SYNTHESIS AND ANTI-MICROBIAL ACTIVITIES OF OXADIAZOLES, THIADIAZOLES AND TRIAZOLES CONTAINING 5-BROMO-3-AMINO BENZOFURAN NUCLEUS FROM 5-BROMOSALICYLONITRILE‡

Parameshwarappa[1] G, Raga[2] B, Omkar Khandre S[1] and Sushila. S. Sangapure[1]*

[1]Department of Post-Graduate Studies and Research in Chemistry, Gulbarga University, Gulbarga-585 106.
[2]Department of Bulk Drugs, KRE Society's, Karnataka college of Pharmacy, Bidar-585 402.
E-mail: sangapure@rediffmail.com

Abstract

5-Bromosalicylonitrile **2** has been prepared from 5-Bromosalicylladehyde **1** and hydroxylamine hydrochloride, which on further treatment with ethyl chloroacetate gave ethyl 5-bromo-3-amino-2-benzofurancarboxylate **4**. The resulting compound **4** was treated with hydrazine hydrate in boiling ethanol gave the hydrazide compound **5**. The resulting hydrazide was reacted with substituted aryl isothiocyanates and offered thiosemicarbazide compounds **6-9**. 5-Bromo-3-amino-2-benzofurothiosemicarbazides underwent cyclization with different reagents under different reaction conditions to furnish benzofuran derivatives possessing oxadiazoles, triazoles and thiadiazoles **10-21** respectively. The structures of all the compounds have been assigned by elemental analysis and spectral studies. The synthesized compounds were screened for their antimicrobial and antifungal activities.

Key words: 5-Bromosalicylonitrile, Oxadiazoles, thiadiazoles, triazoles, antibacterial, antifungal activities.

Introduction

Synthetic benzofuran derivatives have received considerable attention owing to their antimicrobial activity[1,2]. Benzofuran nucleus is widely distributed in natural product, particularly among plant kingdom. Many such compounds have been reported to possess very interesting pharmacological and physiological activities, such as insecticidal, fungicidal, antimicrobial and antioxidant properties[3,4]. The benzofuran ring system itself is a common structural element that appears in a large number of medicinally important compounds[5]. Earlier in our lab the synthesis of pharmacologically potential benzofuran moiety has been condensed with different nitrogen heterocycles were reported[6-9]. The wide range of therapeutic properties reported for 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole compounds[10-11]. As a result such an investigation of several furan derivatives were found to exhibit wide range of biological activities. While searching for more potent compounds than the nature has provided, numerous synthetic analogues are being prepared. This approach seems to be useful in view of the fact that it may combine the physiological action of the group with the well- known biological activity of the compounds containing oxadiazoles, thiadiazoles, thiadiazoles and benzofuran groups. As far as we know this is the first report on these substances.

---

* For Correspondence, e-mail: sangapure@rediffmail.com

‡ Part of this work has been presented in "2nd International Conference on Heterocyclic Chemistry-2006" held at University of Rajasthan, Jaipur, INDIA, during December 16-19, 2006.

Experimental Section:

General procedure: All the reagents were purchased commercially and used with further purification. The melting points were determined on an open capillary method and are uncorrected; IR spectra were recorded on Perkin-Elmer Spectrum ONE FTIR spectrophotometer. ^{1}H NMR spectra were recorded on AMX-400 AV III Solids NMR. The chemical shifts were expressed in the ppm (δ scale) downfield from TMS. Mass spectra were recorded on LCMS-2010A Data Report-Shimadzu and elemental analysis on Flash EA 1112 Series CHNS Report Thermo Finnigan. Silica gel Merck (60-120 mesh) and DC-Alufoline 60 F254 were normally used for column and TLC Chromatography respectively

Synthesis of 5-bromosalicylonitrile 2

5-bromosalicylaldehyde **1** (0.05 mol) was treated with hydroxylamine hydrochloride (0.055 mol) in anhydrous dimethylformamide and refluxed for 20 min. The content was poured into cold water to give solid 5-bromosalicylonitrile **2.**

Synthesis of 5-bromo-2-cyanophenoxyacetate 3

To a solution of compound **2** (0.01 mol) in anhydrous acetone, ethyl chloroacetate (0.01 mol) was added and anhydrous potassium carbonate as a basic catalyst. The reaction mixture was refluxed for 8-10 hrs to furnish the compound **3.**

Synthesis of ethyl-5-bromo-3-amino-2-benzofurancarboxylate 4

Cyclization of compound **3** was carried out with anhydrous dimethylformamide in presence of basic catalyst to give compound **4.**

Synthesis of 5-bromo-3-amino-benzofuran-2-carbohydazide 5

A mixture of ethyl-3-amino-benzofuran-2-carboxylate **4** (0.0079 mol) and hydrazine hydrate (99%, 4 ml) was refluxed in ethanol (6 ml) on a steam bath for 2 hrs. The reaction product was cooled thoroughly and colorless solid separated as compound **5.**

Synthesis of 5-bromo-3-amino-benzofuran-2-thiosemicarbazides 6-9

A suspension of compound **5** (0.006 mol) in alcohol (20 ml) was treated with appropriate amount of alkyl\aryl isothiocyanates (0.006 mol), the reaction mixture was heated under reflux for 2 hrs, on water bath. The product which separated as solid on cooling to room temperature. The solid separated out and was filtered and dried to give the desired compounds **6-9.**

Synthesis of 5-bromo-3-amino- (1,3,4-oxadiazole-2-yl) benzofuran 10-13

To solution of appropriate amount of compounds **6-9** (0.002 mol) in ethanol (60 ml) aqueous NaOH (1.5 ml, 4%) was added. To this a solution of iodine in potassium iodide (aq. 5%) was added in portion with vigorous shaking at room temperature and was heated under reflux for an hour and concentrated under reduced pressure. The oxadiazoles compounds, which separated on dilution with water, was collected and dried to give the desired compounds **10-13.**

Synthesis of 5-bromo-3-amino- (1,3,4-thiadiazole-2-yl) benzofuran 14-17

To a well stirred anhydrous *o*-phosphoric acid (6 ml) with compound **6-9** (0.002 mol) was added in portion during 30 min. The reaction mixture was heated at 110-120^{0}C for 30 min in oil bath. After cooling the reaction mixture was treated with ice-cold water and the product separated was collected, washed with water and dried to give the desired compounds **14-17.**

Synthesis of 5-bromo-3-amino- (1,2,4-triazole-2-yl) benzofuran 18-21

A suspension of compound **6-9** (0.002 mol.) in aqueous NaOH (30 ml, 4%) was heated under gentle reflux for 2-3 hrs. After cooling to room temperature the reaction mixture was filtered. The filtrate was acidified with glacial acetic acid to furnish the compounds **18-21.** Which were collected dried to give the desired compounds **18-21.**

5-bromosalicylonitrile (2). Colorless solid (78.53%), mp 158-159 °C, MS: (M^+) 198; Anal.Calcd for C_7H_4ONBr: C 42.42, H 2.02, N 7.07. Found: C 42.42, H 2.05, N 7.24. IR (KBr cm^{-1}): 2236 (-CN), 3249 (-OH). ^{1}H NMR (δ, DMSO): 6.4 (s, 1H, -OH), 7-8 (m, 3H, Ar-H).

Ethyl-5-bromo-3-amino-2-benzofurancarboxylate (4). Light gray colour solid (76.66%), mp144-146 °C, MS: (M^+) 285; Anal.Calcd for $C_9H_{10}O_3NBr$: C 46.48, H 3.52 and N 4.93. Found: C 46.43, H 3.35, N 4.90. IR (KBr cm^{-1}): 1710 (CO), 3330-3435 (-NH_2). ^1H NMR (δ, DMSO): 1.4 (t, 3H, -CH_3), 4.5 (q, 2H, -CH_2), 7.5 (s, 2H, -NH_2), 7-8 (m, 3H, Ar-H).

5-bromo-3-amino-benzofuran-2-carbohydazide (5). Colorless solid (74.15%), mp238-241 °C, Anal.Calcd for $C_9H_8O_2N_3Br$: C 40.00, H 2.96, and N 15.55. Found: C 40.08, H 2.90, N 15.48. IR (KBr cm^{-1}): 3330-3435 (-NH_2). ^1H NMR (δ, DMSO): 9.2(s, 1H, -NH), 7.3-8.1 (m, 3H, Ar-H).

5-bromo-3-amino-benzofuran-2-thiosemicarbazides (6). Colorless solid (71.22%), mp210-211 °C, Anal.Calcd for $C_{16}H_{13}O_2N_4SBr$: C 47.41, H 3.21, and N 13.83. Found: C 47.40, H 3.28, N 13.76. IR (KBr cm^{-1}): 1676 (-CO), 3330-3405 (-NH).

5-bromo-3-amino-benzofuran-2-thiosemicarbazides (7). Pale yellow solid (70%), solid (71.22%), mp200-202 °C, Anal.Calcd for $C_{17}H_{15}O_2N_4SBr$: C 48.69, H 3.58, and N 13.37. Found: C 48.59, H 3.55, N 13.46. IR (KBr cm^{-1}): 1663 (-CO), 3283 (-NH_2)

5-bromo-3-amino-benzofuran-2-thiosemicarbazides (8). White colour solid (69.02%), mp 213-215 °C, Anal.Calcd for $C_{16}H_{12}O_2N_4SClBr$: C 43.69, H 2.73, and N 12.74. Found: C 43.59, H 2.70, N 12.61 IR (KBr cm^{-1}): 1676 (-CO), 3330-3405 (-NH_2).

5-bromo-3-amino-benzofuran-2-thiosemicarbazides (9). White colour solid (73%), mp 216-220 °C, Anal.Calcd for $C_{16}H_{12}O_2N_4SBr_2$: C 39.67, H 2.48, and N 11.57. Found: C 39.58, H 2.51, N 11.51 IR (KBr cm^{-1}): 1716 (-CO), 3277 (-NH_2).

5-(3-amino-5-bromobenzofuran–2-yl)-*N*-phenyl-1,3,4-oxadiazole-2-amine (10). White solid (75.01%), mp 220-222 °C, MS: (M^+) 370; Anal.Calcd for $C_{16}H_{11}O_2N_4Br$: C 51.89, H 2.70, and N 15.13. Found: C 51.48, H 2.72, N 15.22 IR (KBr cm^{-1}): 3359, 3278 (-NH_2). ^1H NMR (δ): 5.98 (s, 2H, -NH_2), 9.37 (s, 1H, NH). 6.97-8.04 (m, Ar-H).

5-(3-amino-5-bromobenzofuran–2-yl)-*N*-p-tolyl-1,3,4-oxadiazole-2-amine (11). Pale yellow solid (64.21%) mp 240(d) °C, Anal.Calcd for $C_{17}H_{13}O_2N_4Br$: C 52.99, H 3.38, and N 14.55. Found: C 52.88, H 3.322, N 14.49 IR (KBr cm^{-1}): 3319, (-NH_2). ^1H NMR (δ): 5.88 (s, 2H, -NH_2), 9.44 (s, 1H, NH). 2.2 (s, 3H, CH_3) 6.97-8.04 (m, Ar-H).

5-(3-amino-5-bromobenzofuran–2-yl)-*N*-p-chloro-1,3,4-oxadiazole-2-amine (12). Yellow solid (62.84%) mp 270(d) °C, Anal.Calcd for $C_{16}H_{10}O_2N_4ClBr$: C 47.35, H 2.47, and N 13.81. Found: C 47.30, H 2.52, N 13.84 IR (KBr cm^{-1}): 3326, (-NH_2).

5-(3-amino-5-bromobenzofuran–2-yl)-*N*-p-chloro-1,3,4-oxadiazole-2-amine (13). Brown solid (67%) mp 1400(d) °C, Anal.Calcd for $C_{16}H_{10}O_2N_4Br_2$: C 42.67, H 2.44, and N 12.44. Found: C 42.48, H 2.52, N 12.49 IR (KBr cm^{-1}): 3301, (-NH_2).

5-(3-amino-5-bromobenzofuran-2-yl)-*N*-phenyl-1,3,4-thiadiazol-2-amine (14). Yellow solid (61.00%) mp 218-220 °C, MS: (M^+) 387; Anal.Calcd for $C_{16}H_{11}ON_4SBr$: C 49.61, H 2.84, and N 14.47. Found: C 49.66, H 2.85, N 14.42 IR (KBr cm^{-1}): 3241, (-NH_2). ^1H NMR (δ): 6.22 (s, 2H, -NH_2), 9.61 (s, 1H, NH). 7.1-8.1 (m, Ar-H).

5-(3-amino-5-bromobenzofuran-2-yl)-*N*-p-tolyl-1,3,4-thiadiazol-2-amine (15). Yellow solid (65.11%) mp 209-213 °C, Anal.Calcd for $C_{17}H_{13}ON_4SBr$: C 50.87, H 3.24, and N 13.97. Found: C 50.82, H 3.26, N 13.91 IR (KBr cm^{-1}): 3251, (-NH_2). ^1H NMR (δ): 2.2 (s, 3H, CH_3), 10 (s, 1H, NH). 7.1-8.1 (m, Ar-H).

5-(3-amino-5-bromobenzofuran-2-yl)-4-phenyl-4H-1,2,4-trizole-3-thiol (18).

Yellow solid (71%) mp. 255 °C (d), Anal.Calcd for $C_{16}H_{11}N_4SBr$: C 49.48, H 2.84, and N 14.43. Found: C 49.44, H 2.86, N 14.41 IR (KBr cm^{-1}): 3368, (-NH_2). ^1H NMR (δ): 5.84 (s, 2H, NH_2), 14.1 (s, 1H, SH). 6.9-8.1 (m, Ar-H).

5-(3-amino-5-bromobenzofuran-2-yl)-4-(6bromohexa-1,3,5-triynyl)-4H-1,2,4-trizole-3-thiol (21). Yellow solid (70%) mp.300 °C (d), Anal.Calcd for $C_{16}H_{10}ON_4SBr_2$: C 41.20, H 2.15, and N 12.02. Found: C 41.27, H 2.19, N 12.08 IR (KBr cm^{-1}): 3363, (-NH_2). ^1H NMR (δ): 5.81 (s, 2H, NH_2), 13.8 (s, 1H, SH). 6.9-8.1 (m, Ar-H).

Result and Discussion

5-Bromosalicylonitrile **2** was required in the present work has been obtained by a single step method by the reaction of 5-bromosalicylaldehyde **1** with hydroxylamine hydrochloride in dimethylformamide. The conversion of aldehydes into the corresponding nitriles is an important functional group transformation due to the extensive utilities of nitrile compounds in synthetic chemistry[12]. Among numerous methods developed for this purpose[13-15], those based on the synthesis of 5-bromosalicylonitrile **2** is the most widely employed one. In the present study by using very simple method, we synthesized the compound 5-bromosalicylonitrile **2**, by above synthetic route was reporting first time as per our knowledge from the literature survey. The identity of the product was determined by IR and ^{1}H NMR, Mass spectral and elemental analysis studies. The IR spectrum of compound **2** revealed a sharp strong absorption band above 2236 cm^{-1} due to the presence of the nitrile function in the structure. The ^{1}H NMR spectra substantiated the results of the IR analysis. The characteristic signals of a nitrile moiety confirm the presence of singlet at 6.4 δ for –OH and multiplet at δ 7-8 ppm for aromatic protons. Also, its mass spectra revealed a molecular ion peak at m/Z 198 (M^+) corresponding to the molecular formula C_7H_4ONBr. The elemental analysis of the compound **2** shows C- 42.42% (found), (calculated 42.42%), H-2.05% (found), (calculated 2.02%) and N-7.24% (found), (calculated 7.07%) these values also corresponds to the molecular formula C_7H_4ONBr and helps to elucidate the structure of the 5-bromosalicylonitrile **2**. The compound **4** was prepared from 5-bromosalicylonitrile **2** on reacting with ethyl chloroacetate in anhydrous potassium carbonate and dry acetone in acceptable yield. The structure of compound **4** was confirmed by IR, NMR and mass spectral analysis. The IR spectrum of compound **4** revealed a sharp strong absorption band above 1710 cm^{-1} and 3330 cm^{-1} due to presence of the ester amine function in the structure. The ^{1}H NMR characteristic signals of an ester moiety confirm the presence of ester group in the structure by resonating as quartet and triplet for CH_2 and CH_3 at δ 4.5 and 1.4 ppm respectively and one singlet at δ 7.5 ppm for $–NH_2$ group present in the structure. The aromatic protons resonate as multiplet at δ 7–8 ppm. Also its mass spectra revealed a molecular ion peak at 285 (M^+) corresponding to the molecular formula $C_{11}H_{10}O_3NBr$. Thus compound **4** was reacted with hydrazine hydrate in ethanol at reflux temperature to obtain 5-bromo-3-amino-benzofuran-2-carbohydrazide **5** in good yields. The structure of **5** was confirmed by IR, NMR spectral analysis. The IR spectrum of **5** showed the absence of ester stretching frequency, instead it gave a band at 1667 cm^{-1} for carbonyl group and showing two sharp bands in the 3300-3400 cm^{-1} for –NH frequencies. The ^{1}H NMR spectrum of compound **5** exhibited no peak corresponds to ester instead it shows signals at δ 9.2 and 5.9 ppm for –NH and $–NH_2$ of hydrazide respectively.

To prepared 5-bromo-3-amino-benzofuran-2-thiosemicarbazides **6-9**, the compound **5** was treated with various aryl isothiocyanates in ethanol at reflux temperature. Finally the resulting compounds **6-9** were reacted with different reagents with different reaction conditions like NaOH/KI, H_3PO_4 and NaOH respectively to give compounds **10-13, 14-17** and **18-21** respectively. The structural analysis of the newly synthesized compounds 10-21 were confirmed by spectral analysis. The IR spectra of compound **10** revealed 3359 and 3278 cm^{-1} corresponds to –amine group present in compound. Further, ^{1}H NMR spectrum exhibits singlet at δ 5.98 ppm for $-NH_2$ proton, another singlet at δ 9.37 ppm for –NH group and aromatic protons shows a multiplet at δ 7.0-8.04 ppm. Also its mass spectra revealed a molecular ion peak at 370 (M^+) corresponding to the molecular formula $C_{16}H_{10}O_2N_4Br$. The IR and NMR spectrum of compound **14** shows a 3241cm^{-1} for $–NH_2$ function and ^{1}H NMR shows a singlet at δ 6.22 and 9.61 ppm for $–NH_2$ and –NH respectively and one multiplet exhibit at δ 7.1-8.8 ppm for aromatic protons. Also its mass spectra revealed a molecular ion peak at 387 (M^+) corresponding to the molecular formula $C_{16}H_{11}ON_4SBr$. The IR and ^{1}H NMR spectral data's of compound **18** shows a sharp absorption peak at 3368 cm^{-1} for $–NH_2$ function, and two singlet at δ 5.84 ppm for $-NH_2$ and δ 14.1 ppm it shows a –SH function present in the molecule and multiplet occurs at δ 6.9-8.1 ppm for aromatic protons. Similarly, all these compounds were purified by column chromatography and characterized on the basis of spectral studies. The spectral details of all the synthesized compounds are given and results are in agreement with the assigned structures.

Evaluation of anti-microbial activity

Antibacterial and antifungal activity

The *in vitro* antimicrobial activity was carried out against 24 hr old cultures of three bacteria and three fungi by cup-plate method[16]. Compounds **2-20** has been tested for their antimicrobial activity against *S. aureus*, *B. subtilis* and *E. coli* and antifungal activity against *A. niger* and *A. fumigatus* at a concentration of 1000 µg/ml in distilled DMF using cup plate diffusion method. Nutrient agar and potato dextrose agars were used to culture the bacteria and fungus respectively. The solution of Gentamycin 1000 µg/ml and Flucanazole 1000 µg/ml were prepared in sterilized water and used as standards for comparison of antibacterial and antifungal activities respectively.

The compounds **4**, **7**, **9**, **19**, and **20** exhibiting good activity against *S. aureus* and compounds **4**, **7**, **10**,**11** and **20** showing good activity against *E.coli*, and compounds **3**, **5**, **7**, **8** and **18** showing good activity against *B. subtilis*. All remaining compounds exhibited moderate activity against all the organisms used for screening.

The compounds **4**, **6** and **20** exhibited excellent activity against *A. niger* and compounds **10** and **11** exhibiting good activity against *A. fumigatus*. All remaining compounds exhibiting moderate activity against both the organisms used for screening.

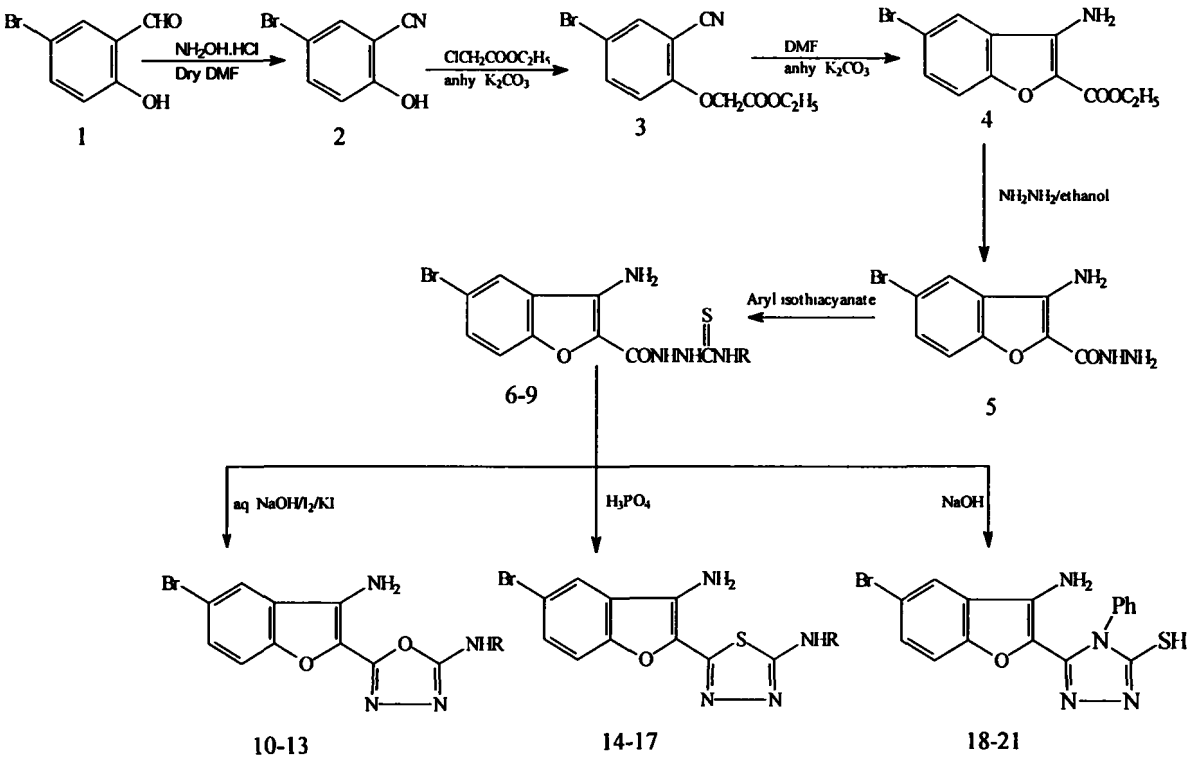
Table 1. Antimicrobial Activities of Synthesized Compounds

Compounds	Zone of inhibition in mm				
	Antibacterial			Antifungal	
	S. aureus	*B. subtilis*	*E. coli.*	*A. niger*	*A. fumigatus*
2	06	07	06	07	06
3	06	08	06	06	05
4	15	07	07	09	06
5	05	09	03	05	04
6	06	08	04	11	06
7	12	14	07	08	08
8	06	10	06	05	07
9	07	04	05	10	05
10	04	03	08	09	10
11	05	07	08	04	10
12	04	04	05	04	05
13	05	06	06	07	04
14	04	04	05	06	03
15	05	07	04	03	05
16	05	05	03	05	07
17	06	04	04	05	08
18	04	08	04	06	07
19	07	05	05	05	06
20	10	06	06	12	05
Std.	07	12	10	14	12
Control	00	00	00	00	00

Standard: *Gentamycin*, (for antibacterial.)

Standard: *Flucanazole*, (for antifungal.)

Control: *DMF*.



R = C_6H_5
R = $C_6H_4CH_3$
R = C_6H_4Cl
R = C_6H_4Br

Scheme 1

References

1. Masubuchi, M.; Kawasaki, K.; Ebiike, H.; Ikeda, Y.; Tsujii, S.; Sogabe, S.; Fujii, T.; Sakata, K.; Shiratori, Y.; Aoki, Y.; Ohtsuka, T.; shimma, N. *Bioorg. Med. Chem. Lett.*, **11**, 1833. (2001)
2. Murat Koca, Misir Ahmedzade, Alaaddin Cukurovali and cavit kazaz. *Molecules.*, **10**, 747.(2005)
3. Dean, *"Naturally Occurring Ring Compounds"* Butterworthe and Co., London, (1963).
4. Ward, R.S. Lignans, Neolignans and Related Compounds. *Nat. Prod. Rep.*, **16**, 75. (1999)
5. Yoo, S.E.; Lee, S.H.; Kim, S.K.; Lee, S.H. *Bioorgn. Med. Chem.* **5**,445. (1997)
6. Vaidya V P and Agasimundin Y S., *Indian J Chem,* **20B**, 780. (1981)
7. Raga Basawaraj and S S Sangapure, *Indian J Heterocyclic Chem.* **15**, 153. (2005)
8. Raga Basawaraj, G. Parameshwarappa and S.S.Sangapure. *Indian J.Hetrocyclic Chem.*Vol.**16**, 75. (2006)
9. Gulhan Turan Zitouni, Zafer Asim Kaplancikli and Demet Kaya, *European J. Med. Chem.*, **40**, 607. (2005).
10. Anil K Sengupta and Anuraga Ateet Gupta, *Indian J Chem.*, **22B**, 263. (1983)

11. Basawaraj K M and Agasimundin Y S., *Indian J Chem.*, **22B**, 458. (1983).
12. Friedrich, K.; Wallensfels, K. *The Chemistry of the Cyano Group*; Rappoport, Z.; Ed.; Wiley- Interscience: New York, **1970.**
13. Coskun, N.; Arikan, N. *Tetrahedron* **55**, 11943. (1999).
14. Boruah, M.; Knowar, D. *J.Org.Chem.*, **67**, 7138. (2002).
15. Jia-Liang Zhu, Fa-Yen Lee, Jen-Dar Wu, Chun-Wei Kuo, Kak-Shan Shia. *Letter.*, 1317.(2007).
16. Saundane, A. R.; Rudresh, K.; Satyanarayan, N. D.; Hiremath, S. P. *Indian. J. Pharm. Sci.*, **60**, 379. (1998).

Received on December 10,2008.