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SYNTHESIS, ANTIBACTERIAL AND ANTI- MYCOBACTERIAL ACTIVITY OF SOME NOVEL3-(6-PHENYL-[1,2,4]TRIAZOLO[3,4-B][1,3,4]THIADIAZOL-3-YL)-2H-CHROMEN-2-ONE

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Keywords:

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ABSTRACT: Several 3, 6-substituted - [1,2,4] Triazolo [3,4-b][1,3,4] Thiadiazole}-2H-Chromen-2-ones(5a-5j) have been synthesized from ethyl-2-oxo-2H-chromene-3-carboxylate (1) through a multistep reaction sequence. Compound 1 reacted with hydrazine hydrate in the presence of ethanol to give 2-oxo-2H-chromene-3-carbohydrazide (2) which on treatment with carbon disulphide and methanolic potassium hydroxide yielded corresponding potassium dithiocarbazates (3). They were then converted in to 3-(4-amino-5-sulfonyl-4H-1, 2, 4-triazol-3-yl)-2H-chromene-2-one (4) by refluxed with aqueous hydrazine hydrate. The title compounds 5a-5j was prepared by condensing 4 with various substituted aromatic acids in the presence of phosphorus oxy chloride. The synthesized compounds have been characterized by the physical (melting point and TLC) and spectral (IR, ^{1}NMR, ^{13}C NMR and MASS) data. All the compounds were screened for their antimicrobial and anti-tubercular activities. The antimicrobial activity was examined against four types of bacteria by cup plate method. Compounds 5b, 5c, 5g, 5e & 5a showed maximum activity at both 50 and 100µg/ml concentrations. Compounds 5a, 5b, 5c, 5d, 5e, 5g, 5h & 5i showed significant activity against ATCC 27294 H37 RV strain and showed MIC at 50µg/mL.

INTRODUCTION: The chemistry of coumarin derivatives continues to draw attention of synthetic organic chemists duo to their varied biological activities [1-5]. Along with this 1,2,4-triazole or 1, 3, 4-thiadiazole moieties are reported to show a broad spectrum of pharmacological properties and have received much attention during recent years.

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The synthesis of coumarins fused to triazolothiadiazole nucleus has attracted attention due to their diverse applications as antibacterial [6], antiviral [7], antifungal [8], analgesic [9] and anti-inflammatory agents [10]. Based upon review the biological importance of thiadiazole and triazole, we planned to synthesize novel derivatives of coumarinyl triazolothiadiazole and evaluated them for their potential as antibacterial and anti-tubercular agents.

Novel coumarinyl triazolothiadiazole derivatives were synthesized by multistep procedure which involved the formation of ethyl ester of coumarin (1) by Knoevenagel condensation of salicylaldehyde and diethyl malonate. This was

then hydrazinolysed by treating with hydrazine hydrate which reacted with Carbon disulfide and potassium hydroxide in ethanol to yield potassium dithiocarbazinate (3) which later cyclized to form 1,2,4-triazole moiety (4) by reacting with hydrazine hydrate. The resulted triazole further converted to triazolothiadiazole by the condensation with different aromatic acids in the presence of phosphorous oxy chloride. The synthesized compounds have been characterized by the physical (melting point and TLC) and spectral (IR, ^{1}NMR, MASS) data and evaluated them for their potential as antibacterial and anti-tubercular agents.

MATERIALS AND METHODS:

General:

Melting points were taken in open capillary tubes using Arson digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on BRUKER AV III, 500 MHz, IIS bangalore.^{13}C NMR from IICT Hyderabad. IR spectra were recorded on BRUKER Alpha FTIR Spectrometer with universal sampling model using KBr pellets.TLC was carried out using pre coated Silica gel plates. All the chemicals and solvents used were of LR grade and obtained from Sd-Fine and Merck.

Chemistry:

Novel coumarinyl triazolothiadiazole derivatives were synthesized by multistep procedure which involved the formation of ethyl ester of coumarin (1) by Knoevenagel condensation of salicylaldehyde and diethyl malonate. This was then hydrazinolysed by treating with hydrazine hydrate which reacted with Carbon disulfide and potassium hydroxide in ethanol to yield potassium dithiocarbazinate (3) which later cyclized to form 1,2,4-triazole moiety (4) by reacting with hydrazine hydrate. The resulted triazole further converted to triazolothiadiazole by the condensation with different aromatic acids in the presence of phosphorous oxy chloride. The synthesized compounds have been characterized by the physical (melting point and TLC) and spectral (IR, ^{1}NMR, MASS) data.

Synthesis:

General procedure for the synthesis of title compounds

Synthesis of ethyl-2-oxo-2H-chromene-3-carboxylate (1):

A mixture of salicylaldehyde (0.05 mole, 6ml) and diethyl malonate (0.05 mole, 7ml) was treated with piperidine (0.02 mole, 2ml) and stirred for 30min, at room temperature. Then it was acidified with 20% HCl to neutral. The precipitated compound was filtered, washed with small portions of cold water and dried. The product was purified by Recrystalization from ethanol.

Synthesis of 2- oxo - 2H – chromene - 3-carbohydrazide (2):

A mixture of ethyl-2-oxo-2H-chromene-3-carboxylate [1] (0.01 mole, 1.9 gm) and hydrazine hydrate (0.02 mol, 2ml) in absolute ethanol was refluxed for 5-6 hr. Completion of the reaction was judged by TLC. After cooling, the reaction mixture was added to the cool water. The solid, thus separated, was collected by filtration, dried and recrystalized from ethanol.

Synthesis of 2- oxo - 2H - chromene-3-carbohydrazide potassium dithio carbazinate (3):

2-oxo-2H-chromene-3-carbohydrazide (2) was added to KOH (0.015mole) solution which is dissolved in absolute ethanol in cool condition. To this, carbon disulfide (0.015M) was added in small portions with constant stirring. The reaction was agitated continuously for a period of 32-35 hrs and product was isolated from diethyl ether. The precipitated potassium dithiocarbazinate was collected by filtration. The precipitate was further washed with anhydrous ether and dried.

Synthesis of 3-(4-amino-5-sulfonyl-4H-1, 2, 4-triazol-3-yl)-2H-chromene-2-one (4):

2-oxo-2H-chromene-3-carbohydrazide potassium dithio carbazinate [3] (0.01mole) and hydrazine hydrate (0.02mmol) was reflux for 8-12 hrs in oil bath. The reaction mixture was poured into crushed ice and treated with concentrated HCl. The product was filtered and dried. Recrystalization was done by using ethanol.

Synthesis of Coumarinyl triazolothiadiazole derivatives (5a-5j):

An equimolar mixture of 3-(4-amino-5-sulfonyl-4H-1, 2, 4-triazol-3-yl)- 2H-chromene-2-one [4]

(0.001M) and substituted aromatic acids (0.001M) in dry Phosphorous oxy chloride (15ml) was refluxed for 10-12 hrs. Completion of the reaction was judged by TLC. The reaction mixture was cooled to room temperature and then gradually poured onto crushed ice with constant stirring. The solid thus separated was filtered, washed with water and recrystalized from ethanol.

5a: 3{6-phenyl [1,2,4] Triazolo [3, 4-b] [1,3,4] Thiadiazole}-2H-Chromen-2-one

IR (KBr) (cm-^{1}): 2921.68 (Ar-CH),1608.72 (C=O), 1460.36 (C-N), 1230.14 (N-N=C), 1009.29 (C-S); **^{1}H NMR (DMSO), δ ppm** : 7.29-7.31 (2H, m, Coumarin ring), 7.54-7.62 (2H, m, Coumarin ring), 7.69-7.93 (4H, m, Ar-H), 8.05-8.07 (1H, s, Coumarin ring) ; **^{13}C NMR(δ ppm):** 90. 10, 116, 118, 130, 134,140,142,148,164,168; GC-**MS (m/z,%)** : 346 $(M+H)^{+}$; Anal. Calcd for $C_{18}H_{10}N_4O_2S$: C, 62.42; H, 2.91; O, 9.24; N, 16.18; S, 9.26. Found: C, 62.36; H, 2.89; O, 9.20; N, 16.14; S, 9.22.

5b: 3{6-(4-chlorophenyl) [1,2,4]Triazolo [3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2918.39 (Ar-CH Str), 1594.50 (C=O Str), 482.97 (C-N Str), 1013.19(C-S Str), 833.13 (C-Cl Str); **^{1}H NMR (DMSO), δ ppm** : 6.93-6.99 (2H, m, Coumarin ring), 7.01-7.04 (3H, m, Coumarin ring), 7.37-7.45 (4H, m, Aromatic ring), 7.77-7.79 (1H, s, Coumarin ring); Anal. Calcd for C18H9N4O2S: C, 56.77; H, 2.38; Cl, 9.31; N, 14.71; O, 8.40; S, 8.42. Found: C, 56.64; H, 2.31; Cl, 9.26; N, 14.69; O, 8.37; S, 8.38.

5c: 3 {6-(4-nitrophenyl) [1,2,4] Triazolo [3,4-b] [1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2955.48, 2920.61 (Ar-CH Str), 1694.61(C=O Str), 1463.35 (C-N Str), 1606.30(C=N Str), 1013.14(C-S Str), 1349.49(Ar NO_2); **^{1}H NMR (DMSO), δ ppm**: 6.94-6.99 (2H,m, Coumarin ring),7.39-7.43 (1H,m, Coumarin ring), 7.69-7.71 (1H,m, Coumarin ring), 8.16-8.40 (4H, m, Aromatic ring), 9.0 (1H, s, Coumarin ring). Anal. Calcd for $C_{18}H_9N_5O_4S$: C, 55.24; H, 2.32; N, 17.89; O, 16.35; S, 8.19. Found: C, 55.20; H, 2.31; N, 17.84; O, 16.30; S, 8.15.

5d: 3{6-(4-methoxyphenyl)[1,2,4] Triazolo[3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2924.52 (Ar-CH Str), 1606.54 (C=O Str), 494.53(C-N Str), 1259.87(N-N=C Str), 025.50(C-S Str). **^{1}H NMR (DMSO), δ ppm**: 6.99-7.0 (4H, m, Aromatic ring), 7.02-7.16 (3H, m, Aromatic ring), 7.89-8.0 (4H,m, Coumarin ring), 8.25 (1H, s, Coumarin ring), 3.75-3.85 (3H, s, OCH_3). Anal. Calcd for $C_{19}H_{12}N_4O_3S$: C, 60.63; H, 3.21; N, 14.89; O, 12.75; S, 8.52. Found: C, 60.59; H, 3.19; N, 14.87; O, 12.73; S, 8.49.

5e: 3 {6-(4-aminophenyl) [1,2,4] Triazolo [3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 3172.54 (Ar-CH Str), 3521.99(NH_2 Str), 1696.59 (C=O Str), 1661.52 (C=N Str), 1486.80 (C-N Str), 1255.52 (N-N=C Str), 1032.63(C-S Str). Anal. Calcd for $C_{18}H_{11}N_5O_2S$: C, 59.82; H, 3.07; N, 19.38; O, 8.85; S, 8.87. Found: C, 59.80; H, 3.04; N, 19.36; O, 8.81; S, 8.86.

5f: 3{6-(4-hydroxyphenyl) [1,2,4] Triazolo[3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2922.08 (Ar-CH Str), 3384.31(OH Str), 1604.34 (C=O Str), 1508.89(C-N Str), 1240.10(N-N=C Str), 1087.41 (C-S Str). **^{1}H NMR (DMSO), δ ppm**: 6.84 (1H,m, Coumarin ring), 6.92-6.98 (1H,m, Coumarin ring), 7.11-7.44 (2H,m, Coumarin ring), 7.94-8.02 (2H, m, Aromatic ring), 8.1-8.26 (2H, m, Aromatic ring), 7.7-7.81 (1H, s, Coumarin ring), 11.31 (1H, s, OH). Anal. Calcd for $C_{18}H_{10}N_4O_3S$: C, 59.66; H, 2.78; N, 15.46; O, 13.25; S, 8.85. Found: C, 59.64; H, 2.74; N, 15.43; O, 13.24; S, 8.82.

5g: 3{6-(3,5-dinitrophenyl) [1,2,4] Triazolo[3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2921.68 (Ar-CH Str), 1608.72 (C=O Str), 1460.36 (C-N Str), 1230.14(N-N=C Str), 1009.29(C-S Str). **^{1}H NMR** (500MHz, DMSO-d6): δ 9.04 (2H, s, Aromatic ring), 8.80 (1H, s, Aromatic ring), 8.42-8.72 (4H, m, Coumarin ring), 7.82 (1H, s, Coumarin ring). Anal. Calcd for $C_{18}H_8N_6O_6S$: C, 49.54; H, 1.85; N, 19.26; O, 22.00; S, 7.35. Found: C, 49.51; H, 1.82; N, 19.24; O, 19.98; S, 7.34.

5h: 3 {6-(3-nitrophenyl) [1,2,4] Triazolo [3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2921.68 (Ar-CH Str), 1608.72 (C=O Str), 1460.36 (C-N Str), 1230.14(N-N=C Str), 1009.29(C-S Str). Anal. Calcd for

$C_{18}H_9N_5O_4S$: C, 55.24; H, 2.32; N, 17.89; O, 16.35; S, 8.19. Found: C, 55.23; H, 2.30; N, 17.87; O, 16.33; S, 8.18.

5i: 3{6-(2-hydroxyphenyl) [1,2,4] Triazolo[3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2919.78, 2850.40 (Ar-CH Str), 3422.87 (OH Str), 1607.13 (C=O Str), 1462.67 (C-N Str), 1021.40 (C-S Str). Anal. Calcd for $C_{18}H_{10}N_4O_3S$: C, 59.66; H, 2.78; N, 15.46; O, 13.25; S, 8.85. Found: C, 59.65; H, 2.76; N, 15.44; O, 13.23; S, 8.84.

5j: 3{6-(2-acetylphenyl) [1,2,4] Triazolo [3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one:

IR (KBr) cm^{-1}: 2923.89 (Ar-CH Str), 1739.46 ($OCOCH_3$ Str), 1602.59 (C=O Str), 1493.59 (C-N Str), 1060.97 (C-S Str). Anal. Calcd for $C_{20}H_{12}N_4O_4S$: C, 59.40; H, 2.99; N, 13.85; O, 15.83; S, 7.93. Found: C, 59.38; H, 2.98; N, 13.83; O, 15.82; S, 7.90.

Pharmacological evaluation:

Antibacterial activity:

All the synthesized compounds were tested for their *in vitro* antimicrobial activity. By using four types of bacteria *E. coli, P. aerugenosa, B. subtili, S. aureus* by agar cup plate method. Streptomycin was used as reference standard for antimicrobial studies. The test compounds were tested at a concentration of 100µg/ml and 200µg/ml. The average zone diameter of the plates was measured and recorded. The results of antibacterial screening studies are reported in **Table 1**.

TABLE 1: ANTIMICROBIAL STUDY OF COUMARINYL TRIAZOLOTHIADIAZOLE (5a-5j)

Compound code	Zone of inhibition							
	S.aureus		***B.subtilis***		***E.coli***		***P.aeruginosa***	
	50µg/ml	**100µg/ml**	**50µg/ml**	**100µg/ml**	**50µg/ml**	**100µg/ml**	**50µg/ml**	**100µg/ml**
5a	13	15	12	16	10	11	13	15
5b	18	23	17	20	19	22	14	19
5c	18	20	18	20	18	22	15	19
5d	12	13	9	11	16	20	15	20
5e	16	18	12	13	18	22	15	19
5f	2	6	–	–	6	6	–	–
5g	18	20	16	19	16	20	13	15
5h	–	1	4	6	–	–	–	–
5i	–	–	9	11	3	4	8	12
5j	12	13	8	9	–	–	11	11
Standard	20	24	18	23	20	25	19	21
Control	–	–	–	–	–	–	–	–

Anti-tubercular activity:

All newly synthesized compounds were screened for their anti-mycobacterial activity. Compounds was taken at concentration of 100 - 0.8 µg/ml against *M. tuberculosis* ATCC 27294 H37 Rv in middle brook 7H9 broth medium by serial dilution method was carried out in department of microbiology, Belgaum institute of medical sciences, Belgaum. Compounds were screened at 100 – 0.8 µg/ml concentration in a broth micro-dilution assay with Alamar blue called as microplate Alamar blue assay [11, 12] against M. tuberculosis ATCC 27294 H37 Rv to determine MIC. The results were comparisons with Pyrazinamide and streptomycin as the reference drug and the results are listed in **Table 2**. To prepare inoculum, microorganism was grown in middle brook 7H9 broth medium until a bacterial density corresponding to 0.5 McFarland turbidity standards. 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.

The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate. The final drug concentrations tested were 0.8-100.0 µg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. After this time, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and

pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

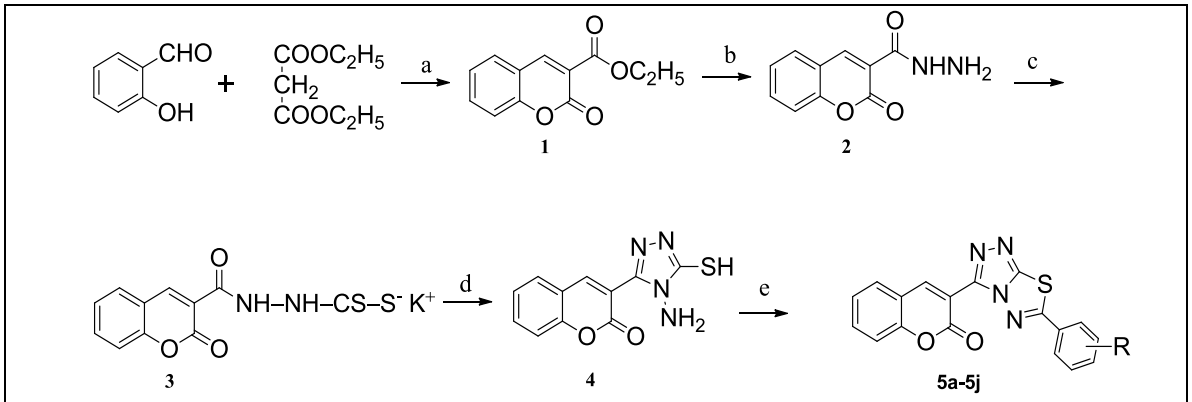
TABLE 2: ANTI-TUBERCULAR ACTIVITY OF COUMARINYL TRIAZOLOTHIADIAZOLE (5a-5j)

Compound code	MIC, µg/ml
5c	50
5d	50
5e	50
5f	100
5g	50
5h	50
5i	50
5j	50
Pyrazinamide	3.125
Streptomycin	6.25

RESULTS AND DISCUSSION:

A facile and efficient approach for the synthesis of title compounds has been developed. A series of titled compounds, i.e., coumarinyl triazolothiadiazoles have been synthesized using appropriate synthetic procedures, In step 1,ethyl ester of coumarin (1) has been synthesized from salicylaldehyde and diethyl malonate by Knoevenagel condensation. In step 2, the formed ethyl ester of coumarin was hydrazinolysed to give hydrazides (2) by treating with hydrazine hydrate, which reacted with carbon disulphide and potassium hydroxide in ethanol to yield potassium dithiocarbazinate (3), which later cyclized to form 1, 2, 4-triazole (4) by reacting with hydrazine hydrate. The resulted triazole moiety further converted to triazolothiadiazoles by the condensation with different aromatic acids in the presence of phosphorous oxy chloride.

Structures were confirmed on the basis of spectral and analytical data. The IR spectra of compound 5a have showed absorption bands at 2921.68 cm^{-1} for Ar-CH Stretching, 1608.72 cm^{-1} for C=O Stretching, 1460.36 cm^{-1} for C-N Stretching, 1230.14 cm^{-1} for N-N=C Stretching, 1009.29 cm^{-1} for C-S Stretching. The ^{1}NMR (δ ppm) showed a singlet at δ 8.24 corresponding to the proton 4th position of Coumarin ring, a multiplet at δ 7.86-8.16 corresponds to 5 protons of the aromatic ring, another multiplet at δ 7.45-7.68 corresponds to the 4 protons of coumarin ring. MASS Spectra exhibited Molecular ion Peak at 346.2. The IR spectra of compound 5b have showed absorption bands at 2918.39 cm^{-1} for Ar-CH Str, 1594.50 cm^{-1} for C=O Str, 1482.97 cm^{-1} for C-N Str, 1013.19 cm^{-1} for C-S Str, 833.13 cm^{-1} for C-Cl Str. The ^{1}NMR (**δ ppm**) showed a singlet at δ 8.32 corresponding to the proton 4th position of Coumarin ring, a multiplet at δ 7.75-8.07 corresponds to 5 protons of the aromatic ring, another multiplet at δ 7.42-7.67 corresponds to the 4 protons of coumarin ring.



Scheme: Conventional synthetic route for 3{6-phenyl[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazole}-2H-Chromen-2-one.

The preliminary in vitro antibacterial and anti-tubercular screening of synthesized compounds revealed that, compounds 5b,5c,5g,5e, & 5a showed maximum activity at both concentrations, where as compounds 5d & 5j showed moderate activity. The results of anti-tubercular activity

revealed that compounds5a,5b,5c,5d,5e,5g,5h & 5i has significant activity against ATCC 27294 H37 Rv strain and showed MIC at 50µg/ml. The overall outcome of these results revealed that triazolothiadiazoles ring is a satisfactory backbone for antibacterial and anti-tubercular activities. The azole derivatives have shown interesting antimicrobial and anti-tubercular activity, inhibiting the bacteria by blocking lipid biosynthesis and additional mechanisms, thus by hybridization of 1,2,4-triazole and 1,3,4-thiadiazole ring enhance the activity.

CONCLUSION: A series of titled compounds, i.e., coumarinyl triazolothiadiazoles have been synthesized using appropriate synthetic procedure. The newly synthesized compounds purity was ascertained by melting point determination and appearance of single spot in the TLC. The structure of the synthesized compounds was confirmed by different spectrophotometrical methods, IR, ^{1}HNMR, ^{13}CNMR and MASS. Biological activity of synthesized compounds results revealed that, all the compounds showed significant antibacterial and anti-tubercular activities.

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