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DRUG SYNTHESIS

SYNTHESIS AND DISCOVERY OF NEW BISADDUCTS DERIVED FROM HETEROCYCLIC ALDEHYDES AND ACTIVE METHYLENE COMPOUNDS AS POTENT ANTITUBERCULAR AGENTS

MOHAMMAD ASAD[1], CHUAN-WEI OO[1], RAJU SURESH KUMAR[1], HASNAH OSMAN[1] and MOHAMED ASHRAF ALI[2*]

[1]School of Chemical Sciences, University Sains Malaysia, Minden 11800, Penang, Malaysia
[2]Institute for Research in Molecular Medicine, University Sains Malaysia, Minden 11800, Penang, Malaysia
[2]Sunrice University, Faculty of Pharmacy, Department of Drug Discovery Research, Alwar, Rajasthan, India-301030

Abstract: A series of some new bisadducts possessing five, six membered and coumarin subunits were synthesized by the condensation of heterocyclic aldehydes with active methylene compounds and characterized by IR, NMR and X-ray crystallographic studies and were assayed as antitubercular agents. Among the bisadducts, 4-hydroxy-3-[(4-hydroxy-2-oxo-2*H*-3-chromenyl)(3-thienyl)methyl]-2*H*-2-chromenone **3a** was found to be the most promising compound, active against *Mycobacterium tuberculosis (Mtb)* H_{37}Rv and isoniazid resistant *Mycobacterium tuberculosis (INHR-Mtb)* with minimum inhibitory concentration 5.22 and 8.34 μM, respectively.

Keywords: heterocyclic aldehydes, active methylene compounds, bisadducts, *Mycobacterium tuberculosis*

Tuberculosis (TB) is making a worldwide resurgence. Several factors may be responsible for the increase in the infection rate like infection with human immunodeficiency virus, changing economic and social circumstances and decline in tuberculosis control programs (1). Modern chemotherapy has played a major role in the control of tuberculosis. Yet tuberculosis still remains a leading infectious disease worldwide, largely owing to persistence of tubercle bacillus and inadequacy of the current chemotherapy. The increasing emergence of drug-resistant tuberculosis along with the HIV pandemic threatens the disease control and highlights the necessity for the understanding of the mechanism of the current drugs and the importance to develop more effective drugs. The next threat for tuberculosis is the emergence of drug resistant strains of *Mycobacterium tuberculosis*. In addition, outbreaks of multi-drug resistant tuberculosis have been identified (2). When the AIDS pandemic began, one third of the world population was infected with *Mycobacterium tuberculosis*. Each year, eight to ten million people are developing active disease and three million people die from tuberculosis. Currently, the available first-line antituberculous agents such as rifampcin, ethambutol, streptomycin and pyrazinamide are highly effective and are generally well tolerated. Problems in the chemotherapy of tuberculosis arise when any patients develop resistance to any of these drugs. This is due to the fact that the second-line drugs such as *p*-aminosalicylic acid, amikacin, cycloserine, capreomycin and ethionamide are less effective and more toxic (3). The global mortality rate for TB is very high and the development of new kinds of TB like MDR and XDR TB alarming for the discovery of new drugs to reduce the potential hazards caused by the fatal disease.

In the past decade, most heterocyclic systems have been used as a source to discover new compounds with varied biological potentials. Especially, nitrogen containing heterocyclic moieties play a vital role in discovering novel candidates having antimicrobial potentials (4, 5). Based on these papers, the current work was designed to synthesize new bis adducts possessing heterocyclic

* Corresponding author: e-mail: asraf80med@rediffmail.com

units. The present investigation of the new compounds shows encouraging antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB) and INH resistant *Mycobacterium tuberculosis* (INHR-MTB).

The oxygen and nitrogen containing active methylene compounds (AMC) have attracted a vast deal of significance due to their association with various kinds of biological properties (6, 7). They are used as precursors for various heterocyclic compounds (8, 9) as well as flavonoids and isoflavonoids and are involved in the biosynthesis of natural products (10, 11). In addition, the different heterocyclic compounds synthesized from AMC have been found to possess diverse biological properties such as cytotoxicity and enzyme inhibitory activities (12), anticonvulsant and antiepileptic (13), whereas tricyclic and tetracyclic moieties were used as anticancer agents (14, 15). The varied biological importance of compounds from AMC and oxygen, sulfur containing heterocyclic aldehydes, and our ongoing work on the synthesis of novel heterocyclic leads and screening them for antimycobacterial activities (16–23), led us to investigate the new bis adducts possessing five, six membered and coumarin subunits as antitubercular agents and to report the results in this paper.

EXPERIMENTAL

Melting points were measured in open capillary tubes and are uncorrected. IR spectra were recorded on a Perkin Elmer system 2000 Fourier-transform (FT)-IR instrument in KBr. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500, 400 and 300 MHz instruments in $CDCl_3$/DMSO and tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are given in parts per million (δ-scale) and the coupling constants are given in hertz. All compounds were synthesized by our new procedure. The purity of all compounds was checked on TLC plates using silica gel (Merck G_{254}), the TLC plates were developed in chloroform : methanol (4:1, v/v) solvent system and visualized by UV lamp and iodine vapors. Single crystal X-ray data set for **1b**, **3a** and **3e** was collected on Bruker APEXII DUO CCD area detector diffractometer with Mo K_α ((λ = 0.71073 Å) radiation. Scan range was $3.3^o \le \Theta \le 32.6^o$ (**1b**), $2.4^o \le \Theta \le 26.0^o$ (**3a**) and $2.6^o \le \Theta \le 30.6^o$ (**3e**). Elemental analyses were performed on a Perkin Elmer 2400 Series II Elemental CHNS analyzer.

Chemistry

General method for the preparation of chromene carbaldehyde (**1b**)

To a well stirred solution of 4-fluoro-2-hydroxyacetophenone (6.5 mmol) in DMF (4 mL), $POCl_3$ (26.1 mmol) was added dropwise with stirring in ice bath. After 15 min, the ice bath was removed and the reaction mixture was continued to be stirred at room temperature for overnight. The resultant reaction mixture was then decomposed by pouring on the crushed ice and the final product was collected by filtration, washed with ethanol-water (1:1, v/v) and recrystallized from acetone to afforded **1b**. Yellow solid; IR (KBr, cm^{-1}): 1712 (CHO), 1654 (C=O), 1625 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.22-7.29 (m, 2H, Ar-H), 8.29-8.32 (m, 1H, Ar-H), 8.52 (s, 1H, H-2), 10.35 (s, 1H, CHO). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 105.6 ($^2J_{CF}$ = 100 Hz), 115.4 ($^2J_{CF}$ = 90 Hz), 120.4, 122.1 ($^4J_{CF}$ = 10 Hz), 128.8 ($^3J_{CF}$ = 45 Hz), 157.2 ($^3J_{CF}$ = 50 Hz), 160.7 ($^1J_{CF}$ = 256.3 Hz), 166.1, 175.0, 188.2. Analysis: calcd. for $C_{10}H_5FO_3$: C, 62.51; H, 2.62%; found: C, 62.38; H, 2.53%.

General method for the preparation of symmetrical bisadducts (**3a**-**i**)

A mixture of heterocyclic aldehyde **1a,b** (1 mmol) and AMC **2a**-**e** (2 mmol) in methanol (20 mL) were stirred at room temperature for overnight. The completion of the reaction was monitored by TLC. After completion of the reaction, the crude product was filtered, washed with methanol and dried. The isolated product was further purified by recrystallization from chloroform-methanol (1:1, v/v) to give the pure compounds (**3a**-**i**) respectively.

4-Hydroxy-3-[(4-hydroxy-2-oxo-2*H*-3-chromenyl)(3-thienyl)methyl]-2*H*-2-chromenone (**3a**)

White solid; IR (KBr, cm^{-1}): 3107, 1660, 1614. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.96 (d, *J* = 2.0 Hz, 1H, CH), 6.88 (dd, *J* = 6.8, 2.0 Hz, 1H, Ar-H), 7.00-7.02 (m, 1H, Ar-H), 7.28-7.31 (m, 1H, Ar-H), 7.37-7.42 (m, 4H, Ar-H), 7.61-7.67 (m, 2H, Ar-H), 7.97-8.12 (m, 2H, Ar-H), 11.30 (brs, 1H, OH), 11.61 (brs, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 34.1, 105.7, 116.9, 117.2, 121.8, 124.7, 125.1, 126.1, 127.0, 127.3, 133.0, 136.7, 152.9, 164.9. Analysis: calcd. for $C_{23}H_{14}O_6S$: C, 66.02; H, 3.37%; found: C, 66.18; H, 3.25%.

3-[(7-Fluoro-4-oxo-4*H*-3-chromenyl)(4-hydroxy-2-oxo-2*H*-3-chromenyl)methyl]-4-hydroxy-2*H*-2-chromenone (**3b**)

Light yellow solid; IR (KBr, cm^{-1}): 3076, 1650, 1626. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.00 (d,

J = 1.8 Hz, 1H, CH), 7.10-7.22 (m, 2H, Ar-H), 7.36-7.50 (m, 4H, Ar-H), 7.60-7.76 (m, 2H, Ar-H), 7.90 (d, *J* = 1.5 Hz, 1H, Ar-H), 8.03-8.15 (m, 3H, Ar-H), 11.50 (brs, 2H, 2OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 30.8, 104.5 (J_{CF} = 27.4 Hz), 114.4 (J_{CF} = 22.8 Hz), 117.0 (J_{CF} = 5.8 Hz), 119.6, 120.8, 124.7, 125.2, 128.8, 129.0, 133.1, 152.7, 153.8, 157.7, 164.4, 164.7, 167.8, 168.4, 176.2. Analysis: calcd. for $C_{28}H_{15}FO_8$: C, 67.47; H, 3.03%; found: C, 67.36; H, 3.24%.

4-Hydroxy-3-[(4-hydroxy-6,7-dimethyl-2-oxo-2*H*-3-chromenyl)(3-thienyl)methyl]-6,7-dimethyl-2*H*-2-chromenone (3c)

White solid; IR (KBr, cm^{-1}): 3150, 1655, 1624. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.35 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.40, (s, 3H, CH_3), 5.92 (d, *J* = 1.6 Hz, 1H, CH), 6.86 (dd, *J* = 4.8, 1.2 Hz, 1H, Ar-H), 6.98-7.00 (m, 1H, Ar-H), 7.20-7.30 (m, 3H, Ar-H), 7.73 (s, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 11.30 (brs, 1H, OH), 11.62 (brs, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 19.7, 20.8, 34.0, 105.4, 117.4, 121.6, 124.5, 126.1, 127.4, 134.2, 137.1, 143.5, 151.2, 165.2, 165.6. Analysis: calcd. for $C_{27}H_{22}O_6S$: C, 68.34; H, 4.67%; found: C, 68.26; H, 4.73%.

3-[(7-Fluoro-4-oxo-4*H*-3-chromenyl)(4-hydroxy-6,7-dimethyl-2-oxo-2*H*-3-chromenyl) methyl]-4-hydroxy-6,7-dimethyl-2*H*-2-chromenone (3d)

Light yellow; IR (KBr, cm^{-1}): 3413, 1645, 1618 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.33 (s, 6H, $2CH_3$), 2.36 (s, 6H, $2CH_3$), 5.92 (d, *J* = 1.8 Hz, 1H, CH), 7.06-7.30 (m, 5H, Ar-H), 7.73-8.12 (m, 4H, Ar-H), 11.52 (brs, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 19.6, 19.9, 20.6, 20.9, 103.4, 114.5, 117.4, 119.6, 120.6, 120.7, 124.5, 134.3, 143.5, 151.2, 154.0, 157.5, 164.3, 165.1, 167.6, 168.7, 176.3. Analysis: calcd. for $C_{32}H_{23}FO_8$: C, 69.31; H, 4.18%; found: C, 69.23; H, 4.41%.

4-Hydroxy-3-[(4-hydroxy-6-methyl-2-oxo-3,6-dihydro-2*H*-3-pyranyl)(3-thienyl)methyl]-6-methyl-3,6-dihydro-2*H*-2-pyranone (3e)

White solid; IR (KBr, cm^{-1}): 3134, 1675, 1621. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.30, (s, 6H, $2CH_3$), 5.64 (d, *J* = 1.5 Hz, 1H, CH), 6.82-6.84 (m, 2H, Ar-H), 6.94-6.95 (m, 1H, Ar-H), 6.96-7.00 (m, 1H, Ar-H), 7.26-7.30 (s, 1H, Ar-H), 10.74 (brs, 2H, OH), 10.96 (brs, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 19.7, 29.9, 32.7, 103.3, 104.3, 121.5, 125.8, 127.3, 136.9, 161.8, 169.3. Analysis: calcd. for $C_{17}H_{14}O_6S$: C, 58.95; H, 4.07%; found: C, 58.74; H, 4.25%.

3-[Di(4-hydroxy-6-methyl-2-oxo-3,6-dihydro-2*H*-3-pyranyl)methyl]-7-fluoro-4*H*-4-chromenone (3f)

White solid; (KBr, cm^{-1}): 3076, 1670, 1638. ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.13, (s, 6H, $2CH_3$), 5.41 (s, 1H, CH), 5.93 (s, 2H, Ar-H), 7.30-8.10 (m, 4H, Ar-H), 11.30 (brs, 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 20.6, 31.5, 100.7, 105.8, 120.7, 124.3, 124.5, 153.5, 157.6, 157.7, 161.0, 163.9, 164.8, 164.9, 166.9, 167.2, 176.1. Analysis: calcd. for $C_{22}H_{15}FO_8$: C, 61.98; H, 3.55%; found: C, 61.83; H, 3.68%.

3-Hydroxy-2-[(2-hydroxy-4,4-dimethyl-6-oxo-2-cyclohexenyl)(3-thienyl)methyl]-5,5-dimethyl-3-cyclohexen-1-one (3g)

White solid; IR (KBr, cm^{-1}): 3422, 1622, 1594. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.11 (s, 6H, $2CH_3$), 1.22 (s, 6H, CH_3), 2.30-2.50 (m, 8H, $4CH_2$), 5.41 (d, *J* = 1.5 Hz, 1H, CH), 6.76 (dd, *J* = 4.8, 1.2 Hz, 1H, Ar-H), 6.78-6.81 (m, 1H, Ar-H), 7.21-7.24 (m, 1H, Ar-H), 12.03 (brs, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 27.5, 30.0, 30.7, 31.7, 46.7, 47.4, 116.5, 120.8, 125.4, 127.8, 139.6, 189.6, 189.9. Analysis: calcd. for $C_{21}H_{26}O_4S$: C, 67.35; H, 7.00%; found: C,67.63; H, 7.21%.

3-Methyl-4-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-4-pyrazolyl)(3-thienyl)methyl]-1-phenyl-4,5-dihydro-1*H*-5-pyrazolone (3h)

White solid; IR (KBr, cm^{-1}): 3347, 1648, 1598. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.10 (s, 6H, $2CH_3$), 3.72 (d, *J* = 1.2 Hz, 1H, H-4), 4.72 (d, *J* = 0.8 Hz, 1H, CH), 6.81(dd, *J* = 4.8, 1.2 Hz, 1H, Ar-H), 6.96-7.00 (m, 1H, Ar-H), 7.10-7.33 (m, 8H, Ar-H), 7.52-7.60 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 11.9, 30.6, 106.5, 120.6, 121.6, 121.7, 122.5, 125.9, 126.6, 127.9, 129.3, 137.4, 142.1, 146.4, 158.2. Analysis: calcd. for $C_{25}H_{22}N_4O_2S$: C, 67.85; H, 5.01; N, 12.66%; found: C,67.52; H, 5.34, N, 12.55%.

4-[(7-Fluoro-4-oxo-4*H*-3-chromenyl)(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-4-pyrazolyl) methyl]-3-methyl-1-phenyl-4,5-dihydro-1*H*-5-pyrazolone (3i)

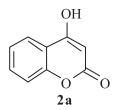
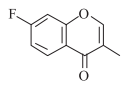
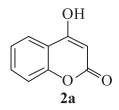
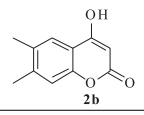
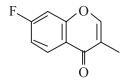
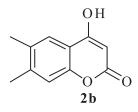
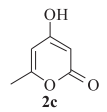
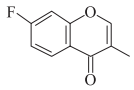
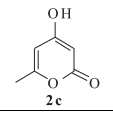
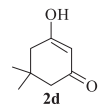
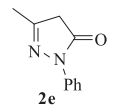
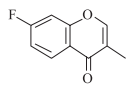
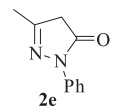
Light yellow solid; IR (KBr, cm^{-1}): 1736, 1651, 1615. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.18 (s, 6H, $2CH_3$), 3.68 (d, *J* = 7.2 Hz, 1H, CH), 5.00 (s, 1H, H-4, H-4'), 7.05-7.12 (m, 4H, Ar-H), 7.23-7.30 (m, 4H, Ar-H), 7.40 (s, 1H, Ar-H), 7.51-753 (m, 4H, Ar-H), 8.13 (m, 1H, Ar-H), 8.35 (s, 1H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 12.1, 25.6, 104.8, 105.2, 113.9, 114.3, 121.1, 121.7, 124.6, 126.5,

128.3, 128.5, 128.6, 129.1, 137.6, 147.6, 155.4, 158.8, 176.6. Analysis: calcd. for $C_{30}H_{23}FN_4O_4$: C, 68.96; H, 4.44; N, 10.72%; found: C, 68.74; H, 4.58, N, 10.63%.

Antitubercular evaluation

The primary screening was conducted at a concentration of 6.25 µg/mL (or molar equivalent of highest molecular weight compound in a series of congeners) against *M. tuberculosis H37Rv* (ATCC27294) and INH resistant *M. tuberculosis* in BACTEC 460 radiometric system (24–26). Compound demonstrating at least 90% inhibition in the primary screen was re-examined at lower concentration (MIC) in broth micro dilution assay with alamar blue. The MIC was defined as the lowest

Table 1. Physical constants and antimycobacterial activity of the synthesized compounds.

Comp. no.	R	Active methylene compound	Yield (%)	M.P (°C)	(MIC) µM	
					MTB[a]	MTB[b]
1b	-	-	75	130-131	> 6.25	> 6.25
3a		 **2a**	70	226-228	5.22	8.34
3b		 **2a**	75	228-230	> 20.0	> 20.0
3c		 **2b**	78	238-240	> 10.0	> 10.0
3d		 **2b**	76	244-246	> 10.0	> 10.0
3e		 **2c**	67	192-194	> 10.0	> 10.0
3f		 **2c**	65	196-198	> 10.0	> 10.0
3g		 **2d**	85	175-177	5.78	9.72
3h		 **2e**	68	146-148	> 10.0	> 10.0
3i		 **2e**	65	160-162	> 10.0	> 10.0
INH	-	-	-	-	0.73	11.37

[a]*Mycobacterium tuberculosis* $H_{37}R_v$; [b]INH resistant *Mycobacterium tuberculosis*

concentration inhibiting 99% of the innoculum. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC_{50}) in VERO at concentration equal to and greater than the MIC for *M. tuberculosis H37Rv* and INH resistant *M. tuberculosis* after 72 h exposure. Viability was assessed on the basis of cellular conversion of MTT (3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan product using the promega cell Titer 96 non radioactive cell proliferation assay (27).

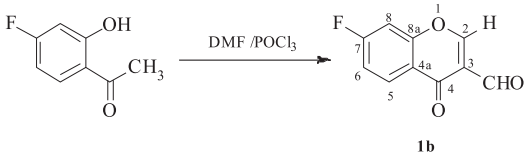
Antimycobacterial assay

The synthesized compounds **3a**-**3i** were tested for their antimycobacterial activity *in vitro* against MTB and INHR-MTB by agar dilution method using double dilution technique similar to that recommended by the National Committee for Clinical Laboratory Standards (24). The MIC was defined as the minimum concentration of compound required to inhibit 90% of bacterial growth and MIC's of the compounds are reported in Table 1 with standard drug INH for comparison.

RESULTS AND DISCUSSION

Chemistry

In the present investigation, the reaction of AMC with heterocyclic aldehydes furnished the new bisadducts **3a**-**i** in good yields. Heterocyclic aldehy-



Scheme 1. Synthesis of 7-fluoro-4-oxo-4*H*-3-chromenecarbaldehyde **1b**

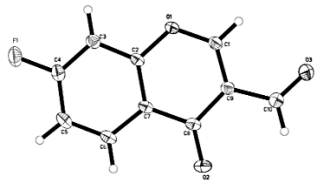
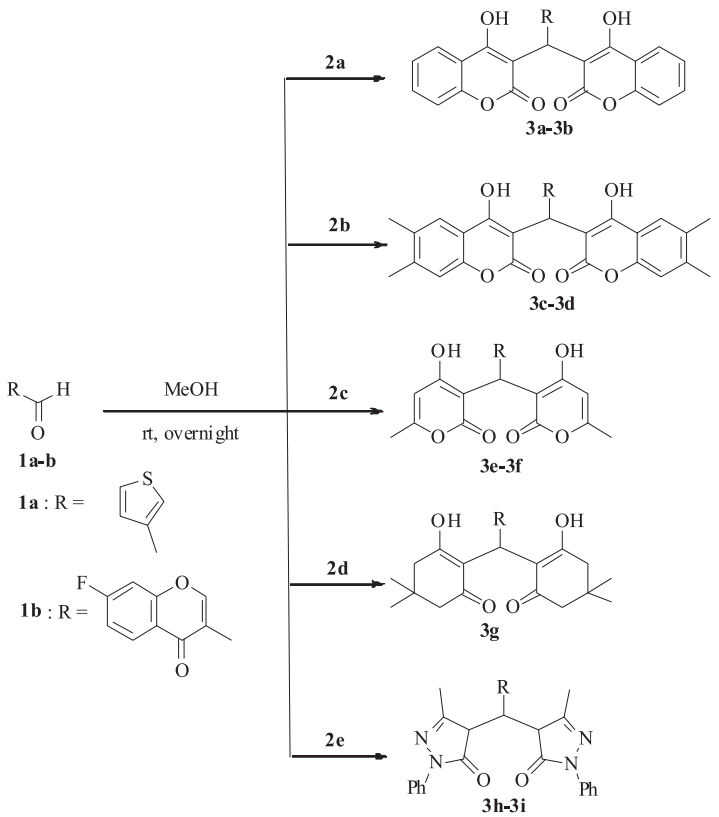
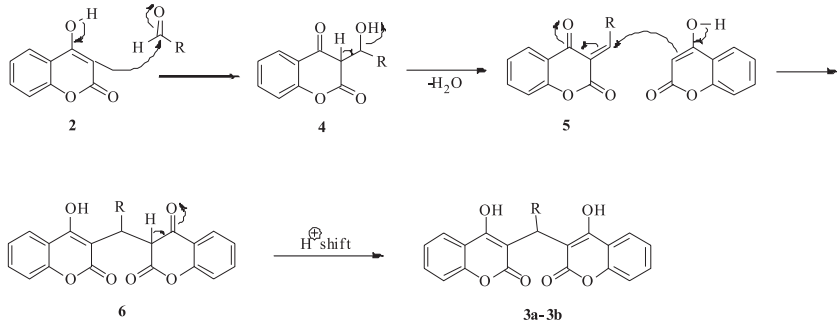


Figure 1. ORTEP diagram of **1b**



Scheme 2. Synthesis of bisadducts **3a**-**i**



Scheme 3. Mechanism for the formation of bisadducts **3a-i**

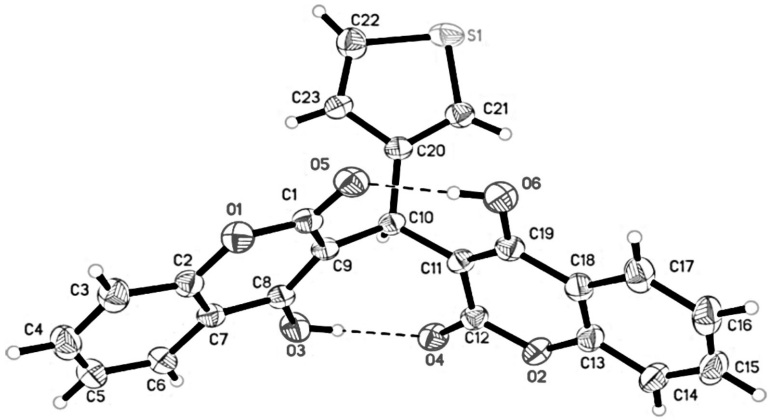


Figure 2. ORTEP diagram of **3a**

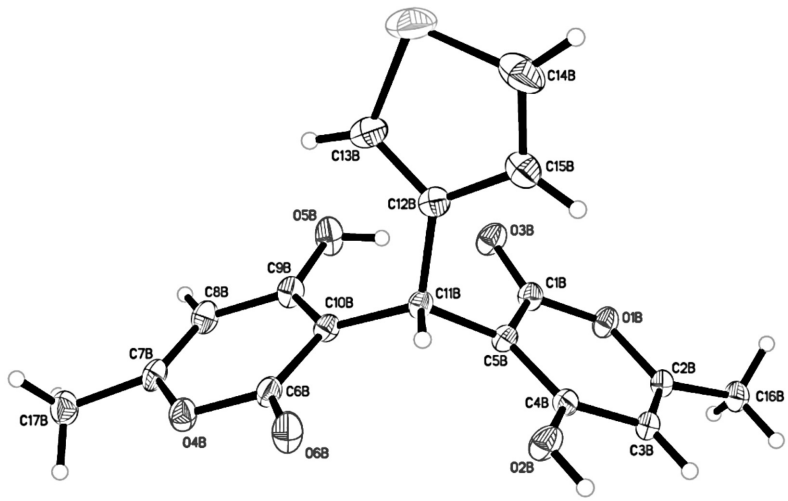


Figure 3. ORTEP diagram of **3e**

des namely, thiophene-3-carboxaldehyde and 7-fluoro-3-formylchromone are used as a scaffold in the synthesis of new fused heterocyclic systems. The aldehyde **1b**, 7-fluoro-3-formylchromone, was synthesized by following the literature procedure (28) from 4-fluoro-2-hydroxyacetophenone using Vilsmeier-Hack reagent (DMF + $POCl_3$) (Scheme 1). The structure of **1b** was confirmed by IR and NMR spectroscopy. In the IR spectrum of **1b**, the absorption band at 1712 cm^{-1} is due to formyl group and the peak at 1654 cm^{-1} is due to strong band of chromone carbonyl group and C=C band appears at 1625 cm^{-1}. In the ^{1}H NMR spectrum of **1b**, the singlets at δ 10.35 and 8.52 ppm is due to the aldehyde and the olefinic (H-2) protons, respectively. The aromatic protons appear as multiplets at 7.22-7.29 ppm and 8.29-8.32 ppm. In the ^{13}C NMR spectrum, the signals at 188.3 and 175.0 ppm are due to formyl C=O and chromene C=O, respectively, and those for C-2 and C-3 appear at 160.7 ppm and 120.4 ppm. The aromatic carbons appear as doublets in the region 105.5-167.1 ppm due to C-F coupling. The structure of **1b** was further confirmed by X-ray crystallographic studies (Fig. 1) (29). In **1b**, the chromenone ring is essentially planar, with a maximum deviation of 0.039 Å (1). The dihedral angle between the fluoro-subsituted benzene ring and the pyran ring is 1.92° (4). In the crystal, molecules are connected *via* weak intermolecular C-H…O hydrogen bonds, forming supramolecular ribbons along the *b* axis. These ribbons are stacked down the *a* axis.

The symmetrical analogues of a variety of the bisadducts were synthesized by the Knoevenagel condensation of AMC and heterocyclic aldehydes in a molar ratio 2:1 in methanol at room temperature stirring for overnight (Scheme 2). The precipitated solid was filtered off and washed with methanol to afford the bisadducts in 65-85% yields. AMC *viz*., 4-hydroxycoumarin **2a**, 6,7-dimethyl-4-hydroxycoumarin **2b**, triacetic acid lactone **2c**, dimedone **2d** and 3-methyl-1-phenyl-pyrazolone-5-one **2e** and heterocyclic aldehydes such as thiophene-3-carboxaldehyde **1a** and 7-fluoro-3-formylchromone **1b** were used for the present study. The structure of all bisadducts was characterized by IR, NMR spectroscopic data and elemental analysis. In the IR spectrum of **3a**, the absorption bands for OH, C=O and C=C appear at 3107, 1660 and 1614 cm^{-1}, respectively. In the ^{1}H NMR spectrum of **3a**, the aliphatic methine proton appears as a doublet at 5.96 ppm with J = 2.0 Hz. The aromatic protons appear as doublet of doublets and multiplets at 5.96-8.12 ppm. The broad singlets at 11.30 and 11.61 ppm are due to two OH groups of the coumarin ring. The structure and stereochemistry of the symmetrical analogue of bisadducts **3** was further confirmed by X-ray crystallographic studies (Fig. 2 and 3) (30, 31). In the crystal of **3a**, the molecules are linked by intermolecular C-H…O interactions, forming chains along the *b* axis. The structure is further stabilized by π-π interactions with centroid–centroid distances of 3.594 (2) and 3.608 (5) Å. In the crystal of **3e**, molecules are linked through intermolecular O-H…O and C-H…O hydrogen bonds, forming a three-dimensional network.

A probable mechanism for the formation of bisadducts **3a**-**3i** is shown in Scheme 3. The nucleophilic addition of active methylene to the C=O of the aldehyde affords the enol **4**, which on dehydration gives the primary product, the unsaturated adduct **5**. Again the addition of active methylene hydrogen of **2** to **5** gives the adduct **6** which on subsequent hydrogen shift affords the symmetrical bisadducts **3**.

Antitubercular activity

Among the ten synthesized compounds, two compounds were found to be the most active with minimum inhibitory concentration of less than 10 μM and were more active than INH against INHR-MTB. Compounds with thiophene group substituted on the ring were showing better activity. Compound **3a** was found to be the most active agent against *Mycobacterium tuberculosis* $H37_{Rv}$ (MTB) and INH resistant *Mycobacterium tuberculosis* (INHR-MTB) with minimum inhibitory concentration of < 10.0 μM, followed by compound **3g** which was found to be active with MIC of 5.78 and 9.72 μM, respectively. The rest of the compounds produced low inhibitory activity against both *Mycobacterium* strains. These reports clearly show that the presence of thiophene ring at this analogue shows remarkable improvement in antimycobacterial activity.

All the compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μM/mL (i.e., 10 times of MIC of the compounds). After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive cell proliferation method. Most of the active compounds were found to be non-toxic till 62.5 μg/mL.

CONCLUSION

The screening of all the bisadduct derivatives identified novel compounds that are endowed with

antimycobacterial activity. It is conceivable that derivatives showing more potency, selectivity and low toxicity make them excellent leads for synthesizing novel derivatives for antimycobacterial activity against MTB and INHR-MTB. Also these derivatives can be further modified to exhibit better potency than the standard drugs. Further studies are ongoing in our laboratory to acquire more information about Quantitative Structure-Activity Relationships (QSAR) and MDR. The bisadduct derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of anti-tubercular diseases.

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