INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL ACTIVITY OF SOME NOVEL AZETIDINONES FROM NAPHTHO [2,1-b]THIOPHENE

K. Veena[1], M. Ramaiah[2*] and VP. Vaidya[3]

[1]Department of Chemistry, Maharani's Science College for Women, Bangalore- 560 001, Karnataka, India.
[2]Department of Chemistry, NMKRV College for Women, Bangalore- 560 011, Karnataka, India.
[3]Department of Chemistry, Kuvempu University, Shankaraghatta- 577 451, Shimoga, Karnataka, India.

ABSTRACT

The reaction of ethyl naphtho[2,1-b]thiophene-2- carboxylate **2** with hydrazine hydrate produced naphtho[2,1-b]thiophene-2-carbohydrazide **3** which upon treatment with various aromatic aldehydes produced Schiff bases i.e. N-(aryl-methylene) substitutednaphtho[2,1-b]thiophene-2-carbohydrazides **4a-f** which served as an excellent intermediate for the synthesis of titled compounds. The Schiff bases **4a-f** readily underwent cyclisation upon reaction with chloroacetyl chloride in presence of triethyl amine to yielded azetidinones **5a-f.** The Structures of the newly synthesized compounds have been established by analytical and spectral studies. All the synthesized compounds were evaluated for antibacterial, antifungal and antioxidant activities.

Keywords: Azetidinone, naphtho [2, 1-b]thiophene, antimicrobial activity, antioxidant activity.

INTRODUCTION

A large number of medicinal compounds which have been discovered belong to a major class of heterocycles containing nitrogen and sulphur. The numerous synthetic applicability and biological activity of these heterocycles has helped medicinal chemist to implement new approach towards the discovery of novel drugs.

These heterocyclic compounds hold a special place among pharmaceutically significant natural products and synthetic compounds. Thiophene based compounds have been a constant matter of investigation due to their wide range of applications[1]. Thiophene can be fused with various heterocyclic systems giving rise to novel series of compounds with enhanced biological activity[2-4]. They are the precursors of many drugs with high therapeutically potential, being used in the treatment of cancers, osteoporosis, hypertension, more specifically thiophene derivatives have shown insecticide activity, antibacterial, antiviral, and antioxidant activity.

It has been demonstrated that thiophene containing β-lactam antibiotics like Cefoxitin, Cephalothin and Cephalorodine exhibit good antibacterial activity. The β-lactam heterocycles are the most prescribed antibiotics used in medicine[5]. They are considered as an important contribution of science to humanity. Hence, with a view to further assess biologically important classes of organic compounds, due to their relevance in both clinical and economic fields, it was thought worthwhile to synthesize some new β-lactam heterocycles by incorporating the naphtho[2,1-b]thiophene nucleus and azetidinone moieties in a single molecular framework. The present work deals with the synthesis of the title compounds starting from ethyl naphtho[2,1-b]thiophene-2-carboxylate, and evaluating them for antimicrobial and antioxidant activities.

MATERIALS AND METHODS

Melting points were recorded in open capillary tube method and are uncorrected. IR Spectra (in KBr pellets) were recorded on Shimadzu FTIR Spectrophotometer. The NMR spectra were recorded on Bruker 300MHz Spectrometer , standard chemical shifts are given in δ ppm values. The ompounds were checked for their purity by TLC. The newly synthesized compounds were separated and purified by column chromatography using silica gel (60-120 mesh).

EXPERIMENTAL

Synthesis of ethyl naphtho[2,1-b]thiophene-2- carboxylate 2

A mixture of ethyl naphtho[2,1-b]furan-2-carboxylate 1 (2.4 g,0.01mol) and Lawesson's reagent (4.02 g,0.01 mol) and toluene (20 ml) was heated under reflux for 10 h. After the completion of the reaction the solvent was evaporated to obtain the product. The solid thus obtained was recrystallised from ethanol.

Synthesis of naphtho[2,1-b]thiophene-2-carbohydrazide 3

A mixture of ethyl naphtho[2,1-b]thiophene-2-carboxylate 2 (2.55 g, 0.01 mol) and hydrazine hydrate (2.5 ml, 99%) in ethanol (10 ml) were stirred on a magnetic stirrer for 3 h at room temperature and the solid thus separated was filtered, washed with ethanol and recrystallised from aqueous DMF.

Synthesis of N-(aryl-methylene) substituted naphtho[2,1-b]thiophene-2-carbohydrazides 4a-f

To a solution of naphtho[2,1-b]thiophene-2-carbohydrazide 3 (0.42 g, 0.001 mol) in DMF (50 ml), 4-methoxy benzaldehyde (1.2ml,0.01 mol) was added, the mixture was refluxed on for 5 h, and then poured into ice-cold water. The product 4b that separated was collected, and recrystallised from aqueous DMF.

Similarly compounds 4a and 4c-f were synthesized using appropriate aromatic aldehydes.

Synthesis of N-(3-chloro-2-oxo-substituted-phenyl-azetidine-1-yl)naphtho[2,1-b]thiophene-2-carboxamides 5a-f.

A solution of chloroacetyl chloride (1.2 ml, 0.01 mol) in dioxane (10ml) was cooled using ice-salt bath and kept for stirring. To this, triethyl amine (1.0 ml) was added drop wise maintaining the temperature below 0^0C, white solid separated out. To this reaction mixture solution of N-(4-methoxybenzylidene)naphtho[2,1-b]thiophene-2-carbohydrazide 4b (3.6 g,0.01 mol) in dioxane (10 ml) was added drop wise regulating the temperature less than 0^0C with stirring. After the addition was over the reaction mixture was refluxed for 16 h. The reaction mixture was poured into ice cold water. The product 5b thus obtained as a solid was filtered and dried. The product was recrystallised from dioxane.

The compounds 5a and 5c-f were synthesized from 4a and 4c-f respectively by the similar method.

The physical data of the newly synthesized compounds are tabulated in Table -1.

Evaluation of Biological activities

Naphthothiophenes in association with other heterocyclic molecules exhibit wide spectrum of biological and pharmacological activities. There are numerous examples where in thiophene and azetidinone derivatives being used for the treatment of various diseases. This fact stimulated us to take up the biological evaluation of newly synthesized derivatives.

Antimicrobial Screening

The synthesized compounds were subjected to antimicrobial screening by adopting the procedure available in the literature[6-9].

The antibacterial screening of both the series **4a-f** and **5a-f** have been carried out against *Staphylococcus aureus* , *Streptococcus fecalis, Escherichia coli Pseudomonas aeruginosa*, and antifungal activity against *Candida albicans and Aspergillus fumigatus by* disc diffusion method. Streptomycin and ciclopiroxcalamine were used as standard drugs for antibacterial and antifungal activity respectively. The zone of inhibition was compared with the standard drug after 18 h of incubation at 25^0 C for antibacterial activity and after 48h at 30^0 C for antifungal activity. The results are tabulated in Table - 2.

Antioxidant activity by DPPH method[10]

Different concentrations (10 µg, 50 µg and 100 µg) of samples 4a-f and 5a-f in dimethyl sulphoxide, andbButylated hydroxy anisole (BHA) were taken in different test tubes. The volume was adjusted to 500µl by adding methanol. Five milliliters of 0.1 mM methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at room temperature for 20 min. The absorbance of the samples was measured at 517 nm.

Radical scavenging activity was calculated using the following formula

% Free radical scavenging activity =

$$\frac{\text{(Control OD - Sample OD) x 100}}{\text{Control OD}}$$

The results of antioxidant activity by DPPH method are tabulated in Table - 3 and the comparison of free radical scavenging activity of the samples with that of butylated hydroxy anisole (BHA) is depicted in the Fig. 1.

RESULT AND DISCUSSION

Ethyl naphtho[2,1-b]furan-2-carboxylate **1** which is the starting material required for the synthesis of novel azetidinones was synthesised by the reaction of 2-hydroxy-1-naphthaldehyde with ethyl chloroacetate in presence of a weak base anhydrous potassium carbonate in dimethylformamide. Thionation of 1 using Lawesson's reagent[11-12] produced mixture of three compounds. These compounds were separated by column chromatography and the required compound ethyl naphtho[2,1-b]thiophene-2-carboxylate 2 was collected and the structure assigned to this compound was confirmed by its spectral data. The IR(KBr) spectrum of 2 exhibited a strong absorption band at 1727 cm^{-1} due to ester carbonyl group. The 300 MHz ^1H NMR spectrum of 2 exhibited a quartet at δ 4.45 and a triplet at δ 1.6 indicating the presence of ester group. A mutliplet which appeared at δ 7.4-8.5 was attributed to seven aromatic protons. The structure assigned was further supported by its mass spectrum which showed a molecular ion peak at m/z 256 corresponding to its molecular weight. The intermediate naphtho[2,1-b]thiophene-2-carbohydrazide 3 was obtained by the condensation of 2 with 99% hydrazine hydrate in ethanol medium at room temperature.

The IR spectrum of 3 exhibited characteristic absorption bands at 3206 cm^{-1} and 3280 cm^{-1} due to NH_2 group and 1665 cm^{-1} due to carbonyl group. The ^1H NMR spectrum displayed a D_2O exchangeable singlet at δ10.5 for NH_2 protons and a multiplet at δ 7.2-8.2 for aromatic and NCH protons. The reaction of 3 with various aromatic aldehydes produced N-(aryl-methylene) substitutednaphtho[2,1-b] thiophene-2-carbohydrazides 4a-f in good yield.

The IR spectrum of 4b exhibited absorption bands at 3474 cm^{-1} and at 1677 cm^{-1} due to NH and C=O stretching frequencies respectively. The ^1H NMR spectrum of 4b showed a singlet at δ 3.58 due to methoxy protons, a multiplet at δ 6.8 - 7.7 integrating for nine aromatic protons and a multiplet at δ 7.9 to 8.0 integrating for three protons was observed which is attributed to two aromatic and one NCH proton. The CONH proton appeared as a D_2O exchangeable singlet at δ 10.4. The spectral data of the compounds 4a and 4c-f is presented in Table-4.

The compounds 4a-f readily underwent cyclisation upon treatment with chloro acetyl chloride in the presence of triethyl amine using dioxane as a solvent resulting in the formation of title compounds N-(3-chloro-2-aryl-4-oxo-azetidine-1-yl)naphtho[2,1-b] thiophene-2-carboxamides 5a-f .The structure of 5b was confirmed by elemental analysis and spectral studies. The IR spectrum of 5b exhibited sharp absorption bands at 1687 cm^{-1} due to amide carbonyl group and at 1646 cm^{-1} due to carbonyl group of β-lactam ring. The broad NH absorption band appeared at 3430 cm^{-1}. ^1H NMR spectrum of 5b exhibited a singlet for three protons at δ 3.45 due to methoxy group. A doublet at δ 6.55 integrating for two protons is assigned to C-5 and C-6 protons of the naphthofuran ring. A multiplet between δ 7.15-7.69 integrating for seven protons is due to C-3, C-4 and C-7 protons of the naphthofuran ring and four aromatic protons of the methoxy substituted phenyl group and a multiplet between δ 7.90-7.95 integrating for two protons is assigned to C-8 and C-9 protons of the naphthofuran ring. A singlet at δ 5.0 and at δ 5.50 was due to proton of CHPh and proton of CHCl. The CONH proton appeared as a singlet, down field at δ 9.95 (D_2O exchangeable).The mass spectrum of 5b showed a molecular ion peak at m/z 436 (M^+) and at 438 (M+2) corresponding to its molecular weight. The IR and ^1H NMR spectral data of compounds 5a and 5c-f are summarized in the Table - 5.

The newly synthesized compounds have been evaluated for antimicrobial activity by disc diffusion method. The compounds 4a, 4c, 4d, 4e and 4f exhibited excellent activity against *Staphylococcus aureus*, *Staphylococcus fecalis* and *Candida albicans*, The compound 5a exhibited better activity against *Pseudomonas aeruginosa* and *Aspergillus fumigates.* The compound 5d exhibited promising activity against *Pseudomonas aeruginosa.* The compounds 5b, 5c and 5e showed better activity against *Staphylococcus fecalis*. The remaining compounds were found to have mild activity against the tested organism.Some of the compounds were found to be inactive.

Antioxidant activity results revealed that compounds 4c, 4d and 4e exhibited good antioxidant activity compared to standard BHA. Other derivatives exhibited mild to moderate activity.

Table 1: Analytical and physical data of the synthesized compounds

Comp	Molecular formula	Yield %	mp⁰ C	Found (Calcd) %		
				C	H	N
4a	$C_{20}H_{14}N_2OS$	70	220	72.7 (72.8)	4.3 (4.4)	8.4 (8.5)
4b	$C_{21}H_{16}N_2O_2S$	75	210	69.2 (69.4)	4.4 (4.5)	7.1 (7.3)
4c	$C_{20}H_{13}N_3O_3S$	63	200	64.1 (64.2)	3.7 (3.8)	11.0 (11.1)
4d	$C_{20}H_{13}N_2OSCl$	68	180	66.6 (66.8)	3.6 (3.8)	7.7 (7.8)
4e	$C_{22}H_{16}N_2OS$	60	215	74.4 (74.5)	4.4 (4.6)	7.9 (8.0)
4f	$C_{18}H_{12}N_2O_2S$	58	185	67.8 (67.9)	3.8 (3.9)	8.7 (8.8)
5a	$C_{22}H_{15}ClN_2O_2S$	62	210	64.9 (65.0)	3.7 (3.8)	6.8 (6.9)
5b	$C_{23}H_{17}ClN_2O_3S$	71	140	63.4 (63.5)	3.4 (3.5)	6.5 (6.6)
5c	$C_{22}H_{14}ClN_3O_4S$	62	133	58.3 (58.4)	3.2 (3.3)	9.2 (9.3)
5d	$C_{22}H_{14}Cl_2N_2OS$	64	125	59.1 (59.3)	3.2 (3.3)	6.5 (6.6)
5e	$C_{25}H_{17}ClN_2O_2S$	63	190	67.7 (67.8)	3.8 (3.9)	6.2 (6.3)
5f	$C_{21}H_{13}ClN_2O_3S$	52	160	61.8 (61.9)	3.2 (3.3)	6.8 (6.9)

Table 2: Antimicrobial activity data of the synthesized compounds

Comp	Zone of inhibition in mm					
	Antibacterial activity				Antifungal activity	
	S. aureus	*S. fecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. fumigatus*
4a	15	14	13	14	14	11
4b	12	11	9	13	13	13
4c	13	12	13	16	12	14
4d	15	15	14	15	14	12
4e	16	10	13	11	12	15
4f	15	13	12	10	13	12
5a	12	12	-	11	-	16
5b	6	11	-	8	-	-
5c	6	12	-	10	-	-
5d	7	10	-	14	-	-
5e	13	12	-	7	-	-
5f	4	6	-	5	-	-
Streptomycin	18	16	19	19	-	-
Ciclopiroxcalamine	-	-	-	-	21	19

Table 3: Antioxidant activity of compounds 4a-f and 5a-f

Comp	% Free radical scavenging activity		
	Concentration		
	10 µg	50 µg	100 µg
4a	16.2	22.4	24.8
4b	17.0	21.4	32.5
4c	14.1	28.4	44.5
4d	20.4	30.6	50.4
4e	19.8	32.8	52.0
4f	20.5	23.0	38.4
5a	4.76	5.06	6.28
5b	1.42	4.55	8.91
5c	4.15	7.59	11.54
5d	4.66	6.98	12.25
5e	4.45	7.59	12.85
5f	4.86	5.97	7.19
Standard (BHA)	23.68	48.89	70.45

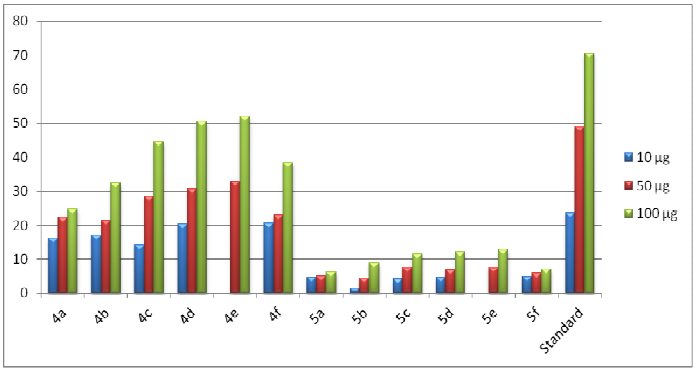
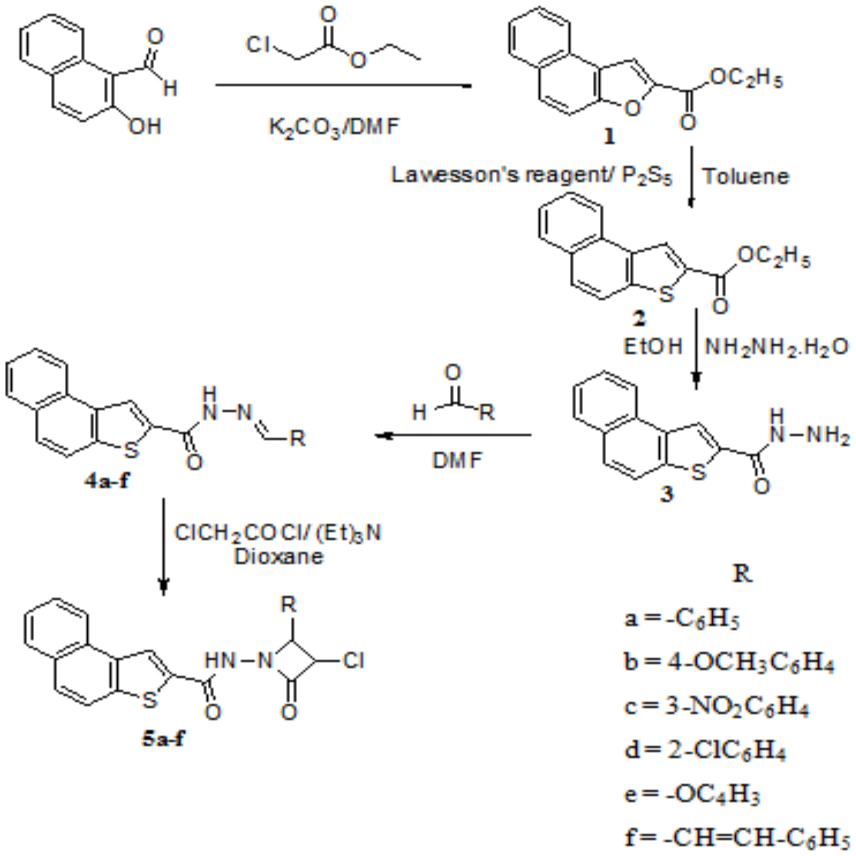


Fig. 1: Comparison of Free radical scavenging activity of samples 4a-f and 5a-f with that of Butylated hydroxy anisole (BHA)



Scheme

Table 4: IR and ^{1}H NMR Spectral data of the compounds 4a and 4c-f

Comp	R	IR (KBr) cm^{-1}	IR (KBr) cm^{-1}	^{1}H NMR δ in ppm
		C=N	C=O	
4a	H	1631	1677	7.2-7.8 (m, 12H, ArH) 8.2 (s,1H, NCH) 10.5 (s, 1H, CONH)
4c	3-NO_2	1610	1655	7.3-2.5 (m, 11H, ArH) 8.6 (s, 1H, NCH), 11.0 (s, 1H, CONH)
4d	2-Cl	1618	1680	7.2-7.9 (m, 11H, ArH) 8.1 (s, 1H, NCH) 11.1 (s, 1H, CONH)
4e	-OC_4H_3	1612	1682	6.2-7.8 (m, 14H, ArH) 7.9 (s, 1H, NCH) 10.1 (s, 1H, CONH)
4f	-CH=CH-C_6H_5	1611	1685	7.2-8.1 (m, 10H, ArH,) 8.4 (s, 1H, NCH) 10.5 (s, 1H, CONH)

Table 5: IR and ^{1}H NMR Spectral data of the compounds 5a and 5c-f

Comp	R	IR (KBr) cm^{-1}	IR (KBr) cm^{-1}	^{1}H NMR δ in ppm
		amide	keto	
5a	H	1582	1672	5.0 (s, 1H, CHPh), 5.4 (d, 1H CHCl), 7.1-7.9 (m, 12H, ArH), 10.2 (s, 1H, CONH)
5c	3-NO_2	1690	1650	5.1 (s, 1H, CHPh) 5.4 (d, 1H, CHCl) 7.3-7.9 (m, 11H, ArH), 8.1 (s, 1H, CONH)
5d	2-Cl	1692	1653	5.0 (s, 1H, CHPh) 5.5 (d, 1H, CHCl), 6.8-7.8 (m, 11H, ArH) 9.2 (s, 1H, CONH)
5e	-OC_4H_3	1675	1680	5.1 (s, 1H, CHPh) 6.0(d, 1H, CHCl) 6.2-7.8 (m, 14H, ArH), 11.1 (s, 1H, CONH)
5f	-CH=CH-C_6H_5	1680	1654	5.0 (s, 1H, CHPh) 5.8 (s, 1H, CHCl) 6.6-7.8 (m, 10H, ArH) 9.0 (s, 1H, CONH)

CONCLUSION

All the synthesized compounds were purified by column chromatography. The synthesized compounds were subjected to spectral analysis such as IR, ^{1}H NMR and Mass spectra to confirm the structure. All the analytical details show satisfactory results.

Many of the synthesized compounds showed good, mild and moderate antimicrobial activity. It is interesting to note that among the synthesized compounds 4a-f series showed better activity compared to 5a-f series. Investigation of the antimicrobial activity revealed that activities are independent of electron withdrawing and electron releasing groups in the newly synthesized compounds.

ACKNOWLEDGMENT

We thank the Principal, NMKRV College for women, Bangalore, for providing laboratory facilities. The authors are also thankful to Principal, SCS College of pharmacy, Harapanahalli, for helping in carrying out biological activities and IISc Bangalore for spectral analysis.

REFERENCES

1. Yoshinori Tominaga, Ram Pratap, N Raymond. Castle, Milton and L. Lee. The synthesis of the monomethyl isomers of benzo[b]naphtho[2,1-d]thiophene. J Heterocyclic Chem. 1982;19(4):859-863.

2. Patel KH and Mehta AG. Synthesis and antifungal activity of azetidinone and Thiazolidinones derivatives of 2-amino-6-(2-naphthalenyl) thiazolo [3, 2-d] thiadiazole. E-J Chem. 2006;13(3):267-273.
3. Ishwar Bhat K, Sunil Chaithanya K and Satyanarayana PD. The synthesis and antimicrobial study of some azetidinone derivatives with the para-anisidine moiety. J Serb Chem Society. 2007;72(5):437-442.
4. Das BP. Alpha-(Alkylaminomethyl)-4-naphtho[2,1-b]thiophenemethanols as antimalarials. J Med Chem. 1972;15(4):370-374.
5. Shashikala devi K, Ramaiah M, Roopa DL and Vaidya VP. Synthesis and Investigation of Antimicrobial and Antioxidant Activity of 3-Nitro-N- (3-chloro-2-oxo-substituted-phenyl-azetidin-1-yl)naphtho [2,1-b]furan-2-carboxamides. E- J Chem. 2010;7(S1):S358-S362.
6. Robbert D and Smyth. Clinical analysis, Microbiology, Remington's Pharmaceutical sciences, 18th ed. Mack Publishing company, Peninisilvenia. 1991;524-527.
7. Shastry CS, Joshi SD, Aravind MB and Veerapur VP. Synthesis and biological activity of substituted benzothiazoles. Indian J Heterocyclic Chem. 2003;13:57-60.
8. Jayachandran E, Nargund LVG, Shivakumar S and Kamal Bhatia. Synthesis of Bioactive molecules fluro substituted Benzothiazole Comparing potent Heterocyclic moieties for Biological and Pharmacological screening. Oriental J Chem. 2003;19(1):139-142.
9. Gurupadaiah BM, Jayachandran E, Shivakumar B, Nagappaand AN and Nargund LVG. Synthesis of fluro substituted benzothiazoles for biological and pharmacological screening. Indian. J Heterocyclic Chem. 1998;7:213-216.
10. Brand-Williams W, Cuvelier ME and Berset C. New parameter for evaluation of free radical scavenging capacity of polyphenols. Evaluation of antioxidant properties of silymarin and its potential to inhibit peroxyl radicals in vitro; lebensm-Wiss Technol. 1995;28:25-30.
11. Kusuma K, Vaidya VP and Mahadevan KM. Thionation of ethyl naphtho [2, 1-b] furan-2-carboxylate. Int J Chemical Sci. Technology. 2011;1(4):177-182.
12. Thomsen I, Clausen K, Scheibye S and Lawesson SO. Thiation with 2, 4-bis (4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide,n-methylthiopyrrolidone. Org Synthesis. 1990;7:372.