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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NOVEL BENZOTRIAZOLES DERIVATIVES CONTAINING PYRAZOLIDINEDIONE MOIETIES

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ABSTRACT

A number of drugs are in the clinical use, search for new NSAIDS is still relevant because the existing molecules suffer from the drawback of adverse effects such as gastric ulceration, inhibition of platelet function, alterations in the renal function, hypersensitivity reactions. The present research work focuses on the diazotization, alkylation with conventional stirring and refluxation methods to form some novel benzotriazole derivatives containing pyrazolidine dione moieties. The characterisation of n=8 derivatives was carried out using I.R, ^{1}H NMR and mass spectral analysis. The percentage yield of final compounds was found to be 71.43 % to 88.06%. Purity of the compounds was checked by using TLC and elemental analysis. These compounds showed a considerable anti-inflammatory activity and analgesic activity using *In vivo* methods compared to Indomethacin and Tramadol respectively.

Keywords: Benzotriazole, Pyrazolidine 3, 5-dione, Anti -inflammatory activity, Analgesic activity.

INTRODUCTION

Benzotriazole derivatives are an important class of nitrogen containing heterocyclic and were reported to possess a wide spectrum of pharmacological activities anti-inflammatory, analgesic antibacterial and antifungal activities. Although number of drugs is available in the market, but the need of discovering the new anti-inflammatory drugs with better pharmacokinetic profile and lesser toxicity has become the main objective in the field of medicinal chemistry, it is also due to the fast microbial resistance to the existing molecules. Despite a number of drugs being in clinical use, search for new NSAIDS is still relevant because the existing molecules suffer from the drawback of adverse affects such as gastric ulceration, inhibition of platelet function, alterations in renal function, hypersensitivity reactions (Beale JM Jr. Wilson *et al*., 2004).

Research on pyrazolidine-3,5-dione and their synthetic analogs have revealed to possess to be anti-inflammatory activity along with wide range of antimicrobial activity (Kini S *et al*., 2008, Cauvin C *et al*., 2001, Suma BV *et al*., 2010). It is our interest to synthesize some new benzotriazole derivatives containing

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pyrazolidine-3,5-dione moieties and to evaluate the *invivo* anti-inflammatory and analgesic activity. The synthesized compounds have shown satisfactory spectral data which are in conformity of the proposed structures along with good analgesic and anti inflammatory activity.

MATERIALS AND METHODS (Suma BV *et al*., 2010, Rakesh Saini *et al*., 2010)

The chemicals and reagents used in this were of AR and LR grade. They were procured from SpectroChem, Hi-Media, Merck, Sigma Aldrich and Ranbaxy.

The melting points of the synthesized compounds were determined by using Thiel's melting point apparatus (open capillary tube method) and all the compounds gave sharp melting points and are uncorrected. Purity of the compounds was ascertained by thin layer chromatography using silica gel-G as stationary phase and appropriate mixtures of the following solvents as mobile phase: Benzene and Ethyl acetate, Hexane and Ethyl acetate. The spots resolved were visualized using iodine chamber. The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrophotometer (model Shimadzu 8400S) in the range of 400-4000 using diffuse reflectance system and values of ν_{max} are reported in cm^{-1}. ^1H NMR spectra were recorded on amx-400 NMR spectrometer and chemical shifts (δ) are reported in ppm downfield from internal reference Tetramethylsilane (TMS). Mass spectra were recorded on Shimadzu LC-MS model 2010A. Elemental analysis of the newly synthesized compounds was carried out using FLASH EA 1112 series elemental analyzer. The Synthetic Procedure involved the following four steps.

Diazotization of benzene-1,2-diamine with Glacial acetic acid: (1A- 1H)

Substituted Benzene-1,2-diamines (0.01M) was dissolved in a mixture of glacial acetic acid (11.5ml) and water (30ml) in a beaker. It is cooled to 12°C. A solution of sodium nitrite (0.01M) in 15ml of water was added with stirring to the above solution. It was stirred continuously for 15 minutes by maintaining the temperature at 12°C in a chilled ice bath, then the solid precipitate was filtered and recrystallised using water. Percentage yield and melting points of all the (1A-1H) derivatives are presented in table 1.

Synthesis of ethyl 1H-benzotriazole-1-yl acetate: (2A-2H)

In 250 ml iodine flask, acetone (60ml), 1H-benzotriazole (0.01M), ethyl chloroacetate (0.01M) and anhydrous potassium carbonate (3gm) was added and stirred for 6 hours at room temperature. The solution was filtered to remove potassium carbonate. The solvent was removed under reduce pressure. The product so obtained was extracted with ether. The ether was removed under reduced pressure to get needle shaped crystals. Percentage yield and melting points of all the (2A-2H) derivatives are presented in table 1.

Synthesis of substituted 2-(1H-benzotriazol-1-yl) aceto hydrazides: (3A- 3H)

In a 250ml iodine flask, an ethanolic solution of ethyl 1-H benzatriazol-1-yl acetate (0.01M) and hydrazine hydrate (20 ml) was stirred for 4 hours at room temperature and then refluxed on a water bath for 3 hours in a 250 ml round bottom flask, the solution was kept overnight in a 250 ml beaker and then excess solvent was removed under reduced pressure. The solid mass so obtained was washed with cold water and recrystallised from ethanol. Percentage yield and melting points of all the (3A-3H) derivatives are presented in table 1.

Synthesis of substituted benzotriazoles derivatives containing pyrazolidine dione moieties: (4A- 4H)

In a 250ml round bottomed flask, 2-(1-H-benzotriazol-1-yl) aceto hydrazide (0.01M) was dissolved in ethanol (50ml) and diethyl propanedioate (0.01M), glacial acetic acid (2-3 drops) were added. The reaction mixture was refluxed for 6 hours. Then the reaction mixture was kept in an open china dish for 3 days. The so obtained crystals were filtered and recrystallised from ethanol. Percentage yield and melting points of all the (4A-4H) derivatives are presented in table 1.

PHARMACOLOGICAL ACTIVITY

1) Anti-inflammatory activity (Gerhard Vogel H 2006, Asati KC *et al*., 2006, Fahmy HH *et al*., 2001, OECD 2001)

Anti-inflammatory activity was carried out by Carrageenan induced paw edema method. Animals were weighed and numbered. A mark was made on the hind paw (left) just beyond tibio-tarsal junction, so that the paw was dipped in the mercury upto the fixed mark. Initial paw volume (left) was noted on each rat by mercury displacement method. Animals were divided into ten groups each comprising of six rats. The first group received di-methyl sulfoxide (vehicle), second group received intraperitoneal injection of indomethacin, third group to tenth group were treated with test compounds (4A-4H) intraperitoneally. After 30 min, 0.1 ml of 1 % (w/v) carrageenan was injected in the plantar region of the left paw subcutaneously of all the groups followed by

measurement of paw volume (left) of all the groups at 0, 15, 30, 60 and 120 min after carrageenan challenge. The increase in paw volume was observed and the percentage inhibition of edema exhibited by standard and test drugs was calculated by the following formula.

% edema inhibition = 100 [1-(Vt-Vo) $_T$ / (Vt-Vo) $_C$]
V_0= Initial paw volume at 0 minutes.
V_t= Final paw volume at 120 minutes

2) Analgesic activity (Gerhard Vogel H 2006, Asati KC *et al*., 2006, Fahmy HH *et al*., 2001, OECD 2001)

Analgesic activity was carried out by Eddy's hot plate method. Animals were divided into ten groups each comprising of six mice. The mice were treated with di-methyl sulfoxide (control), Tramadol (standard) and substituted benzotriazoles [4A- 4H] half an hour prior to analgesic screening.

Eddy's hot plate method is used for screening of central analgesic property. The mice were placed on analgesiometer that is eddy's hot plate kept and maintained at constant temperature (55±1^{O}C). The time of response i.e. time taken by the animal to lick its hind paw or jump after placing it on the hot plate, is recorded as the reaction of painful stimuli. A cut off period of 15 sec was considered to avoid the damage to the paw.

RESULTS AND DISCUSSION

Synthesis of 1A-1H & 2A-2H was carried out using diazotization & alkylation reactions, further it was converted to hydrazides 3A-3H and to the final compounds, physical data of which is provided in table 1. Elemental analysis of all the final 4A-4H compounds showed that they were highly pure and reported in table 2; purity was also checked using TLC. Spectral results for all the intermediates and final compounds are summarized in table 3.

A considerable anti-inflammatory and analgesic activity was shown by all the synthesized derivatives except the compound 4G which did not exhibit anti-inflammatory activity. Compound 4D was found to possess a better anti-inflammatory activity and Compound 4G was observed to exhibit a better analgesic activity than the other synthesized compounds.

Table 1. Physical Data of Synthesized Compounds

Compounds	R	R^1	%Yield	Melting Point (°C)
1A-1D	H	-	84	93-98
1E-1H	Cl	-	78.43	85
2A-2D	H	-	90.24	55-58
2E-2H	Cl	-	79.17	75
3A	H	H	78.53	130-135
3B	H	C_6H_5	76.78	158-160
3C	H	$C_6H_4N_2O_2$	72.83	140
3D	H	C_6H_5Cl	88.04	155
3E	Cl	H	88.89	128
3F	Cl	C_6H_5	91.06	160
3G	Cl	$C_6H_4N_2O_2$	76.73	170
3H	Cl	C_6H_5Cl	82.74	144
4A	H	H	71.43	200-220
4B	H	C_6H_5	88.06	248-250
4C	H	$C_6H_4N_2O_2$	77.17	235
4D	H	C_6H_5Cl	82.43	270
4E	Cl	H	73.13	216
4F	Cl	C_6H_5	75.13	232
4G	Cl	$C_6H_4N_2O_2$	81.52	240
4H	Cl	C_6H_5Cl	83.17	280

Table 2. Elemental Analysis

Sl. No.	Compound	Elements		
		C(Calculated)	H(Calculated)	N(Calculated)
1	**4A**	48.68(50.97)	2.55(3.50)	26.50(27.02)
2	**4B**	57.75(60.89)	3.68(3.91)	16.85(20.89)
3	**4C**	46.85(48.01)	2.04(2.61)	22.70(23.05)
4	**4D**	54.26(55.22)	2.97(3.27)	17.64(18.94)
5	**4E**	44.26(44.99)	1.57(2.75)	22.46(23.85)
6	**4F**	52.16(55.22)	2.57(3.27)	17.36(18.94)
7	**4G**	43.38(44.41)	2.07(2.19)	20.22(21.33)
8	**4H**	48.84(50.51)	1.88(2.74)	15.87(17.33)
9	**4I**	40.62(43.43)	2.15(2.65)	27.22(27.62)

Table 3. Characterization of Compounds (William Kemp 2004, Silverstein RM *et al*., 2004)

Compound IR (cm^{-1}), ^{1}HNMR, Mass

1A-1D 3255(N-H str), 3045 (Ar. C-H str), 1500 (N-H bend)

1E-1H 3074 (N-H str), 2950 (Ar. C-H str), 1496 (-N-H bend), 802 (-CCl str)

2A-2D 3056.96 (Ar. C-H str), 2983.67 (-CH_2 str), 1749.32 (Ester, >C=O str)

2E-2H 3093. 61 (Ar. C-H str), 2985.60 (-CH_2 str). 1473.53 (Ester. >C=O str), 811.98 (-CCl str)

3A 3303.83 (-NH_2 str), 2956.67 (Ar. C-H str), 1612.38(Amide, >C=O str), 1660.60 (>C=O str), 2902.67 (-CH_2 str), 1542.95 (-N-H bend), 3053.11 (N-H str)

3B 3049.25 (Ar. C-H str), 1598.88(Amide, >C=O str), 1683.74 (>C=O str), 1535.23 (-N-H bend), 3321.19 (N-H str)

3C 3053.11 (Ar. C-H str), 1612.38(Amide, >C=O str), 1749.46 (>C=O str), 2989.46 (-CH_2 str), 1450.37(-N-H bend), 3317.34 (N-H str), 1278.72 (-NO_2 str.)

3D 3051.18 (Ar. C-H str), 1739.67 (>C=O str), 2987.53 (-CH_2 str), 3143.75 (N-H str), 750.26 (C-Cl str)

3E 3006.82 (Ar. C-H str), 1614.31(Amide, >C=O str), 1693.38 (>C=O str), 2950.89 (-CH_2 str), 1483.16 (-N-H bend), 3296.12(N-H str), 815.83 (-CCl str)

3F 3043.25 (Ar. C-H str), 1596.95 (Amide, >C=O str), 1685.67 (>C=O str), 2950.89 (-CH_2 str), 1490.87 (-N-H bend), 3323.12(N-H str), 800.40 (-CCl str)

3G 3056.96 (Ar. C-H str), 1600 (Amide, >C=O str), 1745.46 (>C=O str), 2987.53 (-CH_2 str), 1494.73 (-N-H bend), 3313.48(N-H str), 754.12 (-CCl str), 1271 (-NO_2)

3H 3096 (Ar. C-H str), 1598.88 (Amide, >C=O str), 1793.67 (>C=O str), 2983.67 (-CH_2 str), 1492.80 (-N-H bend), 3319.26(N-H str), 752.19 (-CCl str)

4A 3058.89 (Ar. C-H str), 3190.04 (N-H str), 1681.17 (pyrazolidinedione, >C=O str), 1677.95 (amide, >C=O), 2821.66 (alkanes, C-H str), 1496.66 (N-H bend), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.7-8.1 (Ar), 5.5(NH), 260 (M+1) and other important peak is 205.

4B 2985.60 (Ar. C-H str), 1755.10 (pyrazolidinedione, >C=O str), 2898.66 (alkanes, C-H str), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.5-8.1 (Ar), 337 (M+2) and other important peaks are 267,205.

4C 3056.96 (Ar. C-H str), 1739.67 (pyrazolidinedione, >C=O str), 2989.46 (alkanes, C-H str), 1278.72 (-NO_2 str), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.5-8.1 (Ar), 426(M+1) and other important peak is 357.

4D 3018.39 (Ar. C-H str), 1772.46 (pyrazolidinedione, >C=O str), 2879.52 (alkanes, C-H str), 775.33 (-CCl str), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.3-8.1 (Ar), 370(M^+) and other important peaks are 302, 210.

4E 3051.18 (Ar. C-H str), 1712.67 (pyrazolidinedione, >C=O str), 2881.45 (alkanes, C-H str), 827.41 (-CCl str), 1485.09 (N-H str), 3190.04(N-H bend), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.8-8.1 (Ar), 5.6 (NH), 295(M+2) and other important peak is 240.

4F 3093.61 (Ar. C-H str), 1741.60 (pyrazolidinedione, >C=O str), 2931.60 (alkanes, C-H str), 729.04 (-Ccl str), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.6-8.1 (Ar), 372(M+2) and other important peaks are 240,302.

4G 2991.39 (Ar. C-H str), 1826.46 (pyrazolidinedione, >C=O str), 2875.67 (alkanes, C-H str), 811.98 (-Ccl str), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.4-8.0 (Ar), 461 (M+2) and other important peak is 390.

4H 3093.61 (Ar. C-H str), 1739.67 (pyrazolidinedione, >C=O str), 2983.67 (alkanes, C-H str), 811.98 (-CCl str), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.4-8.0 (Ar), 405(M+1) and other important peak is 250.

Table 4. Anti-inflammatory activity

Carrageenan Induced Rat Paw Edema				
Treatment	**Volume (Vo)**	**Maximum displacement (Vt)**	**Percentage increase in paw volume (%)**	**Percentage inhibition of edema (%)**
Control	0.4283±0.0030	0.695±0.0080	60	**-**
Standard	0.38±0.0025	0.4433±0.0033	16[a]	**77**
4A	0.4033±0.0049	0.6083±0.0070	52[a]	**20**
4B	0.39±0.0073	0.635±0.0147	61[c]	**8**
4C	0.38±0.0057	0.5833±0.0120	53[a]	**23**
4D	0.3916±0.0070	0.5833±0.0294	49[a]	**27**
4E	0.3683±0.0087	0.60±0.0139	62[b]	**12**
4F	0.385±0.0042	0.61±0.0089	60[b]	**12**
4G	0.3666±0.0061	0.655±0.0189	76	**0**
4H	0.375±0.0076	0.6116±0.014	65[c]	**8**

n=6 animals in each group. Values expressed as Mean ± SEM
[c]P < 0.05, [b]P < 0.01, [a]P < 0.001, as compared with control.

Table 5. Analgesic activity

Eddy's Hot Plate Method		
Sl. No	**Groups**	**Response time (seconds)**
1	**Control**	**2.08±0.3250**
2	**Standard**	**12.02±0.3619[a]**
3	**4A**	**11.42±0.4053[a]**
4	**4B**	**10.2±0.5910[b]**
5	**4C**	**11.1±0.6298[a]**
6	**4D**	**11.28±0.4037[a]**
7	**4E**	**11.43±0.8057[a]**
8	**4F**	**11.13±0.6844[a]**
9	**4G**	**11.77±0.5667[a]**
10	**4H**	**10.28±0.7968[b]**

n=6 animals in each group. Values expressed as Mean ± SEM
[c]P < 0.05, [b]P < 0.01, [a]P < 0.001, as compared with control.

Fig1. Bar graph for Carregeenan induced paw edema method for anti-inflammatory activity

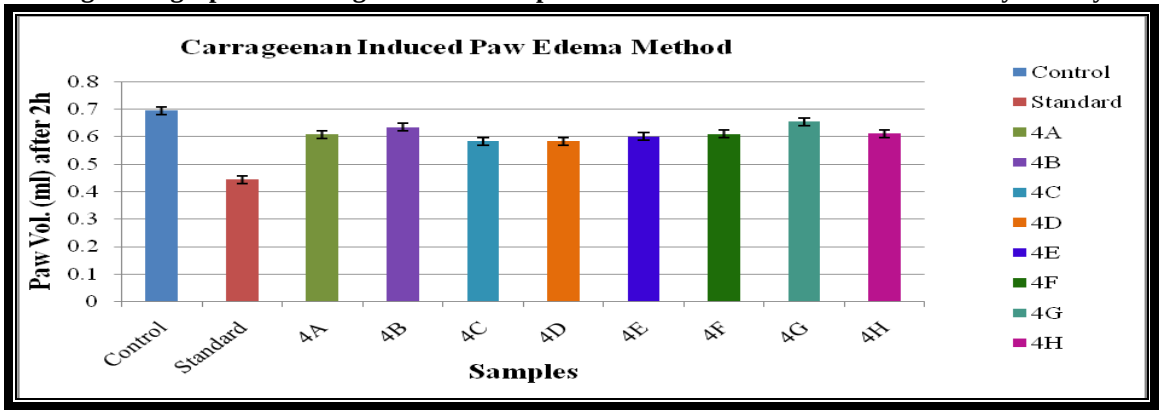


Fig 2. Pi-graph for Carregeenan induced paw edema method for anti-inflammatory activity

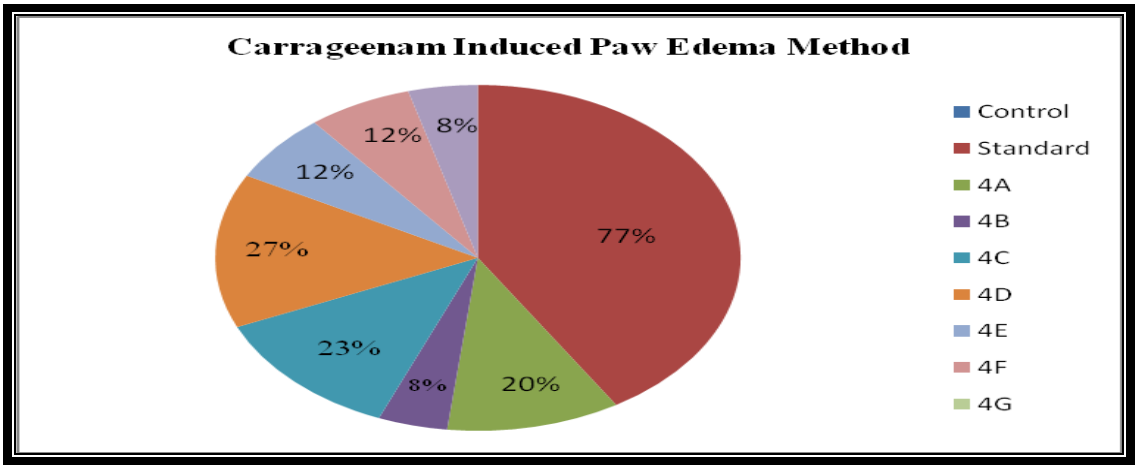
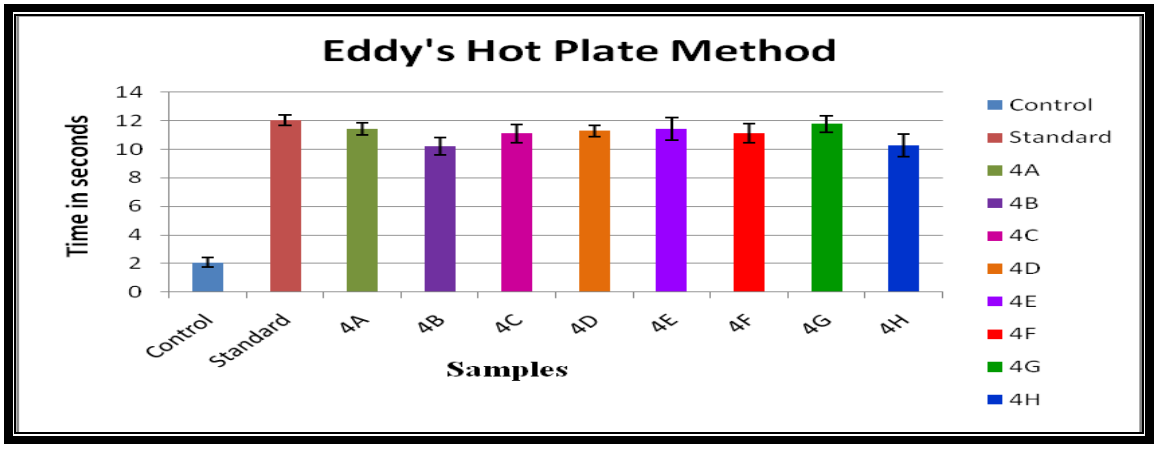


Fig 3. Eddy's Hot plate method for analgesic activity



Statistical analysis

The data was expressed as Mean ± SEM and was statistically analysed by one way ANOVA followed by Tukey-Kramer multiple comparison test for *in-vivo* anti – inflammatory and analgesic activity. P<0.05 (vs) control is considered to be significant.

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REFERENCES

Asati KC, Srivastava SK and Srivastava SD. Synthesis of 5-arylidene-2-aryl-3-(benzotriazloacetamidyl)-1,3-thiazololidin-4-ones as analgesic and antimicrobial agents. *Indian Journal of Chemistry*, 45B, 2006, 526-531.

Beale JM Jr. Wilson and Gisvold's textbook of organic and pharmaceutical chemistry. 11thEd. A Wolters Kluwer Company (NY), Lippincott Williams & Wilkins, 731, 2004, 753-763.

Cauvin C, Bourdonnec BL, Norberg B, Henichart JP and Durant F. Pyrazolidine-3,5-dione angiotensin-II receptor antagonists. *Acta Crystallographica*, 57, 2001, 1330-1332.

Fahmy HH and Soliman GA. Synthesis of new salicylamide derivatives with evaluation of their anti-inflammatory, analgesic and antipyretic activities. *Archibes of Pharmacal Research*, 24(3), 2001, 180-189.

Gerhard Vogel H. Drug discovery and evaluation, pharmacological assays. 2nd Ed. Springer: 2006, 696.

Kini S and Gandhi AM. Novel 2-pyrazoline derivative as potential anti-bacterial and anti- fungal agents. *Indian Journal of Pharmaceutical Sciences*, 70, 2008, 105-108.

OECD/OCDE, OECD guidelines for testing of Chemical-423. 17 Dec, 2001.

Rakesh Saini, Saurabh Chaturvedi, Achyut Narayan Kesari, Swatrantra Kushwaha. Synthesis of 2-(substituted)-5-(benzotriazomethyl)-1,3,4-oxadiazole. *Der pharma chemical*, 2(2), 2010, 297-302.

Silverstein RM, Webster FX. Spectrometric identification of organic compounds. 6th ed. NY. John Wiley & Sons, 2004.

Suma BV, Ankit Rochani K, Venkataramana CHS, Judy Jays, Madhavan V. Synthesis, characterization, invitro antibacterial, anti-inflammatory evaluations of novel 4- quinolone containing pyrazolidinedione derivatives. *International Journal of Chem Tech Research*, 2(4), 2010, 2156-2162.

William Kemp. Organic spectroscopy. 3rd edition Palgrave, New York, 2004, 182-184.