Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

SYNTHESIS AND ANTIBACTERIAL OF SOME NEW 1, 2, 3 BENZOTRIAZOLES DERIVATIVES CONTAINING PYRAZOLIDINEDIONE MOIETIES

B.V SUMA*†, N.N NATESH†, C.H.S VENKATARAMANA†, JUDY JAYS†, V. MADHAVAN††

*Department of Pharmaceutical Chemistry, M.S Ramaiah College of Pharmacy, M.S.R.I.T Post, M.S R. Nagar, Bengaluru-560054, Karnataka, India, ††Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, M.S.R.I.T. Post, Bengaluru 560054, India.
Email: bvs332@yahoo.co.in, bvs332@gmail.com

Received: 12 Feb 2011, Revised and Accepted: 18 May 2011

ABSTRACT

Benzotriazole derivatives have shown several pharmacological activities, which are antimicrobial activity, anti-inflammatory, analgesic activity, anticancer. On the basis of our observation the present research work was carried out to synthesize substituted benzotriazole derivatives containing pyrazilidin-3,5dione moieties. The various benzotriazole derivatives were prepared by diazotisation using substituted benzene 1, 2 diamine with glacial acetic acid **(1A-1I)** the various benzotriazole derivatives were treated with ethyl chloroacetate and anhydrous potassium carbonate to yield its ethyl 1 H benzotriazole-1-yl acetate **(2A-2I)**. The formed acetate is converted into hydrazide **(3A-3I)** by reacting with various substituted hydrazines. Finally the resulted hydrazides were condensed with diethyl propanedioate to yield some new 1, 2, 3, benzotriazole derivative containing pyrazolidine 3, 5 dione moieties. The entire synthesized compounds were characterized by IR, 1H-NMR, Mass spectroscopy and elemental analysis. Purity of the compounds was checked by using TLC and elemental analysis. The antimicrobial activity of the synthesized compounds was evaluated by cup plate diffusion method. These compounds had a considerable anti-bacterial activity against *S. aureus, B. subtilis, E. coli, Proteus vulgaris* compared to Ciprofloxacin, Amoxicillin respectively. Similarly compounds had considerable antifungal activity against *A. niger*, *C. albicans* compared to Ketoconazole, Clotrimazole respectively.

Keywords: Benzotriazole, Pyrazolidine 3, 5-dione, Antibacterial activity, Antifungal activity

INTRODUCTION

Benzotriazole derivatives are an important class of nitrogen containing heterocyclic and were reported to possess a wide spectrum of biological and pharmacological activities such as antibacterial and antifungal, anticancer, anti-inflammatory and analgesic activities. Although number of drugs is available in the market, but the need of discovering the new antimicrobial 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[1].

Research on pyrazolidine-3, 5-dione and their synthetic analogs has revealed to possess to be angiotensin II receptor antagonist along with wide range of antimicrobial activity[2-4]. It is our interest to synthesize some new benzotriazole derivatives containing pyrazolidine-3,5-dione moieties and to evaluate the invitro antimicrobial activity. The synthesized compounds have shown satisfactory spectral data which are in conformity of the proposed structures.

MATERIALS AND METHODS[4, 5]

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- 1I)

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-1I)** derivatives are presented in table 1.

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

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-2I)** derivatives are presented in table 1.

Synthesis of substituted 2-(1H-benzotriazol-1-yl) acetohydrazides: (3A- 3I)

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-3I)** derivatives are presented in table 1.

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

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-4I)** derivatives are presented in table 1.

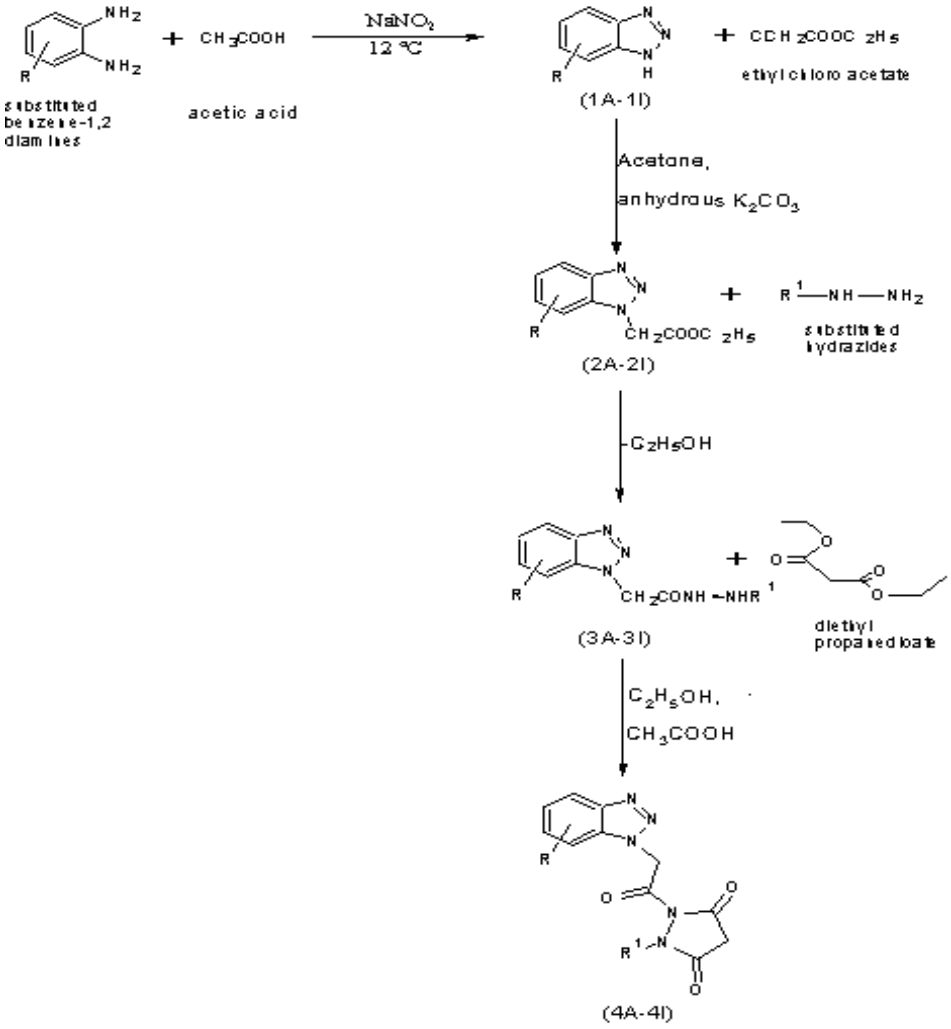

Table 1: Physical Data of Synthesized Compounds

Compounds	R	R^1	%Yield	Melting Point (°C)
1A-1D	H	-	84	93-98
1E-1H	Cl	-	78.43	85
1I	NO_2	-	91.46	210
2A-2D	H	-	90.24	55-58
2E-2H	Cl	-	79.17	75
2I	NO_2	-	80	70
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
3I	NO_2	H	88.98	172
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
4I	NO_2	H	80.59	242

Anti-bacterial activity[6-9]:

All the synthesized compounds were screened for antibacterial activities, against gram-positive organisms like *S. aureus & B. subtilis* & gram-negative organisms like *E.Coli & P. vulgaris* by diffusion agar media using nutrient agar as cultural medium. After agar was solidified, cups were made in the nutrient agar. The Anti-microbial test drugs **4A-4I** were placed in the cups (1000µg/ml). The plates were incubated at a temp of 37ºC ± 1ºC for 24hrs for bacterial culture. Dimethylsulphoxide was used as solvent. Ciprofloxacin and Amoxicillin (100 µg/ml) were used as standard for antibacterial screening.

Antifungal activity[6-9]

All the synthesized compounds were screened for antifungal activity against *A. niger*, *C. albicans*, by cup plate diffusion method by measuring the zone of inhibition in mm. The fungal activity was performed in Sabouraud's Dextrose Agar medium at a concentration of 1000 µg/ml. The plates were incubated at 25ºC ± 1ºC for 48 hrs Ketoconazole and Clotrimazole were used as a standard. Dimethylsulphoxide (DMSO) was used as a solvent for all the compounds and as a control.

RESULTS AND DISCUSSION

Synthesis of **1A-1I** & **2A-2I** was carried out using diazotization & alkylation reactions, further it was converted to hydrazides **3A-3I** and to the final compounds, physical data of compounds are presented in table 1. Elemental analysis of all the final **4A-4I** 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 antibacterial and antifungal activity was shown by all the synthesized derivatives compared to standard drugs. The biological activity data are presented in table 4. Compound **4H** was found to be more effective against *S. aureus*, compound **4F** was found to have good activity against *B.subtilis*. Compound **4B** found to have good activity against *E.coli*. Compound **4G** was found to have good activity against *P. vulgaris*. Accordingly, in Antifungal activity, Compounds **4E, 4H** and **4I** were found to have good activity against *A.niger* while compounds **4C** and **4G** were found to have good activity against *C. albicans*

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[10, 11]

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)
1I 3085.89 (N-H str), 3018.39 (Ar. C-H str), 2790.80 (-CH_2 str), 1494.73 (N-H bend), 1278.72 (-NO_2 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)
2I 3085.89 (Ar. C-H str), 2904.60 (-CH_2 str), 1747.39 (Ester, >C=O str), 1211.21 (-NO_2 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)
3I 3053.11 (Ar. C-H str), 1544.88 (Amide, >C=O str), 1660.60 (>C=O str), 2902.67 (-CH_2 str), 1450.37 (-N-H bend), 303.83(N-H str), 1278.72 (-NO_2 str)
4A 3058.89 (Ar. C-H str), 3190.04 (N-H str), 1681.17 (pyrazolidine dione, >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 (pyrazolidine dione, >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 (pyrazolidine dione, >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 (pyrazolidine dione, >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 (pyrazolidine dione, >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 (pyrazolidine dione, >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 (pyrazolidine dione, >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 (pyrazolidine dione, >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.

4I 3026.10 (N-H str), 1743.53 (pyrazolidine dione, >C=O str), 2923.88 (alkanes, C-H str), 1261.36 (-NO str), 2952.81 (Ar. C-H str), 1492.80 (N-H bend), 1.8-2.1 (alkyl CH_2), 1.2-1.6 (acyclic), 7.8-8.0 (Ar), 5.6 (NH), 306(M+2) and other important peaks are 250, 163.

Table 4: Antimicrobial activity of synthesized compounds (4A- 4I)

S. No.	Compound	Antibacterial activity zone of inhibition in (mm)				Antifungal activity	
		S.aureus	*B.Subtilis*	*E.Coli*	*P. Vulgaris*	*A.niger*	*C.Albicans*
1.	4A	07	06	07	04	07	10
2.	4B	11	07	08	03	04	12
3.	4C	08	04	03	07	06	13
4.	4D	13	08	07	06	07	14
5.	4E	10	03	04	01	09	11
6.	4F	14	09	06	02	07	12
7.	4G	12	03	03	08	05	13
8.	4H	16	06	05	04	09	12
9.	4I	08	02	02	06	09	10
10.	Ciprofloxacin	17	19	15	16	-	-
11.	Amoxicillin	15	14	17	13	-	-
12.	Ketoconazole	-	-	-	-	18	17
13.	Clotrimazole	-	-	-	-	15	12
14.	Control (DMSO)	NI	NI	NI	NI	NI	NI

Note: All the values are mean of triplicates, NI: no inhibition, - : not tested

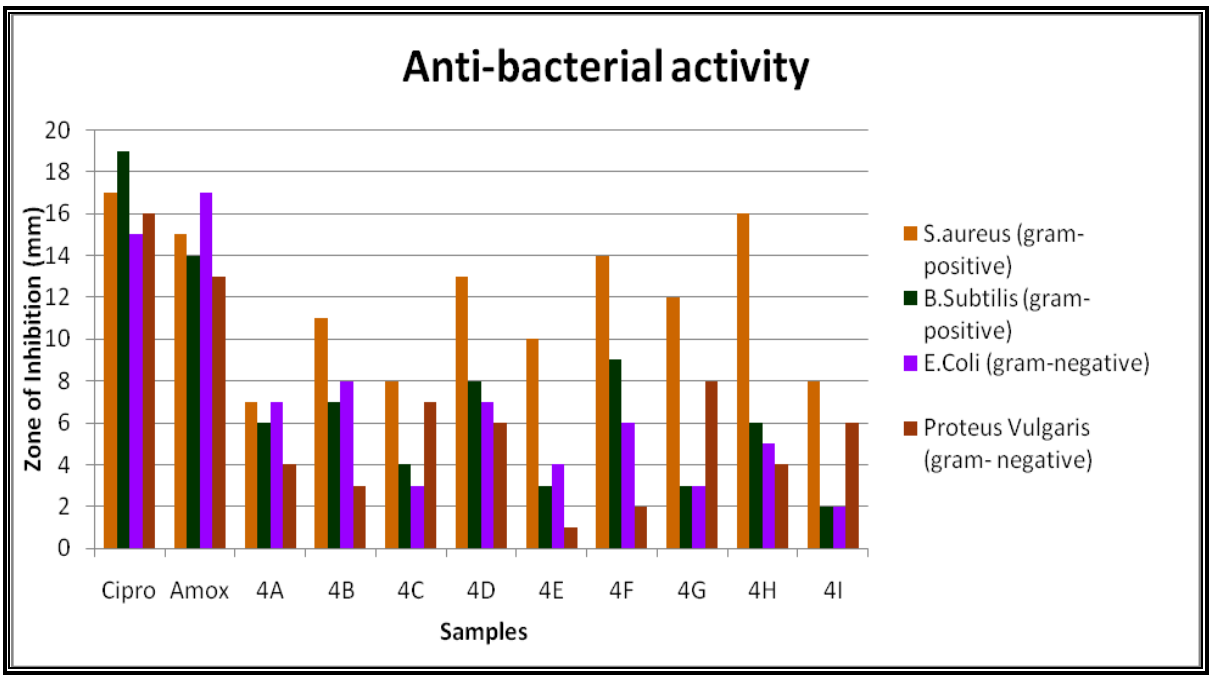

Fig. 1: Antibacterial Activity

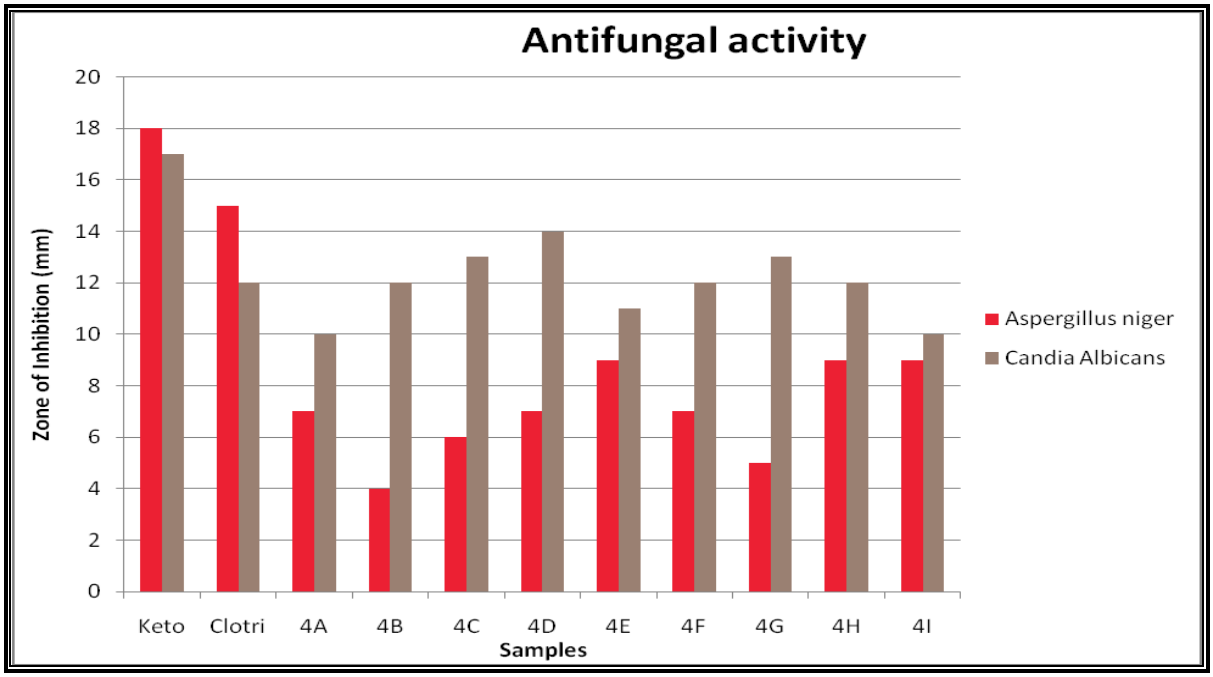

Fig. 2: Antifungal activity

CONCLUSION

All the compounds were synthesized with an objective of developing better antimicrobial molecules with maximum percentage of yield. All the synthesized compounds were effective against all the four strains and two strains, antibacterial activity and antifungal activity might be due to the presence Benzotriazole and pyrazolidine-3,5dione moieties.

Further investigation with appropriate structural modification of title compound may result in therapeutically useful products.

ACKNOWLEDGMENT

The authors are thankful to Gokula Education Foundation for providing facilities encouragement and financial support throughout the work. The authors are thankful to Gokula Education Foundation for providing facilities encouragement and financial support throughout the work. Authors are also thankful to Indian Institute of Sciences (IISc), Bengaluru, for providing ^{1}H NMR reports, Indian Institute of Chemical Technology, Hyderabad for providing Mass spectrum reports and to Uwin Global Services, Bengaluru for providing Elemental analysis reports of the synthesized compounds.

REFERENCES

1. Beale JM Jr. Wilson and Gisvold's textbook of organic and pharmaceutical chemistry. 11thEd. A Wolters Kluwer Company (NY): Lippincott Williams & Wilkins, 2004, 731;753-763.
2. 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 J. of Chem. Tech. Research 2010; 24:2156-2162.
3. Rakesh Saini, Saurabh Chaturvedi, Achyut Narayan Kesari, Swatrantra Kushwaha. Synthesis of 2-(substituted)-5-(benzotriazomethyl)-1, 3, 4-oxadiazole. Der. pharma chemical 2010;2, (Suppl.2): 297-302.
4. Kini S. and Gandhi A. M., Novel 2-pyrazoline derivative as potential anti-bacterial and anti- fungal agents. Indian J of Pharmaceutical Sciences, 2008; 70:105-108.
5. Cauvin C, Bourdonnec B.L., Norberg B., Henichart J.P. and Durant F., Pyrazolidine-3, 5-dione angiotensin-II receptor antagonists. Acta Crystallographica, 2001; 57:1330-1332.
6. Mrunmayee P Toraskar, Vilasrao J Kadam , Vithal M Kulkarni. Synthesis and antifungal activity of azetidinones. International J. of Chem. Tech. Research 2009; 1(Suppl 4): 1194-1199.
7. Shukhla DK and Srivastava SD. Synthesis of some new 5-[2-{(1,2,3-benzotriazole) -1-yl-methyl} -1-(4substituted aryl-3-chloro-2-oxo azetidine)] -amino-1,3,4-thiadiazoles: Antifungal and antibacterial agents. Indian J of Chemistry 2008; March, 47B: 463-469.
8. Ewa Augustynowicz-Kopec, Zofia Zwolska, Andrzej Orzeszko. Synthesis and antimycobacterial activity of selected nitrobenzyloxylated benzotriazoles. Acta Poloniae Pharmaceutica Drug Research 2008; 65(Suppl 4): 435-439.
9. Hemavathi S, Vishnu kumar BK, and. Lokanatha rai KM Synthesis and biological screening of some new 2, 5-disubstituted 1, 3, 4-oxadiazoles, International J. of Pharmacy and Pharmaceutical Sciences, 2011;3 (Suppl 4): 110-114
10. William Kemp. Organic spectroscopy. 3rd ed. Palgrave (Newyork): 2004: 182-184.
11. Silverstein RM, Webster FX. Spectrometric identification of organic compounds. 6th ed. NY. John Wiley & Sons, 2004.