World Journal of Chemistry 5 (1): 52-56, 2010
ISSN 1817-3128

Greener and Expeditious Synthesis of 2-Azetidinone Derivative from 2-Mercaptobenzothiazole and Their Pharmacological Screening of the Synthesized Compounds Using Microwave Irradiation

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Abstract: A new selective methods has been developed for 2-(4-substituted aryl-3-chloro-2-oxo-azetidine)-2-iminobenzothiazoles 3(a-n) by the heterocyclization of 2-substituted arylidene hydrazino benzothiazoles, 2(a-n) with chloroacetyl chloride in the presence of triethylamine under microwave irradiation (MWI) is described. The reaction rate and yield is enhanced tremendously under MWI as compared to conventional methods. The structures of the synthesized compounds have been characterized on the basis of their elemental analysis in IR, HNMR and Mass spectral data. The synthesized compounds have been screened *in vitro* for their antimicrobial activity against *Bacillus substilis, Escherichia coli, Streptococcus aureus, Klebsiella pneumoniae* bacteria and antifungal activity against *Aspergillus niger, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride* fungi respectively.

Key words: 2-Mercaptobenzothiazole · Arylidene · 2-azetidinone · Antimicrobial activity

INTRODUCTION

For more than a century, heterocycles have constituted one the largest areas of research in organic chemistry. They have contributed to the development of society from a biological and industrial point of view as well as to the understanding of life processes and to the efforts to improve the quality of life [1]. Conventional methods of organic synthesis are orders of magnitude too slow to satisfy the demand for generation of such compounds.The fields of combinatorial and automated medicinal chemistry have emerged to meet the increasing requirement of new compounds for drug discovery, where speed is of the essence. The synthetic chemical community has been under increased pressure to produce, in an environmentally benign fashion, the myriad of substances required by society in short periods of time and the best option to accelerate these synthetic processes is to use microwave (MW) technology. The efficiency of MW flash-heating has resulted in dramatic reductions in reaction times. The time saved by using the MW heating approach is potentially important in traditional organic synthesis and assembly of heterocyclic systems. In this study, we have summarized our recent activity in the area of greener and expeditious synthesis of bioactive heterocycles using microwave irradiation [2].

Azetidinones, which are part of the antibiotic structure, are known to exhibit interesting biological activities [3]. A large number of 3-chloro monocyclic ß-lactams possess powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant and antitubercular activity [4-8]. They also function as enzyme inhibitors and are effective on the central nervous system [9]. Similarly, benzothiazole derivatives have attracted considerable attention as they are also enrolled with wide range of pharmacological activities like schistosomicidal [10], anthelminitic [11], antiparasitic [12], anticancer [13], anticonvulsants [14] and antimicrobial [15,16] activities. In continuation of our studies on benzothiazole we have synthesized 2-azetidinone moiety linked to bioactive benzothiazole ring to analyse their biological profile. Hence, in the present study, the 2nd position in 2-mercapto benzothiazole moiety having thiol (-SH) group, was used as the target for chemical modification Scheme-I. A comparative study in term of yield and reaction period is shown (Table 1).

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Table 1: Characterization data of the compounds 2b-n and 3b-n

Comp	Ar	% Yield (Reaction time) MW (mins)	% Yield (Reaction time) Conv. (hrs.)	M.P. (°C)	Molecular formula	Found % (Calcd) C	Found % (Calcd) H	Found % (Calcd) N
2b	2-ClC_6H_4	91 (2)	66 (4)	139-40	$C_{14}H_{10}N_3SCl$	58.41 (58.43)	3.47 (3.50)	14.57(14.60)
2c	3-ClC_6H_4	86 (2)	54 (4)	143-45	$C_{14}H_{10}N_3SCl$	58.42 (58.43)	3.46 (3.50)	14.55(14.60)
2d	4-ClC_6H_4	83 (2)	58 (4)	140-42	$C_{14}H_{10}N_3SCl$	58.39 (58.43)	3.47 (3.50)	14.58(14.60)
2e	2-BrC_6H_4	79 (3)	70 (5)	121-23	$C_{14}H_{10}N_3SBr$	50.58 (50.61)	2.99 (3.03)	12.61(12.64)
2f	3-BrC_6H_4	74 (3)	65 (5)	119-21	$C_{14}H_{10}N_3SBr$	50.56 (50.61)	2.97 (3.03)	12.63(12.64)
2g	4-BrC_6H_4	78 (3)	68 (5)	132-34	$C_{14}H_{10}N_3SBr$	50.57 (50.61)	3.01 (3.03)	12.62(12.64)
2h	2-$OCH_3C_6H_4$	83 (5)	61 (6)	172-74	$C_{15}H_{13}N_3OS$	63.54 (63.58)	4.58 (4.62)	14.76(14.82)
2i	3-$OCH_3C_6H_4$	87 (5)	60 (6)	168-70	$C_{15}H_{13}N_3OS$	63.54 (63.58)	4.59 (4.62)	14.79(14.82)
2j	4-$OCH_3C_6H_4$	87 (5)	59 (6)	176-78	$C_{15}H_{13}N_3OS$	63.56 (63.58)	4.60 (4.62)	14.77(14.82)
2k	2-$NO_2C_6H_4$	94 (1)	65 (2)	109-11	$C_{14}H_{10}N_4O_2S$	56.34 (56.37)	3.35 (3.37)	18.76(18.78)
2l	3-$NO_2C_6H_4$	93 (1)	86 (2)	128-30	$C_{14}H_{10}N_4O_2S$	56.35 (56.37)	3.34 (3.37)	18.75(18.78)
2m	4-$NO_2C_6H_4$	97 (1)	84 (2)	117-19	$C_{14}H_{10}N_4O_2S$	56.36 (56.37)	3.33 (3.37)	18.74(18.78)
2n	4,4- $(CH_3)_2NC_6H_4$	86 (2)	79 (4)	194-96	$C_{16}H_{15}N_4S$	65.0 (65.07)	5.06 (5.11)	18.94(18.97)
3b	2-ClC_6H_4	89 (6)	76 (12)	154-56	$C_{16}H_{11}N_4OSCl_2$	54.38 (54.41)	5.9 (3.11)	11.86(11.89)
3c	3-ClC_6H_4	87 (6)	82 (11)	152-54	$C_{16}H_{11}N_4OSCl_2$	54.36 (54.41)	3.7 (3.11)	11.84(11.89)
3d	4-ClC_6H_4	90 (6)	78 (12)	169-71	$C_{16}H_{11}N_4OSCl_2$	54.38 (54.41)	3.08 (3.11)	11.85(11.89)
3e	2-BrC_6H_4	89 (5)	74 (9)	167-69	$C_{16}H_{11}N_3OSClBr$	41.98 (42.02)	2.66 (2.69)	10.25(10.28)
3f	3-BrC_6H_4	86 (5)	72 (11)	165-67	$C_{16}H_{11}N_3OSClBr$	41.96 (42.02)	2.64 (2.69)	10.23(10.28)
3g	4-BrC_6H_4	90 (5)	75 (10)	169-71	$C_{16}H_{11}N_3OSClBr$	41.95 (42.02)	2.65 (2.69)	10.24(10.28)
3h	2-$OCH_3C_6H_4$	98 (7)	81 (12)	162-64	$C_{17}H_{13}N_3O_2SCl$	56.79 (56.82)	3.58 (3.62)	11.67(11.69)
3i	3-$OCH_3C_6H_4$	95 (7)	84 (10)	157-59	$C_{17}H_{13}N_3O_2SCl$	56.80 (56.82)	3.60 (3.62)	11.66(11.69)
3j	4-$OCH_3C_6H_4$	91 (7)	86 (12)	152-54	$C_{17}H_{13}N_3O_2SCl$	56.78 (56.82)	3.59 (3.62)	11.64(11.69)
3k	2-$NO_2C_6H_4$	95 (5)	75 (8)	156-58	$C_{16}H_{11}N_4O_3SCl$	51.24 (51.27)	2.91 (2.93)	14.91(14.94)
3l	3-$NO_2C_6H_4$	94 (5)	74 (8)	170-72	$C_{16}H_{11}N_4O_3SCl$	51.23 (51.27)	2.89 (2.93)	14.93(14.94)
3m	4-$NO_2C_6H_4$	93 (5)	79 (8)	166-68	$C_{16}H_{11}N_4O_3SCl$	51.25 (51.27)	2.90 (2.93)	14.92(14.94)
3n	4,4'-$(CH_3)_2NC_6H_4$	81 (6)	74 (10)	173-75	$C_{18}H_{17}N_4SCl$	57.96 (57.99)	4.53 (4.56)	14.99(15.02)

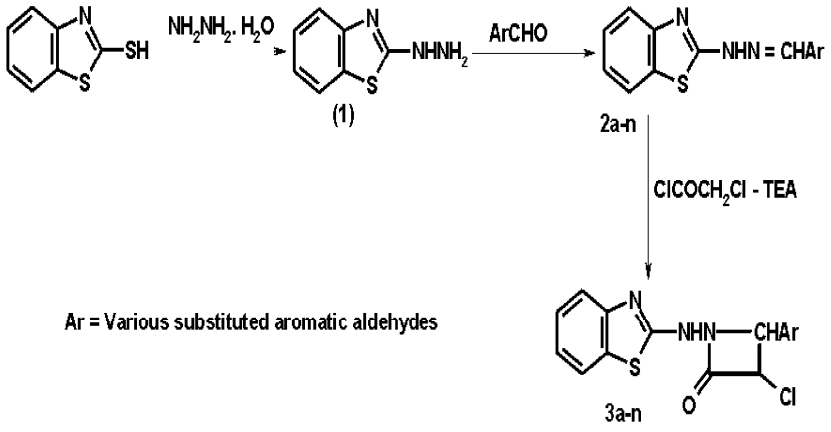
MW: Microwave; Conv.: Conventional

RESULTS AND DISCUSSION

2-Hydrazinobenzothiazole 1 was synthesized by using 2-mercaptobenzothiazole as a starting material using hydrazine hydrate as one of the reactant. Compound 1 on condensation with various substituted aromatic aldehydes yielded compounds 2a-n which on reaction with chloroacetyl chloride in the presence of triethyl amine afforded 4-substituted aryl-3-chloro-2-oxo-azetidine-2-iminobenzothiazoles 3a-n (Scheme-1).

The structures of all the synthesized compounds were established by spectral and analytical method (Table 1).

All the synthesized compounds 2(a-n) and 3(a-n) have been screened *in vitro* for their antibacterial activity against *B. subtilis* (Bs), *E. coli* (Ec), *S. aureus* (Sa) and *K. pneumoniae* (Kp) at two concentrations (50 and 100 ppm) and antifungal activity against *A. niger* (An), *A. flavus* (Af), *F. oxisporium* (Fo) and *T. viride* (Tv) at two concentrations (50 and 100 ppm).



Standard antibacterial Streptomycin and fungicide Griseofulvin were also screened under the similar conditions for comparison. The following compounds were found active against the tested bacteria: 4d(Ec,Sa), 4f(Bs,Kp), 4g(Ec), 4h(Bs,Ec,Sa), 2i,2j(Kp), 3b,3c,3d,3e,3g(Bs,Ec,Kp,Sa),3i(Ec,Kp,Sa),3h(Kp),3k(Ec), 3n(Kp,Sa) and fungi: 2f(Af,Tv), 2g(An), 2n(Fo), 3b(Tv), 3c (Af, Fo), 3d(An), 3e, 3f, 3g(An, Af, Fo,Tv), 3i(Af, Fo), 3j (An,Af), 3h(Fo,Tv), 3k(An,Af), 3l, 3m, 3n (An).

CONCLUSION

On the basis of structural activity relationship it has been observed that among the substituents present on the phenyl ring, halo derivatives were found to be highly active against in the series. Further study reveals that bromo derivatives are highly active.

Experimental: Melting points were taken in open capillaries. Purity of compounds was monitored on silica gel "G" coated TLC plates. All instrumental analysis was performed at the Central Drugs Research Institute, Lucknow (India). IR spectra were recorded in KBr disc on a Schimadzu 8201 PC, FTIR spectrophotometer (ν_{max} in cm^{-1}) and ^{1}HNMR spectra were measured on a Brucker DRX-300 spectrometer in $CDCl_3$ at 300 MHz using TMS as an internal standard. All chemical shifts were reported as δ (ppm) values. The FAB mass spectra were recorded on a Jeol SX-102 mass spectrometer. Elemental analyses were performed on a Carlo Erba-1108 analyzer. The analytical data of all the synthesized compounds were highly satisfactory. For chromatographic purification Merck silica Gel 60 (230-400 Mesh) was used. Microwave assisted reaction were carried out in a Qpro-M-modified microwave oven. The reagent grade chemicals were purchased Merck and Aldrich Chemical Co. Ltd. were used. Anhydrous silica gel 60 (0.063-0.2 mm) was used as solid support after dehydration under microwave irradiation for 4 minutes.

General Procedure for the Synthesis of the New Compounds

Preparation of 2-Hydrazinobenzothiazole (1): Equimolar mixture of 2-mercaptobenzothiazole (0.2 mole, 21.408 g) and hydrazine hydrate (0.2 mole, 9.72 g) in methanol (100 ml) was refluxed on a water bath for about 10 hr. It was cooled, filtered and solvent was distilled off under reduced pressure and the solid obtained was passed through a column of silica gel using chloroform: methanol (8:2 v/v) mixture as eluent. The eluate (250 ml) was concentrated to give a product which was recrystallised with ethanol to give compound 1, yield 86%, m.p. 202-204°C. Anal Calcd for $C_7H_7N_3S$(M.wt. 218), C, 50.88, H, 4.27, N, 25.43%, found: C, 50.85, H, 4.24, N, 25.39%, IR: 3392, 3276, (-NH_2),3355(-NH), 3020, 1597, 1035(sub.aryl ring), 1650 (C=N), 1192, 1078, 669(C-S-C);^{1}HNMR: 6.92-7.79 (m, 4H, Ar-H); 7.90 (s, 1H,-NH); 4.76 (s, 2H,-NH_2); MS: 218 (M^+).

Microwave Method: A mixture of 2-mercaptobenzothiazole (0.2 mole, 21.408 g) and hydrazine hydrate (0.2 mole, 9.72 g) was added and mixed thoroughly. The mixture was air dried and subjected to microwave irradiation for 1 minute (completion of reaction as indicated by TLC). The reaction mixture was cooled to room temperature and the separated solid was extracted with ethanol. On standing the filtrate afforded colourless crystalline solid. The product was purified by column chromatography and recrystallised from ethanol, yield 89%. Spectral and analytical data were found to similar as reported for conventional method.

Preparation of 2-benzylidene-hydrazinobenzothiazoles (2a): A mixture of the compound 1 (0.02 mole, 3.304 g) and benzaldehyde (0.02 mole, 2.02 g) and 2-3 drops of glacial acetic acid in benzene (40 ml) was refluxed on a water bath for about 1 hr. It was cooled, filtered, dried and passed through a column of silica gel using chloroform: methanol (6:4 v/v) mixture as eluent. The eluate was concentrated to give a product which was recrystallised from $CHCl_3$ to give compound 2a, yield 80%, m.p. 198-200°C. Anal. Calcd. For $C_{14}H_{11}N_3S$ (M.wt. 253),C, 66.37, H, 4.37, N, 16.58%, found C, 66.35, H, 4.31, N, 16.56%. IR: 3356, (-NH), 1547 (-N=CH), 3023, 1597, 1035(sub.aryl ring),1649(C=N), 1192, 1078, 668 (C-S-C); ^{1}HNMR: 6.91-7.80 (m, 9H, Ar-H); 4.88 (s, 1H,-N=CH), 7.95 (s, 1H,-NH).).Other compounds 2(b-n) were synthesized in the similar manner using compound 1 and various aromatic aldehydes.

Microwave Method: Equimolar mixture of compound 1 (0.02 mole, 3.304 g) and benzaldehyde (0.02 mole, 2.02 g) in methanol (20 ml) with 4-5 drops of glacial acetic acid was kept at room temperature. Anhydrous microwave transparent solid support silica gel was added and the solvent was removed under vacuum. The adsorbed reaction mixture was introduced in an open quartz tube which was then subjected to microwave irradiation in the resonance cavity of the microwave power system for 1.30 minutes. The sample was cooled in an ice bath and TLC was used to monitor the reaction progress. The reaction

product was extracted with methanol, filtered and dried over anhydrous sodium sulphate and then the solvent was removed. The product was purified by column of silica gel and recrystallised with ethanol to give compound 2a. Yield 94%.

Other compounds 2(b-n) were synthesized in the similar manner using compound 1 and various aromatic aldehydes. Characterization data are presented in Table-1.

Preparation of 2-(4-phenyl-3-chloro-2-oxo-azetidine)-2-imino-benzo thiazoles *(3a):* Compound 2a (0.005 mole, 1.233 g) and triethylamine (0.005 mole, 0.696 g) in acetone (30 ml) with chloroacetyl chloride (0.005 mole, 0.398 g) was stirred for about 1 hr followed by refluxation on a water bath for about 8 hr and cooled, filtered, dried and passed through a column of silica gel using chloroform: methanol (7:3 v/v) mixture as eluent. The eluate was concentrated which was recrystallised with ethanol to give compound 3a, Yield 60%, m.p. 174-176°C. Anal. Calcd for $C_{16}H_{12}N_3OSCl$ ((M.wt. 409),C, 64.55, H, 4.03, N, 14.11%, found C, 64.53, H, 4.01, N, 13.99. IR: 3355 (-NH), 3027, 1597, 1034 (sub.aryl ring), 1652(C=N), 1191, 1078, 665 (C-S-C), 1775 (>C=O), 763 (CH-Cl); ^{1}HNMR: 6.90-7.78 (m, 9H, Ar-H), 7.85 (s, 1H,-NH), 5.15 (d, J=7.00 Hz, 1H,-CHCl), 4.18 (d, J = 7.00 Hz, N-CH-Ar);Mass(FAB): 409(M^+). Other compounds 3(b-n) were synthesized in the similar manner using compounds 2(b-n) and chloroacetyl chloride.

Microwave Method: Compound 2a (0.005 mole, 1.233 g) and triethylamine (0.005 mole, 0.696 g) with chloroacetyl chloride (0.005 mole, 0.398 g) in methanol (20 ml) in an open quartz tube which was then subjected to microwave irradiation for 8 minutes. Initial and final sample temperature was increased. The sample was cooled in an ice bath and the irradiation was repeated 4 times. TLC was used to monitor the reaction progress. The solvent was removed under vacuum and the product was purified by column chromatography and recrystallized with ethanol to give compound 3a, yield 96%.

Other compounds 3(b-n) were synthesized in the similar manner using compounds 2(b-n). Characterization data are presented in Table-1

Antimicrobial Activity

Antibacterial Activity: All the synthesized compounds were evaluated *in vitro* for antibacterial activity by using filter paper disc method [17, 18] against different strains of bacteria viz. *B. substilis, E. coli, S. aureus* and *K. pneumoniae*. All the compounds along with standard antibacterial Streptomycin were used at 50 and 100 ppm concentrations.

Procedure: Solution of known concentration (50 and 100 ppm) of the test sample were made by dissolving in DMSO. Dried and sterilized filter paper discs (6mm in diameter) soaked with known amount of test agents were placed on the nutrient agar media solidified in petridishes (120 mm diameter) and inoculated with the test organisms. These plates were then kept at low temperature (4°C) for 24 hours to allow maximum growth of the organisms. The antibacterial activity was determined by measuring the diameter of zone of inhibition in mm.

Antifungal Activity: All the compounds were assayed *in vitro* for antifungal activity against *A. niger, A. flavus, F. oxisporium and T. viride* fungi employing the filter paper disc method [19] by measuring inhibition zone in mm. All the tested compounds along with standard fungicide Griseofulvin were used at 50 and 100 ppm concentrations.

Procedure: The test samples were dissolved in DMSO to make 50 and 100 ppm concentration solutions. Sterilized symmetrical filter paper discs of 6 mm diameter were taken in a blank petridishes sample solution 10 µl /discs were applied on the discs with the help of a micropipette in an aseptic condition. The discs were left for a few minutes in the aseptic condition for complete removal of the solvent. Isolated spore (4-6 similar) of pure fungus was inoculated in screw capped tube containing equal amount of potato dextrose agar (PDA) media and incubated at 28°C for 5-7 days for development of new pure culture that was used as inoculum. PDA medium was steamed to dissolve and dispersed 4 ml amount of it into a petridish. It was then autoclaved at 121°C for 15 minutes. It was allowed to cool to 30°C until the media became solid. Each petridish was inoculated with different types of inoculums removed from a seven days old culture fungus. Dried and sterile sample discs and standard (Fungal) disc were placed on nutrient agar plates seeded with the test organism. These were then kept at low temperature (4°C) for 24 hours to allow maximum diffusion. Finally the petridishes were inoculated at 27-28°C for 5-7 days. The activity was justified by measuring the diameter of zone of inhibition in mm.

ACKNOWLEDGEMENT

The authors are thankful to SAIF, CDRI Lucknow for providing spectral and analytical data of the compounds. We are also grateful to Dr. Mrs. Archana Tiwari, Department of Biotechnology, Dr. H.S. Gour University Sagar for providing help in carrying out the antimicrobial

screening. We are also thankful to Head, Department of this University for giving the facilities to carryout the work.

REFERENCES

1. Valverde, M.G. and T. Tomas, 2005. Sulfur-Nitrogen Heterocycles. Molecules, 10: 318-320.
2. Varma, R.S., 2006. Kirk-Othmer On-line Encyclopedia of Chemical Technology, 5th ed., John Wiley, Hoboken, New Jersey, 16: 538.
3. Durckheimer, W., J. Blumbach, R. Lattrell and K.H. Scheunemann, 1985. Recent Developments in the Field of ß-Lactam Antibiotics. Angew. Chem. Int. Ed. Engl., 24: 180-202.
4. Abdulla, R.F. and K.H. Fuhr, 1975. Monocyclic antibiotic beta-lactams. J. Med. Chem., 18: 625-627.
5. Feigelson, G.B., W.V. Curran and C.B. Ziegler, 1994. 4-Substituted azetidinones as recursors to 2-substituted-3-carboxy carbapenem antibiotics and a method of producing them. US 5, 371: 215.
6. Doherty, J.B., C.P. Dorn, P.L. Durette, P.E. Finke, M. Mac Coss, S.G. Mills, S.K. Shah, S.P. Sahoo, S.A. Polo and W.K. Hagmann, 1994. Substituted azetidinone as anti-inflammatory and antidegenerative agents. Pub. No.WO 1994/ 010143.
7. Khalafallah, A.K., M.A. Selim, R.M.A. El-Hamd, M.A. Elmaghraby, H.A. Soleiman and M.A. Raslan, 1995. Novel synthesis of some new fused/spiro heterocyclic compounds and their biological activity. Indian J. Chem., 34B: 1066-1070.
8. Parikh, K.A., P.S. Oza and A.R. Parikh, 2000. Synthesis of some new 2- azetidinones as potential antitubercular agents. Indian J. Chem., 39B: 716-722.
9. Vashi, B.S., D.S Mehta and V.H. Shah, 1995. Synthesis and biological activity of 4-thiazolidinones,2-azetidinones, 4-imidazolinone derivatives having thymol moiety. Indian J. Chem., 34B: 802-808.
10. Mahran, M.A., S. William, F. Ramzy, A.M. Sembel, 2007. Synthesis and *in vitro* evaluation of new benzothiazole derivatives as schistosomicidal agents. Molecules, 12(3): 622-33.
11. Sreenivasa, G.M., E. Jayachandran, B. Shivakumar, K. Jayaraj and M.M.J. Vijay Kumar, 2009. Synthesis of Bioactive Molecule Fluoro Benzothiazole comprising Potent Heterocyclic Moieties for Anthelmentic Activity. Arch. Pharm. Sci. Res., 1(2): 150-157.
12. Mahran, M.A., S.M. El-Nassry, S.R. Allam and L.A. El-Zawawy, 2003. Synthesis of Some new benzothiazole derivatives as potential antimicrobial and antiparasitic agents. Pharmazie, 58(8): 527-30.
13. Stanton, H.L.K., *et al*, 2008. Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines Bioorganic Med. Chem., 16(7): 3626-3631.
14. Siddiqui, N. and W. Ahsan, 2009. Benzothiazole incorporated barbituric acid derivatives: Synthesis and anticonvulsant screening. Arch. Pharm., 342(8): 462-468.
15. Rajeeva, B., N. Srinivasulu and S.M. Shantakumar, 2009. Synthesis and AntimicrobialActivity of Some New 2-Substituted Benzothiazole Derivatives, E-J. Chem., 6(3): 775-779.
16. Kaur, H., S. Kumar, I. Singh, K.K. Saxena and A. Kumar, 2010. Synthesis, Characterization and Biological Activity of Various Substituted Benzothiazole Derivatives, Digest J. Nanomaterials and Biostructures, 5(1): 67-76.
17. Koneman, E.W., S.D. Allen, W.M. Janda and P.C. Schreckenberger, 1997. Colour Atlas and Text Book of Diagnostic Microbiology, 5th ed., Jt. Editor W.C. Winn, Lippincott, Philadelphia, P.A., pp: 185-196.
18. Cleidson, V., M. Simone, F.A.S. Elza and Jr. S. Artur, 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol., 38(2): 369-380.
19. Franziska, W., M. Kurt and R. Max, 1980. A Screening Method for the Estimation of Filter Paper Activity. J. Gen. Micro., 117: 267-269.