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RESEARCH ARTICLE

Synthesis of Coumarin-benzotriazole Hybrids and Evaluation of their Anti-tubercular Activity

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Abstract: ***Background***: Tuberculosis is one of the top ranked airborne infectious diseases caused by the bacillus *Mycobacterium tuberculosis* with high mortality rate from a single infectious agent. In the present article, we aimed to synthesize oxadiazole-coumarin-triazole based small molecules and evaluate for their possible anti-mycobacterial activity.

Method: Herein, we describe the facile synthesis of 5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol-tethered substituted 4-(bromomethyl)-7-methyl-2H-chromen-2-one derivatives and evaluated for their anti-mycobacterial activity against H37Rv strain of *M. tuberculosis*. We also evaluated the cytotoxic effect of new compounds on normal cells.

Results: Among the 14 novel oxadiazole-coumarin-triazole derivatives, 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-6-methoxy-2H-chromen-2-one (5f) displayed good antimycobacterial activity towards *M. tuberculosis* with an MIC value of 15.5 µM. Pyrazinamide was used as reference drug. Our investigation also revealed that, 5f is not cytotoxic to normal cells.

Conclusion: In summary, the findings suggested that novel 1,3,4-oxadiazole coumarin-triazole hybrids are promising antimycobacterial agents against *M. tuberculosis.*

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1. INTRODUCTION

Tuberculosis is an infectious and airborne bacterial disease caused by the bacillus *Mycobacterium tuberculosis* (Mtb) and one of the leading killers globally [1]. According to the 2014's report of World Health Organization, 9.6 million new TB cases were identified with the mortality rate of 1.5 million deaths worldwide [2]. Based on the affected organ, TB is broadly classified into pulmonary TB (lungs) and extrapulmonary TB (pleura, central nervous system, bones, lymphatic system) [3]. Several studies have indicated the development of drug-resistance by Mtb against first-line and second-line drugs including isoniazid, rifampicin, ethambutol and pyrazinamide is making the regimen complicated [4]. In multidrug-resistance TB (MDR-TB), Mtb do not respond to first-line drugs. On the other hand, in extensively drug-resistance TB (XDR-TB), the bacillus no longer responds to the most effective second-line anti-TB drugs [2]. Development of resistance by mycobacterial strains against the conventional anti-TB agents demands the discovery of new therapeutic agents which can effectively target Mtb.

Benzotriazoles have been considered as privileged structures in medicinal chemistry because of their diverse pharmacological properties including anticancer, antitubercular, antibacterial, antiviral, antiparasitic and antioxidants [5]. Evidently, benzotriazole-oxazolidinone conjugates have displayed excellent anti-mycobacterial activity against drug-

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resistant clinical isolates of Mtb with the MIC value superior to isoniazid [6]. In an another study, benzotriazolyl-acrylonitriles showed promising growth inhibition of Mtb up to 99% at lower micromolar concentrations [7]. In addition, azetidinone derivatives of benzotriazole inhibited the growth of H37Rv Mtb strain effectively with the MIC values comparable to ethambutol [8]. Several studies have reported the anti-TB activity of benzotriazoles conjugated with broad range of heterocycles [9]. Moreover, high-throughput screening of 87,000 small compounds presented oxadiazoles as potent agents against M. tuberculosis [10]. Recently, oxadiazole conjugated with benzimidazole reported to possess good anti-tubercular activity against H37Rv Mtb strain [11]. On the other hand, coumarins are also an important pharmacophore that tend to have broad range medicinal properties including antitubercular activity. Synthesis of 7-amino-4-methylcoumarin derivatives and evaluation for their anti-TB activity revealed that the new compounds significantly inhibited the growth of H37Rv and MDR clinical isolates. Notably, 7-amino-4-methylcoumarin displayed synergetic effect with first-line drugs such as isoniazid and rifampicin [12]. Therefore, it is expected to have additive effect against Mtb when these pharmacophores are hybridised. In continuation of our research to design synthetic small molecules [13], in the present investigation, we attempted to synthesize benzotriazole-coumarins hybrids and evaluated for their anti-tubercular efficacy against Mtb H37Rv strain. The hypothetical interaction model of two pharmacophores is represented as Fig. (**1**). Herein, we describe an efficient and facile synthesis of coumarin–benzotriazole hybrids (**5a-n**) under three different conditions.

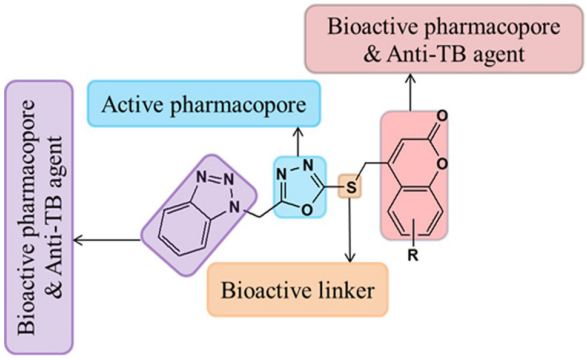


Fig. (1). Design strategy for key structural requirements of benzotriazole-coumarin hybrids to enhance antitubercular activity (hypothetical interaction model).

2. EXPERIMENTAL SECTION

Melting points were determined with open capillary method on a Buchi apparatus. IR spectra were recorded on a Nicolet 5700 Fourier transform infrared spectroscopy instrument (Nicolet, Madison, WI, USA) as KBr discs. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer using $CDCl_3$ as solvent and TMS was used as internal standard. All chemical shifts were reported as d values (ppm). Mass spectra were recorded using Shimadzu GCMS QP2010S. The elemental analyses were carried out using Hereaus CHN rapid analyzer.

2.1. General Procedure for the Preparation of 4-((5-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-2,5-dihydro-1,3,4-oxadiazol-2-ylthio)methyl)-7-methyl-2*H*-chromen-2-ones (5a-n)

2.1.1. Reaction Condition A

Sodium metal (1 mmol) was dissolved in absolute ethanol (25 ml) followed by treatment with 5-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol and substituted 4-bromo methyl coumarins (1 mmol). The mixture is allowed to reflux for 16-18 h and the progress of reaction was monitored by TLC. Thereafter, reaction mass was poured into crushed ice. The solid mass obtained was filtered, dried and recrystallized using methanol-chloroform mixture (3:1) to get the final compound.

2.1.2. Reaction Condition B

5-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (1 mmol) was dissolved in absolute ethanol (25 ml) followed by the addition of dried K_2CO_3 (1.5 eq) and stirred for 1 h. To this solution, substituted 4-bromo methyl coumarins (1 mmol) were added and refluxed for 18-24 h. The completion of reaction was monitored by TLC. Thereafter, reaction mass was poured into crushed ice. The solid mass obtained was filtered, dried and recrystallized using ethanol-chloroform mixture (3:1) to get the final compound.

2.1.3. Reaction Condition C

5-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (1 mmol), N,N-Diisopropylethylamine (5-10 mol%) and substituted 4-bromo methyl coumarins (1 mmol) were mixed in acetone : MDC (80:20; 10 ml) solvent in round bottom flask and stirred at room temperature for 30-36 h under nitrogen atmosphere. The completion of reaction was monitored by TLC. Thereafter, solvent was removed under vacuo and the residue was extracted twice with dichloromethane and dried over anhydrous $MgSO_4$. The solvent was evaporated and the compound was recrystallized from ethanol to get the final compound.

2.1.3.1. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-7-methyl-2H-chromen-2-one (5a)

Light pale yellow solid crystals; Mp 126-127 °C; IR (K-Br) (v_{max}/cm^{-1}): 1711(C=O of coumarin), 1614 (C=N of triazole) cm^{-1}, 949 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.38 (s, 3H, CH_3), 4.70 (s, 2H,-CH_2), 6.38 (s, 2H,CH_2), 6.52 (s, 1H), 7.32 (d, 1H, *J* = 8.4 Hz), 7.44-7.50 (m, 2H, Ar-H), 7.59 (t, 1H, *J* = 7.5 Hz), 7.76 (s, 1H, Ar-H), 7.86 (d, 1H, Ar-H, *J* = 8.4 Hz), 8.09 (d, 1H, Ar-H, *J* = 8.4 Hz), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 20.36, 32.00 , 41.97, 115.59, 115.66, 117.12, 117.94, 119.32, 124.37, 124.88, 127.12, 127.95, 132.87, 133.12, 133.78, 145.12, 149.68, 151.38, 159.42, 163.60, ESI-MS: 406.43 $[M]^+$: Anal. calcd for $C_{20}H_{15}N_5O_3S$: C, 59.25; H, 3.73; N, 17.27%. Found: C, 59.21; H, 3.76; N, 17.23%.

2.1.3.2. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-7,8-dimethyl-2H-chromen-2-one (5b)

Off-white solid; Mp 141-142 °C; IR (KBr) (v_{max}/cm^{-1}): 1704 (C=O of coumarin), 1611 (C=N of triazole) cm^{-1}, 971 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.31 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 4.83 (s, 2H, CH_2,), 6.40 (s, 2H, CH_2), 6.50 (s, 1H, Ar-H), 7.07 (d, 1H, Ar-H), 7.43-7.49 (m, 1H, Ar- H), 7.60-7.61 (m, 1H, Ar-H), 7.88 (d, 1H, *J* = 10.8 Hz, Ar-H), 8.09 (d, 1H, *J* = 5.6 Hz, Ar-H),^{13}C NMR (300 MHz, $CDCl_3$, δ ppm):): 20.56, 23.27, 36.13, 41.98, 110.44, 114.61, 115.71, 117.94, 119.31, 124.37, 127.12, 127.94, 129.89, 132.88, 135.84, 142.21, 150.72, 154.81, 159.00, 163.05; ESI-MS: 421.12[M+2]$^+$: Anal calcd for $C_{21}H_{17}N_5O_3S$: C, 60.13; H, 4.09; N, 16.70%. Found: C, 60.19; H, 4.16; N, 16.56%.

2.1.3.3. Ethyl 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-2-oxo-2H-chromen-7-ylcarbamate (5c)

Off-white colour powder; Mp 129-130°C; IR (KBr) (v_{max}/cm^{-1}): 3284 (NH), 1703 (C=O of coumarin), 1686 (NHC=O); 1615 (C=N of triazole) cm^{-1}, 946 (C-S),; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.28 (t, 3H, *J* = 6.9 Hz, CH_3), 4.18 (q, 2H, *J* = 7.2 Hz, CH_2), 4.65 (s, 2H, CH_2, C4), 6.38 (s, 2H, CH_2), 6.40 (s, 2H, CH_2), 7.39-7.47 (m, 2H, H), 7.57-7.59 (m, 2H, H), 7.83-7.84 (m, 2H, H), 8.10 (d, 1H, *J* = 8.2 Hz, H), 10.19 (s, 1H, N-H); ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 14.35, 31.92, 41.97, 60.73, 104.67, 110.44, 112.03, 112.94, 114.26, 119.32, 124.37, 125.79, 127.95, 132.87, 143.13, 145.11, 149.80, 153.28, 154.28,159.58, 162.93, 163.61. ESI-MS: 477.08 [M-1]$^+$: Anal. calcd for $C_{22}H_{18}N_6O_5S$: C, 55.22; H, 3.79; N, 17.56 %. Found: C, 55.13; H, 3.86; N, 17.51%.

2.1.3.4. Methyl 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-2-oxo-2H-chromen-7-ylcarbamate (5d)

Off-white solid; Mp 136-137°C; IR (KBr) (v_{max}/cm^{-1}): 3281 (NH), 1707 (C=O of coumarin carbonyl), 1691 (NHC=O); 1619 (C=N of triazole) cm^{-1}, 941 (C-S),; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.23 (s, 1H, N-H), 8.09 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.76 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.63-7.57 (m, 2H, H), 7.40 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.55 (s, 1H, H), 6.42 (s, 2H, CH_2), 5.36 (s, 2H, CH_2, C4), 3.73 (s, 3H, CH_3), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm) : 29.29, 35.51, 45.13, 105.17, 110.22, 112.63, 113.02, 114.13, 115.03, 116.49, 119.42, 124.23, 127.11, 128.34, 131.12, 132.18, 133.52, 143.12, 145.10, 153.03, 154.49, 159.76, 162.04, 162.70; ESI-MS: 465.39 [M]$^+$: Anal. calcd for **$C_{21}H_{16}N_6O_5S$-**C, 54.31; H, 3.47; N, 18.09 %. Found: C, 54.22; H, 3.53; N, 18.15%.

2.1.3.5. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-7-hydroxy-2H-chromen-2-one (5e)

Light brown colour: Mp 146-147°C; IR (KBr) (v_{max}/cm^{-1}): 1719 (C=O of coumarin), 1611 (C=N of triazole) cm^{-1}, 971 (C-S),; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 4.52 (s, 2H, CH_2), 6.30 (s, 2H, CH_2), 6.62 (s, 1H, H), 7.27-7.35 (m, 1H, H), 7.41-7.46 (m, 3H, H), 7.57 (d, 1H, H), 7.63 (d, 1H, *J* = 5.4 Hz, H), 8.02 (d, 1H, *J* = 6.6 Hz, H), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 32.19, 42.54, 116.13, 117.31, 119.53, 119.92, 120.36, 124.25, 130.09, 132.31, 133.05, 134.21, 144.37, 150.04, 152.64, 154.99, 160.51, 161.12, 162.14 ESI-MS: 409 [M+1]: Anal. calcd for **$C_{19}H_{13}N_5O_4S$-** C, 56.01; H, 3.`22; N, 17.19%. Found: C, 56.11; H, 3.14; N, 17.27%.

2.1.3.6. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-6- methoxy-2H-chromen-2-one (5f)

Off white solid: Mp 152-153 °C; IR (KBr) (v_{max}/cm^{-1}): 1706 (C=O of coumarin), 1617 (C=N of triazole) cm^{-1}, 947 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.82 (s, 3H, -OCH_3), 4.73 (s, 2H, -CH_2), 6.39 (s, 2H, -CH_2), 6.54 (s, 1H), 7.23-7.27 (m, 1H), 7.37-7.48 (m, 3H), 7.49-7.56 (m, 1H), 7.86 (d, 1H, *J* = 8.4 Hz), 8.10 (d, 1H, *J* = 11.4 Hz, H), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 32.33. 41.99, 55.65, 108.20, 110.40, 110.51, 116.06, 117.89, 119.32, 119.49, 125.08, 128.077, 132.67, 144.81, 147.52, 149.71, 155.85, 159.62, 163.75; ESI-MS: 422.2 [M+2]$^+$: Anal calcd for **$C_{20}H_{15}N_5O_4S$**: C, 57.00; H, 3.59; N, 16.62%, Found: C, 56.90; H, 3.64; N, 16.70%.

2.1.3.7. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-5,7-dimethyl-2H-chromen-2-one (5g)

Pale yellow powder: Mp 129-130 °C; IR (KBr) (v_{max}/cm^{-1}): 1702 (C=O of coumarin), 1623 (C=N of triazole) cm^{-1}, 963 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): **:** 2.35 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 6.39 (s, 2H, CH_2), 6.49 (s, 1H, Ar-H), 7.05-7.12 (d, 2H, Ar-H), 7.42-7.49 (m, 1H, Ar-H), 7.56-7.60 (m, 1H, Ar-H), 7.86 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.08 (d, 1H, *J* = 8.0 Hz, Ar-H), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 20.56, 23.27, 36.13, 41.98, 110.44, 114.61, 115.71, 117.94, 119.31, 124.37, 127.12, 127.94, 129.89, 132.89, 135.84, 142.21, 145.11, 150.72, 154.82, 159.00, 163.05; ESI-MS: 418.27 [M+2]$^+$: Anal calcd for **$C_{21}H_{17}N_5O_3S$**: C, 60.13; H, 4.09; N, 16.70%. Found: C, 60.23; H, 4.13; N, 16.61%.

2.1.3.8. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-6-chloro-2H-chromen-2-one (5h)

Pale yellow powder: Mp 146-147°C; IR (KBr) (v_{max}/cm^{-1}): 1721 (C=O of coumarin), 1609 (C=N of triazole) cm^{-1}, 976 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.42 (s, 2H, CH_2), 6.38 (s, 2H, CH_3), 6.58 (s, 1H), 7.26 (d, 1H, *J* = 3.2 Hz), 7.38-7.48 (m, 2H), 7.64 (s, 1H, Ar-H), 7.78 (d, 1H, *J* = 7.2 Hz), 8.06 (d, 1H, *J* = 12.6 Hz), ^{13}C NMR (400 MHz, CD-Cl_3, δ ppm): 32.12, 43.14, 116.82, 117.54, 118.51, 119.02, 120.26, 124.35, 130.29, 132.41, 133.85, 144.27, 150.35, 154.01, 160.51, 162.24. 163.01; ESI-MS: 425.4 [M]$^+$, 426.4 [M+1]$^+$: Anal calcd for **$C_{19}H_{12}ClN_5O_3S$:** C, 53.59; H, 2.84; N, 16.45% . Found: C, 53.68; H, 2.76; N, 16.38%.

2.1.3.9. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-7-chloro-2H-chromen-2-one (5i)

Off-white powder: Mp 116-117°C; IR (KBr) (v_{max}/cm^{-1}): 1718 (C=O of coumarin), 1606 (C=N of triazole) cm^{-1}, 969 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.52 (s, 2H,

CH_2, C4), 6.33 (s, 2H, CH_2), 6.49 (s, 1H, Ar-H), 7.26 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.35-7.39 (m, 2H, Ar-H), 7.25 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.33-7.39 (m, 1H, Ar-H), 7.41 (d, 1H, *J* = 2.8 Hz, Ar-H), 7.68 (d, 1H, Ar-H), 7.74 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.90 (d, 1H, *J* = 8.0 Hz, Ar-H), ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 32.19, 42.54, 116.13, 117.31, 119.53, 119.92, 120.36, 125.25, 130.09, 132.31, 133.05, 134.22, 144.37, 150.04, 152.64, 154.99, 160.10, 161.12, 162.14; ESI-MS: 424.48 $[M]^+$, 426.57 $[M+2]^+$: Anal calcd for **$C_{19}H_{12}ClN_5O_3S$**: C, 53.59; H, 2.84; N, 16.45 % Found: C, 53.65; H, 2.72; N, 16.38%.

2.1.3.10. 1-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-3H-benzo[f] chromen-3-one (5j)

Off-white solid: Mp 156-157°C; IR (KBr) (ν_{max}/cm^{-1}): 1701 (C=O of coumarin), 1609 (C=N of triazole) cm^{-1}, 958 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.70 (s, 2H, CH_2), 6.35 (s, 2H, CH_2), 6.61 (s, 1H Ar-H), 7.25-7.30 (m, 1H, Ar-H), 7.53-7.66 (m, 4H), 7.80-784. (m, 1H), 7.85-7.93 (m, 1H), 8.08 (s, 1H, Ar-H), 8.11 (s,1H, Ar-H), 8.48 (d, 1H, *J* = 8.8 Hz), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 32.59, 41.99, 113.56, 115.21, 116.57, 117.13, 117.81, 119.77, 123.21, 124.31, 124.83, 127.61, 128.82, 132.21, 132.61, 133.78, 133.94, 144.75, 149.03, 151.01, 151.07, 160.60, 163.53; ESI-MS: 442.2 $[M]^+$: Anal calcd for $C_{23}H_{15}N_5O_3S$: C, 62.58; H, 3.42; N, 15.86%, Found: C, 62.47; H, 3.70; N, 15.71%.

2.1.3.11. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-6-methyl-2H-chromen-2-one (5k)

Pale yellow solid: Mp 142-143°C; IR (KBr) (ν_{max}/cm^{-1}): 1711 (C=O of coumarin), 1603 (C=N of triazole) cm^{-1}, 976 (C-S),; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.82 (s, 3H, CH_3), 4.73 (s, 2H, CH_2, C4), 6.38 (s, 2H, CH_2), 6.54 (s, 1H, Ar-H), 7.23-7.26 (m, 1H, Ar-H), 7.27-7.47 (m, 3H, Ar-H), 7.49-7.56 (m, 1H, Ar-H), 7.86 (d, 1H, *J* = 8.4 Hz), 8.11 (d, 1H, *J* = 9.0 Hz), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 20.13, 32.11, 41.98, 115.29, 115.66, 116.55, 117.12, 117.94, 120.12, 124.37, 124.89, 127.12, 127.96, 132.88, 133.77, 145.13, 149.68, 151.37, 159.42, 163.23; ESI-MS: 406 $[M+1]^+$, 407.4 $[M+2]^+$, Anal calcd for $C_{20}H_{15}N_5O_3S$: C, 59.25; H, 3.73; N, 17.27% Found: C, 59.22; H, 3.76; N, 17.32%.

2.1.3.12. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-7-methoxy-2H-chromen-2-one (5l)

Off-white solid: Mp 138-139 °C; IR (KBr) (ν_{max}/cm^{-1}): 1714 (C=O of coumarin), 1612 (C=N of triazole) cm^{-1}, 961 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.49 (s, 3H, O-CH_3), 6.62 (s, 2H, CH_2, C4), 6.81 (s, 1H, Ar-H), 7.24 (d, 1H, Ar-H), 7.41-7.46 (m, 2H), 7.60-7.63 (m, 1H), 7.80 (d, 1H, *J* = 6.0Hz), 8.10 (d, 1H, *J* = 6.0 Hz), ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 32.13, 41.97, 55.95, 108.61, 110.43, 113.94, 116.06, 117.89, 118.01, 119.38, 119.50, 124.38, 133.95, 134.07, 147.56, 153.27, 155.65, 159.43, 162.05, 163.66; ESI-MS: 420.05 $[M]^-$, Anal calcd for $C_{20}H_{15}N_5O_4S$: C, 57.00; H, 3.59; N, 16.62%. Found: C, 56.90; H, 3.64; N, 16.70%.

2.1.3.13. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-6-bromo-2H-chromen-2-one (5m)

Off-white powder: Mp 139-140°C; IR (KBr) (ν_{max}/cm^{-1}): 1714 (C=O of coumarin), 1619 (C=N of triazole) cm^{-1}, 971 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.62 (s, 2H, CH_2, C4), 6.63 (s, 2H, CH_2), 6.49 (s, 1H), 7.23 (d, 1H, *J* = 6.0 Hz), 7.28-7.55 (m, 3H), 7.58 (d, 1H, *J* = 8.0 Hz), 7.88 (d, 1H, *J* = 12.4 Hz), 8.00 (d, 1H, *J* = 8.4 Hz), ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 34.01, 45.13, 117.09, 117.99, 118.55, 119.02, 124.13, 127.38, 129.09, 133.29, 134.71, 137.18, 144.09, 149.49, 152.12, 154.39, 159.82, 162.11, 163.20; ESI-MS: 471$[M]^+$, Anal calcd for $C_{19}H_{12}BrN_5O_3S$: C, 48.52; H, 2.57; N, 14.89% Found: C, 48.43; H, 2.63; N, 14.98%.

2.1.3.14. 4-((5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-2H-benzo [h]chromen-2-one (5n)

Off-white solid: Mp 138-139 °C; IR (KBr) (ν_{max}/cm^{-1}): 1711 (C=O of coumarin), 1607 (C=N of triazole) cm^{-1}, 962 (C-S),; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.40 (s, 2H, CH_2), 6.37 (s, 1H, 2H, CH_2), 6.75 (s, 1H), 7.04-7.28 (m,1H), 7.30- 7.36 (m, 2H), 7.38-7.61 (m, 3H), 7.62-7.70 (m, 1H), 7.91 (d, 1H, *J*=8.4Hz), 8.02 (s, 1H), 8.30 (d, 1H, *J* = 8.0 Hz), ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 20.56, 23.27, 36.13, 41.99, 110.44, 114.61, 115.71, 117.94, 119.31, 124.37, 127.12, 127.94, 129.89, 132.89, 135.84, 142.21, 150.72, 154.82, 159.00, 163.05, ; ESI-MS: 442 $[M]^+$, 443M+1$]^+$, Anal calcd for $C_{23}H_{15}N_5O_3S$: C, 62.58; H, 3.42; N, 15.86%, Found: C, 62.51; H, 3.36; N, 15.92%.

2.2. Pharmacology

All the newly prepared compounds were screened for their potential anti-tubercular activity against Mtb H37Rv strain by Microplate Alamar Blue Assay (MABA). The active compounds were tested for their cytotoxicity against Vero cells by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

2.3. Antitubercular Activity

All the newly synthesized compounds were evaluated for their anti-tubercular activity against *M. tuberculosis* H37Rv strain (ATCC 27294) using a microplate Alamar Blue assay (MABA). Briefly, 200 µL of sterile deionized water was added to all outer-perimeter wells of sterile 96-well plates to minimize evaporation of the medium in the test wells during incubation. The wells in rows B to G in columns 3 to 11 received 100 µL of Middle brook 7H9 broth. 100 µL of 2X drug solutions were added to the wells in rows B to G in columns 2. 100 µL was transferred from column 2 to column 3, and the contents of the wells were mixed well. Identical serial 1:2 dilutions were continued through column 10, and 100 µL of excess medium was discarded from the wells in column 10 in order to get the final concentration of 0.2 µg/mL. 100 µL of Mtb inoculum was added to the wells in rows B to G in columns 2 to 11 (yielding a final volume of 200 µL per well). Thus, the wells in column 11 served as drug-free control. The plate was sealed with parafilm and incubated at 37°C for 5 days. Thereafter, 50 µL of freshly prepared 1:1 mixture of Alamar Blue reagent and 10%

tween-80 was added to each well and incubated for 24 h. Appearance of blue colour was interpreted as no bacterial growth, and pink colour was used as an indicator of bacterial growth. The inhibitory activity of new compounds against Mtb swaps expressed as the minimum inhibitory concentration (MIC) in μM. MIC was defined as lowest drug concentration which prevented the colour change from blue to pink. Pyrazinamide was used as a positive control.

3. RESULTS

3.1. General Synthesis of Coumarin-Benzotriazole Hybrid Derivatives

In this study, we synthesized novel 1,3,4-oxadiazoles containing benzotriazole and coumarin moiety and the synthetic route for the preparation of title compounds is outlined in Fig. (**2**). Initially, benzotriazole was esterified with ethyl chloroacetate in acetone in the presence of potassium carbonate to give compound **1**. Next, compound **1** was made to react with 85% hydrazine hydrate in methanol at 4ºC to get compound **2**. Further refluxing of compound **2** with carbon disulfide in the presence of potassium hydroxide and anhydrous ethanol gives compound **3**. The substituted 4-bromomethyl coumarins (4a-n) were synthesized using a Pechman cyclisation of the phenols with 4-bromoethyl-acetoacetate [14]. We attempted the synthesis of compounds 5a-n in three different reaction conditions as indicated in materials and methods. Halogen substituted 4-(bromomethyl)-7-methyl-2H-chromen-2-one in the presence of sodium metal, potassium carbonate and DIPEA in dry EtOH, MeOH & anhydrous N,N-dimethylformamide gave title compounds in 75–84% yields. Among these reaction conditions, use of diisopropylethylamine **(**DIPEA) as base and reagent found to be more efficient compared to other conditions (reaction condition C). All the newly synthesized compounds were characterized using Fourier transform infrared spectroscopy (FTIR), ^{1}H NMR, ^{13}C NMR, mass, elemental analysis and the physical data is provided in Table **1**. ^{1}H NMR and ESI-MS spectra were consistent with the assigned structures. The spectral data of the newly synthesized compounds (5a-n) are provided as supplementary information.

3.2. Anti-TB Activity of New Coumarin-Benzotriazole Hybrids

Coumarin and benzotriazole heterocycles have been demonstrated to possess good anti-mycobacterial activity. Therefore, we next investigated the anti-TB activity of new coumarin-benzotriazole derivatives against the Mtb H37Rv strain using Alamar Blue assay as described previously [15]. The Mtb was incubated with different concentrations of new compounds and minimum inhibitory concentration (MIC) was recorded and results are tabulated in Table **1**. Most of the pharmacophore hybrids displayed good anti-mycobacterial activity suggesting that new compounds bear substantial anti-TB activity. Compound 4-((5-((1H-benzo[d]-[1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-7-hydroxy-2H-chromen-2-one (**5e**) and 4-((5-((1H-benzo[d]-[1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazol-2-ylthio)methyl)-6-methoxy-2H-chromen-2-one (**5f**) showed the relatively low MIC of 18.3 and 15.5 μM respectively than the other structural analogues.

Investigation of structure-activity relationship (SAR) of the screened molecules suggests that introduction of hydroxyl group at 7th position of coumarin enhances the inhibitory activity. Furthermore, introduction of hydrophobic and electron withdrawing groups like halogens at 6th position of coumarins decreases the activity. Addition of methyl group at 8th position of coumarin decreases the antimycobacterial activity of compounds.

We next evaluated the cytotoxic effect of new compounds on the viability of Vero (monkey kidney epithelial) cells using MTT assay as described earlier [16]. However, new compounds did not induce significant cytotoxicity towards normal cells up to 72 h at the concentration of 50 μM. From these results, it was apparently clear that coumarin-benzotriazole hybrids are capable of selectively inhibiting the Mtb and does not induce cytotoxicity in host cells.

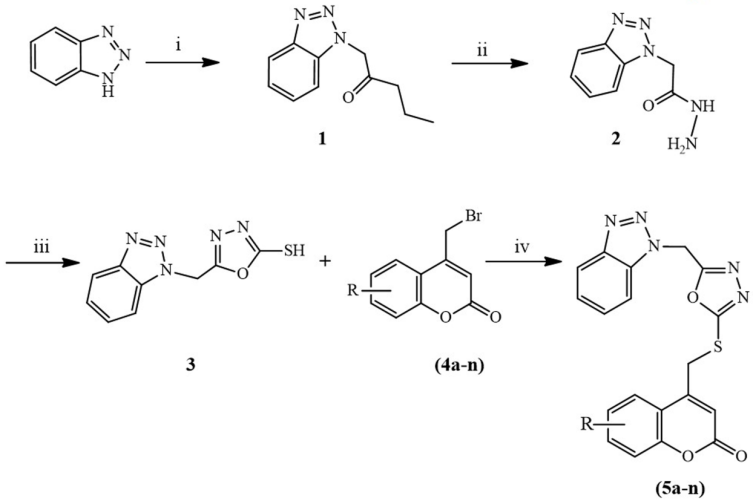
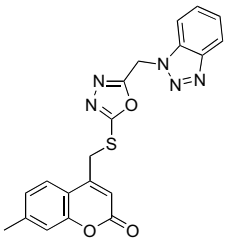
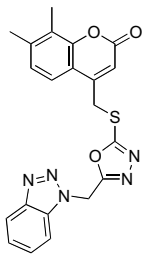
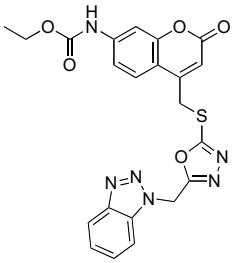
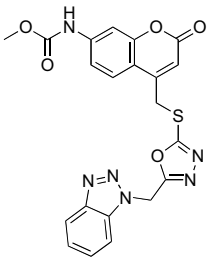
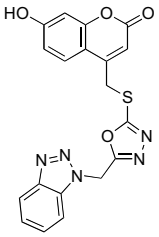
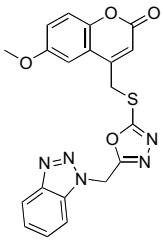
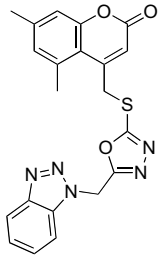
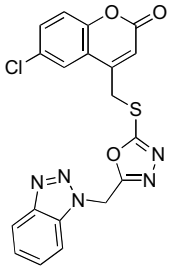
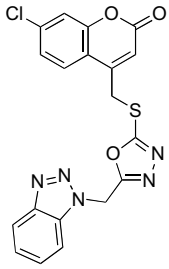
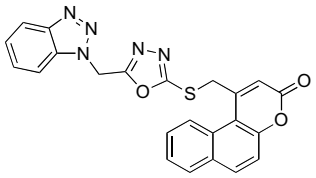
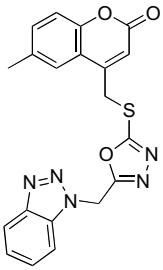
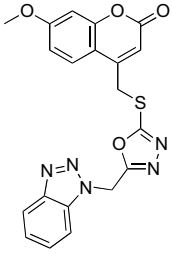


Fig. (2). General synthesis of compounds (**5a-n**). Reagents and conditions: (i) ethyl chloroacetate, acetone, K_2CO_3, reflux, 8 h; (ii) $NH_2NH_2.H_2O$ (85%), methanol, 4°C, 12 h; (iii) CS_2/KOH, ethanol, reflux 24 h (iv) Method A, B, C.

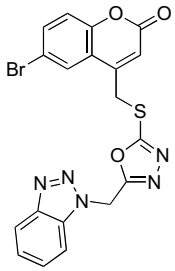
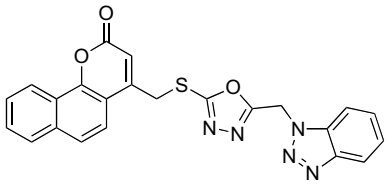
Table 1. Physical data of the coumarin-benzotriazole hybrids and MIC values towards the H37Rv strain of *M.tuberculosis*.

Entry	Compounds (5a-n)	Reaction Conditions	Yield (%)	Time (h)	MIC (µM)
5a		A	88	16	20.7
5b		B	84	24	>50
5c		B	89	24	>50
5d		B	82	26	>50
5e		C	91	30	18.3
5f		C	94	31	15.5

(Table 1) Contd….

Entry	Compounds (5a-n)	Reaction Conditions	Yield (%)	Time (h)	MIC (µM)
5g		C	90	34	30.1
5h		B	85	22	>50
5i		B	80	20	>50
5j		C	87	28	>50
5k		C	90	30	21.3
5l		C	92	28	28.9

(Table 1) Contd….

Entry	Compounds (5a-n)	Reaction Conditions	Yield (%)	Time (h)	MIC (µM)
5m		B	83	26	>50
5n					>50
Pyrazinamide					12.5

Reaction conditions- A: Na Metal, EtOH, Reflux 16-18 h; Method B: K_2CO_3, MeOH, Reflux 18-24 h; Method C: Diisopropylethylamine (DIPEA) 10mol%, Acetone: MDC, Stir, 26-36 h.

DISCUSSION AND CONCLUSION

Development of new therapeutic agents to treat tuberculosis has emerged as one of the major challenges due to the complexity and toxicity, and development of resistance by Mtb against the conventional treatment strategies. Hence, considerable attention has been paid to design new anti-mycobacterial agents against multi-drug resistant and extensively drug-resistance TB. Notably, treatment outcome of MDR-TB is generally associated with poor prognosis projecting the need of discovery of new therapeutic agents [17]. New drugs were not introduced to clinical practice against TB, since the approval of rifampicin in 1960s [18]. Recently, bedaquiline (quinoline derivative) and delamanid (imidazole derivative) have entered clinical practice. Bedaquiline is an inhibitor of bacterial ATP synthetase and delamanid targets cell wall synthesis of Mtb. In order to develop anti-TB agents, several chemical agents have been prepared by various research groups and some of them displayed promising results. AZD5847, SQ109, sutezolid, BTZ043 and PA-824 are some of the small molecules that have been tested at various levels as anti-TB agents. It is evident that, there is a requirement of new, inexpensive, shorter treatment period and safe drug to fight against TB.

In conclusion, herein we comprehensively report the synthesis and antimycobacterial potential of 5-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol substituted -2H-chromen-2-one derivatives. The uniqueness of the study relies in the usage of simple, cost effective base reagent like DIPEA in the preparation of coumarin-benzotriazole hybrids without impurities and with excellent yield compared to conventional methods. Some of the newly synthesized compounds showed good antimycobacterial activity against Mtb H37Rv strain at low micromolar doses. Therefore, this report presents coumarin conjugated benzotriazoles as a new class of anti-mycobacterial pharmacophores.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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