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Synthesis and antimicrobial activity of novel 1,2,4-triazole derivatives

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ABSTRACT

*The title compounds were synthesized by cyclization of 1-(2-(3-chloro-4-methyl-2-oxo-2H-chromen-7-yloxy)acetyl)-4-substituted phenyl thiosemicarbazides **(5a-i)** in alkaline medium. Compounds **(5a-i)** were obtained by the reaction of 2-(3-chloro-4-methyl-2-oxo-2H-chromen-7-yloxy)acetohydrazide **(3)** with substituted phenyl isothiocyanates. The newly synthesized compounds were characterized by ^{1}H NMR, IR and Mass spectral data. Further all the compounds were screened for antibacterial activity against Bacillus substilis, Escherichia coli, Pseudomonas aeruginosa and antifungal activity against Aspergillus niger* and *Aspergillus flavus.*

Keywords: 1-(2-(3-chloro-4-methyl-2-oxo-2H-chromen-7-yloxy)acetyl)-4-substituted phenyl thiosemicarbazides, 2-(3-chloro-4-methyl-2-oxo-2H-chromen-7-yloxy)acetohydrazide, phenyl isothiocyanates, antibacterial, antifungal activity.

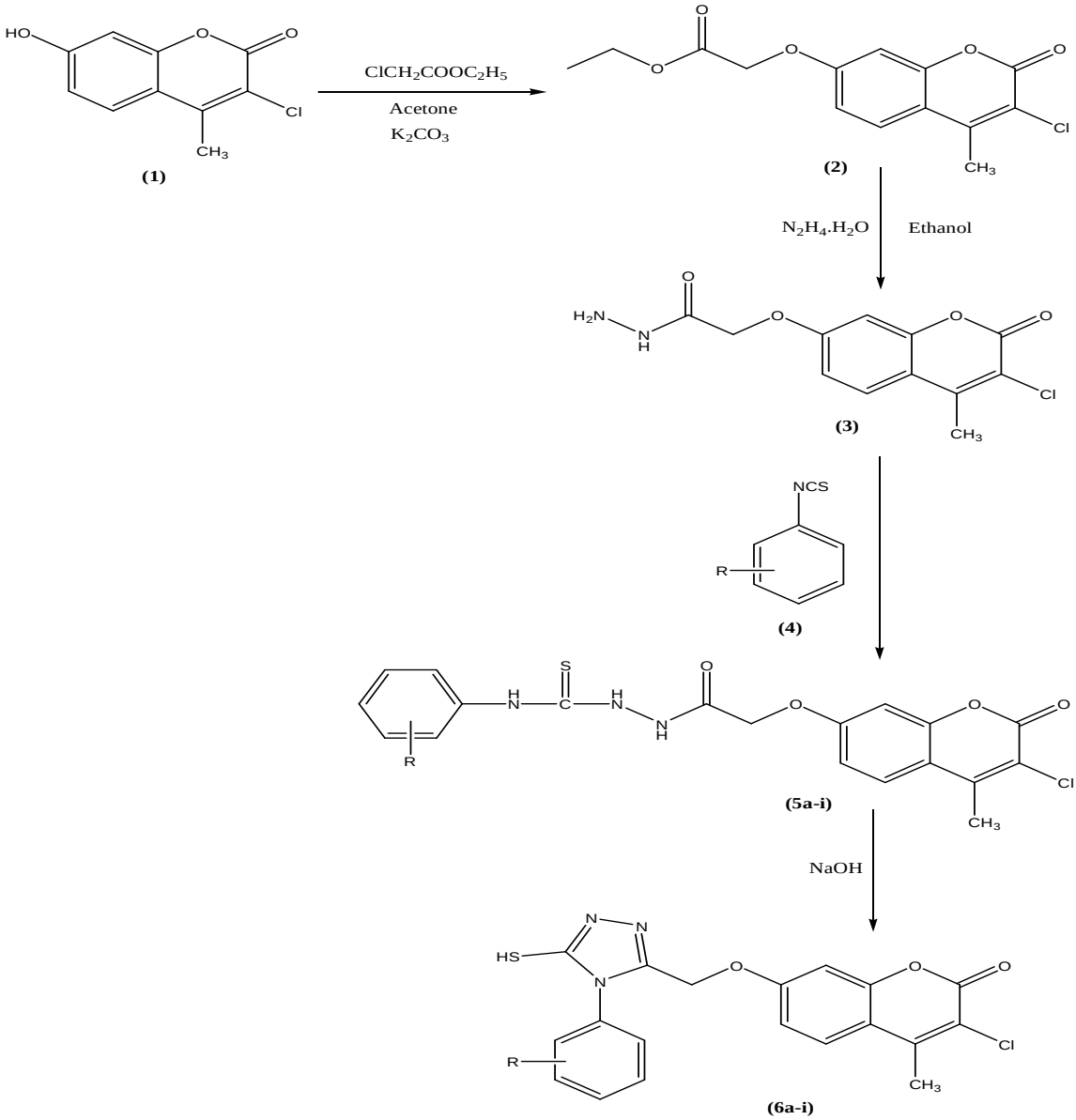
INTRODUCTION

The large number of biologically active molecules that contain heterocyclic rings has made development of different synthetic approaches for the synthesis of new heterocyclic systems. Heterocyclic compounds containing nitrogen paved the way for active research in pharmaceutical chemistry. Moreover these compounds constitute the core structure of a number of pharmacologically and biologically active interesting compounds. Triazole derivatives have attracted particular attention due to their diverse pharmacological activities. A wide range of therapeutic applications have been obtained using triazole system. They have been reported to possess antimicrobial [1-6], antiviral [7], anti-inflammatory [8-11], antidepressant [12], anticonvulsant [13,14], anticancer [15,16], analgesic [17], antihypertensive [18], antitubercular [19,20] activities. 1,2,4-Triazole is incorporated in a large number of drugs such as itraconazole, fluconazole, voriconazole, anastrozole (antifungal), ribavirin (antiviral), rizatriptan (antimigraine). On the other hand, coumarin and its derivatives represent one of the important class of heterocyclic compounds possessing a wide range of biological activities. These include antibacterial [21], antifungal [22,23], antitumor [24,25], herbicidal, anti-inflammatory [26] activities. The interesting biological activity of these coumarins made these compounds attractive in organic synthesis. Several synthetic strategies for their synthesis have already been developed. Coumarins are oxygen containing heterocycles widely distributed in nature. They are also used as additives in food, perfumes, agrochemicals, pharmaceuticals and in the preparation of insecticides, optical brighteners, dispersed fluorescent and dye lasers.

In view of the above increasing biological importance of these compounds and in continuation of our interest in the synthesis of biologically active heterocyclic compounds, it was aimed to synthesize some 1,2,4-triazole derivatives containing coumarin moiety and evaluated for antimicrobial activity.

MATERIALS AND METHODS

All the solvents and reagents were obtained from commercial sources and were used without further purification. Melting points were determined in open capillaries and were uncorrected. TLC was used to monitor the progress of all reactions and to check the purity of compounds. The IR spectra (KBr pellets) were recorded on Perkin-Elmer spectrum BX series FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer with TMS as an internal standard. Mass spectra were recorded on LCMS-2010A, SHIMADZU spectrometer.



Scheme-1: Synthetic route for the compounds (6a-i)

Compound	R
6a	H
6b	3-Cl
6c	4-Cl
6d	3-CH_3
6e	4-CH_3
6f	2-OCH_3
6g	3-OCH_3
6h	4-OCH_3
6i	4-NO_2

Procedure for the synthesis of ethyl 2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetate (2):
A mixture of 3-chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one **1** (0.05 mol), ethyl chloroacetate (0.05 mol) and potassium carbonate in dry acetone was refluxed for 30 hrs. The progress of the reaction was monitored by TLC up to completion. The reaction mixture was filtered hot and the solvent was distilled off from the filtrate. The solid thus obtained was purified by recrystallization from ethanol [27]. Yield: 92%; m.p: 110-112°C.

Procedure for the synthesis of 2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetohydrazide (3):
A mixture of compound **2** (0.05 mol) and hydrazine hydrate (0.05 mol) in ethanol was refluxed for 4-5 hrs. The reaction mixture was poured into ice cold water [28]. The solid product was collected by filtration, washed with water, dried and finally recrystallized from ethanol.

Yield: 92%; m.p: 185-187°C; ^{1}H NMR (400 MHz, DMSO): δ 2.60 (s, 3H, CH_3), 4.41 (s, 2H, NH_2), 4.69 (s, 2H, OCH_2), 7.09-7.87 (m, 3H, Ar-H), 9.46 (s, 1H, NH); LCMS (m/z): 283 $(M+H)^+$.

Procedure for the synthesis of 1-(2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetyl)-4-substituted phenyl thiosemicarbazides (5a-i):
An equimolar mixture of hydrazide **3** (0.01 mol) and substituted phenyl isothiocyanates **4** (0.01 mol) in ethanol was heated to reflux for 6 hrs. The reaction mixture was cooled to room temperature and the solid obtained was filtered, dried and recrystallized from ethanol.

Procedure for the synthesis of triazole derivatives (6a-i):
A mixture of substituted phenyl thiosemicarbazides **5a-i** (0.001 mol) and NaOH (8 %, 4 ml) in ethanol was refluxed for 7-8 hrs. After cooling, the reaction mixture was poured onto crushed ice and acidified with dilute HCl. The separated product was filtered, dried and recrystallized from ethanol.

Spectral data of synthesized compounds
Synthesis of 7-((5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6a):
Yield: 57%; m.p: 235-237°C; IR (KBr): 3030 (Ar C-H), 2667 (S-H), 1604 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.3 (s, 3H, CH_3), 5.49 (s, 2H, OCH_2), 7.08-7.86 (m, 8H, Ar-H), 9.72 (s, 1H, SH); LCMS (m/z): 400 $(M+H)^+$; Anal. Calcd for $C_{19}H_{14}ClN_3O_3S$: C, 57.07; H, 3.53; N, 10.51; Found: C, 57.09; H, 3.57; N, 10.54.

Synthesis of 7-((4-(3-chlorophenyl)-5-mercapto-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6b):
Yield: 54%; m.p: 212-214°C; IR (KBr): 3017 (Ar C-H), 2610 (S-H), 1611 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.2 (s, 3H, CH_3), 5.8 (s, 2H, OCH_2), 7.25-7.99 (m, 7H, Ar-H), 9.85 (s, 1H, SH); Anal. Calcd for $C_{19}H_{13}Cl_2N_3O_3S$: C, 52.55; H, 3.02; N, 9.68; Found: C, 52.52; H, 3.05; N, 9.72.

Synthesis of 7-((4-(4-chlorophenyl)-5-mercapto-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6c):
Yield: 52%; m.p: 205-207°C; IR (KBr): 2958 (Ar C-H), 2853 (S-H), 1616 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.6 (s, 3H, CH_3), 5.62 (s, 2H, OCH_2), 7.18-7.89 (m, 7H, Ar-H), 10.65 (s, 1H, SH); LCMS (m/z): 434 $(M+H)^+$; Anal. Calcd for $C_{19}H_{13}Cl_2N_3O_3S$: C, 52.55; H, 3.02; N, 9.68; Found: C, 52.59; H, 3.07; N, 9.71.

Synthesis of 7-((5-mercapto-4-*m*-tolyl-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6d):
Yield: 60%; m.p: 187-189°C; IR (KBr): 3055 (Ar C-H), 2592 (S-H), 1634 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.4 (s, 3H, CH_3), 3.1 (s, 3H, CH_3), 5.6 (s, 2H, OCH_2), 7.30-8.01 (m, 7H, Ar-H), 10.20 (s, 1H, SH); LCMS (m/z): 414 $(M+H)^+$; Anal. Calcd for $C_{20}H_{16}ClN_3O_3S$: C, 58.04; H, 3.90; N, 10.15; Found: C, 57.99; H, 3.86; N, 10.11.

Synthesis of 7-((5-mercapto-4-*p*-tolyl-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6e):
Yield: 56%; m.p: 202-204°C; IR (KBr): 3060 (Ar C-H), 2625 (S-H), 1625 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.1 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 4.85 (s, 2H, OCH_2), 7.15-7.85 (m, 7H, Ar-H), 9.93 (s, 1H, SH);

LCMS (m/z): 414 $(M+H)^+$; Anal. Calcd for $C_{20}H_{16}ClN_3O_3S$: C, 58.04; H, 3.90; N, 10.15; Found: C, 57.98; H, 3.84; N, 10.12.

Synthesis of 7-((5-mercapto-4-(2-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6f):
Yield: 59%; m.p: 241-243°C; IR (KBr): 3020 (Ar C-H), 2580 (S-H), 1618 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.4 (s, 3H, CH_3), 4.12 (s, 3H, OCH_3), 4.75 (s, 2H, OCH_2), 7.02-7.77 (m, 7H, Ar-H), 10.60 (s, 1H, SH); LCMS (m/z): 430 $(M+H)^+$; Anal. Calcd for $C_{20}H_{16}ClN_3O_4S$: C, 55.88; H, 3.75; N, 9.77; Found: C, 55.91; H, 3.81; N, 9.79.

Synthesis of 7-((5-mercapto-4-(3-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6g):
Yield: 52%; m.p: 219-221°C; IR (KBr): 3036 (Ar C-H), 2572 (S-H), 1630 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.8 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 4.62 (s, 2H, OCH_2), 7.18-7.89 (m, 7H, Ar-H), 9.18 (s, 1H, SH); Anal. Calcd for $C_{20}H_{16}ClN_3O_4S$: C, 55.88; H, 3.75; N, 9.77; Found: C, 55.84; H, 3.73; N, 9.83.

Synthesis of 7-((5-mercapto-4-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6h):
Yield: 52%; m.p: 228-230°C; IR (KBr): 3042 (Ar C-H), 2786 (S-H), 1598 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.7 (s, 3H, CH_3), 3.98 (s, 3H, OCH_3), 4.92 (s, 2H, OCH_2), 7.20-7.93 (m, 7H, Ar-H), 10.15 (s, 1H, SH); LCMS (m/z): 430 $(M+H)^+$; Anal. Calcd for $C_{20}H_{16}ClN_3O_4S$: C, 55.88; H, 3.75; N, 9.77; Found: C, 55.83; H, 3.78; N, 9.72.

Synthesis of 7-((5-mercapto-4-(4-nitrophenyl)-4*H*-1,2,4-triazol-3-yl)methoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (6i):
Yield: 51%; m.p: 192-194°C; IR (KBr): 3019 (Ar C-H), 2556 (S-H), 1621 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.5 (s, 3H, CH_3), 5.69 (s, 2H, OCH_2), 7.28-7.99 (m, 7H, Ar-H), 9.54 (s, 1H, SH); LCMS (m/z): 445 $(M+H)^+$; Anal. Calcd for $C_{19}H_{13}ClN_4O_5S$: C, 51.30; H, 2.95; N, 12.59; Found: C, 51.23; H, 2.99; N, 12.54.

Biological Evaluation
Antimicrobial activity
The antimicrobial activity of newly synthesized compounds was determined using agar well diffusion method [29]. All the compounds were tested invitro for their antibacterial activity against *Bacillus substilis* (Gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative bacteria) using nutrient agar medium **(Table 1)**. Antifungal activity was carried out against *Aspergillus niger* and *Aspergillus flavus* using potato dextrose agar medium **(Table 2)**. Streptomycin and Fluconazole were used as standard drugs for antibacterial and antifungal activity respectively. DMSO was used as solvent control. The compounds were tested at a concentration of 100 µg/ml against both bacterial and fungal strains.

Preparation of Nutrient agar medium
To prepare 1 lit of nutrient agar medium 3 g of beef extract, 3 g of peptone, 15 g of agar was used. The ingredients were accurately weighed and dissolved in a liter of distilled water before the addition of agar. The P^H of the medium was adjusted to 7.0 by adding few drops of 0.1 N NaOH/HCl. Later this medium was transferred to conical flasks and plugged with nonabsorbent cotton. Medium was then sterilized by autoclaving at 15lbs pressure for 15 mins, cooled and used for the study.

Preparation of Potato dextrose agar medium
200 g of potato slices were boiled with distilled water. Dextrose and agar were weighed separately. 20 g of dextrose was mixed with potato infusion. 20 g of agar was added as a solidifying agent. These constituents were mixed thoroughly and later this medium was transferred to conical flasks and plugged with nonabsorbent cotton. Medium was then sterilized by autoclaving at 15lbs pressure for 15 mins, cooled and used for the study.

Method of testing
The sterilized media was poured onto the sterilized petri dishes (20-25 ml, each petri dish) and allowed to solidify. Wells of 6 mm diameter was made in the solidified media with the help of sterile borer and solutions of the test compounds were added with the help of micropipette. A sterile swab was used to evenly distribute microbial

suspension over the surface of solidified media. The plates were incubated at 37°C for 24 hrs in case of antibacterial activity and 72 hrs at 25°C for antifungal activity. The zone of inhibition was measured in mm scale.

Table 1. Antibacterial activity of synthesized compounds (6a-i)

Compound	Zone of inhibition (mm) at 100 µg/ml Concentration		
	B.subtilis	***E.coli***	***P.aeruginosa***
6a	15	11	12
6b	10	14	11
6c	13	18	13
6d	16	14	17
6e	18	11	14
6f	20	16	18
6g	14	19	13
6h	21	22	19
6i	16	19	18
Streptomycin	24	24	22
Control (DMSO)	-	-	-

Table 2. Antifungal activity of synthesized compounds (6a-i)

Compound	Zone of inhibition(mm) at 100 µg/ml concentration	
	Aspergillus niger	***Aspergillus flavus***
6a	12	12
6b	11	15
6c	14	19
6d	16	11
6e	20	18
6f	13	22
6g	19	16
6h	23	21
6i	11	13
Fluconazole	25	25
Control (DMSO)	-	-

RESULTS AND DISCUSSION

Chemistry

The new triazole derivatives **(6a-i)** were prepared following the reaction sequences depicted in scheme-1. Reaction of compound **(1)** with ethyl chloroacetate and potassium carbonate in dry acetone afforded ethyl 2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetate **(2)**. Compound **(2)** on treatment with hydrazine hydrate in presence of ethanol yielded hydrazide compound **(3)** in good yields. The compound **(3)** on treatment with various substituted phenyl isothiocyanates **(4)** gave 1-(2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetyl)-4-substituted phenyl thiosemicarbazides **(5a-i)**. The thiosemicarbazides were cyclised with NaOH (8%) to triazole derivatives **(6a-i)**. The synthesized compounds were purified by recrystallization using appropriate solvents and some purified by column chromatography. The structures of all the newly synthesized compounds were established by ^{1}H NMR, IR and mass spectral data. The IR spectrum of compound **6c** exhibited absorption bands for S-H, C=N at 2853, 1616 cm^{-1} respectively. The ^{1}H NMR spectrum of compound **6c** showed a singlet at δ 2.6 due to CH_3 protons. Another singlet at δ 5.62 corresponds to OCH_2 protons. Aromatic protons appeared as multiplet at δ 7.18-7.89. A singlet at δ 10.65 indicated the presence of SH proton in the triazole ring, confirmed the structure of the compound. Further the spectral data of other synthesized compounds was also in agreement with their structures.

Biological activity

Antimicrobial studies

All the synthesized compounds were screened for antimicrobial activity by agar well diffusion method. The results showed that among the tested compounds **6h** exhibited very good activity against all the three bacteria. Compounds **6e, 6f** showed good activity against *Bacillus subtilis*, whereas compounds **6c, 6g, 6i** showed good activity against *Escherichia coli*. Compounds **6d, 6f, 6i** showed good activity against *Pseudomonas aeruginosa*. Compounds **6e, 6g, 6h** exhibited good activity against *Aspergillus niger* and compounds **6c, 6e, 6f, 6h** exhibited good activity against *Aspergillus flavus*.

CONCLUSION

This study reports the synthesis, characterization and antimicrobial activity of new series of triazole derivatives containing coumarin moiety. All the compounds were tested for antibacterial activity against *Bacillus subtilis* (Gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative bacteria) using streptomycin as standard drug and antifungal activity was performed against *Aspergillus niger* and *Aspergillus flavus* using fluconazole as standard drug. The screening results revealed that most of the compounds were found to exhibit significant antimicrobial activity.

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