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Research Article

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Synthesis and Antimicrobial Study of Triazolo[3,4-*b*][1,3,4]Thiadiazole and Triazolo-[3,4-*b*][1,3,4]Thiadiazine derivatives of Chromeno [4,3-*b*]pyridin-5-one moiety

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ABSTRACT

*The series of new Triazolo[3,4-b][1,3,4]thiadiazole (**7a-e**) and Triazolo[3,4-b][1,3,4]thiadiazine (**8a-e**) derivatives of chromeno[4,3-b]pyridin-5-one were successfully synthesized by multistep synthesis of 4-amino-3-Formyl-2-oxo-2H-chromene. The newly synthesized compounds were well characterized by IR, ^{1}HNMR, ^{13}CNMR, Mass and Elemental analysis. The compounds were evaluated for their antimicrobial activity against three antibacterial species namely Staphylococcus aureus, Escherichia coli and Bacillus cereus and two fungal species namely Candida albicans and Aspergillus clavatus. Most of the compounds show very good antimicrobial inhibition while compared with the standard drug such as Ciprofloxacin and Ketoconazole.*

Keywords: Coumarin, Triazolothiadiazole, Triazolothiadiazine, Antimicrobial activity.

INTRODUCTION

Heterocyclic system containing Coumarin nuclei are the most versatile bioactive compounds. Presence of lactone framework in the coumarin makes it hydrophobic which is responsible to exhibit the biological potency. The incorporation of other groups alters the Pharmacological property of parent Coumarin and converts it into more useful products [1]. Natural Coumarins are well known to have antidiabetic activity [2]. The Natural product Lamellarins belongs to the coumarin nucleus [3]. The potent antibiotic like Novobiocin, Coumaromycin and Chartesium containing coumarin nucleus. Many Coumarin derivatives are applied as anticoagulant [4], anti-HIV [5], Antifungal [6], antiviral [7], antitumor [8], Cytotoxic [9] and antioxidant activity [10]. Triazole and Thiadiazole derivatives are well known for their antimicrobial [11-15], anti-inflammatory [16], anti-leishmanial [17] and anticancer activity [18].

In the present communication we have reported the synthesis and antimicrobial evaluation of Triazolothiadiazole and Triazolothiadiazine encouraged from our ongoing project [19]. We have to know the effect of incorporation of various biologically active heterocyclic entities such as sulfur in the form of pentacyclic heterocycles such as thiadiazole and six membered heterocycles such as thiadiazine and secondary amine such as morpholine in the target molecule. The reported compounds are synthesized for first time obtained in good yield by using readily available materials.

EXPERIMENTAL SECTION

Unless otherwise stated, all materials were obtained from commercial suppliers and were used without further purification. All reactions were monitored by thin layer chromatography (TLC) on 25mm silica gel 60 F254 plates (Merck, Darmstardt, Germany) using UV light (254 & 366 nm) for detection. Compounds were purified by column

chromatography using appropriate solvents. Melting points are uncorrected. The ^{1}H NMR (300MHz) and ^{13}C NMR (75MHz) spectra were measured on a Varian NMR Mercury 300 spectrometer in $CDCl_3$. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), or m (multiplet). IR spectra were recorded using a Shimadzu IR 408 spectrophotometer as a potassium bromide pellets. Mass spectra were recorded on an Agilent 1100 LC-Q-TOF mass spectrophotometer with an ionization potential of 70eV. The obtained products were moisture and oxygen stable at ambient temperature.

Synthesis of Ethyl-2, 5-dihydro-2,5-dioxo-1H-chromeno[4,3-b]pyridine-3-carboxylate (**2**)
1 mmol 4-amino-2-oxo-2*H*-chromene-3-cabaldehyde (**1**) was taken in 25mL round bottom flask in 15mL ethanol to which 1.1 mmol of diethylmalonate was added along with 2-3 drops of piperidine and heated to reflux for 4 hours**.** Reaction progress was monitored by TLC (Ethylacetate:n-Hexane, 1:1). After completion of reaction the solvent was removed under vacuum and the obtained solid was recrystallized in ethanol.
Yield- 78%; IR (in KBr) ῡ 3220 cm^{-1} (-NH), 1730 cm^{-1} (C=O), 1645 cm^{-1}(C=C); ^{1}HNMR (DMSO): δ 8.1 (bs, -NH, 1H), 7.95 (s, 1H, Ar-H), 7.6 (d, 1H, Ar-H), 7.5(m, 1H, Ar-H), 7.15 (d, 2H, Ar-H), 4.35 (q, 2H, -CH_3), 2.81 (s, 3H, -CH_2); MS MS: m/z 286 (M+1); Anal. Calcd for $C_{15}H_{11}NO_5$: C, 63.16; H, 3.89; N, 4.91; O, 28.04, Found: C, 63.03; H, 3.61; N, 4.83; O, 27.76.

Synthesis of Ethyl 2-chloro-5-oxo-5H-chromeno[4,3-b]pyridine-3-carboxylate (**3**)
1 mmol of Ethyl-2, 5-dihydro-2, 5-dioxo-1*H*-chromeno[4,3-*b*]pyridine-3-carboxylate (**2**) taken into the 25mL round bottom flask. To this 10mL of Phosphorus oxychloride has been added and heated on water bath for 2 hours at 70°C undergoes chlorination of amide functionality yielded Ethyl 2-chloro-5-oxo-5H-chromeno[4,3-*b*]pyridine-3-carboxylate (**3**). The product was recrystallized in ethanol.
Yield-83%; IR (in KBr) ῡ 1738cm^{-1}(C=O) lactone, 1655 (C=C); ^{1}HNMR ($CDCl_3$): δ 7.81 (s, 1H, Ar-H), 7.54(d, 1H, Ar-H), 7.48 (m, 1H, Ar-H), 7.29 (d, 2H, Ar-H), 4.40 (q, 2H, -CH_3), 2.88 (s, 3H, -CH_2); MS: m/z 305 (M+2); Anal. Calcd for $C_{15}H_{10}ClNO_4$: C, 59.32; H, 3.32; Cl, 11.67; N, 4.61; O, 21.07, Found: C, 58.88; H, 3.225; Cl, 11.55; N, 4.81; O, 21.18.

Synthesis of Ethyl-2-morpholino-5-oxo-5H-chromeno[4,3-b]pyridine-3-carboxylate (**4**)
1 mmol of compound **3** when reacted with 1.1 mmol of morpholine in presence of base K_2CO_3 stirred at 25° C for 2 hours yielded compound **4** in good yield. The reaction progress was monitored by TLC (Ethyl acetate: n-Hexane, 1:3),
Yield-75%; IR (in KBr) ῡ 1732 cm^{-1}(C=O), 1695 cm^{-1}(C=C); ^{1}HNMR ($CDCl_3$): δ 7.74 (s, 1H, Ar-H), 7.62(d, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.40 (d, 2H, Ar-H), 4.35 (q, 2H, -CH_3), 3.78 (t, 4H, 2xCH_2), 3. 14 (t, 4H, 2xCH_2), 2.73 (s, 3H, -CH_2); MS: m/z 355 (M+1); Anal. Calcd for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.91; O, 22.58, Found: C, 64.32; H, 5.22; N, 7.83; O, 22.44.

Synthesis of 2-Morpholino-5-oxo-5H-chromeno[4,3-b]pyridine-3-carbohydrazide (**5**).
1 mmol of Ethyl-2-morpholino-5-oxo-5*H*-chromeno[4,3-*b*]pyridine-3-carboxylate (**4**) was taken into the 25mL round bottom flask containing 20mL of methanol. Add to it 1.1mmol of hydrazine hydrate and reflux the reaction mixture for 4 hours. Reaction progress was monitored by TLC (Ethyl acetate: n-Hexane; 1:1) and obtained solid was recrystallized in Methanol.
Yield-68%; IR (in KBr) ῡ 3315 cm^{-1}(NH), 3260 cm^{-1}(NH), 1735 cm^{-1}(C=O); ^{1}H NMR (DMSO): δ 8.78 (bs, 1H, -NH), 7.81 (s, 1H, Ar-H), 7.55 (d, 1H, Ar-H), 7.39 (m, 1H, Ar-H), 7.33 (d, 2H, Ar-H), 4.48 (bs, 2H, -NH_2), 3.66 (t, 4H, 2xCH_2), 3. 25 (t, 4H, 2xCH_2); MS: m/z 341 (M+1); Anal. Calcd for $C_{17}H_{16}N_4O_4$: C, 59.99; H, 4.74; N, 16.46; O, 18.80, Found: C, 59.74; H, 4.62; N, 16.31; O, 18.72.

Synthesis of 2-Morpholino-3-[1,2,4]triazolo[3,4-b]-5H-chromeno[4,3-b]pyridin-5-one (**6**)
1 mmol of compound **5** was taken in 25mL round bottom flask, add to it 1.2mmol of carbon disulfide and KOH (1mmol) and heated it to reflux. Reaction progress was monitored by TLC (Ethyl acetate: n-Hexane, 2:1). As the reaction progresses it turns to cyclization after this 1.2mmol of hydrazine hydrate was added to it, in order to hydrozinolysis of oxadiazole into Triazole in good yield. Yield: 79%; IR (in KBr) ῡ 3380 cm^{-1}(NH), 1731 cm^{-1} (C=O), 1649 cm^{-1} (C=C).
^{1}H NMR (DMSO): δ 7.73 (s, 1H, Ar-H), 7.60 (d, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.39 (d, 2H, Ar-H), 4.73 (bs, 2H, -NH_2), 3.53 (t, 4H, 2xCH_2), 3.29 (t, 4H, 2xCH_2) 3.07 (s, 1H, -SH); MS: m/z 397 (M+1); Anal. Calcd for $C_{18}H_{16}N_6O_3S$: C, 54.54; H, 4.07; N, 21.20; O, 12.11; S, 8.09, Found: C, 54.43; H, 4.15; N, 21.05; O, 11.94; S, 7.96.

Synthesis of 2-morpholino-3-(6-aryl-[1,2,4]triazolo[3,4-b][1,3,4]oxadiazol-3-yl)-5H-chromeno [4,3-b]pyridin-5-one (**7a-e**)

To a 25mL round bottom flask 1 mmol 2-Morpholino-3-[1,2,4]triazolo[3,4-*b*]-5*H*-chromeno[4,3-*b*]pyridin-5-one (**6**) and 1.1 mmol of aryl acid chloride was added in 10mL of Phosphorus oxychloride. The suspension was refluxed for 1.5-4 hours. Reaction progress was monitored by TLC (Ethyl acetate: n-Hexane: 1:1).

2-morpholino-3-(6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]oxadiazol-3-yl)-5H-chromeno [4,3-b]pyridin-5-one (**7a**)

Yield: 78%; IR (in KBr) ῡ 1739 cm^{-1} (C=O), 1643 cm^{-1} (C=C); ^{1}H NMR (DMSO): δ 7.82 (s, 1H, Ar-H), 7.68 (d, 1H, Ar-H), 7.59 (d, 2H, Ar-H), 7.48 (m, 1H, Ar-H), 7.43 (d, 2H, Ar-H), 7.36 (dd, 3H, Ar-H), 3.61 (t, 4H, 2xCH_2), 3.24 (t, 4H, 2xCH_2); MS: m/z 483 (M+1); Anal. Calcd for $C_{25}H_{18}N_6O_3S$: C, 62.23; H, 3.76; N, 17.42; O, 9.95; S, 6.65, Found: C, 62.04; H, 3.63; N, 17.35; O, 9.83; S, 6.56.

Synthesis of 2-Morpholino-3-(6-aryl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-5H-chromeno [4,3-b]pyridin-5-one (**8a-e**)

1 mmol of 2-Morpholino-3-[1,2,4]triazolo[3,4-*b*]-5*H*-chromeno[4,3-*b*]pyridin-5-one (**6**) and 1.1 mmol aroylacyl bromide was taken into the 25mL round bottom flask in 10mL glacial acetic acid with catalytic sodium acetate. Heat the reaction mixture to reflux for 2.5-4.5hours. Reaction progress was monitored by TLC (Chloroform: Methanol, 9:1). The obtained solid was crystallized Ethanol.

2-Morpholino-3-(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-5H-chromeno [4,3-b] pyridin -5-one (**8**a)

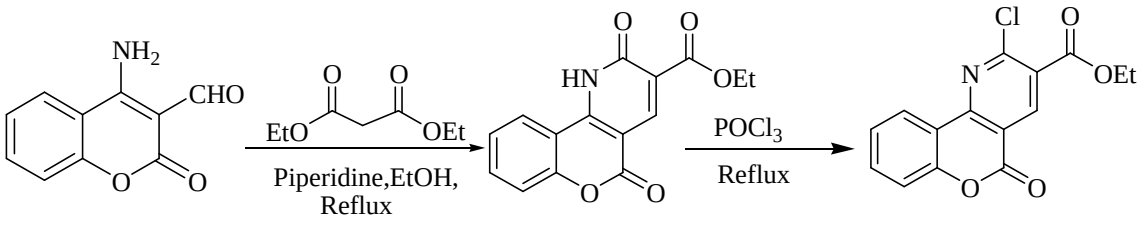
Yield: 82%; IR (in KBr) ῡ 1735 cm^{-1} (C=O), 1655 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 7.81 (s, 1H, Ar-H), 7.64 (d, 1H, Ar-H), 7.53 (dd, 2H, Ar-H), 7.41 (m, 1H, Ar-H), 7.36 (d, 2H, Ar-H), 7.29 (d, 3H, Ar-H), 3.46 (t, 4H, 2xCH_2), 3.28 (s, 2H, -CH_2), 3.18 (t, 4H, 2xCH_2); MS: m/z 498 (M+1); Anal. Calcd for $C_{26}H_{20}N_6O_3S$: C, 62.89; H, 4.06; N, 16.93; O, 9.67; S, 6.46, Found: C, 62.73; H, 3.95; N, 16.85; O, 9.55; S, 6.35.

RESULTS AND DISCUSSION

In the present communication we have synthesize Ethyl-2,5-dihydro-2,5-dioxo-1*H*-Chromeno[4,3-*b*]pyridine-3-carboxylate (**2**) using 4-amino-2-oxo-2*H*-chromene-3-carbaldehyde (**1**) by Friedlander condensation of amino-aldehyde functionality (Compound **1**) with active methylene compound such as diethylmalonate in ethanol and 2-3 drops of piperidine gives a product i.e. ethyl 2, 5-dihydro-2, 5-dioxo-1*H*-chromeno[4,3-*b*]pyridine-3-carboxylate (**2**). The ^{1}HNMR of Compound **2** shows broad singlet at 8.1 ppm, due to NH proton. Compound **2** undergoes chlorination with phosphorus oxychloride furnished chloro-ester functionality (compound **3**) (**Scheme- 1**).

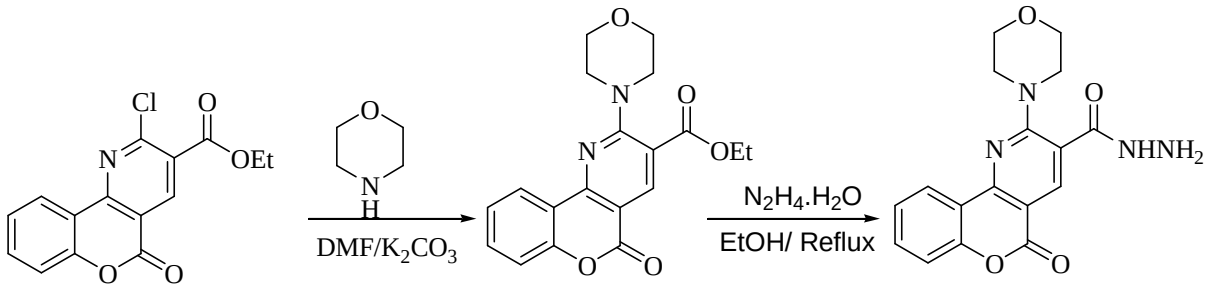
Compound reaction with morpholine in at room temperature yielded ethyl 2-morpholino-5-oxo-5*H*-chromeno[4,3-*b*]pyridine-3-carboxylate (**4**). Compound **4** undergoes nucleophilic substitution with hydrazine hydrate gives 2-morpholino-5-oxo-5*H*-chromeno[4,3-*b*] pyridine-3-carbohydrazide (**5**). This carbohydrazide functionality exhibited IR band at 3410cm^{-1}, 3380cm^{-1}, 3245cm^{-1} due to NH stretching. The ^{1}HNMR shows broad singlet at 4.1 ppm and 8.5 for –NH_2 and –NH respectively (**Scheme 2**).

Compound **5** when refluxed with carbon disulphide in ethanol and KOH for 4 hours and then hydrazine hydrate was added to the reaction mixture and reflux for another 6 hours leads to get compound **6.** Compound **6** shows IR band at 3225cm^{-1} and 3140cm^{-1} due to -NH_2 stretching. ^{1}HNMR shows distinct peak at 4.7 ppm due to NH_2 protons, also the peak appeared at 3.9 ppm corresponding to the –SH proton. Compound **6** when reflux with aromatic acid in Phosphorus oxychloride it leads to the cyclized product i.e. Triazolothiadiazole (Compound **7a-e).** Also Compound **6** treated with various derivatives of aroylacyl bromide in Acetic acid with catalytic amount of sodium acetate furnished Triazolothiadiazine (Compound **8a-e**). The IR shows stretching frequency at 1735 cm^{-1} due to lactone C=O. ^{1}HNMR shows distinct multiplet at 8.40 ppm and 8.25 ppm due to protons present on newly fused aromatic ring (**Scheme 3**)



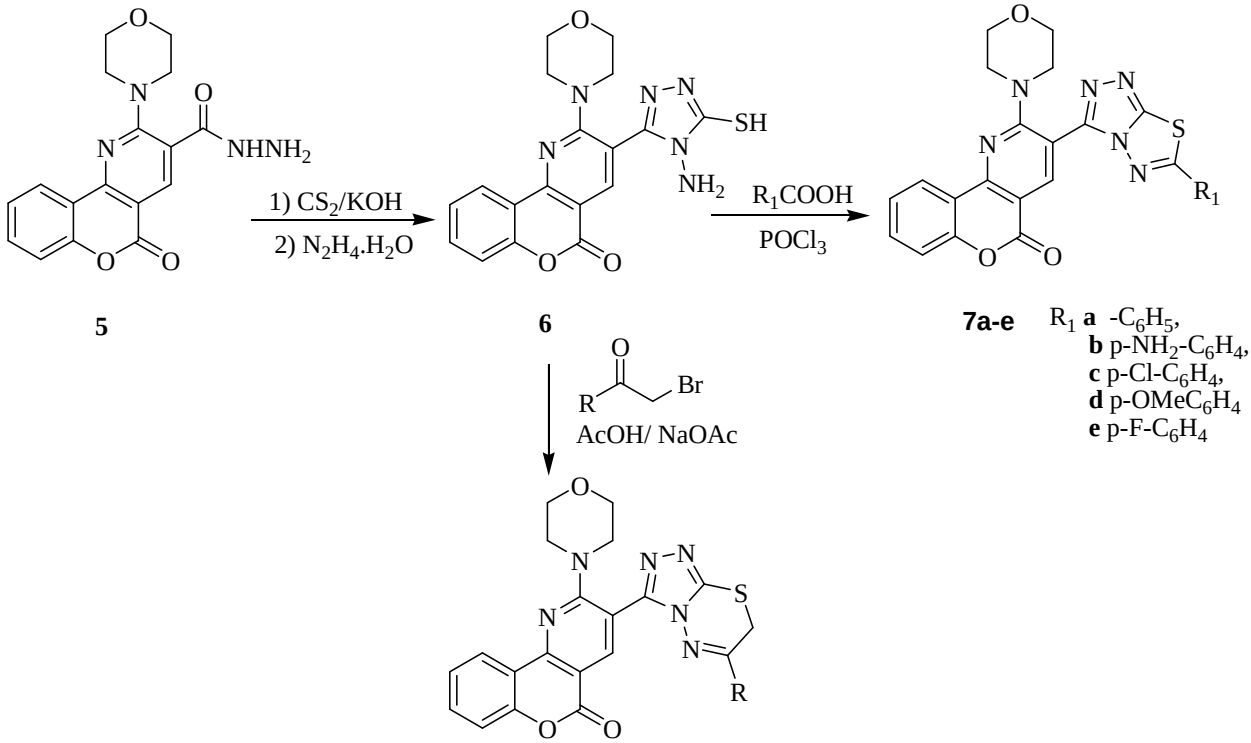
1 **2** **3**

SCHEME-1



3 **4** **5**

SCHEME-2



5 **6** **7a-e** R_1 **a** -C_6H_5, **b** p-NH_2-C_6H_4, **c** p-Cl-C_6H_4, **d** p-$OMeC_6H_4$ **e** p-F-C_6H_4

8a-e R **a** -C_6H_5, **b** p-Br-C_6H_4, **c** p-Cl-C_6H_4, **d** p-$OMeC_6H_4$ **e** p-F-C_6H_4

SCHEME-3

The antimicrobial activity was assessed by agar well diffusion method using Mueller Hinton agar medium. Zone of Inhibition and MIC's were measured for compounds **6a-e**, **8a-e** and **9a-e** against bacterial strains such as *Staphylococcus aureus, Escherichia coli, Bacillus cereus* and fungal strains as *Candida albicans, Aspergillus*

clavatus with reference to standard drugs Ciprofloxacin and Ketoconazole. The data presented in Table- 1 shows that, Compounds **7c** and **7e** showed excellent activity against *Staphylococcus aureus* at the MIC of 5µg/mL. In case of *Escherichia coli* compounds **7c** and **8b** shows good activity at the 5µg/mL concentration. Compounds **7d**, **7e** and **8d** shows excellent inhibitory activity at 5µg/mL concentration against one of the bacterial strain *viz*., *Bacillus cereus*. The fungal strain *Candida albicans* compounds **7c**, **7e** and **8c** shows good microbial inhibition at 5µg/mL concentration. Compound **7e**, **8d** and **8e** shows good inhibitory activity at 5µg/mL concentration against fungal strain *Aspergillus clavatus*.

Table-1: In vitro antimicrobial activity of compounds 7a-e and 8a-e as zone of inhibition in mm and MIC (µg/mL) in parentheses

Entry	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus cereus*	*Candida albicans*	*Aspergillus clavatus*
7a	13.7 (10)	15.5 (15)	11.9 (10)	14.5 (15)	13.8 (10)
7b	13.5 (10)	14.1 (10)	**13.3 (05)**	14.5 (10)	13.2 (10)
7c	**14.8 (05)**	**13.5 (05)**	14.03(10)	**14.1 (05)**	14.5 (10)
7d	13.9 (10)	12.8 (10)	**15.2 (05)**	13.2 (10)	14.1 (10)
7e	**15.1 (05)**	14.4 (10)	15.8 (10)	**13.5 (05)**	**14.7 (05)**
8a	15.9 (15)	12.8 (10)	14.8 (15)	12.6 (10)	13. 2 (10)
8b	14.3 (10)	**12.7 (05)**	13.1 (10)	15.8 (15)	13.7 (10)
8c	13.5 (10)	14.5 (10)	15.5 (10)	**12.9 (05)**	**14.2 (05)**
8d	12.5 (15)	15.8 (15)	**14.6 (05)**	13.8 (10)	**13.2 (05)**
8e	13.8 (15)	14.7 (10)	14.3 (10)	15.3 (10)	14.1 (10)
Cipro.	18.5 (05)	16.9 (05)	17.8 (05)	n.t.	n.t.
Ketok.	n.t.	n.t.	n.t.	16.7(05)	17.1(05)

Cipro.: Ciprofloxacin (100 µg/disc) ; Ketok. : Ketokonazole (100 µg/disc); Test Compound: (100 µg/disc); n.t. not tested

CONCLUSION

The Triazolothiadiazole and Triazolothiadiazine derivatives of chromeno[4,3,*b*]pyridine (**7a-e** and **8a-e**) were synthesized successfully with good yields and using usual methods of synthesis. Most of the synthesized compounds exhibited good activity. These results suggest that, compounds with halogen or methoxy substituent showed excellent activity against the various kinds of microorganism.

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