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Synthesis and biological activity of 7-(2-(1*H*-1,2,4-triazol-1-yl)ethoxy)-4-(styryl/4-substituted styryl)-2*H*-chromen-2-one

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Incorporation of other hetero-compounds to parent coumarin increases its effectiveness towards its bioactivity. In view of this finding we have synthesized coumarin triazole derivatives. The key synthon used for this reaction pathway are 7-hydroxy-4-methyl-2*H*-chromen-2-one. This substituted coumarin has been refluxed with 1-bromo-2-chloroethane in presence of anhydrous K_2CO_3 to afford 7-(2-chloroethoxy)-4-methyl-2*H*-chromen-2-one, which has been condensed with triazole to yield 4-methyl coumarin triazole derivative by optimising solvent/base pair. 4-Methyl group of coumarin triazole derivative has been condensed with aromatic aldehydes to afford 7-(2-(1*H*-1,2,4-triazol-1-yl) ethoxy)-4-(styryl/4-substituted styryl)-2*H*-chromen-2-one **7a-e**. All the synthesized products are characterized using IR and, ^{1}H, ^{13}C NMR, mass spectroscopy and elemental analysis. Final synthesized compounds **7a-e** have been evaluated for their anti-bacterial and anti-fungal activity.

Keywords: Coumarin triazole, anti-bacterial, anti-fungal activities, solvent/base pair

2*H*-1-Benzopyran-2-ones as an elite class of naturally occurring compounds that possess promising therapeutic perspective due to diversity in their structural complication. It also belongs to the flavonoid class of plants, secondary metabolites, natural and synthetic origin, such as substituted coumarins to polysubstituted polycyclic/fused coumarins[1,2]. Warfarin is a 4-hydroxy coumarin moiety. Warfarin has been isolated from woodruff as well as from lavender and used to prevent clotting of blood in the veins, lungs or heart[3]. Hydroxycoumarins are known to be powerful chain-breaking anticoagulants and anti-oxidants which can prevent free radical injury by scavenging reactive oxygen species[4]. Methylcoumarin derivatives, have demonstrated growth-inhibitory activity against two human tumour cell lines, breast carcinoma MCF-7 and hepatocellular carcinoma HePG-2 [5,6]. Also, it has been reported as anti-proliferative activity in prostate cancer, malignant melanoma and metastatic renal cell carcinoma in clinical trials[5].

Some classes of triazole compounds are now available in the market or in the final stage of clinical trials[7]. Because of broad spectrum of triazole and improved safety profile, triazoles play a principal role in the treatment of systemic fungal infections. But the widespread use of these compounds has led to the development of resistance in recent years. As a consequence, development of more effective antifungal azoles with fewer adverse effects, is a main area of antifungal drug research[8]. 1,2,4-Triazole moieties have exhibited antibacterial[9], antifungal[10], antioxidant[11], antituberculosis, anti-HIV and antiviral[12] activities. Substituted coumarins are known to be biologically versatile compounds possessing several biological properties[13,14]. It has been reported that coumarin compounds bearing other heterocyclic systems possess a number of interesting biological activities such as anti-tumor[5], antimicrobial[15] and anticancer[16].

The insertion of other heterocyclic moiety either as substituent group or as a fused component into parent coumarin modifies the property of parent coumarin and converts it into a more useful product[17]. In light of these findings, we efficiently synthesized substituted coumarin and successfully introduce 1,2,4-triazol moiety in coumarin scaffold afford coumarin triazole derivatives. The resulting compounds have been analysed for their antimicrobial activitiy.

Results and Discusssion

Coumarin constitutes one of the major class of naturally occurring compounds and attention in its chemistry continues unabated because of its usefulness

in various fields. In view of its valuable biological importance and in continuation of our interest in the synthesis of heterocyclic compounds, it was aimed to synthesize some coumarin containing 1,2,4-triazole moiety and evaluate their antimicrobial activity.

The precursor compound **1** was prepared from resorcinol and ethyl acetoacetate in presence of iodine[18].The structure of compound **1** was confirmed by spectral analysis. FTIR spectra recorded broad peak at 2951-3500 cm^{-1} due to -OH stretching frequency and peak at 1681 cm^{-1} indicates C=O group of coumarin. This data is also supported by ^{1}H NMR spectra, one proton shows singlet at δ 10.31 ppm indicating presence of -OH group. Substituted coumarin **1** on condensation with 1-bromo-2-chloro ethane in acetonitrile in presence of anhydrous K_2CO_3 affords compound **3** in good yield. In ^{1}H NMR spectra of **3**, two protons shows triplets at δ 3.84 and 4.28 ppm indicating presence of $–CH_2Cl$ and $–CH_2O$- groups respectively. Compound **3** shows absence of –OH peak, indicating substitution of ethane chloride at that position.

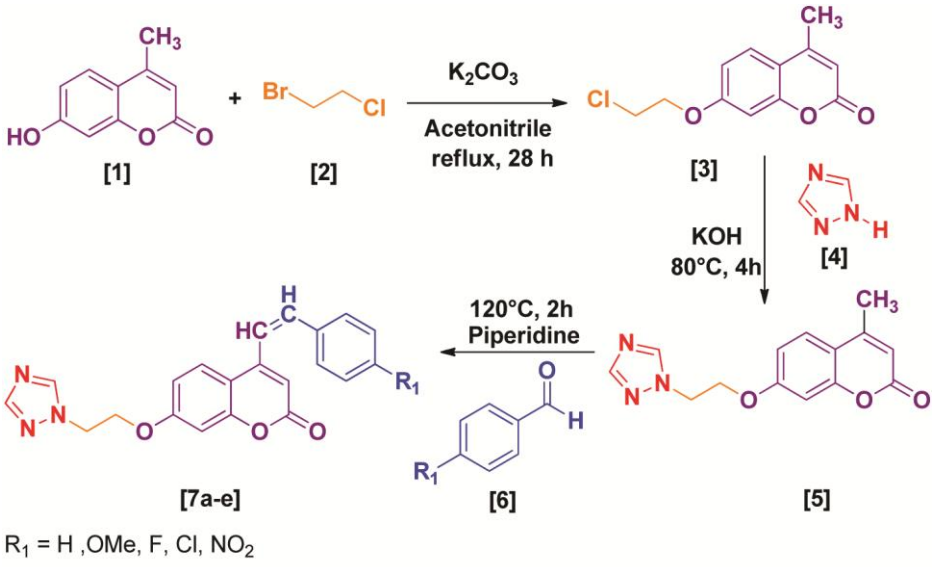
For the preparation of coumarin triazole derivative **5**, we used initially K_2CO_3/acetonitrile pair for condensation of triazole **4** with 4-methylcoumarin derivative **3** to afford a very low yield product (Table I, entry 1). Therefore, we optimized this step with different solvents and bases (Table I). In next attempt we used ethanol/KOH pair and reflux for 26 h but coumarin remains as unreacted, product was not formed (Table I, entry 2). Very poor yields were obtained in acetone/ K_2CO_3 and acetone/KOH, with extended reaction time (Table I, entries 3, 4). So, we turned towards the system dichloromethane/KOH and surprisingly, we got product within 4 h with shiny appearances. It revealed that dichloromethane/KOH was the most regimented solvent/base pair for the formation of coumarin triazole **5** (Scheme I).

Formation of compound **5** is fully supported by ^{1}H NMR spectra, one proton shows singlet at δ 7.91 and 8.49 ppm indicates two protons of triazole ring. Three protons shows singlet at δ 2.39 ppm indicating methyl group of coumarin. ^{13}C NMR also shows peak at δ 144.3 and 152.5 ppm indicating two carbons of triazole ring. The 4-methyl group of compound **5** undergo condensation with aromatic aldehydes in presence of piperidine at 120°C to afford 7-(2-(1*H*-1,2,4-triazol-1-yl)ethoxy)-4-(4-substitutedstyryl)-2*H*-chromen-2-one **7a-e** (Scheme I). ^{1}H NMR spectra of coumarin triazole derivative **7a-e** shows disappearances of peak at δ 2.39 ppm indicating condensation of $–CH_3$ group with aromatic aldehydes.

Table I — Optimization of solvents and basic condition for formation of compound[a] **5**

Entry	Solvent	Base	Time (h)	Yield[b] (%)
1	Acetonitrile	K_2CO_3	28	40
2	Ethanol	KOH	26	–
3	Acetone	K_2CO_3	30	21
4	Acetone	KOH	31	32
5	dichloromethane	KOH	04	78

[a] Reaction conditions: (3 mmol) compound 3, (6 mmol) triazole 4, (6 mmol) base and (3 mL) solvent, [b] Isolated Yield.



Scheme I — Synthesis of 7-(2-(1*H*-1,2,4-triazol-1-yl)ethoxy)-4-(4-substitutedstyryl)-2*H*-chromen-2-one **7a-e**

All the synthesized compounds have been thoroughly characterized by IR, ^{1}H and ^{13}C NMR, mass spectroscopy, elemental analysis and screened for their antimicrobial activities.

Antimicrobial Activity

Antimicrobial activity was evaluated by well diffusion method[19]. All the compounds were screened for their antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhi* and antifungal activity against *C. albicans* and *A. flavus*, using Streptomycin and Greseofulvin as the reference drug respectively. All these compounds showed about 44-51% inhibition against *Escherichia coli* and *Staphylococcus aureus* while they did not exhibit any activity against *Salmonella typhi* (Table II). Only three compounds are active against *Pseudomonas aeruginosa* (44-52%). Antifungal data revels that compounds **7c** and **7d** demonstrated the broadest spectrum of inhibitory activity (74.07% and 66.66% respectively) against *Aspergillus flavus.* Compound **7c**, **7d** and **7e** are inactive against *Candida albicans* while compound **7a** and **7b** were also inactive against *Aspergillus flavus*, other compounds show 40-44% activity compared to standard drug Greseofulvin. Zone of inhibition of antibacterial and antifungal activity is shown in Figure 1.

Materials and Methods

Melting points was measured in open capillaries and are uncorrected. Progress of reaction was monitored by silica gel-G coated TLC plates in *n*-hexane: ethyl acetate system (1:1). The spot was visualized by exposing dry plate in UV chamber. IR spectra were recorded on Schimadzu IR affinity model-1 spectrometer using KBr. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE II 400 NMR spectrometer at frequencies 400 MHz and 100 MHz respectively (SAIF, Panjab University Chandigarh). $CDCl_3$/DMSO-d_6 was used as solvents and TMS as internal standard. Mass spectra were recorded on WATERS Q-TOF MICROMASS (ESI-MS) (SAIF/CIL, Panjab University, Chandigarh). Elemental analysis was done from ThermoFinnigan, CHNS analyser (SAIF, Panjab University Chandigarh).

Experimental Section

Procedure for the preparation of 7-(2-chloroethoxy)-4-methyl-2*H*-chromen-2-one, 3

To a stirred solution of 7-hydroxy-4-methyl coumarin (17mmol) and potassium carbonate (10mmol) in 30 mL of acetonitrile, 1-bromo-2-chloro ethane (17mmol) was added and refluxed for 25 h (monitored by TLC). After removal of solvent, the content was extracted with dichloromethane (30 mL), washed with10% potassium hydroxide solution (2×20 mL) and dried over magnesium sulphate. The solvent was evaporated under reduced pressure and recrystallized with methanol to yieldcompound **3**.

7-(2-Chloroethoxy)-4-methyl-2*H*-chromen-2-one, 3: White solid, m.p. 168-169°C; Yield 75%; IR (KBr): 854 (C-Cl), 1610 (C=C), 1720 (C=O), 2958 (-CH aliphatic), 3074 (-CH aromatic) cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, $-CH_3$), 3.84 (t, 2H, J=5.6Hz, $-CH_2$-Cl), 4.28 (t, 2H, J=5.7Hz, $-CH_2$-O), 6.14 (s, 1H), 6.80 (d, 1H, J=2.3Hz), 6.88 (dd, 1H), 7.50 (d, 1H, J=8.8Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.6, 41.6, 68.3, 101.6, 112.2, 112.4, 114.0, 125.7, 152.4, 155.1, 161.0*;* MS: *m/z*$[M+H]^+$: 239.

Procedure for the preparation of 7-(2-(1*H*-1,2,4-triazol-1-yl)ethoxy)-4-methyl-2*H*-chromen-2-one, 5

A mixture of 7-(2-chloroethoxy)-4-methyl-2*H*-chromen-2-one **3**(12 mmol), triazole**4**(25 mmol) in dichloromethane (30 mL) and KOH (25 mmol) was taken in round bottom flask. The reaction mixture was refluxed for 4 hr. The progress of reaction was monitored by TLC. The cooled reaction mixture was then poured into ice-cold water with stirring. The

Table II — Results of *in vitro* antimicrobial activity of compounds **7a-e**

	Percent inhibition (Zone of inhibition in mm)					
	Antibacterial activity				Antifungal activity	
Entry	*E. coli*	*S.aureus*	*P.aeruginosa*	*S. typhi*	*C.albicans*	*A.flavus*
7a	57.69 (15)	51.85.(14)	48.00 (12)	–	44.00 (11)	–
7b	53.84 (14)	40.74 (11)	–	–	32.00 (08)	–
7c	53.84 (14)	51.85 (14)	44.00 (11)	–	–	74.07 (20)
7d	38.46 (10)	44.44 (12)	–	–	–	66.66 (18)
7e	46.15 (12)	44.44 (12)	52.00 (13)	–	–	44.44 (12)
Strepto-mycin	100 (26)	100 (27)	100 (25)	100 (25)	–	–
Greseo-fulvin	–	–	–	–	100 (25)	100 (27)

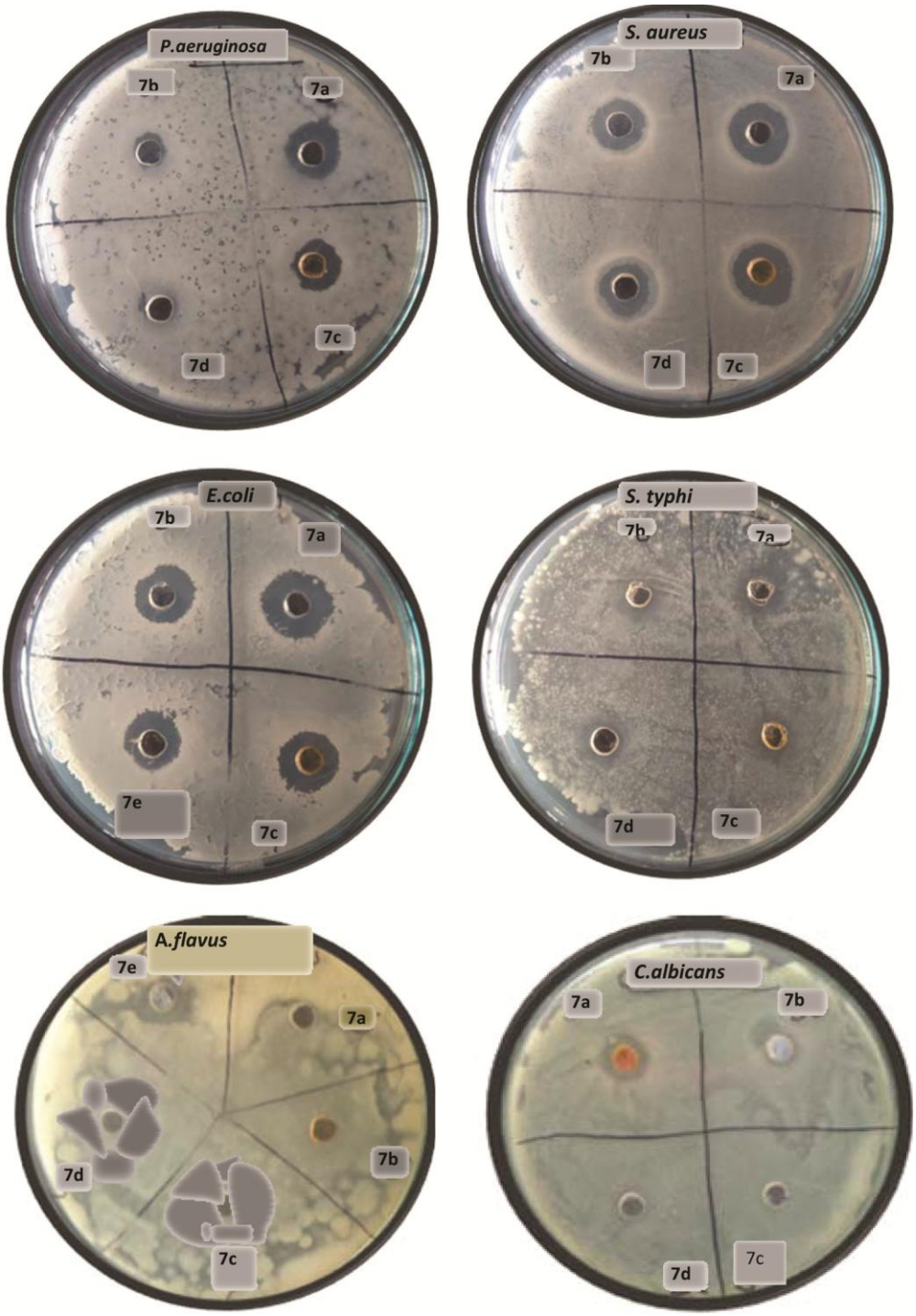


Figure 1 — Antimicrobial activity of compounds **7a-e**against selected micro-organisms

solid was filtered, washed with water several times(check by litmus) and recrystallized from MeOH to yield compound **5**.

7-(2-(1*H*-1,2,4-Triazol-1-yl)ethoxy)-4-methyl-2*H*-chromen-2-one, 5: White solid, m.p. 160-162°C; Yield 78%; IR (KBr): 1512 (C=C), 1614 (C=N), 1710 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H,-CH_3), 4.45 (t, 2H, -CH_2-Cl), 4.62 (t, 2H, -CH_2-O), 6.13 (s, 1H), 6.87 (d, 2H), 7.58 (d, 1H), 7.91 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.1, 48.1, 66.0, 101.2, 111.4,

112.0, 113.4, 125.9, 144.3, 152.5, 154.5, 159.9, 160.6; MS: *m/z*$[M+H]^+$: 272.

General procedure for the preparation of 7-(2-(1*H*-1,2,4-triazol-1-yl)ethoxy)-4-(4-substitutedstyryl)-2*H*-chromen-2-one, 7a-e

To a mixture of 7-(2-(1*H*-1,2,4-triazol-1-yl) ethoxy)-4-methyl-2*H*-chromen-2-one **5** (1.2 mmol), aromatic aldehyde (1.2 mmol),2-3 drops of piperidine was added and fused at 120°C for 2 h. After completion of reaction, add 10 mL cold water, stir well and poured over crushed ice. The precipitate was separated by filtration, washed with water and purified by recrystallization with methanol to obtain the final product **7a-e**.

7-(2-(1*H*-1,2,4-Triazol-1-yl)ethoxy)-4-styryl-2*H*-chromen-2-one, 7a: Yellow solid, m.p. 156-157°C; Yield 61%; IR (KBr): 1506 (C=C), 1616 (C=N), 1710 (C=O) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 4.45 (t, 2H), 4.63 (t, 2H), 6.14 (s, 1H), 6.80 (d, 4H), 6.88 (m, 4H), 7.50 (d, 2H), 7.90 (s, 1H), 8.45 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.1, 70.6, 103.2, 111.5, 112.5, 118.8, 125.0, 125.9, 128.6, 128.9, 135.4, 143.0, 150.5, 151.5, 154.6, 159.8 ; MS: *m/z*$[M+H]^+$: 360. Anal.calcd for $C_{21}H_{17}N_3O_3$: C, 70.15; H, 4.43; N, 11.70. Found: C, 69.84; H, 4.24; N, 11.46%.

7-(2-(1*H*-1,2,4-Triazol-1-yl)ethoxy)-4-(4-methoxystyryl)-2*H*-chromen-2-one, 7b: Dark yellow solid, m.p. 186-188°C; Yield 71%; IR (KBr): 1520 (C=C), 1614 (C=N), 1714 (C=O) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 4.33 (t, 2H), 4.59 (t, 2H), 6.11 (s, 1H), 6.70 (d, 2H), 6.86 (d, 4H), 7.02 (dd, 2H), 7.55 (d, 4H), 7.81 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 49.3, 54.6, 69.8, 111.5, 105.4, 105.8,114.5, 118.1, 125.7, 127.5, 130.5, 130.8, 131.3, 143.5, 150.1, 151.7, 157.3, 157.7; MS: *m/z*$[M+H]^+$: 390. Anal.calcd for $C_{22}H_{19}N_3O_4$: C, 67.87; H, 4.92; N, 10.80. Found: C, 67.64; H, 4.57; N, 10.69%.

7-(2-(1*H*-1,2,4-Triazol-1-yl)ethoxy)-4-(4-fluorostyryl)-2*H*-chromen-2-one, 7c: Red solid, m.p. 147-149°C; Yield 69%; IR (KBr): 1516 (C=C), 1608 (C=N), 1710 (C=O) cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$): δ 4.39 (t, 2H), 4.60 (t, 2H), 6.15 (s, 1H), 6.77-6.81 (m, 2H), 6.88 (d, 3H), 7.47 (d, 1H), 7.71 (d, 3H), 7.97 (s, 1H), 8.22 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.3, 68.4, 104.5, 111.3, 116.3,118.2, 125.6, 130.5, 130.9, 131.6, 143.6, 150.2, 151.3, 159.2, 159.8 ; MS: *m/z*$[M+H]^+$: 378. Anal.calcd for $C_{21}H_{16}N_3O_3F$: C, 66.85; H, 4.29; N, 11.15. Found: C, 66.92; H, 3.89; N, 11.24%.

7-(2-(1*H*-1,2,4-Triazol-1-yl)ethoxy)-4-(4-chlorostyryl)-2*H*-chromen-2-one, 7d: Yellow solid, m.p. 128-131°C; Yield 59%; IR (KBr): 1519 (C=C), 1611 (C=N), 1717 (C=O) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 4.48 (t, 2H), 4.58 (t, 2H), 6.18 (s, 1H), 6.88-6.94 (m, 4H), 7.25-7.45 (m, 3H), 7.61 (d, 2H), 7.94 (s, 1H), 8.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 49.9, 70.7, 111.0, 104.8, 119.7, 127.6, 125.8, 129.1, 130.5, 131.3, 133.1, 133.8, 143.4, 149.7, 150.4, 158.5 ; MS: *m/z*$[M]^+$393, $[M+2H]^+$ 395. Anal.calcd for $C_{21}H_{16}N_3O_3Cl$: C, 64.06; H, 4.09; N, 10.65. Found: C, 63.91; H, 3.83; N, 10.24%.

7-(2-(1*H*-1,2,4-Triazol-1-yl)ethoxy)-4-(4-nitrostyryl)-2*H*-chromen-2-one, 7e: Dark yellow solid, m.p. 148-150°C; Yield 69%; IR (KBr): 1554 (C=C), 1614 (C=N), 1710 (C=O) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 4.45 (t, 2H), 4.63 (t, 2H), 6.12 (s, 1H), 6.86-6.89 (m, 4H), 7.57-7.59 (m, 4H), 7.90 (s, 1H), 8.47 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 50.1, 69.7, 111.2, 104.2, 118.6, 123.5, 125.6, 129.1, 130.8, 131.7, 141.3, 142.7, 147.3, 150.8, 151.8, 158.7, 159.2; MS: *m/z*$[M+H]^+$405. Anal.calcd for $C_{21}H_{16}N_4O_5$: C, 62.37; H, 3.99; N, 13.84. Found: C, 62.41; H, 3.54; N, 13.54%.

Antimicrobial Assay

Antimicrobial activity evaluated by agar well diffusion method[19]. Initially agar medium and petri plates are sterilised in autoclave. Hot 12-15 mL of Muller Hinton agar medium was poured into previously sterilized and labelled petri plates. After diffusion these petri plates were swabbed with 100µL inocula of each test bacterium. Using sterile cork borer of 6 mm diameter, wells were bored into seeded agar plates and these were loaded with a 100µL volume with concentration of 100µg/mL of each compound constituted in dimethylsulphoxide (DMSO). The plates were incubated for 24 h at 37°C under aerobic conditions. After incubation, confluent bacterial growth was observed.

For antifungal activity evaluation Potato dextrose agar (PDA) medium was prepared. Apply same procedure and concentration as mention above and plates were incubated at room temperature for 48 h. After incubation, confluent fungal growth was observed and measured in mm. All the final compounds were tested and diameter of zone of inhibition was compared against the zone shown by standard drug Greseofulvin.

Conclusion

Plentiful source of potential drugs candidate in relation to its safety and efficacy is coumarin and coumarin related compounds. Coumarin and triazole both having biological activity in order to explore their activities, we have combined coumarin and triazole moiety together, by optimising solvent as well as basic condition. Condensation of methyl group of coumarin triazole derivatives with aromatic aldehydes yield final products **7a-e** in good to moderate yield. Anti-bacterial properties of the synthesized compounds have showed near about 50% inhibition against selected bacterial culture as compared to Streptomycin. Halogen substitution at fourth position show remarkable antifungal activity. The compounds **7c** having 4-flurophenyl and **7d** having 4-chlorophenyl substituent have been exhibited noticeable activity (74.07% and 66.66% respectively) towards *Aspergillus flavus*.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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