [5]. Pyrazole and Benzimidazole compounds possess biological activities as antimicrobial, antitumor anti-inflammatory, analgesic, antiviral, anti-Alzheimer's, antiulcer, antidiabetic [6]. "Pyrazole-Benzimidazole combined compounds are reported to possess antimicrobial properties"- is the conclusion from the recent literature [7-8].

2. MATERIALS AND METHODS

The processes requiring anhydrous conditions were carried out using well-dried equipment. Distillation and crystallisation were used to purify laboratory reagent grade solvents and reagents as necessary. The synthesis was carried out using a Catalyst scientific microwave synthesiser (CATA-R, 32 litre, 850 W, 2450 MHz). The melting temperatures of freshly synthesised compounds were determined using the open capillary technique. Recrystallization was used to purify the final products, and micro TLC was used to ensure purity. Using a KBr pressed pellet, the IR spectra of the compounds were recorded on a JASCO FT/IR-5300 spectrometer. TMS was used as an internal standard in a BRUKER DPX-200MHz spectrometer to record 1H NMR spectra. Perkin Elmer 2400 elemental analyzer was used for analysis of C, H and N which were found within ± 0.4 % of the theoretical values.

3. SYNTHETIC SCHEME

A series of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-arylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-ones (IIIa3 to IIIe3) was prepared by the reaction between [(4*Z*)-4-arylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl]acetic acids (IIIa2 to IIIe2) and o-phenylene diamine by refluxing it in presence of absolute ethanol. (IIIa2 to IIIe2) were prepared by reaction of (IIIa1 to IIIe1) with chloroacetic acid by refluxing with absolute ethanol. (IIIa1 to IIIe1) were obtained by reacting compound II (5-methyl-2,4-dihydro- 3H-pyrazol-3-one) with glacial acetic acid in attendance of merged sodium acetate. Compound II was obtained by cyclization of ethyl acetoacetateate (I) with hydrazine hydrate by rousing in absolute ethanol.

4. PROCEDURES FOR SYNTHESIS

Conventional synthesis method was preferred for the study. The purpose was to synthesize non-toxic/ less toxic derivatives with good activity, high yield and purity, less solvent requirement, less reaction time and novelty.

Conventional method for the synthesis of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II):

A crystalline deposit was formed when ethyl acetoacetate (1.3g, 0.01mol) was put in a conical flask and swirled magnetically during the gradual dropwise addition of hydrazine hydrate (98 percent, 0.5 ml, 0.01 mol) in absolute ethanol (1ml) at a temperature of roughly 600 C. The reaction mixture was cooled in an ice bath for full recrystallization after 1 hour of stirring at ambient temperature, filtered, washed with ice-cold ethanol, and dried, with a m.p. of 2220 C. 90 percent yield (0.88g) [1].

General procedure for the synthesis of (4E)-4-arylidene-5-methyl-2,4–dihydro-3*H*-pyrazol-3-ones (IIIa1-e1):

A mixture of 5-methyl-2,4 dihydro-3H-pyrazol-3-one (II) (0.98g,0.01mol), appropriate aldehyde (0.01 mol), anhydrous sodium acetate (0.82g,0.01mol), and glacial acetic acid (40ml) was heated under reflux for 4 hours on a heating mantle, cooled to room temperature, poured in ice cold water, filtered, washed with water, and rec The yield as well as the m. p. were recorded [1,2,3].

General procedure for the Synthesis of compounds (IIIa2-e2):

Compounds (IIIa1-e1) (0.01 mol) were added in methanol (20 ml) and stirred well to dissolve. To this chloroacetic acid (0.01mol) was added drop wise with continuous stirring to get clear solution and then refluxed for 2 hours on water bath to get solid residue of compounds (IIIa2-e2). The yield and m. p. were reported [4].

General procedure for the Synthesis of compounds (IIIa3-b3):

Compounds (IIIa2-b2) (0.01 mol) were refluxed with O-Phenylene diamine (0.01 mol) for 4 hours in absolute ethanol (20 ml). The solvent was reduced to one third of its volume and then acidified with 10% HCl to yield final products (IIIa3-b3). The yield and m. p. were reported [5].

Characterization of (4*Z*)-4-benzylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIa1):

The compound **IIIa1** with melting point 197-199^0 C was analysed for $C_{11}H_{10}N_2O$. It exhibits intense bands at 3416 cm^{-1}(aromatic N-H str), 3095 cm^{-1} (aromatic C-H str), 2903 cm^{-1} (C-H str in CH_3), 1680 cm^{-1} (C=O) 1613 cm^{-1}, 1585 cm^{-1} (C=C and C=N), 1134 cm^{-1}, 1052 cm$^{-1,}$ 787 cm^{-1} (monosubstituted benzene ring).

Characterization of (4Z)-4-(2-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIb1):

The compound **IIIb1** with melting point 175-177^0 C was analysed for $C_{11}H_9ClN_2O$. It exhibits intense bands at 3456 cm^{-1} (aromatic N-H str), 3000 cm^{-1} (aromatic C-H str), 2921 cm^{-1} (C-H str in CH_3), 1687 cm^{-1} (C=O) 1613 cm^{-1}, 1556 cm^{-1} (C=C and C=N), 1120 cm^{-1}, 1051 cm$^{-1,}$ 757 cm^{-1} (1, 2-disubstituted benzene ring).

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-benzylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIa3):

The compound **IIIa3** with melting point 226-227^0 C was analyzed for $C_{19}H_{16}N_4O$. It exhibits intense bands at 3416 cm^{-1}(aromatic N-H str), 3095 cm^{-1} (aromatic C-H str), 2903 cm^{-1} (C-H str in CH_3), 1680 cm^{-1} (C=O), 1513 cm^{-1} and 1585 cm^{-1} (C=C and C=N), 1052 cm^{-1}, 787 cm^{-1}. (monosubstituted benzene ring).The ^{1}H NMR spectrum in $CDCl_3$ is given in Fig. 1. It shows peaks at δ: 7.25 (d, 1H, =C<u>H</u>- on C4), 4.22 (s, 2H, -C<u>H</u>$_2$), 7.53- 7.99 (m, 9H, Ar-<u>H</u>), 12.1832 (bs, 1H, benzimidazole N-<u>H</u>) and 2.348 (s, 3H,-C<u>H</u>$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(72.13%) H(5.10%) N(17.71%) found: C(72.10%) H (5.14%) N(17.72%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-(2-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIb3):

The compound **IIIb3** with melting point 215-216^0 C was analyzed for $C_{19}H_{15}ClN_4O$. It exhibits intense bands at 3423 cm^{-1}(aromatic N-H str), 3056 cm^{-1} (aromatic C-H str), 2856 cm^{-1} (C-H str in CH_3), 1716 cm^{-1} (C=O)1581 cm^{-1} and 1466 cm^{-1} (C=C and C=N), 1265 cm^{-1} (C-N str),1052 cm^{-1}, 787 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in Fig. 1. It shows peaks at δ: 7.91 (d, 1H, =C<u>H</u>- on C4), 4.22 (s, 2H, -C<u>H</u>$_2$), 7.12- 7.58 (m, 8H, Ar-<u>H</u>), 12.1833 (bs, 1H, benzimidazole N-<u>H</u>) and 2.342 (s, 3H,-C<u>H</u>$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(65.05%) H(4.31%) N(15.97%) found: C(65.08%) H(4.29%) N(15.94%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-[(1*H*-benzimidazol-2-yl)methyl]-4-[(2-hydroxyphenyl) methylidene]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIc3):

The compound **IIIc3** with melting point 206-208^0 C was analyzed for $C_{19}H_{16}N_4O_2$. It exhibits intense bands at 3510 cm^{-1} (O-H str), 3315 cm^{-1} (aromatic N-H str), 3140 cm^{-1} (aromatic C-H str), 2894 cm^{-1} (C-H str in CH_3), 1723 cm^{-1} (C=O), 1579 cm^{-1} and 1653 cm^{-1} (C=C and C=N), 1338 cm^{-1} (C-N str), 1047 cm^{-1}, 1097 cm^{-1}, 780 cm^{-1} (1,2- disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in Fig. 1. It shows peaks at δ: 11.85 (s, 1H, OH), 7.75 (d, 1H, =C<u>H</u>- on C4) , 4.22 (s, 2H, -C<u>H</u>$_2$), 6.65- 7.56 (m, 8H, Ar-<u>H</u>), 12.1833 (bs, 1H, benzimidazole N-<u>H</u>) and 2.497 (s, 3H,-C<u>H</u>$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(68.66%) H(4.85%) N(16.86%) found: C(68.69%) H(4.82%) N(16.89%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-(2,4-dichlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIId3):

The compound IIId3 with melting point 223-2240 C was analyzed for C19H14Cl2N4O. It exhibits intense bands at 3442 cm-1(aromatic N-H str), 3083 cm-1 (aromatic C-H str), 2922 cm-1 (C-H str in CH3), 1733 cm-1 (C=O), 1579 cm-1 and 1653 cm-1 (C=C and C=N), 1315 cm-1 (C-N str), 1097 cm-1, 780 cm-1 (1,2,4-trisubstituted benzene ring). The 1H NMR spectrum in CDCl3 is given in Fig. 1. It shows peaks at δ: 7.72 (d, 1H, =CH- on C4), 4.22 (s, 2H, -CH2), 7.04- 7.61 (m, 7H, Ar-H), 12.1856 (bs, 1H, benzimidazole N-H) and 2.496 (s, 3H,-CH3). Elemental analysis for composition of C, H and N is given as calculated: C(59.24%) H(3.66%) N(14.54%)

found: C(59.26%) H(3.64%) N(14.51%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-[4(dimethylamino) benzylidene]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIe3):

The compound IIIe3 with melting point 244-2460 C was analyzed for $C_{21}H_{21}N_5O$. It exhibits intense bands at 3428 cm-1(aromatic N-H str), 3092 cm-1 (aromatic C-H str), 2923 cm-1 (C-H str in CH3), 1702 cm-1 (C=O), 1482 cm-1 and 1682 cm-1 (C=C and C=N), 1323 cm-1 (C-N str), 1108 cm-1, 781 cm-1(1,4-disubstituted benzene ring). The 1H NMR spectrum in CDCl3 is given in Fig. 1. It shows peaks at δ: 7.75 (d, 1H, =CH- on C4), 4.22 (s, 2H, -CH2), 6.91- 8.25 (m, 8H, Ar-H), 12.1823 (bs, 1H, benzimidazole N-H), 3.065 (s, 6H,-N (CH3)2) and 2.345 (s, 3H,-CH3). Elemental analysis for composition of C, H and N is given as calculated: C(70.17%) H(5.89%) N(19.48%) found: C(70.14%) H(5.87%) N(19.53%). The data confirms the structure of the compound.

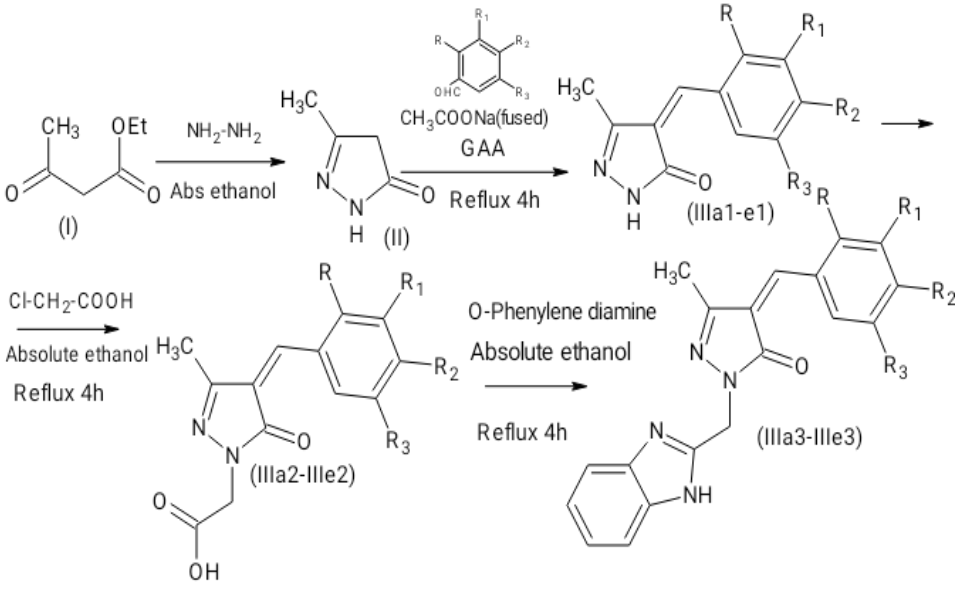

Fig. 1. Synthesis scheme

Table 1. Physical constants of compounds (IIIa3-e3)

Compound	Recrystalization Solvent	% yield	m.p. (^{0}c)	Molecular formula	Molecular weight	*Rf
IIIa3	Ethanol	58	226-227	$C_{19}H_{16}N_4O$	316.356	0.61
IIIb3	Glacial acetic acid + Ethanol(1:1)	70	215-216	$C_{19}H_{15}ClN_4O$	277.709	0.39
IIIc3	Glacial acetic acid	65	206-208	$C_{19}H_{16}N_4O_2$	259.263	0.58
IIId3	Glacial acetic acid	82	223-224	$C_{19}H_{14}Cl_2N_4O$	312.154	0.61
IIIe3	Ethanol	80	244-246	$C_{21}H_{21}N_5O$	286.332	0.43

5. BIOLOGICAL ACTIVITY

The LD_{50} values of synthesized compounds (IIIa3-e3) have been determined by the Karber's method [6]. Analgesic activity of synthesized compounds was studied by acetic acid induced writhings method [7,8]. Carrageenan-induced rat paw oedema was used to test the anti-inflammatory efficacy of produced compounds [7,8]. The disc diffusion technique [8] was used to assess the antibacterial activity of produced compounds. The diffusion of an antibiotic from a filter paper disc through the solidified culture material of a Petri dish employed in the investigation is the basis for this procedure. In a circular region "zone" around the filter paper disc containing a solution of the antibiotic and the test chemicals, the growth of injected microorganisms is completely stopped.

5.1 Antimicrobial Assay

The paper disc (No. 2 Whatmann) was cut into a tiny disc (6 mm in diameter) and autoclave sterilised before being impregnated with the test and reference solutions. The dried discs were put on the medium's surface. Petri plates were kept at room temperature for 30 minutes after disc placement as a period of pre-incubation diffusion to reduce the impact of temporal differences between applications of different solutions. All Petri plates were incubated for 24 hours at the specified temperatures, 370°C for bacteria and 250°C for fungi. After incubation, the diameters of the circular inhibition zones were measured.

6. RESULTS

All the results of this research work are summarized in tables from 2-5.

Table 2. ED_{50} values of synthesized compounds IIIa3-e3)

S. No.	Compound	ED_{50} (mg/kg)
1	IIIa3	140
2	IIIb3	130
3	IIIc3	145
4	IIId3	135
5	IIIe3	120

Table 3. Analgesic activity of compounds (IIIa3-e3)

S. No.	Design of treatment (Groups)	Dose (mg/kg, p.o.)	Number of writhings in 5 minutes	% Inhibition
1	Control(CMC, 0.25%, 1ml)	-	180.00 ±0.60	-
2	Aspirin	100	41.83 ±0.047**	76.76
3	Compound IIIa3	140	82.33±0.073**	54.26
4	Compound IIIb3	130	67.33±0.043**	**62.59**
5	Compound IIIc3	145	74.00±0.047**	58.88
6	Compound IIId3	135	57.00±0.060**	**68.33**
7	Compound IIIe3	120	69.33±0.046**	**61.48**

*Values are expressed as mean ± SEM, N=6, When compared with control, *= P< 0.05, **= P< 0.01, ***= P< 0.001 (One way ANOVA followed by Dunnett's multiple comparison test)*

Table 4. Anti-inflammatory activity of compounds (IIIa3-e3)

S. No.	Design of treatment (Groups)	Dose (mg/kg, p.o.)	Change in paw edema at the end of 3h (mm)	% Inhibition
1	Control (CMC, 0.25%,1ml)	-	0.85±0.0067	-
2	Indomethacin	10	0.22±0.0060**	74.11
3	Compound IIIa3	143	0.53±0.0043**	37.64
4	Compound IIIb3	127	0.38±0.0055**	**55.29**
5	Compound IIIc3	148	0.50±0.0055**	41.17
6	Compound IIId3	131	0.32±0.0043**	**62.35**
7	Compound IIIe3	117	0.43±0.0040**	49.41

Values are expressed as mean ± SEM, N=6, When compared with control,= P< 0.05, **= P< 0.01, ***= P< 0.001 (One-Way ANOVA followed by Dennett's multiple comparison test)*

Table 5. Antimicrobial (Antibacterial and Antifungal) activity of compounds (IIIa3-e3)

S. No.	Design of treatment (1mg/ml)	Diameter of zone of inhibition (mm)					
		Escherichia coli	***Staphylococcus aureus***	***Shigella dysenteriae***	***Streptococcus mutans***	***Candida albicans***	***Rhizopus oryzae***
1	Standard*	20	14	24	16	26	22
2	Compound IIIa3	**13**	**8**	**17**	**14**	10	7
3	Compound IIIb3	11	NA	11	**14**	12	11
4	Compound IIIc3	6	4	NA	4	6	12
5	Compound IIId3	10	4	2	5	4	NA
6	Compound IIIe3	4	6	14	**13**	**18**	**16**

NA: No activity at this amount of test compound or standard

**Standard drugs: Amoxicillin-clavulanic acid (for Gram Positive Bacteria), Cefixime (for Gram Negative Bacteria), Ketoconazole (for Fungi)*

7. DISCUSSION AND CONCLUSION

Synthesis of two new chemical entities incorporating the two active pharmacophores namely pyrazoline and heteronucleus (another Pyrazole and benzimidazole) in a single molecular framework was successfully carried out. Conventional synthesis of new series of pyrazole-benzimidazoles, characterization of synthesized compounds by spectral methods viz. Infra Red, Nuclear Magnetic Resonance spectroscopy and elemental analysis and screening for the analgesic, anti-inflammatory and antimicrobial activity are the major highlights of the research work [7].

Pyrazoles with benzimidazole derivatives can be synthesized by conventional method. The yield is almost quantitative.

All of the compounds that were created have analgesic, anti-inflammatory, antibacterial, and antifungal properties. Compounds IIIb3, IIId3, and IIIe3- were shown to have excellent analgesic properties. Compounds IIIb3 and IIId3- were shown to have potent anti-inflammatory properties. Compound IIIa 3 was shown to be effective against *E. coli, S. aureus, Shigella dysenteriae,* and *Streptococcus mutans*. *Streptococcus mutans* was shown to be resistant to IIIb3-. *Streptococcus mutans*, *Candida albicans,* and *Rhizopus oryzae* all demonstrated high efficacy against IIIe3- [8].

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The prior permission of Institutional Animal Ethics Committee [IAEC Registration No. 1153/PO/Re/S/08/CPCSEA (present), 1153/PO/ac/08/CPCSEA (previous)] was taken before conducting activity on animals.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vogel AI. Text book of practical org. chem., 5th Edition, ELBS Publication, London, 1989;807-808.
2. Mariappan G, Saha BP, Sutharson L, Haldar A. Synthesis and bioactivity evaluation of pyrazolone derivatives, Indian journal of Chemistry B. 2010;49B: 1671-1674.
3. Rao R. Mallikarjunna, M. Musthak Ahamad, Sreeramulu J. Synthesis and antimicrobial activity of linked heterocycles containing pyrazolyle-indole derivatives. Journal of Pharmacy Research. 2012;5: 1518-1521.
4. Khanage Shantaram G, Mohite Popat B, Pandhare Ramdas B, Raju S. Appala, Study of analgesic activity of novel 1, 2, 4-triazole derivatives bearing pyrazole and tetrazole moiety. Journal J Pharm Res. 2011;4: 3609-3611.
5. Jumat Salimon, Nadia Salih, Hasan Hussien, Emad Yousif. Synthesis and characterization of new heterocyclic compounds derived from 2-amino pyridine. European Journal of Scientific Research. 2009;31:256-264.
6. Enegide Chinedu, David Arome, Fidelis Solomon Ameh. A new method for determining acute toxicity in animal models. Toxicol Int. 2013;20:224–226. DOI: 10.4103/0971-6580.121674

7. Kulkarni SK. Handbook of experimental pharmacology; 3rd Edition, Vallabh Prakashan, Delhi. 2003;127-128, 190, 193.

8. Robert A. Turner, Peter Hebborn, Screening methods in Pharmacology, Academic press, New York and London. 1965;60-62.

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