Propylene-1*H*-1,2,3-triazole-4-methylene-tethered Isatin-coumarin Hybrids: Design, Synthesis, and *In Vitro* Anti-tubercular Evaluation

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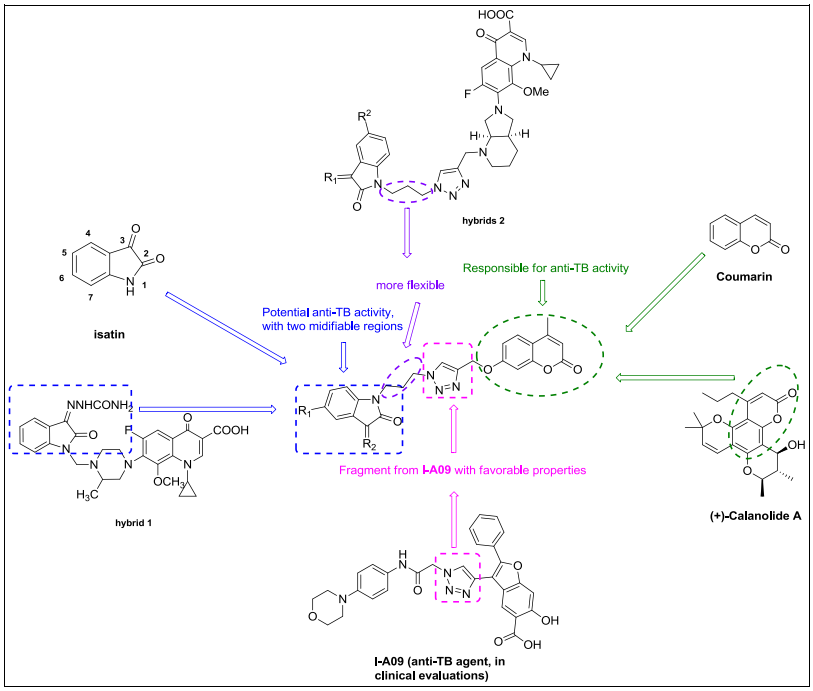

A series of novel propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids **7a–l** that were composed of three anti-tubercular bioactive substances/pharmacophore coumarin, isatin, and **I-A09** were designed, synthesized, and assessed for their in vitro anti-tubercular activity against *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$. In spite of the hybrids were inactive against the tested MTB $H_{37}Rv$, the structure–activity relationship was enriched, and these hybrids may act as an ideal starting point for developing new isatin-coumarin anti-TB candidates with various linkers.

INTRODUCTION

Tuberculosis (TB), caused predominantly by *Mycobacterium tuberculosis* (MTB), is one of the most deadly infectious diseases throughout the world [1]. The drug-susceptible TB can be cured by a 6- to 12-month treatment regimen [a combination of three or four first-line anti-TB agents, comprising isoniazid (**INH**), rifampicin (**RIF**), and pyrazinamide (**PZA**), with or without ethambutol (**EMB**)], but this long-term treatment is accompanied by challenges including the operational hurdles of ensuring uninterrupted drug availability and patient compliance for the full duration of therapy as well as toxicity of prolonged drug therapy [2].

The evolution of MTB new virulent forms like drug-resistant TB (DR-TB, strains that are resistant to at least one of the first-line anti-TB agents), multidrug-resistant TB (MDR-TB, strains resistant to at least two front-line drugs such as **INH** and **RIF**), and extremely drug-resistant TB (XDR-TB, strains that are resistant to **INH**

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and **RIF**, as well as any fluoroquinolones and at least one of three injectable second-line drugs, such as amikacin, kanamycin, or capreomycin) as well as totally drug-resistant TB (TDR-TB, resistant to all first-line and second-line anti-TB agents, is virtually untreatable using current therapeutics and without strengthening of the current TB controls measures) [3–6] and MTB co-infection with HIV has further aggravated the mortality and spread of this disease [7]. All the aforementioned facts creating an urgent need to develop novel, fast acting, high effective, and short therapy duration anti-TB drugs against both drug-susceptible and drug-resistant strains of MTB, particularly in its hard-to-kill MDR-TB, XDR-TB, and TDR-TB strains, to prevent the spread of TB.

Heterocyclic compounds are ubiquitous and play vital role in drugs discovery, among them, isatin, 1*H*-1,2,3-triazole, and coumarin (Fig. 1) as well as their derivatives occupy an important position in medicinal chemistry ascribed to their fascinating array of pharmacological properties. Numerous isatin, 1*H*-1,2,3-triazole, and coumarin derivatives have been screened for their anti-TB activities [8–13], and some of them exhibited excellent potency that are exemplified by **(+)-Calanolide A**, **I-A09,** and hybrid **1**. All **(+)-Calanolide A**, **I-A09,** and hybrid **1** possess great in vitro and in vivo anti-TB potency, in particular, **(+)-Calanolide A** was not only an inhibitor of HIV-1 reverse transcriptase but also has shown promising potency against MTB including drug-resistant strains. Thus, hybridization of two or three of these pharmacophores into one single molecule may provide more effective candidates.

Our previous research results demonstrated that the linker between 1*H*-1,2,3-triazole and isatin or coumarin has significant influence on the anti-TB activity [14–19], and some hybrids tethered via propylene-1*H*-1,2,3-triazole-4-methylene such as hybrids **2** exhibited excellent potency. Based on the aforementioned results and as a part of an ongoing program to optimize 1*H*-1,2,3-triazole-tethered hybrids as anti-TB agents, a

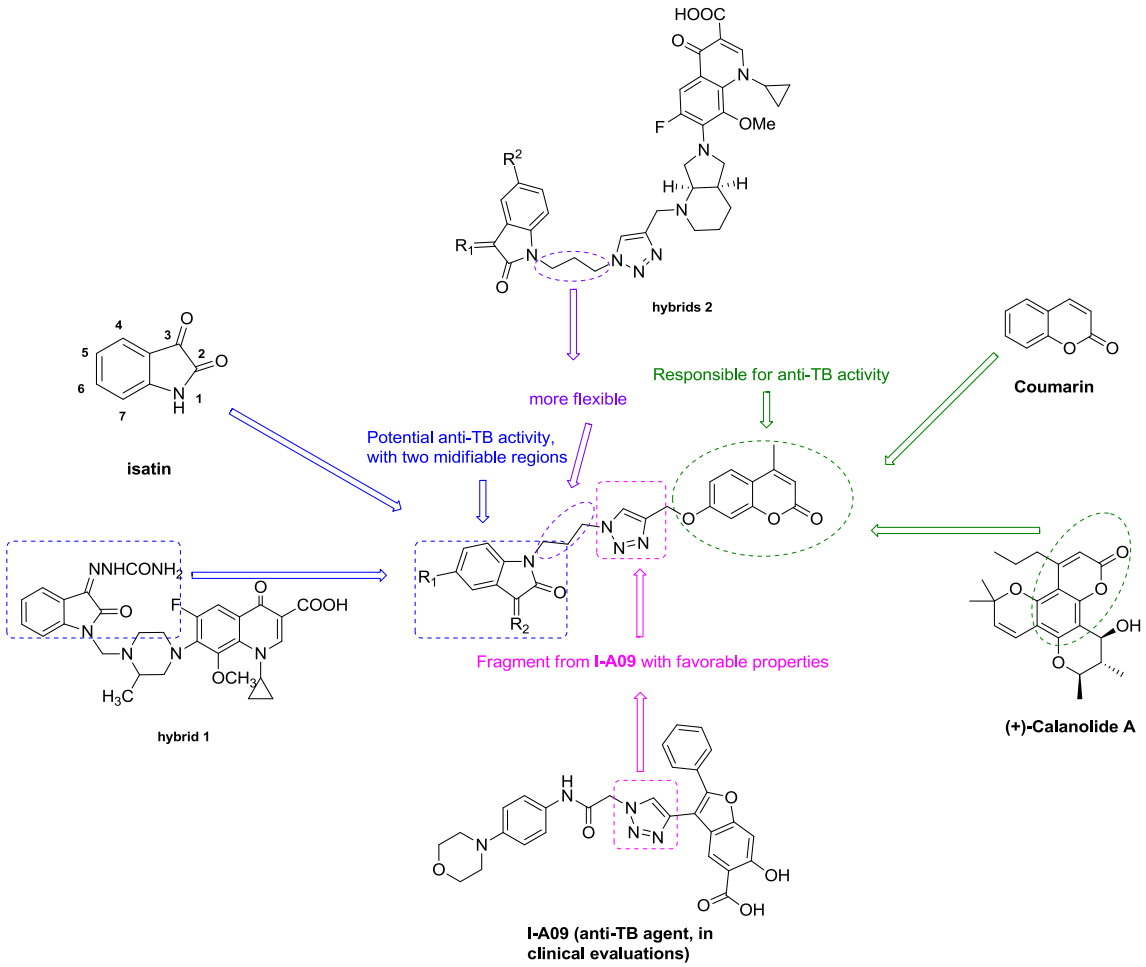


Figure 1. Illustration of the design strategy for propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids. [Color figure can be viewed at wileyonlinelibrary.com.]

series of propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids was designed, synthesized, and evaluated for their in vitro anti-TB activity against MTB $H_{37}Rv$ in this study. Illustration of the design strategy for propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids is depicted in Figure 1.

RESULTS AND DISCUSSION

The synthetic route for propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids **7a–l** is outlined in Scheme 1. C-5 substituted isatins were alkylated with 1,3-dibromopropane in the presence of potassium carbonate to afford the corresponding *N*-(3-bromopropyl)isatins **2a**–**d** (yield: 31–43%) via literature methods [17,18]. Subsequently, treatment of *N*-(3-bromopropyl)isatins **2a**–**d** with sodium azide at 60°C provided the desired azido precursors **3a**–**d** (yield: 58–66%) [17]. The 4-methyl-7-(prop-2-ynyloxy)-2*H*-chromen-2-one **6** (yield: 89%) was obtained by alkylation of 7-hydroxy-4-methyl-7-2*H*-chromen-2-one **5** with propargyl bromide at 50°C in the presence of K_2CO_3 [18]. The precursors **3a**–**d** and **6** were utilized for the synthesis of desired propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids **7a**–**d** via Cu-promoted azide-alkyne cycloaddition reaction in the presence of $Cu(OAc)_2$ in DMF (yield: 36–52%) [18]. Introduction of imines condensations of targets **7a**–**d** with the corresponding amines hydrochloride in the presence of sodium bicarbonate provided targets **7e–l** (51–65%) [4].

The synthesized hybrids were preliminarily screened for in vitro activity against the MTB $H_{37}Rv$ ATCC27294 strain, using the microplate Alamar blue assay [20]. The minimum inhibitory concentration is defined as the lowest concentration affecting a reduction in fluorescence of >90% relative to the mean of replicate bacterium-only controls. The MIC values of the compounds along with moxifloxacin, Linezolid, **INH**, and **RIF** for comparison are presented in μg/mL in Table 1.

The data reveal that the activity of the propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids **7a**–**l** was generally inactive (MIC: >32 μg/mL) against this strain, which was far less active than the references moxifloxacin, linezolid, **INH**, and **RIF** (MIC: 0.082, 0.254, 0.033, and 0.077 μg/mL, respectively). In spite of that, the SAR of isatin-coumarin hybrids was enriched, and the results warrant further development of the anti-TB properties of this kind of hybrids.

Scheme 1. Synthesis of propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids **7a–l.**

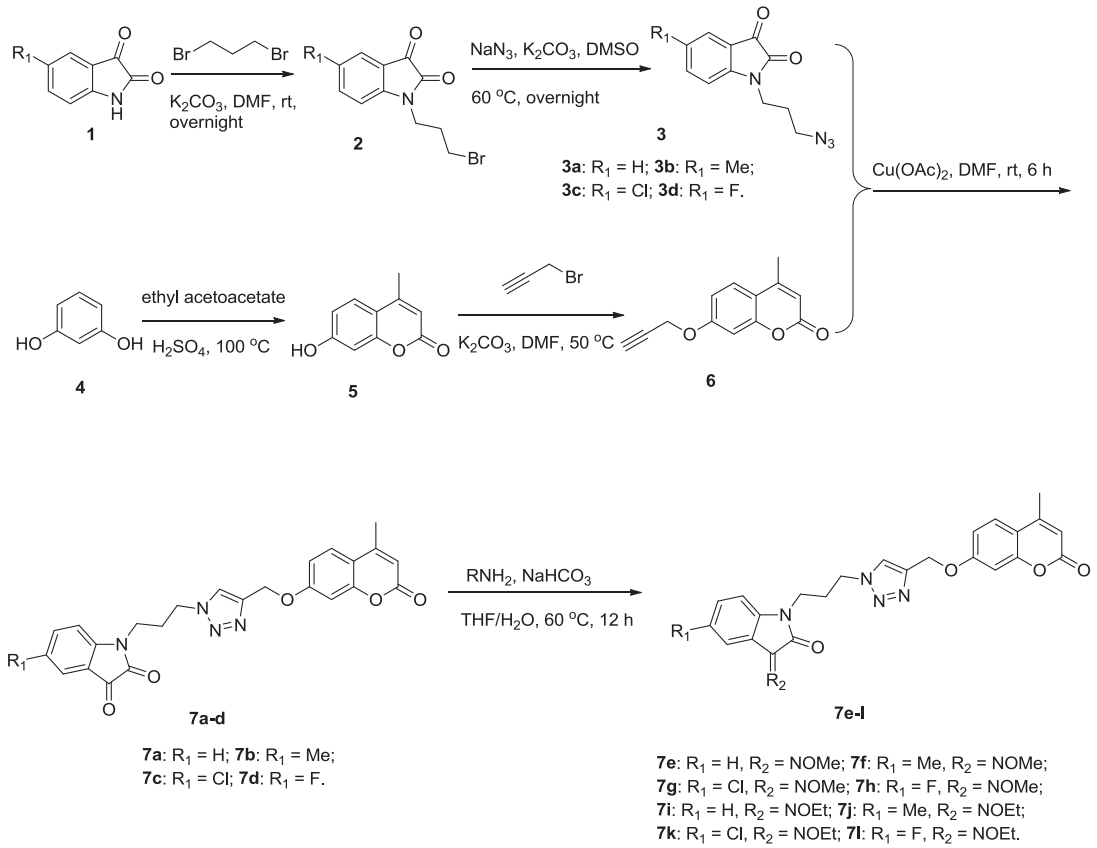
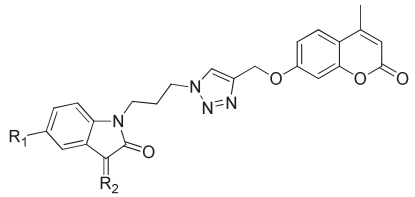


Table 1

Structures and anti-TB activity propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids **7a–l.**



Compound	R_1	R_2	MIC (μg/mL)
7a	H	O	>32
7b	Me	O	>32
7c	Cl	O	>32
7d	F	O	>32
7e	H	NOMe	>32
7f	Me	NOMe	>32
7g	Cl	NOMe	>32
7h	F	NOMe	>32
7i	H	NOEt	>32
7j	Me	NOEt	>32
7j	Cl	NOEt	>32
7l	F	NOEt	>32
Moxifloxacin			0.072
Linezolid			0.254
INH			0.033
RIF			0.077

CONCLUSIONS

A new class of propylene-1*H*-1,2,3-triazole-4-methylene-tethered isatin-coumarin hybrids **7a**–**l**, which integrate three anti-TB bioactive substances/pharmacophoric units coumarin, isatin, and **I-A09**, was designed, synthesized, and evaluated for their in vitro anti-TB activity. All hybrids were inactive against MTB $H_{37}Rv$ ATCC27294 strain, while these hybrids may act as an ideal starting point for developing new isatin-coumarin anti-TB candidates with various linkers.

EXPERIMENTAL

General procedure for the preparation of 7a–d. *N*-(2-Azidopropyl)isatins **3a**–**d** and 4-methyl-7-(prop-2-ynyloxy)-2*H*-chromen-2-one **6** were prepared via literature methods [17,18]. A mixture of *N*-(2-azidopropyl)isatins **3a**–**d** (1.0 mmol), 4-methyl-7-(prop-2-ynyloxy)-2*H*-chromen-2-one **6** (1.0 mmol) and $Cu(OAc)_2$ (100 mg) in DMF (20 mL) was stirred for 6 h at room temperature under N_2 atmosphere. After removal of the solvent, the residue was purified by silica gel column chromatography eluted with PE to *v*(PE): *v*(EA) = 1:1.

1-(3-(4-((((4-Methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione (7a). Yield: 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22–2.24 (2H, m, ─CH_2─), 2.42 (3H, s, ─CH_3), 3.77 (2H, t, ─CH_2 of linker), 4.53 (2H, t, ─CH_2 of linker), 5.30 (2H, s, ─CH_2O─), 6.25 (1H, s, Ar─H), 7.06 (1H, d, Ar─H), 7.14–7.19 (3H, m, Ar─H), 7.58 (1H, d, Ar─H), 7.66–7.73 (2H, m, Ar─H), 8.30 (1H, s, Ar─H). ESI-MS *m/z*: 445 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{24}H_{20}N_4O_5$: C, 64.86; H, 4.54; N, 12.61; found: C, 64.65; H, 4.39; N, 12.48.

5-Methyl-1-(3-(4-((((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione (7b). Yield: 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20–2.23 (2H, m, ─CH_2─), 2.31 (3H, s, ─CH_3), 2.42 (3H, s, ─CH_3), 3.74 (2H, t, ─CH_2 of linker), 4.51 (2H, t, ─CH_2 of linker), 5.29 (2H, s, ─CH_2O─), 6.24 (1H, s, Ar─H), 7.05–7.07 (2H, m, Ar─H), 7.16 (1H, s, Ar─H), 7.40 (1H, s, Ar─H), 7.47 (1H, d, Ar─H), 7.71 (1H, d, Ar─H), 8.30 (1H, s, Ar─H). ESI-MS *m/z*: 459 $[M + H]^+$. $C_{25}H_{22}N_4O_5$: C, 65.49; H, 4.84; N, 12.22; found: C, 65.27; H, 4.79; N, 12.03.

5-Chloro-1-(3-(4-((((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione (7c). Yield: 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20–2.22 (2H, m, ─CH_2─), 2.43 (3H, s, ─CH_3), 3.78 (2H, t, ─CH_2 of linker), 4.52 (2H, t, ─CH_2 of linker), 5.30 (2H, s, ─CH_2O─), 6.26 (1H, s, Ar─H), 7.07 (1H, d, Ar─H), 7.17–7.24 (2H, m, Ar─H), 7.65 (1H, s, Ar─H), 7.72–7.73 (2H, m, Ar─H), 8.29 (1H, s, Ar─H). ESI-MS *m/z*: 479 $[M + H]^+$, 481 $[M + 2 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{24}H_{19}ClN_4O_5$: C, 60.19; H, 4.00; N, 11.70; found: C, 60.01; H, 3.87; N, 11.63.

5-Fluoro-1-(3-(4-((((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione (7d). Yield: 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20–2.23 (2H, m, ─CH_2─), 2.42 (3H, s, ─CH_3), 3.77 (2H, t, ─CH_2 of linker), 4.52 (2H, t, ─CH_2 of linker), 5.39 (2H, s, ─CH_2O─), 6.25 (1H, s, Ar─H), 7.06 (1H, d, Ar─H), 7.16 (1H, s, Ar─H), 7.22 (1H, d, Ar─H), 7.50–7.57 (2H, m, Ar─H), 7.72 (1H, d, Ar─H), 8.29 (1H, s, Ar─H). ESI-MS *m/z*: 463 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{24}H_{19}FN_4O_5$: C, 62.34; H, 4.14; N, 12.12; found: C, 62.19; H, 4.08; N, 12.06.

General procedure for preparing targets 7e–l. A mixture of amines hydrochloride (5 mmol), sodium bicarbonate (5 mmol), and **7a–d** in a mixture of in THF (20 mL) and water (5 mL) was stirred at 60°C for 12 h. After cooling to room temperature, the mixture was extracted with EA (10 mL*3). The combined organic layers were concentrated under reduced pressure, and the residue was purified by column chromatography (silica gel) eluted with PE to *v*(PE):*v*(EA) = 1:1 to give the title compounds **7e–l** (51–65%).

3-(Methoxyimino)-1-(3-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indolin-2-one (7e). Yield: 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20–2.22 (2H, m, ─CH_2─), 2.42 (3H, s, ─CH_3), 3.78 (2H, t, ─CH_2 of linker), 4.23 (3H, s, $NOCH_3$), 4.49 (2H, t, ─CH_2 of linker), 5.29 (2H, s, ─CH_2O─), 6.24 (1H, s, Ar─H), 7.05–7.16 (4H, m, Ar─H), 7.46–7.49 (1H, m, Ar─H), 7.71 (1H, d, Ar─H), 7.91 (1H, d, Ar─H), 8.32 (1H, s, Ar─H). ESI-MS *m/z*: 474 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{25}H_{23}N_5O_5$: C, 63.42; H, 4.90; N, 14.79; found: C, 63.21; H, 4.85; N, 14.67.

3-(Methoxyimino)-5-methyl-1-(3-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indolin-2-one (7f). Yield: 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.21–2.23 (2H, m, ─CH_2─), 2.28 (3H, s, ─CH_3), 2.42 (3H, s, ─CH_3), 3.75 (2H, t, ─CH_2 of linker), 4.25 (3H, s, $NOCH_3$), 4.53 (2H, t, ─CH_2 of linker), 5.29 (2H, s, ─CH_2O─), 6.25 (1H, s, Ar─H), 7.00 (1H, d, Ar─H), 7.04–7.06 (1H, m, Ar─H), 7.14 (1H, s, Ar─H), 7.38 (1H, s, Ar─H), 7.46 (1H, d, Ar─H), 7.69 (1H, d, Ar─H), 8.28 (1H, s, Ar─H). ESI-MS *m/z*: 488 $[M + H]^+$. $C_{26}H_{25}N_5O_5$: C, 64.06; H, 5.17; N, 14.37; found: C, 63.87; H, 5.10; N, 14.19.

5-Chloro-1-(2-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)indoline-2,3-dione (7g). Yield: 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20–2.22 (2H, m, **─CH_2─**), 2.42 (3H, s, ─CH_3), 3.76 (2H, t, ─CH_2 of linker), 4.26 (3H, s, $NOCH_3$), 4.52 (2H, t, ─CH_2 of linker), 5.30 (2H, s, ─CH_2O─), 6.27 (1H, s, Ar─H), 7.05 (1H, d, Ar─H), 7.20–7.24 (2H, m, Ar─H), 7.63 (1H, s, Ar─H), 7.72 (1H, s, Ar─H), 7.74 (1H, s, Ar─H), 8.30 (1H, s, Ar─H). ESI-MS *m/z*: 508 $[M + H]^+$, 510 $[M + 2 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{25}H_{22}ClN_5O_5$: C, 59.12; H, 4.37; N, 13.79; found: C, 58.89; H, 4.13; N, 13.58.

5-Fluoro-3-(methoxyimino)-1-(3-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indolin-2-one (7h). Yield: 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.21–2.23 (2H, m, ─CH_2─), 2.41 (3H, s, ─CH_3), 3.77 (2H, t, ─CH_2 of linker), 4.25 (3H, s, $NOCH_3$), 4.53 (2H, t, ─CH_2 of linker), 5.39 (2H, s, ─CH_2O─), 6.28 (1H, s, Ar─H), 7.07 (1H, d, Ar─H), 7.18 (1H, s, Ar─H), 7.22 (1H, d, Ar─H), 7.50–7.56 (2H, m, Ar─H), 7.70 (1H, d, Ar─H), 8.32 (1H, s, Ar─H). ESI-MS *m/z*: 492 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{25}H_{22}FN_5O_5$: C, 61.10; H, 4.51; N, 14.25; found: C, 61.03; H, 4.39; N, 14.03.

3-(Ethoxyimino)-1-(3-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indolin-2-one (7i). Yield: 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (3H, t, $OCH_2\underline{CH_3}$), 2.22–2.24 (2H, m, ─CH_2─), 2.40 (3H, s, ─CH_3), 3.76 (2H, t, ─CH_2 of linker), 4.43 (2H, q, $O\underline{CH_2}CH_3$), 4.52 (2H, t, ─CH_2 of linker), 5.30 (2H, s, ─CH_2O─), 6.26 (1H, s, Ar─H), 7.06 (1H, d, Ar─H), 7.15–7.20 (3H, m, Ar─H), 7.56 (1H, d, Ar─H), 7.74 (1H, d, Ar─H), 7.81 (1H, d, Ar─H), 8.28 (1H, s, Ar─H). ESI-MS *m/z*: 488 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{26}H_{25}N_5O_5$: C, 64.06; H, 5.17; N, 14.37; found: C, 63.89; H, 4.94; N, 14.13.

3-(Ethoxyimino)-5-methyl-1-(3-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indolin-2-one (7j). Yield: 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (3H, t, $OCH_2\underline{CH_3}$), 2.20–2.22 (2H, m, ─CH_2─), 2.32 (3H, s, ─CH_3), 2.42 (3H, s, ─CH_3), 3.75 (2H, t, ─CH_2 of linker), 4.45 (2H, q, $O\underline{CH_2}CH_3$), 4.52 (2H, t, ─CH_2 of linker), 5.28 (2H, s, ─CH_2O─), 6.24 (1H, s, Ar─H), 7.06 (1H, d, Ar─H), 7.10 (1H, s, Ar─H), 7.16 (1H, s, Ar─H), 7.38 (1H, s, Ar─H), 7.45 (1H, d, Ar─H), 7.74 (1H, d, Ar─H), 8.32 (1H, s, Ar─H). ESI-MS *m/z*: 502 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{27}H_{27}N_5O_5$: C, 64.66; H, 5.43; N, 13.96; found: C, 64.39; H, 5.32; N, 13.87.

5-Chloro-3-(ethoxyimino)-1-(3-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indolin-2-one (7k). Yield: 54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (3H, t, $OCH_2\underline{CH_3}$), 2.20–2.22 (2H, m, ─CH_2─), 2.42 (3H, s, ─CH_3), 3.78 (2H, t, ─CH_2 of linker), 4.43 (2H, q, $O\underline{CH_2}CH_3$), 4.52 (2H, t, ─CH_2 of linker), 5.30 (2H, s, ─CH_2O─), 6.26 (1H, s, Ar─H), 7.06 (1H, d, Ar─H), 7.15–7.19 (2H, m, Ar─H), 7.60 (1H, s, Ar─H), 7.68 (1H, s, Ar─H), 7.73 (1H, d, Ar─H), 8.29 (1H, s, Ar─H). ESI-MS *m/z*: 522 $[M + H]^+$, 524 $[M + 2 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{26}H_{24}ClN_5O_5$: C, 59.83; H, 4.63; N, 13.42; found: C, 58.71; H, 4.39; N, 13.27.

3-(Ethoxyimino)-5-fluoro-1-(3-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indolin-2-one (7l). Yield: 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (3H, t, $OCH_2\underline{CH_3}$), 2.20–2.22 (2H, m, ─CH_2─), 2.42 (3H, s, ─CH_3), 3.76 (2H, t, ─CH_2 of linker), 4.45 (2H, q, $O\underline{CH_2}CH_3$), 4.52 (2H, t, ─CH_2 of linker), 5.34 (2H, s, ─CH_2O─), 6.27 (1H, s, Ar─H), 7.08 (1H, d, Ar─H), 7.16 (1H, s, Ar─H), 7.21 (1H, d, Ar─H), 7.50–7.56 (2H, m, Ar─H), 7.70 (1H, d, Ar─H), 8.32 (1H, s, Ar─H). ESI-MS *m/z*: 506 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{26}H_{24}FN_5O_5$: C, 61.78; H, 4.79; N, 13.85; found: C, 61.59; H, 4.57; N, 13.77.

MIC determination. MICs against replicating *M. tuberculosis* were determined by the microplate Alamar blue assay [20]. CPFX, MXFX, and INH were included as positive controls. The range of the final testing concentrations of the targets was 32 to 0.125 μg/mL. *Mycobacterium tuberculosis* H37Rv was grown to late log phase (70 to 100 Klett units) in Difco Middlebrook 7H9 Broth supplemented with 0.2% (*v*/*v*) glycerol, 0.05% Tween 80, and 10% (*v*/*v*) albumin-dextrosecatalase (BBL Middlebrook ADC Enrichment, catalog No. 212352) (7H9-ADC-TG). The cultures were centrifuged, washed twice, and then re-suspended in phosphate buffered saline. The suspensions were then passed through an 8μ*M*-pore-size filter to remove clumps, and aliquots were frozen at −80°C. Twofold dilutions of the targets were prepared in 7H9-ADC-TG in a volume

of 100 μL in 96-well, black, clear-bottom microplates (BD Biosciences, Franklin Lakes, NJ, USA). *Mycobacterium tuberculosis* (100 μL containing 2×10^5 CFU) was added, yielding a final testing volume of 200 μL. The plates were incubated at 37°C; on day 7 of incubation, 12.5 μL of 20% Tween 80 and 20 μL of Alamar blue were added to all of the wells. After incubation at 37°C for 16 to 24 h, the fluorescence was read at an excitation of 530 nm and an emission of 590 nm. The MIC was defined as the lowest concentration affecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacterium-only controls. MICs against nonreplicating *M. tuberculosis* were determined using a low-oxygen-recovery assay.

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