# SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NITROGEN HETEROCYCLES INCORPORATION INTO COUMARIN

NAHED FATHALLA ABD EL-GHAFFAR

*Faculty of Science, Chemistry Department, Girls Branch, Al-Azhar University, Nasr City. e-mail prof_nahed@yahoo.com*

**Abstract**

3-Carbethoxycoumarin derivatives were reacted with some neucleophilic reagents such as amines, hydrazines, urea derivatives and amino acids in order to study their effect on coumarin nucleus especially carbonyl coumarin and synthesis of new derivatives which have biological effects on microorganisms such as bacteria and fungi. The structural assignments of the new compounds were based on analytical and spectral data.

**Results and discussion:**

3-Carbethoxycoumarin **(Ia)** and 5-bromo-3-carbethoxy coumarin **(Ib)** reacted with aromatic primary amines e.g. aniline and o-toludine to give N-substituted carboxamides (**IIa,b)**[1,2], respectively

Also, when 3-carbethoxycoumarin derivatives **(Ia-c)** were reacted with aromatic heterocyclic amines such as 2-aminopyridine, 2-aminothiazole, 2-minobenzothiazole and 3-methyl-5-ethoxypyroyle the products were N-substituded carboxamidocoumarins, **(IIIa-e)** and **(IV)**, respectively**.**

The mass spectrum of the compound **(IIId)** shows ion peaks fragmentation at m/z 400/402 (5.3%) and other peaks at m/z 251/252 (1.1%) m/z 172 and m/z 74 (5.6%).

Alcoholic ferric chloride test didn't give any definite colour of phenol[3] i.e. the α-pyrone ring is not cleavage.

The previous studies[4] reported that when coumarin derivatives reacted with secondary amines the α-pyrone ring may be opened. In the present investigation[5] when 3-carbethoxycoumarin derivatives are reacted with diethyl amine in the presence of ethanol it give N-diethyl coumarin carboxamide **(V)** which does not give any phenolic colour with alcoholic ferric chloride[3].

3-Carbethoxycoumarin condensed with ethyl acetoacetate in the presence of ammonium acetate to give chemoselective products according to the conditions of the reaction[6]. Thus, when 5-bromo-3-carbethoxycoumarin **(Ib)** is reacted with ethyl acetoacetate and ammonium acetate in boiling ethanol it gave 5-bromo-3(6-ethoxy-4'-methyl pyridozine-3'-yl)-2H-chromen-2-one **(VI).**

A previous studies[2] proved that coumarin derivatives react with hydrazine hydrate through opening of α-pyrone ring to give different phenolic derivatives. Then it was reported[3] that 3-carbethoxycoumarin derivatives react the with hydrazine hydrate to give different hydrazide derivatives, the reaction of 3-carbethoxy coumarin derivatives **(Ia-c)** with phenylhydrazine, 4-nitrophenyl-hydrazine and 2,4-dinitrophenyl hydrazine gave the hydrazide derivatives **(VIIa-f)**, respectively without α-pyrone ring opening products.

Previous studies[7,8] showed that when 3-bromoacetyl coumarin derivative was condensed with aryl thiourea derivatives, the thiazolylcoumarin derivatives were obtained.

Now the reaction of the 3-carbethoxycoumarin **(Ib)** with thiourea in presence of boiling acetic acid gave 3-(6'-amino-4'-thioxo-4',5'-dihydro1',3',5'-triazin-2'-yl)-5-bromo-2H-chromen-2-one **(VIII)**.

The previous studies[9] proved that 3-aminocoumarin derivatives react with phthalic anhydride to give benzopyrano-benzoxazines.

In this study 3-carbethoxycoumarin **(Ia)** reacted with aliphatic amides such as formamide to give [1] benzopyran [3,2-d] chroman 2,4-dione **(IX)**.

Also, 3-carbethoxycoumarins **(Ia-c)** react with succinimide and/or phthalimide in basic medium to give N-[3-2H-(1) benzopyran-2-one] carbonylsuccinamide **(Xa-c)** and benzopyran-3-carbonyl phthalimide **(Xd),** respectively.

3- Carbethoxycoumarin **(Ib)** reacted with anthranilic acid in the presence of sodium ethoxide in boiling ethanol to give 2-(5-bromo-2-oxo-2H-chromen-3-yl)-4H-3,1-benzoxazine-4- one **(XI**).

The reaction of **(IIId)** with chlorosulphonic acid gave the corresponding 6-sulphonyl chloride derivative[10] **(XII),** which is used for preparing dyes especially

flourscence dye[10]. Compound **(XII)** is reacted with secondary aliphatic amine such as diethylamine to give N-alkyl sulphonamide derivative **(XIII).**

**Biological screening**

The prepared compounds were tested against different types of Gram positive, Gram negative bacteria, Unicellular yeast and Film entous fungi using agar-diffusion technique and/or agar plate diffusion techniques[11], as shown in table (1)

Table (1) : Antimicrobial Activity of Some Newly (1) benzopyran derivatives

| **Tested organism** | ***Staphylococcus aureus*** | | | ***Bacillus subtilis*** | | | ***Salmonella typhi*** | | | ***Aspergillus flavus*** | | | ***Aspergillus niger*** | | | ***Candida albicans*** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **concentration** | **1** | **2.5** | **5** | **1** | **2.5** | **5** | **1** | **2.5** | **5** | **1** | **2.5** | **5** | **1** | **2.5** | **5** | **1** | **2.5** | **5** |
| **compd. No.** | **(mg/ml)** | | | **(mg/ml)** | | | **(mg/ml)** | | | **(mg/ml)** | | | **(mg/ml)** | | | **(mg/ml)** | | |
| **IId** | 0 | 0 | 0 | + | + | + | + | + | + | + | + | + | ++ | ++ | ++ | ++ | ++ | ++ |
| **IIe** | 0 | + | + | + | + | + | + | + | + | ++ | ++ | ++ | 0 | 0 | 0 | 0 | 0 | 0 |
| **V** | ++ | ++ | ++ | 0 | + | + | + | + | + | + | + | + | + | + | ++ | 0 | 0 | 0 |
| **VII** | 0 | 0 | 0 | ++ | ++ | ++ | + | + | + | + | + | + | 0 | 0 | 0 | + | + | + |
| **IXb** | 0 | + | + | + | + | + | + | + | + | + | + | + | 0 | 0 | 0 | + | + | ++ |
| **IXc** | + | + | + | + | + | + | + | + | ++ | + | + | + | + | + | + | 0 | 0 | 0 |
| **X** | 0 | + | + | + | + | + | 0 | + | + | ++ | ++ | ++ | + | + | + | + | + | ++ |
| **XIII** | 0 | + | + | ++ | ++ | ++ | 0 | + | + | ++ | ++ | ++ | + | + | + | + | + | + |

Well diameter : 1 cm (100 μl of each conc. was tested).
Inhibition values = 0.1-05 cm beyond control = + ;
Inhibition values = 0.6-1.0 cm beyond control = ++
Inhibition values = 1.1-1.5 cm beyond control = +++ ;
0 = not detected.

**Experimental**

All melting points are uncorrected. The IR spectra were detected on a Shimadzu FT-IR 8201 PC spectrophotometer, Wafer technique in KBr discs and $\overline{\upsilon}$ in $cm^{-1}$. The $^1$H-NMR spectra were run on a Bruker proton NMR Avance (300 Mz) using DMSO-$d_6$ as solvent and TMS as internal standard. The mass spectra were measured on a Varian Mat 112 spectrophotometer (70 eV).

Compounds **I, II , III** and **V** were prepared according to Sammour *et al.*[2]

***3-[(3'-Ethoxy-5'-methyl-1H-pyrazol-yl)carbonyl[-2H-chromen-2-one (IV):***

To 3-carbethoxycoumarin **(Ia)** (0.01 mole) in (30 ml) of absolute alcohol was added 3-methyl-5-ethoxypyrazol (0.01 mole) and the mixture was refluxed for 6 hours, then filtered while hot, left to cool, yellow crystals were separated which were crystallized from ethanol (Table 2).

***5-Bromo-3-(6-ethoxy-4-methyl pyridazin-3-yl)-2H-chromen-2-one (VI).***

Heating a mixture of of 3-carbethoxy coumarin **(Ib)**, (0.01 mole), ethyl acetoacetate(0.01 mole) and ammonium acetate (0.04 mole) in 30 ml of absolute ethanol until clear solution. The reaction mixture was left at room temperature for 72 hours, then concentrated and the solid product that separated out was filter and wash with acetone, then recrystallized from the proper solvent (Table 2).

***Reaction of 3-carbethoxycoumarin (Ia-c) derivatives with hydrzines*. Formation of the hydrazide *derivatives* (VIIa-f):**

To 3-carbethoxycoumarin derivatives (0.01 mole) **(Ia-c)** was added phenylhydrazine (0.01 mole), 4-nitrophenylhydrazine or 2,4-dinitriophenyl hydrazine in absolute ethanol (30 ml). The mixture was refluxed for 6 hours, then filtered off on hot, cooled to room temp and filter. The precipitates obtained were filtrated off and recrystallized from the proper solvent (Table 2).

***3-(6-Amine-4-thioxo-4,5-dihydro-1,3,5-triazin-2-yl)-5-bromo-2H-chromen-2-one (VIII).***

Heating a mixture of 3-carbethoxycoumarin derivative **(Ib)** (0.005 mole) and thiourea (0.005 mole) in (10 ml) of acetic acid and (5 ml) of absolute ethyl alcohol under reflux for 6 hours. The mixture was filtered off while hot and then cooled. The solid product obtained was filtered off, washed with cold water and then recrystallized from ethanol (c.f. Table 2).

***[1] benzopyran [3,2-d] Chroman 2,4-dione (IX)***

A mixture of of 3-carbethoxy coumarin **(Ia)** (0.01 mole) in (50 ml) of ethanol of formamide solution (0.01 mole) and 5 drops of dry pyridine was heated under reflux for 6 hours. The mixture was filtered off while hot and poured onto ice/HCl (99%).

The product obtained was filtered off and then recrystallized of from ethanol (c.f. Table 2).

***Reaction of 3-carbethoxycoumarin derivatives (Ia-c) with succinimide. Formation of (1) benzopyran-2-carbonylsuccinamide (Xa-c).***

Heating a mixture of 3-carbethoxycoumarin **(Ia-c)** (0.01 mole) and succinimide (0.01 mole) in (25 ml) of ethanol and 5 drops of pyridine under reflux for 6 hours. The mixture was filtered off and add ice/HCl. The, separated solid was filtered off, washed well with water and recrystallized from ethanol (Table 2).

***2-[2-oxo-2H-chromen-3-yl) carbonyl]-1H-isoindole-1,3(2H) dione (Xd):***

(0.01 mol) at 3-carbethoxycoumarin derivative **(Ia)** was dissolved in (50 ml) of hot ethanol, then (0.01 mole) of phthalimide was added and (0.03 mole) of anhydrous potassium carbonate, then the mixture was heated under reflux for 6 hours. The mixture was filtered as and ice/HCl was added. The product obtained was filtered off and washed with water, then recrystallized from ethanol (Table 2).

***2-(5-Bromo-2-oxo-2H-chromen-3-yl)-4H-3,1-benzoxazine-4-one (XI)***

A mixture of 3-carbethoxycoumarin **(Ib)** (0.01 mole) in (30 ml) of absolute ethanol, sodium ethoxide (0.01 mol), and (0.01 mol) of anthranilic acid under reflux for 6 hours after cooling ice/HCl was added. The product filtered off and washed with water, then recrystallized from ethanol (Table 2).

***2-{(5-Bromo-2-oxo-2H-chromen-3-yl) carbonyl) amino}-1,3-benzo-thiazole 6-sulfonyl chloride (XII).***

A mixture of **(IIId)** (0.01 mol) and (0.25 mol) of chlorosulphonic acid was heated was at 130˚C while stirring for 3 hours. The mixture was cooled to 10˚C and poured into cold water and filtered. The precipitate obtained was filtered off, washed with water (the pH is 5).

***5-BromoN-[(6-diethylsulfamoyl-benzothiazol-2-yl) amide]-2-oxo-2H- chromene-3-carboxamide (XIII).***

To the solution mixture from step (1) **(XII)** add (5 ml) of water as a solvent, then, (0.02 mol) of diethylamine stirring. The mixture was left at room temperature

for 2 hours, the wash the product with water and recrystallized with ethanol (Table 2)

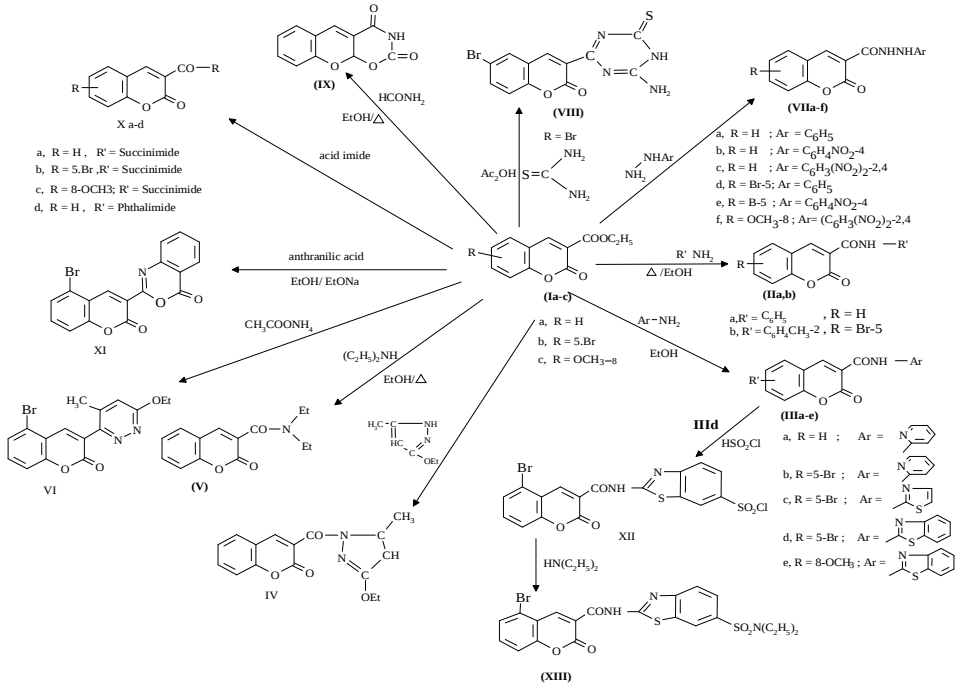


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**Table (2): Analytical and spectral data of the newly prepared compounds**

| Comp. No. | M.P. °C yield % | Solvent of crystallization colour crystals | molecular formula (Mol.wt.) | IR | $^1$HNMR- (δ ppm) |
|---|---|---|---|---|---|
| **IIa** | 128°C 70 | EtOH Dusty powder | $C_{16}H_{11}NO_3$ (265) | υNH at 3220 $cm^{-1}$, υC–H at 2916.2, 2846.7 $cm^{-1}$, υC=O of lactone at 1705 $cm^{-1}$ and υCONH carboxamide at 1635.5 $cm^{-1}$. | 4.12 (s, 1H, NH) ; 6.25 (s, 1H, olefinic H); 6.39-6.37 (m, 9H, Ar–H) . |
| **IIb** | 150 60 | EtOH beige crystals | $C_{17}H_{12}BrNO_3$ (358) | υNH at 3120$cm^{-1}$, υC–H at 2846.7, 2916 $cm^{-1}$, υ$cm^{-1}$, υC=O at 1705 $cm^{-1}$ and υC=N at 1651 $cm^{-1}$. | |
| **IIIa** | 114 40 | EtOH Dusty reddish powder | $C_{15}H_{10}N_2O_3$ (266) | υNH at 3240$cm^{-1}$, υC–H at 3031, 2916.2, 2846.7 $cm^{-1}$ υC=O for δ lactone at 1743.5 $cm^{-1}$, υC=O carboxamide at 1681.8 $cm^{-1}$ and υC=N at 1630 $cm^{-1}$ | 7.39 (m, 9H, Ar–H and olefinic proton) at 8.73 . |
| **IIIb** | 245 55 | EtOH light beige crystals | $C_{15}H_9BrN_2O_3$ (345) | υNH at 3140 $cm^{-1}$, υC–H at 2916.2, 2846.7 $cm^{-1}$, υC=O for δ lactone at 1720.0 $cm^{-1}$, υC=O carboxamide at 1643.2 $cm^{-1}$. | |
| **IIIc** | 215 50 | EtOH dark beige crystals | $C_{13}H_7BrN_2O_3S$ (351) | υNH at 3140, 3240 $cm^{-1}$, υC–H at 3039.6, 2916.2, 2816.7 cm, υC=O δ lactone at 1735.8 $cm^{-1}$ υC=O carboxamide at 1674.1 and υC=N at 1630 $cm^{-1}$. | |
| **IIId** | 217 40 | EtOH light beige powder | $C_{17}H_9BrN_2O_3S$ (401) | υNH at 3225.5 $cm^{-1}$, υC–H at 2916.2, 2846.7 $cm^{-1}$, υC=O δ lactone at 1743.5 $cm^{-1}$, υC=O carboxamide at 1681.8 $cm^{-1}$ and υC=N at 1640 $cm^{-1}$. | |
| **IIIe** | 125 40 | EtOH Beige yellowish powder | $C_{18}H_{12}N_2O_4S$ (352) | υNH at 3240$cm^{-1}$, υC–H at 2918, 2848 $cm^{-1}$, υC=O δ lactone at 1710.0 $cm^{-1}$, υC=O carboxamide at 1670 $cm^{-1}$ and υC=N at 1610 $cm^{-1}$. | |
| **IV** | 146 65 | EtOH yellow crystals | $C_{16}H_{14}N_2O_4$ (298) | υC–H at 2846.7, 2916.2 $cm^{-1}$, υC=O at 1705 $cm^{-1}$ and υC=N at 1635,5 $cm^{-1}$ | 0.98(s,3H, $CH_3$) (t, 3H, $CH_2CH_3$) 1.1 (q, 2H, $CH_2CH_3$) 4.1 (m, 4H, Ar–H) at 7.69–6.g |
| **V** | 152 60 | EtOH Dusty flakes | $C_{14}H_{15}NO_3$ (245) | C–H at 2846.7, 2916.2 $cm^{-1}$, υC=O at 1705 $cm^{-1}$ and υC–N at 1635.5 $cm^{-1}$. | |
| **VI** | 277 35 | EtOH yellow crystals | $C_{14}H_{11}BrN_2O_3$ (335) | υC=O δ lactone at 1710 $cm^{-1}$, υC=N at 1620$cm^{-1}$, υC–H at 2940, 2918, 2850 $cm^{-1}$ and υNH at 3420, 3268, 3143 $cm^{-1}$ | |
| **VIIa** | 180 75 | EtOH Light orange crystals | $C_{16}H_{12}N_2O_3$ (280) | υNH at 3301.9 $cm^{-1}$, υC–H at 3055, 2916.2, 2846.7 $cm^{-1}$, υC=O hydrazide at 1697.2 $cm^{-1}$ , υC=C at 1596.9 and υC=O lactone at 1720$cm^{-1}$ | 9.43 (s,  NH ) at 9.43, 8.31-7.17 (m, 9H, Ar–H) 6.79 (s, 1H, CH=C) at 6.79 ppm. |

**Table (2) : Continued**

| | | | | | |
|---|---|---|---|---|---|
| **VIIb** | 222 | EtOH | $C_{16}H_{11}N_3O_5$ | υNH at 3268.5, υC–H at 3050, 2916.2, 2846.7 $cm^{-1}$, υCO δ lactone at | |

| | | | | | |
|---|---|---|---|---|---|
| | 80 | yellow crystals | (325) | 1720 $cm^{-1}$, υC=O at 1697.2 $cm^{-1}$ and υC=C at 1604.2 $cm^{-1}$ | |
| **VIIc** | 175<br>90 | EtOH<br>yellow white crystals | $C_{16}H_{10}N_4O_7$<br>(370) | υNH at 2380 $cm^{-1}$, υC–H at 3030, 2916.2, 2846.7 $cm^{-1}$, υC=O δ lactone at 1743.5 $cm^{-1}$, υC=O hydrazide at 1697.2 $cm^{-1}$ and υC=C 1605 $cm^{-1}$ | |
| **VIId** | 192<br>20 | EtOH<br>light yellow crystal | $C_{16}H_{11}$ $BrN_2O_3$<br>(359) | υNH 3450 $cm^{-1}$, υC–H at 3010, 2918, 2848 $cm^{-1}$, υC=O δ lactone at 1686 $cm^{-1}$ and C=C at 1602$cm^{-1}$ | |
| **VIIe** | 241<br>20 | EtOH<br>light yellow crystal | $C_{16}H_{10}$ $BrN_3O_5$<br>(404) | υNH at 3440 $cm^{-1}$, υC–H at 3010, 2956, 2918, 2848 $cm^{-1}$ υC=O δ lactone at 1710 $cm^{-1}$, υC=O hydrazide at 1680 $cm^{-1}$ and υC=C at 1598 $cm^{-1}$ | |
| **VIIf** | 148<br>50 | EtOH<br>Orange powder | $C_{17}H_{12}N_4O_8$<br>(400) | | 3.35 (s, $OCH_3$), 4.99 (d, NH-NH) and 8.98-6.6 (m, 8H, Ar–H and olefinic proton) |
| **VIII** | 211<br>50 | EtOH<br>pale brown dusty powder | $C_{12}H_7$ $BrN_4OS$<br>(351) | υC=S at 1280 $cm^{-1}$ υC=N at 1676 $cm^{-1}$ , C=O δ lactone at 1738 $cm^{-1}$ and υCH, $\upsilon NH_2$ at 3042, 3250, 2850, 2918, 3310 and 3390 $cm^{-1}$ | |
| **IX** | 149<br>50 | EtOH<br>pale brown light dusty powder | $C_{11}H_7NO_4$<br>(217) | υNH/OH at 3417.6 $cm^{-1}$, stretching absorption band for C=H at 2846.7, 2916.2$cm^{-1}$υC=O δ lactone at 1705 $cm^{-1}$ and υC=O carbooxamid at 1635.5 $cm^{-1}$ | 7.05-8.97 (4H, Aromatic protons), 6.61 (olefinic protons, 4.11 (NH-proton) 9.0 and 11.21 (s.20H groups) . |
| **Xa** | 165<br>60 | EtOH<br>brown crystals | $C_{14}H_9NO_5$<br>(271) | υC–H at 2846.7, 2916.2, 3040 $cm^{-1}$ υC=O δ lactone at 1705 $cm^{-1}$ and υC=O imide at 1643.2, 1680 $cm^{-1}$. | 7.09-8.31 (4H-Aromatic protons) , 2.57 (d,7Hz) and 6.92 (s, 1H, olefinic) . |
| **Xb** | 215<br>30 | EtOH<br>dusty crystalline | $C_{14}H_8BrNO_5$<br>(350) | υC–H at 2850, 2918, 3042 $cm^{-1}$ υC=O δ lactone at 1736$cm^{-1}$ and υC=O imide at 1676, 1680$cm^{-1}$ | 7.4-8.6 (3H–Aromatic protons), 13 (d, 2H of methylene) and 2.45 (t, 2H of the second methylene group) . |
| **Xd** | 133<br>40 | EtOH<br>dusty powder | $C_{15}H_{11}NO_6$<br>(301) | υN–H at 2848, 2918, 3020$cm^{-1}$, υC=O δ lactone at 1718 $cm^{-1}$, υC=O imide at 1670, 1680 $cm^{-1}$. | |
| **XI** | 190<br>70 | EtOH<br>dark yellow crystals | $C_{17}H_8$ $BrNO_4$<br>(370) | υC–H at 2850, 2920, 3050$cm^{-1}$, υC=O δ lactone at 1692 $cm^{-1}$ and υC=N at 1628 $cm^{-1}$ | 6.65-7.9 (m, 8H–Aromatic protons) . |
| **XII** | 134<br>70 | EtOH<br>light brown crystal | ($C_{17}H_8BrN_2O_5S_2Cl$)<br>() | υC–H at 3050, 2920, 2850 $cm^{-1}$ υC=O δ lactone at 1692$cm^{-1}$, υC=N at 1628 $cm^{-1}$ | 79-6.65 (8H-Aromatic protons) |
| **XIII** | 209<br>50 | EtOH<br>grey circle crystals | $C_{21}H_{18}BrN_3O_5S_2$<br>(536) | υOH/NH at 3146, 3296 $cm^{-1}$ υC–H at 2860, 2980, 3020$cm^{-1}$ υC=O δ lactone at 1710$cm^{-1}$ υC=S at 1638 $cm^{-1}$ and υC–S–C at 1284 $cm^{-1}$ | 1.2 (t, 6H) 2.99 (q, 4H) 7.91-71.7 (m, 6H-Aromatic protons) . |

It is satisfactory for microanalysis C, H, N, S and Br.