Novel indole-thiazolidinone conjugates: Design, synthesis and whole-cell phenotypic evaluation as a novel class of antimicrobial agents

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ABSTRACT

In connection with our research program on the development of novel anti-tubercular candidates, herein we report the design and synthesis of two different sets of indole-thiazolidinone conjugates (**8a,b**; **11a-d**) and (**14a-k**; **15a-h**). The target compounds were evaluated for their *in vitro* antibacterial and antifungal activities against selected human pathogens *viz*. *Staphylococcus aureus* (Gram positiveve), *Pseudomonas aeruginosa, Escherichia coli* (Gram negative), *Mycobacterium tuberculosis* (Acid-fast bacteria), *Aspergillus fumigates and Candida albicans* (fungi). Moreover, eukaryotic cell-toxicity was tested *via* an integrated *ex vivo* drug screening model in order to evaluate the selective therapeutic index (SI) towards antimicrobial activity when microbes are growing inside primary immune cells. Also, the cytotoxicity towards a panel of cancer cell lines and human lung fibroblast normal cell line, WI-38 cells, was explored to assure their safety. Compound **15b** emerged as a hit in this study with potent broad spectrum antibacterial (MIC: 0.39-0.98 µg/ml) and antifungal (MIC: 0.49-0.98 µg/ml) activities, in addition to its ability to kill mycobacteria *M. aurum* inside an infected macrophage model with good therapeutic window. Moreover, compound **15b** displayed promising activity towards resistant bacteria strains MRSA and VRE with MIC values equal 3.90 and 7.81 µg/mL,

respectively. These results suggest compound **15b** as a new therapeutic lead with good selectivity for further optimization and development.

Keywords: Antimicrobial resistance (AMR); Anti-tubercular agents; Indole and Isatin; Macrophage Infection Model; MRSA and VRE; Thiazolidinone.

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1. Introduction

With the regular rise of antimicrobial resistance (AMR) to our best available last stage of antibiotics, our advance medical system is rapidly reaching towards apocalypse by having no available treatment and millions of death along with a serious threat of a return to the pre-antibiotic era. If there is no action taken place then there is an estimate of drug-resistant infections that will kill 10 million people a year by 2050 [1]. It is truly difficult to treat an ever-increasing range of infections caused by superbugs (resistant bacteria, parasites, viruses and fungi) due to serious lack of effective antibiotics.

Although antibacterial resistance is a widely recognized public health threat, but the burden of drug-resistant fungal infections are also an emerging issue [2]. Other than ESKAPE and MRSA, Tuberculosis (TB) is still having continuous global impact on the health worldwide, as nearly one-third of the world`s population is directly and indirectly infected with TB [3]. In 2016, Asia occupied the first rank in new TB cases with about 45% of the total new cases followed by Africa with 25% of the new cases [4]. Nevertheless, multi-drug resistant (MDR) TB and extremely drug resistant (XDR) TB are rapidly emerged, increasing the urgency to develop new antiTB agents based on innovative scaffolds, thus lacking the cross resistance with current therapeutics. Continuous efforts in all different directions to develop broad spectrum as well as specific and targeted drugs against different microbes, are required. A recent strategy to design new agents based on hybridization of different pharmacophores binding diverse biomolecular targets to gain an effective therapy against drug resistant infectious diseases [5].

As per recent report, only eight out of the 51 new biologicals in clinical development are new class of drugs that could add value to the current therapies while most of others are only short-term solutions [6]. During the last two decades, thiazolidinone ring has emerged as novel important core in many chemotherapeutic agents special when conjugated with substituted aminothiazoles derivatives. For example, the aminothiazole derivative **I** was found to exhibit promising antibacterial and antifungal activities [7]. Moreover, ethyl 2-amino-4-methylthiazole-5-carboxylate derivative **II** revealed potent activity against the virulent species of *M. tuberculosis* H37Rv [8]. Therefore, our research team was motivated to synthesize some thiazolidinone/2-amino-4-methylthiazole hybrids bearing 5-acetyl/5-ethyl carboxylate functionalities as potential anti-tubercular agents [9]. Among the synthesized hybrids, compound

III exhibited good antitubercular effect (MIC=1.56 μg/mL) with broad spectrum antibacterial and antifungal activities [9]. It possessed an excellent safety profile with high selectivity toward *M. tubercolosis* over normal human lung cells (selectivity index= 52.11) and considered as good lead for further optimization. Literature survey revealed that, the antimicrobial spectrum and potency of thiazolylamino-thiazoledinone derivatives can be manipulated by changing type or nature of arylidene substituent at position 5 of thiazoledinone ring. It was reported that the incorporation of indolyl moiety as in *N*'-[(1*H*-indol-3-yl)methylene]-isonicotinohydrazide derivative **(IV)** or 2-oxindolyl moiety as in (1,3,4-thiadiazol-2-yl)imino)indolin-2-one derivative **(V)** showed excellent to good anti-tubercular activity [10, 11]. Thus, in continuation of our team work to obtain an optimized scaffold with potent anti-tubercular, antibacterial and antifungal activities [12-15], two series of thiazolylamino-thiazolidinone hybrids were designed in which the 5-pyridylidene moiety in the hybrid **III** was replaced by *N*-(un)substituted 5-[(1*H*-indol-3-yl)methylene] moiety in compounds **8a,b** and **11a-d** or by (un)substituted-2-oxindole in series **14a-k** and **15a-h** to study the effect of such structure modifications on activity (**Figure 1**). As a part of the urgent focus on the discovery of novel lead molecules to fuel the pipeline of antibiotic design, discovery and development in order to tackle the alarming emergence of antimicrobial resistance, we aim to explore the potential of few novel chemical entities with their promising antimicrobial activities and broad-spectrum mode of antibiotic action. The target compounds **8a,b**, **11a-d, 14a-k** and **15a-h** will be evaluated for their *in vitro* anti-tubercular, antibacterial and antifungal activities against selected human pathogens *viz. M. tuberculosis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus fumigates and Candida albicans.* Furthermore, an integrated *ex vivo* drug screening model will be used to evaluate certain selected conjugates for their intracellular inhibition and selectivity towards *Mycobacterium aurum in* mouse macrophages RAW 264.7. Moreover, the cytotoxicity of the most active conjugate towards a panel of cancer cell lines and human lung fibroblast normal cell line, WI-38 cells, will be explored to assure its safety.

Figure 1

2. Results and Discussion

2.1. Chemistry

The synthetic pathways employed to prepare the new target conjugates are depicted in **Schemes 1-3**. *α*-Chloro-*β*-dicarbonyl derivatives **2a,b** were obtained *via* chlorination of acetyl acetone **1a** or ethyl acetoacetate **1b**, using sulfuryl chloride in dry toluene. Subsequent heterocyclization of compounds **2a,b** with thiourea furnished the corresponding 2-aminothiazoles **3a**,**b**. The latter were acetylated with chloroacetyl chloride in dry DMF to furnish compounds **4a,b**, which were cyclized with ammonium thiocyanate in absolute ethanol to give the key intermediates **5a,b** in 75 and 70% yield (**Scheme 1**).

Scheme 1

In **Scheme 2**, the synthesis of the *N*-substituted-indole-3-carbaldehyde derivatives **10a**,**b** was accomplished *via* formylation of 1*H*-indole **6** with phosphorus oxychloride and DMF to afford 1*H*-indole-3-carbaldehyde **7**, which subsequently alkylated with bromomethane **9a** or benzyl bromide **9b** in DMF in the presence of sodium hydride. The first series of the target conjugates **8a,b** and **11a-d** were obtained in good yields (75-83%) through Knoevenagel condensation of intermediates **5a**,**b** with 1*H*-indole-3-carbaldehyde **7** or *N*-substituted-indole-3-carbaldehydes **10a, b** in glacial acetic acid in the presence of anhydrous sodium acetate.

Scheme 2

Finally, alkylation of isatins **12a**,**c** with bromomethane **9a** or benzyl bromide **9b** was carried out *via* heating in DMF in the presence of potassium carbonate to furnish *N*-alkylated isatins **13a-d**. The target compounds (**14a-k** and **15a-h**) were obtained through condensation of the intermediates **5a**,**b** with the appropriate isatin derivative (**12a-f** and **13a-d**) in glacial acetic acid

in the presence of anhydrous sodium acetate under reflux temperature with 64-75% yields (**Scheme 3**).

Scheme 3

Postulated structures of the newly prepared conjugates **8a**,**b**, **11a-d**, **14a-k** and **15a-h** were in full agreement with their spectral and elemental analyses data. IR spectra of the target compounds showed the absorption bands due to the (NH) and (C=O) groups in the regions 3117-3395 and 1651-1740 cm^{-1}, respectively. The ^{1}H NMR spectra of all new compounds showed D_2O-exchangeable singlet signals corresponding to thiazolidinone NH proton at range δ = 11.92-13.21 *ppm*, additionally, ^{1}H NMR spectra of compounds **8a**,**b** displayed another D_2O-exchangeable singlet signals attributable for indole NH at δ 12.11 and 12.13 *ppm*, respectively, whereas the oxindole NH protons of compounds **14a-k** appeared at range δ 10.86-11.42 *ppm*. Moreover, the ^{1}H NMR spectra of the prepared conjugates revealed the presence of singlet signals around δ 2.60 *ppm* attributed to the CH_3 group at position 4 of the thaizole ring . The acetyl protons (CH_3C=O) of conjugates **8a**, **11a**,**b**, **14a-f** and **15a-d** appeared as singlet signals around δ 2.49 *ppm*, while the ethyl ester protons (CH_3CH_2COO-) of conjugates **8b**, **11c**,**d**, **14g-k** and **15e-h** displayed as triplet and quartet signals around δ 1.24 and 4.21 *ppm*, respectively. Furthermore, ^{1}H NMR spectra of compounds **8a**,**b** and **11a-d** confirmed the presence of the singlet signal of the olefinic protons (CH=N) around δ 8.0 *ppm*. The *N*-methyl_substituted derivatives **11a**,**c** and **15a**,**b**,**e**,**f** showed single signals at the range δ= 3.94-3.23 *ppm attributed to* *N*-$C\underline{H_3}$, while those with *N*-benzyl substituent **11b**,**d** and **15c**,**d**,**g**,**h** revealed singlet signals at δ range 5.01-5.60 *ppm* due to the benzylic (-CH_2) protons.

Moreover, ^{13}C NMR spectra of conjugates **8a**,**b**, **11a-d**, **14a-k** and **15a-h** displayed signals resonating in the range δ 18.1-19.1 *ppm* attributable for the carbon of the thiazole CH_3, while the carbons of the acetyl groups (-CH_3C=O) of conjugates **8a**, **11a**,**b**, **14a-f** and **15a-d** appeared as two signals around δ 30.6 and 190.8 *ppm*, respectively, and the carbons of the ethyl ester groups (CH_3CH_2COO-) in conjugates **8b**, **11c**,**d**, **14g-k** and **15e-h** appeared as three signals around δ 14.6, 61.0 and 168.0 *ppm*, respectively. Also, signals attributable to (*N*-CH_3) carbon, as

compounds **11a,c**, and benzylic carbon (-CH_2), as compounds **11b,d**, appeared around δ 33.9 and 50.2 *ppm*, respectively.

2.2. Biological Evaluation

2.2.1. Antimicrobial activity

Anti-mycobacterial, antibacterial and antifungal activities for the target conjugates (**8a,b**, **11a-d**, **14a-k** and **15a-h**) and reference drugs were *in vitro* evaluated at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The results are displayed in **Tables 1-3**.

2.2.1.1. Anti-tubercular Activity; M. tuberculosis (RCMB 010126)

Target conjugates (**8a**,**b**, **11a-d**, **14a-k** and **15a-h**) were examined for their anti-tubercular activity against *M. tuberculosis* (RCMB 010126) using the Microplate Alamar Blue Assay (MABA) [16]. Isoniazid was used as reference drug. The results of such *in vitro* anti-mycobacterial screening are summarized in **Table 1**, as percent inhibition and minimum inhibitory concentration (MIC). As indicated in **Table 1**, compounds **14i, 14k, 15a, 15b, 15d, 15e, 15g** and **15h** (MIC = 0.39 µg/mL) exhibited the highest potency against *M. tuberculosis* (RCMB 010126), with 2-fold increased activity than the reference drug isoniazid (MIC = 0.78 µg/mL). Also, compounds **14c, 14g** and **15f** (MIC = 0.78 µg/mL), displayed potent activity which is equipotent to isoniazid.

Table 1

2.2.1.1.1. Structure activity relationship (SAR)

From the obtained anti-tubercular results, the following structure activity relationship can be concluded: the 1*H*-indole containing compounds **8a,b** exhibited moderate antitubercular activity**.** The 5-acetyl derivatives **8a** revealed better activity than its ester analog **8b** (MIC= 3.12 and 6.24 µg/ml, respectively), this activity was increased by two folds upon *N*-benzylation of the indole as

observed in compound **11b**. On the other hand, *N*-methylation (**11c**) or *N*-benzylation (**11d**) on the indole ring of ethyl carboxylate derivative **8b** abolished the activity. Direct attachment of 5-(un)substituted 2-oxindole to the lead scaffold in **14a-k** resulted in compounds with moderate to potent activity. Generally the ethyl carboxylate derivatives **14g-k** (MIC range = 0.39-1.56 μg/ml) revealed higher activity than their acetyl counterparts **14a-e** (MIC range = 0.78-12.48 μg/ml). The highest activity in this series was associated with the 5-chloro substituent on the 2-oxindole ring. *N*-methylation or ***N***-benzylation of the 2-oxindole in acetyl derivatives **15a-d** enhanced the activity by 4 and 2 folds, respectively, as compared to the *N*-unsubstituted analogs, **14a** and **14c**. However, applying the same structure modification in ethyl carboxylate derivatives **15e-h** resulted in 2 fold increase in activity of the unsubstituted 2-oxindole **14g** only.

2.2.1.2. Antibacterial Activity

All the prepared conjugates (**8a**,**b**, **11a-d**, **14a-k** and **15a-h**) were evaluated for their potential antimicrobial activity against one Gram-positive bacteria: *S. aureus* (RCMB 010028, Sa), and two Gram-negative bacteria: *P. aeruginosa* (RCMB010043, Pa) and *E. coli* (RCMB 010052, Ec). The results are displayed in **Table 2**, as inhibition zone (I.Z) and minimum inhibitory concentration (MIC). From the obtained results, it can be observed that compounds **11b, 14c, 14e, 14g, 14i, 14k** and **15a-h** displayed broad-spectrum antibacterial activity against the tested Gram positive and Gram negative bacteria as compared with ciprofloxacin.

Regarding Gram positive bacteria, compounds **14c, 14g, 14i, 14k, 15a, 15b, 15d** and **15h** (MIC = 0.98 μg/mL) were two times more active than ciprofloxacin. On the other hand compounds **11b, 14e, 15c**, and **15e-g** were equipotent to the ciprofloxacin. As for the Gram negative bacteria *P. aeruginosa,* compounds **14c, 14i, 15a, 15b** and **15d** (MIC = 0.98 μg/mL) were four times more potent than ciprofloxacin, while compounds **14e, 14g, 14k** and **15e-h** (MIC = 1.95 μg/mL) were two times more active than ciprofloxacin, whereas compounds **14a, 14h, 14j** and **15c** were equipotent to the ciprofloxacin. Regarding Gram negative bacteria *E. coli,* the compounds **14c, 15a, 15b** and **15d** (MIC = 0.49 μg/mL) exhibited the highest potency, they were four times more potent than the reference drug ciprofloxacin (MIC = 1.95 μg/mL). Moreover, compounds **14a, 14i, 14k, 15c** and **15f-h** (MIC = 0.98 μg/mL) were two times more potent than ciprofloxacin. While compounds **11a, 11b, 14e, 14g, 14h, 14j** and **15e** were equipotent to the

ciprofloxacin. Thus, 2-oxindole based compounds exhibited better broad spectrum antibacterial activity than indole congeners.

Table 2

2.2.1.3. Antifungal activity

Target conjugates and Amphotericin B, a reference antifungal drug, were evaluated for their antifungal activity, by I.Z. technique and MIC, towards *A. fumigates* and *C. albicans*, **Table 3**. Aspergillus and Candida spp. account for the majority of documented fungal infections. Clinically, candidiasis and aspergillosis account for between 80% and 90% of systemic fungal infections in immunocompromised patients. Data in **Table 3** revealed that conjugates **14c, 14i, 15a, 15b, 15d** and **15g** (MIC = 0.49 μg/mL) exhibited the highest potency against *C. albicans* organism being two times more active than the reference drug Amphotericin B (MIC = 0.98 μg/mL). While conjugates **14a, 14g, 14k, 15c** and **15e** were equipotent to the Amphotericin B. On the other hand, compounds **14c, 14g, 15a, 15b, 15d** and **15g** revealed two fold increase in activity than Amphotericin B, with MIC = 0.98 μg/mL against *A. fumigatus* organism. Moreover, conjugates **14a, 14e, 14h, 14i, 14k, 15c, 15e** and **15h** were equipotent to Amphotericin B.

Table 3

2.2.1.4. Whole-cell phenotypic evaluation and anti-tubercular selectivity

Compounds **14g, 14i, 14k, 15a, 15b, 15e, 15g, 15h** which displayed the most potent anti-tubercular activity were predicted to have acceptable ADME parameters (Supplementary Materials), therefor they were selected for further investigation of their anti-TB activity against fast growing non-pathogenic *M. aurum* in an *ex vivo* drug screening model using SPOTi method (**Figure 2**) [17]. *M. aurum* is a good drug screening surrogate proved to be close towards pathogenic *M. tuberculosis* because of its similar cell wall components and intracellular therapeutic targets. An integrated *ex vivo* drug screening model was used to provide additional information about intracellular inhibition inside host's primary immune cells and to evaluate the

selected conjugates towards their permeability to macrophages RAW 264.7, their stability inside macrophages over the time of incubation, cytotoxicity against RAW 264.7 and their selective killing towards *M. aurum* [18]. Intracellular growth inhibition of *M. aurum* was observed in the presence and absence of selected conjugates. All the selected conjugates were found to be permeable through eukaryotic cells RAW 264.7 and stable over the time of incubation.

Figure 2

Table 4 displays the results of the anti-tubercular screening, cytotoxicity and the selectivity index (SI) towards intracellular killing of mycobacterium. As shown, in **Table 4**, compound **15b** produced an effective killing of intracellular *M. aurum* at 25 µg/mL, while compounds **15a** and **15h** killed mycobacteria at 50 µg/mL (**Table 4**). Also, compounds **14g** displayed inhibitory activity at higher MIC value (100 µg/mL). On the other hand, the other tested compounds are showing inhibition towards intracellular bacilli at higher concentrations. These results, also, suggested that *N*-methylation of the 2-oxindole in acetyl derivatives enhanced the intracellular inhibition.

SI, the ratio between growth inhibition concentration values (GIC_{50}) in macrophages and MIC values in bacteria, indicates the safety and druggability of the target conjugates. The tested conjugates showed moderate cytotoxic activity towards macrophage RAW 264.7 cells, with GIC_{50} values ranging from 62.5 to 500 µg/mL (**Table 4**). However, the most potent compound **15b** exhibited the highest selectivity among the tested conjugates (SI = 5).

Table 4

2.2.1.5. Antibacterial Activity against MRSA and VRE

Compounds **15a** and **15b**, which produced an effective killing of intracellular *M. aurum*, were evaluated for their potential antibacterial activity against two resistant bacteria; methicillin-resistant *Staphylococcus aureus* (MRSA ATCC 33592) and vancomycin- resistant *Enterococcus*

faecium (ATCC 700221), using Vancomycin and Ciprofloxacin as reference drugs, respectively. The results were displayed in **Table 5** as minimum inhibitory concentration (MIC in µg/mL).

Table 5

As shown in **Table 5**, conjugate **15b** displayed potent activity towards both MRSA and VRE (MIC = 3.90 and 7.81 µg/mL, respectively) with two-fold decreased activity than the reference drugs Vancomycin (MIC = 1.95 µg/mL) and Ciprofloxacin (MIC = 3.90 µg/mL). Whereas, compound **15a** showed modest activity with MIC values equal 15.63 and 62.50 towards MRSA and VRE strains, respectively.

2.2.2. In Vitro Cytotoxicity

2.2.2.1. In vitro cytotoxic activity against human normal WI-38 cells

Compounds **15a**, **15b** and **15h** were evaluated for their ability to induce cytotoxic effect against human normal lung fibroblast cell line (WI-38 cells), to investigate their safety [19]. The results were expressed as half maximal inhibitory concentration (IC_{50}), then selectivity index was calculated (**Table 6**). In particular, compounds **15b** and **15h** showed non-significant cytotoxic action with IC_{50} values of 19.5 and 52.1 µg/mL, respectively, (S.I. = 50 and 133, respectively).

Table 6

2.2.2.2. NCI, USA Cytotoxicity assay towards 60 cancer cell lines

Compounds **15b** and **15h** were screened by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for their cytotoxic activity *in vitro*. The cytotoxicity assays were performed in accordance with the protocol of the Drug Evaluation Branch, NCI, Bethesda [20-22]. Compounds **15b** and **15h** were examined at one dose primary cytotoxicity assay towards a panel of approximately 60 cancer cell lines (concentration 10^{-5} M). A 48 hrs drug exposure protocol was used and sulforhodamine B (SRB) protein assay was applied to estimate the cell viability and growth [23]. The data represented as mean growth

percentage over 60 cell lines (**Table 6**). The tested compounds **15b** and **15h** showed non-significant cytotoxic action with mean growth = 101.5 % and 101.2, respectively. Therefore these results ruled out nonspecific binding of compounds **15b** and **15h** to the basic cell macromolecules like proteins, plasma membrane, carbohydrates and also biocompatibility to the eukaryotic cells.

3. Conclusion

In summary, conjugating thiazolylamino-thiazolidinone with either indole **8a,b** and **11a-d** or 2-oxindole **14a-k** and **15a-h** resulted in the discovery of promising scaffold with good antimicrobial activities. It was concluded that compound **15b** revealed the best activity profile as a broad spectrum agent with antimicrobial activity against Gram positive and Gram negative bacteria (MIC: 0.39-0.98 µg/ml) as well as antifungal activity (MIC: 0.49-0.98 µg/ml). Furthermore, it has *in vitro* and *ex vivo* anti-tubercular activity. An integrated *ex vivo* drug screening model depicted the intracellular inhibition of mycobacterium against selected conjugates, their permeability through primary immune cells and stability inside mouse macrophages over the time of incubation. Compound **15b** produced an effective killing of intracellular *M. aurum* at 25 µg/mL, with weak cytotoxic activity towards macrophage RAW 264.7 cells (GIC_{50} = 125 µg/mL). Furthermore, compound **15b** displayed promising activity towards both MRSA and VRE (MIC = 3.90 and 7.81 µg/mL, respectively) with two-fold decreased activity than the reference drugs Vancomycin (MIC = 1.95 µg/mL) and Ciprofloxacin (MIC = 3.90 µg/mL). Also, safety of compound **15b** was approved *via* investigating its cytotoxicity towards cancer cell lines and human lung fibroblast normal cell line, WI-38 cells. The results suggest some potential molecules with greater SI values to be developed further for new therapeutic leads. Noteworthy, the broad-spectrum antimicrobial activities of **15b** with MIC spanning from 0.49 to 0.98 µg/mL indicate the possible endogenous target for compound **15b** could be a fundamental mechanism of bacterial cells (i.e. RNA, DNA, protein synthesis and/or cellular respiration) which must be common in various cell-types. So, further mechanistic studies will be in progress in our laboratories and will be reported upon in the near future.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were measured with a Stuart melting point apparatus and were uncorrected. Infrared (IR) Spectra were recorded as KBr disks using Schimadzu FT-IR 8400S spectrophotometer. Mass spectral data are given by GCMS-QP1000 EX and Helwett Packard 5988 spectrometers at 70 e.V. NMR Spectra were recorded on a Varian Mercury NMR spectrometer or Bruker spectrophotometer. ^{1}H spectrum was run at 400 MHz and ^{13}C spectrum was run at 100 MHz in deuterated dimethylsulfoxide (DMSO-d_6) and deuterated trifluoroacetic acid (TFA-d_1). Chemical shifts are expressed in values (*ppm*) using the solvent peak as internal standard. All coupling constant (*J*) values are given in hertz. The abbreviations used are as follows: *s*, singlet; *d*, doublet; *m*, multiplet. Elemental analyses were carried out at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products.

4.1.2. Synthesis of compounds **2a,b, 3a,b** and **4a,b**

Compounds **2a,b**, **3a**,**b** and **4a**,**b** were prepared according to the literature procedure [24, 25].

4.1.3. Synthesis of 2-[(4-Methylthiazol-2-yl)amino]thiazol-4(5H)-one derivatives **5a,b**

To a solution of 2-chloro-*N*-(4-methylthiazol-2-yl)acetamide derivatives **4a,b** (5 mmol) in absolute ethanol (15 mL), ammonium thiocyanate (0.76 g, 10 mmol) was added. This mixture was heated under reflux for 6 hrs. The formed precipitate was filtered off while hot, and washed with ethanol and petroleum ether, and then recrystallized from dioxane to obtain the targeted compounds **5a,b** [9, 26].

4.1.3.1. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino)thiazolidin-4-one (**5a)**.

Yellow powder (yield: 0.87 g, 75 %), m.p. 220-221 °C; IR (KBr, ν cm^{-1}): 3117 (NH) and 1740, 1717 (2 C=O); ^{1}H NMR (DMSO-*d*6, 400 MHz) δ *ppm*: 2.49 (s, 3H, CH_3CO), 2.60 (s, 3H, CH_3),

4.02 (s, 2H, CH_2), 12.27 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*6, 100 MHz) *δppm*: 19.0 (CH_3), 30.6 ($\underline{C}H_3CO$), 35.7 (CH_2), 132.1, 155.9, 166.8, 170.9, 174.6 (C=O amide), 190.9 (C=O acetyl); MS, *m/z* [%]: 255 [M^+, 19.94], 71 [100]; Anal. Calcd. for $C_9H_9N_3O_2S_2$ (255.31): C, 42.34; H, 3.55; N, 16.46; Found C, 41.98; H, 3.71; N, 16.70.

4.1.3.2. Ethyl-4-methyl-2-[(4-oxothiazolidin-2-ylidene)amino]thiazole-5-carboxylate (**5b)**.

Yellow powder (yield: 1.0 g, 70 %), m.p. 210-211 °C; IR (KBr, ν cm^{-1}): 3128 (NH), 1708, 1697 (2 C=O); ^1H NMR (DMSO-*d*6, 400 MHz) *δ ppm*: 1.24 (t, 3H, $C\underline{H_3}$-CH_2, *J* = 7.20 Hz), 2.57 (s, 3H, CH_3), 4.02 (s, 2H, CH_2), 4.21 (q, 2H, CH_3-$C\underline{H_2}$, *J* = 7.20 Hz), 12.24 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-*d*6, 100 MHz) *δ ppm*: 14.6 ($\underline{C}H_3CH_2$), 17.8 (CH_3), 35.7 (CH_2), 61.3 ($CH_3\underline{C}H_2$), 117.9, 158.0, 162.1, 166.7, 171.1 (C=O ester), 174.5 (C=O amide); MS, *m/z* [%]: 285 [M+, 67.30], 71 [100]; Anal. Calcd. for $C_{10}H_{11}N_3O_3S_2$ (285.34): C, 42.09; H, 3.89; N, 14.73; found C, 41.87; H, 4.02; N, 14.98.

4.1.4. Synthesis of 1H-indole-3-carbaldehyde **7**

Compound **7** was prepared according to the literature procedures [27].

4.1.5. General procedure for synthesis of N-substituted-1H-indole-3-carbaldehydes **10a,b**.

To a stirred suspension of NaH (60 percent in mineral oil, 1 gm, 2.5 mmol) and 1*H*-indole-3-carbaldehyde **7** (3.0 g, 2 mmol) in DMF, bromomethane **9a** or benzyl bromide **9b** (2 mmol) was added drop wise at 0^0C. The reaction mixture was stirred for 24 hrs at room temperature after complete addition of alkyl halide. The reaction mixture was poured into ice water, the formed precipitate was filtered off, washed with water and petroleum ether then recrystallized from ethanol to obtain compounds **10a**,**b** [28].

4.1.6. General procedure for synthesis of target compounds **8a,b** and **11a-d**

To a well-stirred hot solution of the key intermediates **5a,b** (3 mmol) and sodium acetate (0.49 g, 6 mmol) in acetic acid (15 mL), the appropriate 1*H*-indole-3-carbaldehyde derivative **7**, **10a** or **10b** (4.5 mmol) was added. The reaction mixture was heated under reflux for 3 hrs. The formed solid was filtered off while hot, washed with hot ethanol, and crystallized from DMF to furnish the target compounds **8a, b** and **11a-d**, respectively.

4.1.6.1. 5-[(1H-Indol-3-yl)methylene]-2-((5-acetyl-4-methylthiazol-2-yl)imino)thiazolidin-4-one (**8a**).

Yellow powder (yield: 0.51 g, 45%), m.p. >300 °C; IR (KBr, *v* cm^{-1}): 3244, 3190 (2 NH) and 1701, 1651 (2 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.48 (s, 3H, C$\underline{H_3}$C=O), 2.68 (s, 3H, C$\underline{H_3}$), 7.17-7.26 (m, 2H, H-5, H-6 of indolyl), 7.50 (d, 1H, H-7 of indolyl, *J*=8.0 Hz), 7.79 (s, 1H, C-2 of indol), 7.89 (d, 1H, H-4 of indolyl, *J*=8.0), 8.01 (s, 1H, -C$\underline{H}$=), 12.11 (s, 1H, NH indol exchanged with D_2O of indolyl), 12.62 (s, 1H, NH exchanged.D_2O.); ^{13}C.NMR (DMSO-d_6, 100 MHz) δ *ppm*: 19.0 ($\underline{C}H_3$), 30.6 ($\underline{C}H_3C$=O), 111.1, 112.8, 112.9, 117.1, 118.9, 121.6, 123.2, 123.6, 125.9, 127.3, 129.6, 136.1, 136.7, 156.3 (aromatic carbons), 167.3 ($\underline{C}$=O amide), 190.8 ($\underline{C}$=OCH_3); Anal. Calcd. for $C_{18}H_{14}N_4O_2S_2$ (382.46): C, 56.53; H, 3.69; N, 14.65; found C, 56.38; H, 3.78; N, 14.92.

4.1.6.2. Ethyl 2-{[-5-((1H-indol-3-yl)methylene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**8b**).

Yellow powder (yield: 0.49 g, 40%), m.p. 257-260°C; IR (KBr, *v* cm^{-1}): 3309 (NH) and 1701, 1678 (2C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.26 (t, 3H, $\underline{CH_3}$-CH_2-, *J* = 6.80 Hz), 2.66 (s, 3H, CH_3), 4.24 (q, 2H, CH_3-C$\underline{H_2}$-, *J*=6.80 Hz), 7.24 (t, 1H, H-6 of indolyl, *J*=7.32), 7.32 (t, 1H, H-5 of indolyl, *J*=7.6Hz), 7.50 (d, 1H, H-7 of indolyl, *J*=8.0), 7.79 (s, 1H, C-2 of indolyl), 7.88 (d, 1H, H-4 of indolyl, *J*=8.0), 8.0 (s, 1H, -CH=), 12.13 (s, 1H, NH of indolyl exchanged with D_2O), 12.63 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (DMSO-d_6, 100 MHZ) δ *ppm* : 14.6 ($\underline{C}H_3CH_2$), 18.0 (CH_3), 61.3 ($\underline{C}H_2CH_3$), 111.1, 112.9, 117.1, 118.9, 121.6, 123.6, 125.8, 127.3, 129.6, 136.7, 158.3, 159.2, 162.1 (aromatic carbons), 167.3 (C=O ester), 170.6 (C=O amide); Anal. Calcd. for $C_{19}H_{16}N_4O_3S_2$ (412.48): C, 55.33; H, 3.91; N, 13.58; found C, 55.49; H, 3.98; N, 13.67

4.1.6.3. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-((1-methyl-1H-indol-3-yl)methylene)thiazolidin-4-one (**11a**).

Yellow powder (yield: 0.62 g, 52%), m.p. 295-297 °C ; IR (KBr, *v* cm^{-1}) 3395 (NH) and 1701, 1659 (2C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.48 (s, 3H, C$\underline{H_3}$C=O), 2.62 (s, 3H, C$\underline{H_3}$), 3.95 (s, 3H, *N*-C$\underline{H_3}$ of indolyl), 7.21-7.33 (m, 2H, H-5, H-6 of indolyl), 7.56 (d, 1H, H-7 of indol, *J* = 8.0), 7.82 (s, 1H, C-2 of indol), 7.91 (d, 1H, H-4 of indolyl, *J* = 8.0 Hz), 7.99 (s, 1H, -C$\underline{H}$=),

12.59 (s, 1H, NH exchanged with D_2O); Anal. Calcd. for $C_{19}H_{16}N_4O_2S_2$ (396.48): C, 57.56; H, 4.07; N, 14.13; found C, 57.17; H, 4.05; N, 14.25.

4.1.6.4. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-((1-benzyl-1H-indol-3-yl)methylene)thiazolidin-4-one (**11b**).

Yellow powder (yield: 0.55 g, 39%), m.p. 297-300°C; IR (KBr, ν cm^{-1}): 3150 (NH) and 1701, 1658 (2 C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.48 (s, 3H, C$\underline{H_3}$C=O), 2.68 (s, 3H, C$\underline{H_3}$), 5.60 (s, 2H, C$\underline{H_2}$-Ar of indol), 7.20-7.36 (m, 7H, H-5, H-6 of indolyl and 5H of phenyl ring), 7.59 (d, 1H, H-7 of indolyl, J = 7.20), 7.91-7.94 (m, 2H, C-2, H-4 of indol), 8.00 (s, 1H, -CH=), 12.62 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 19.1 (CH_3), 30.6 ($\underline{C}H_3C$=O), 50.2 (CH_2), 111.8, 117.8, 119.3, 122.0, 123.8, 125.2, 127.9, 128.3, 129.1, 132.0, 136.7, 137.3, 156.3, 159.3, 167.3 (aromatic carbons), 170.4 (C=O amide), 190.8 ($\underline{C}$=OCH_3); Anal. Calcd. for $C_{25}H_{20}N_4O_2S_2$ (472.58): C, 63.54; H, 4.27; N, 11.86; found C, 63.50; H, 4.52; N, 11.42

4.1.6.5. Ethyl 4-methyl-2-{[-5-((1-methyl-1H-indol-3-yl)methylene)-4-oxothiazolidin-2-ylidene]amino}thiazole-5-carboxylate (**11c**).

Yellow powder (yield: 0.51 g, 40%), m.p. 280-282 °C ; IR (KBr, ν cm^{-1}): 3120 (NH), 1701, 1678 (2C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.27 (t, 3H, C$\underline{H3}$-CH2-, J = 7.20 Hz), 2.69 (s, 3H, CH_3) , 3.94 (s, 3H, *N*-C$\underline{H3}$ of indolyl), 4.24 (q, 2H, CH_3-$\underline{CH_2}$-, J=7.2 Hz), 7.23 (t, 1H, H-6 of indol, J=7.60 Hz), 7.31 (t, 1H, H-5 of indolyl, J=7.60 Hz), 7.56 (d, 1H, H-7 of indolyl, J=8.0), 7.81 (s, 1H, C-2 of indolyl), 7.90 (d, 1H, H-4 of indol, J=8.0), 7.98 (s, 1H, -C$\underline{H}$=), 12.58 (s, 1H, NH exchanged with D2O); ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 14.64 ($\underline{C}H_3CH_2$), 18.1 (CH_3), 33.9 (*N*-CH_3), 61.3 ($CH_3\underline{C}H_2$), 110.0, 111.3, 117.2, 119.0, 121.9, 123.6, 125.3, 127.7, 133.0, 136.8, 137.3, 158.5, 159.1, 162.1 (aromatic carbons), 167.30 (C=O ester), 170.5 (C=O amide); MS *m/z* [%]: 426 [M^+, 17.15], 187 [100]; Anal. Calcd. for $C_{20}H_{18}N_4O_3S_2$ (426.5): C, 56.32; H, 4.25; N, 13.14; found C, 56.08; H, 4.41; N, 13.29.

4.1.6.6. Ethyl 2-{[-5-((1-benzyl-1H-indol-3-yl)methylene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**11d**).

Yellow powder (yield: 0.6 g, 40%), m.p. 260-263 °C; IR (KBr, ν cm^{-1}): 3150 (NH) and 1701, 1678 (2C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.28 (t, 3H, C$\underline{H_3}$-CH_2-, J = 7.20 Hz), 2.66

(s, 3H, CH_3), 4.24 (q, 2H, CH_3-C$\underline{H_2}$-, *J*=6.80 Hz), 5.60 (s, 2H, CH_2-Ar of indol), 7.20-7.35 (m, 7H, H-5, H-6 of indolyl and 5*H* of phenyl ring), 7.59 (d, 1H, H-7, *J*=8.80 Hz), 7.91-7.96 (m, 2H, H-2, H-4 of indol), 8.00 (s, 1H, -CH=), 12.62 (s, 1H, NH exchanged with D_2O), ^{13}C NMR (DMSO-d_6, 100 MHz) δ *ppm*: 14.63 ($\underline{C}H_3CH_2$), 18.0 (CH_3), 50.2 ($\underline{C}H_2Ph$), 61.3 ($CH_3\underline{C}H_2$), 110.8, 111.7, 117.2, 117.8, 119.2, 122.0, 123.8, 125.1, 127.7, 127.9, 128.3, 129.20, 132.0, 136.7, 137.4, 158.4, 159.20, 162.0 (aromatic carbons), 167.2 (C=O ester), 170.5 (C=O amide); MS *m/z* [%]: 502 [M^+, 44.70], 263 [100]; Anal. Calcd. for $C_{26}H_{22}N_4O_3S_2$ (502.61) : C, 62.13; H, 4.41; N, 11.15; found C, 62.01; H, 4.38; N, 11.35.

4.1.7. General procedure for synthesis of N-substituted isatins **13a-d**.

To a mixture of isatins **12a**,**c** (3 mmol) and anhydrous potassium carbonate (1.24 g, 9 mmol) in dry DMF (15 mL), bromomethane **9a** or benzyl bromide **9b** (6 mmol) was added. The mixture was stirred at room temperature for 24 hrs. The reaction mixture was poured into ice water. The formed precipitate was filtered off and washed with water and petroleum ether and recrystallized from ethanol to afford compounds **13a-d** [29, 30].

4.1.8. General procedure for synthesis of target compounds **14a-k** and **15a-h**.

To a well-stirred hot solution of 2-(thiazol-2-ylimino)thiazolidin-4-one derivatives **5a,b** (0.003 mol) and sodium acetate (0.49 gm, 6 mmol) in acetic acid (15 mL), the appropriate isatins **12a-f** or **13a**, **d** (4 mmol) were added. The reaction mixture was heated under reflux for 3 hrs. The formed precipitate was filtered off while hot, washed with hot ethanol and crystallized from DMF to furnish the target compounds **14a-k** and **15a-h**, respectively.

4.1.8.1. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-2-oxoindolin-3-ylidene)thiazolidin-4-one (**14a**).

Red powder (yield: 0.57 g, 50%), m.p. > 300°C; IR (KBr, *v* cm^{-1}): 3178, 3128 (2NH) and 1716, 1693, 1663 (3 C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ *ppm*: 2.49 (s, 3H, C$\underline{H_3}$C=O), 2.64 (s, 3H, $\underline{CH_3}$), 6.93 (d, 1H, H-7 of isatin, *J* = 7.80 Hz), 7.06 (t, 1H , H-5 of isatin, *J* = 7.80 Hz), 7.37 (t, 1H, H-6 of isatin, *J* = 7.80 Hz), 8.84 (br s, 1H, H-4 of isatin , 11.10 (s, 1H, NH of isatin exchanged with D_2O), 13.05 (s, 1H, NH exchanged with D_2O); MS *m/z* [%]: 384 [M^+, 42.78],

175 [100]; Anal. Calcd. for $C_{17}H_{12}N_4O_3S_2$ (384.43): C, 53.11; H, 3.15; N, 14.57; found C, 53.28; H, 3.27; N, 14.81

4.1.8.2. 2-[(5-Acetyl-4-methylthiazol-2-yl)amino]-5-(-5-fluoro-2-oxoindolin-3-ylidene)thiazolidin-4-one (**14b**).

Red powder (yield 0.54 g, 45%), m.p. >300°C; IR (KBr, ν cm^{-1}): 3159, 3116 (2NH) and 1716, 1701, 1654 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 2.47 (s, 3H, CH_3C=O), 2.61 (s, 3H, CH_3), 6.87-6.90 (m, 1H, H-6 of isatin), 7.19 (t, 1H , H-7 of isatin, J = 8.80 Hz), 8.60 (s, 1H, H-4 of isatin), 11.11 (s, 1H, NH of isatin exchanged with D_2O), 13.07 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 13.11, 27.71, 116.66, 116.94, 120.05, 121.84, 122.09, 127.35, 128.77, 133.45, 139.14, 146.90, 159.42, 165.89, 168.87, 171.17, 193.50; MS *m/z* [%]: 402 [M^+, 93.21], 193 [100]; Anal. Calcd. for $C_{17}H_{11}FN_4O_3S_2$ (402.42): C, 50.74; H, 2.76; N, 13.92; found C, 50.98; H, 2.67; N, 14.25.

4.1.8.3. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-5-chloro-2-oxoindolin-3-ylidene)thiazolidin-4-one (**14c**).

Red powder (yield 0.54 g, 43%), m.p. > 300 °C; IR (KBr, ν cm^{-1}): 3120, 3105 (2NH) and 1701, 1680, 1666 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 2.47 (s, 3H, CH_3C=O), 2.61 (s, 3H, CH_3), 6.89 (d, 1H, H-6 of isatin, J = 8.40 Hz), 7.37 (d, 1H, H-7 of isatin, J = 7.60 Hz), 8.81 (s, 1H, H-4 of isatin), 11.25 (s, 1H, NH of isatin exchanged with D_2O), 13.13 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δppm: 18.97, 33.58, 120.98, 123.21, 124.33, 126.57, 133.35, 134.71, 137.55, 143.89, 147.58, 152.79, 171.74, 174.76, 175.17, 177.04, 199.38; MS *m/z* [%]: 420 [M^++2, 1.21], 418 [M^+, 4.68], 96 [100]; Anal. Calcd. for $C_{17}H_{11}ClN_4O_3S_2$ (418.87): C, 48.75; H, 2.65; N, 13.38; found C, 48.91; H, 2.78; N, 13.52

4.1.8.4. *2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-5-bromo-2-oxoindolin-3-ylidene)thiazolidin-4-one* (**14d**).

Red powder (yield 0.52 g, 38%), m.p. > 300 °C; IR (KBr, ν cm^{-1}): 3178, 3124 (2 NH) and 1693, 1670, 1647 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 2.47 (s, 3H, CH_3C=O), 2.59 (s, 3H, CH_3), 6.86 (d, 1H, H-6 of isatin, J = 7.60 Hz), 7.49 (br s, 1H, H-7 of isatin), 8.98 (s, 1H, H-4 of isatin), 11.18 (s, 1H, NH of isatin exchanged with D_2O), 13.04 (s, 1H, NH exchanged with D_2O);

MS *m/z* [%]: 464 [M^++2, 63.88], 462 [M^+, 57.48], 265 [100]; Anal. Calcd. for $C_{17}H_{11}BrN_4O_3S_2$ (463.32): C, 44.07; H, 2.39; N, 12.09; found C, 44.23; H, 2.06; N, 12.43.....

4.1.8.5. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-5-methoxy-2-oxoindolin-3-ylidene)thiazolidin-4-one (**14e**).

Reddish black powder (yield 0.63 g, 50%), m.p. > 300 °C; IR (KBr, *v* cm^{-1}): 3152, 3113 (2 NH) and 1720, 1693, 1670 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.48 (s, 3H, C<u>H</u>$_3$C=O), 2.61 (s, 3H, C<u>H</u>$_3$), 3.73 (s, 3H, OCH_3), 6.78 (d, 1H, H-6 of methoxy isatin, *J* = 8.40 Hz), 6.93 (d, 1H, H-7 of methoxy isatin, *J* = 8.40 Hz), 8.48 (s, 1H, H-4 of methoxy isatin), 10.86 (s, 1H, NH of methoxy isatin exchanged with D_2O), 12.97 (s, 1H, NH exchanged with D_2O); Anal. Calcd. for $C_{18}H_{14}N_4O_4S_2$ (414.45): C, 52.16; H, 3.40; N, 13.52; found C, 52.44; H, 3.53; N, 13.79.

4.1.8.6. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-2-oxo-5-(trifluoromethoxy)indolin-3-ylidene)thiazolidin-4-one (**14f**).

Red powder (yield 0.71 g, 51%), m.p. > 300 °C; IR (KBr, *v* cm^{-1}): 3150, 3124, (2 NH) and 1720, 1708, 1651 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.48 (s, 3H, CH_3C=O), 2.65 (s, 3H, CH_3), 6.99 (d, 1H, H-6 of isatin, *J* = 8.80 Hz), 7.33 (d, 1H, H-7 of isatin, *J* = 8.80 Hz), 8.84 (s, 1H, H-4 of isatin), 11.35 (s, 1H, NH of isatin exchanged with D_2O), 13.16 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 19.06, 33.68, 118.35, 119.31, 124.44, 125.99, 129.06, 130.84, 133.77, 134.86, 137.93, 147.30, 152.15, 152.88, 171.87, 174.88, 177.05, 199.45; MS *m/z* [%]:468 [M^+, 47.97], 259 [100]; Anal. Calcd. for $C_{18}H_{11}F_3N_4O_4S_2$ (468.43): C, 46.15; H, 2.37; N, 11.96; found C, 46.28; H, 2.29; N, 12.21.

4.1.8.7. Ethyl 4-methyl-2-{[-4-oxo-5-(-2-oxoindolin-3-ylidene)thiazolidin-2-ylidene]amino}thiazole-5-carboxylate (**14g**).

Red powder (yield 0.72 g, 52%), m.p. > 300 °C; IR (KBr, *v* cm^{-1}): 3174, 3140 (2 NH) and 1720, 1708, 1693 (3 C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ *ppm*: 1.28 (t, 3H, C<u>H</u>$_3$-CH_2-, *J* = 6.8 Hz), 2.62 (s, 3H, C<u>H</u>$_3$), 4.24 (q, 2H, CH_3-C<u>H</u>$_2$-, *J* = 6.3 Hz), 6.91 (d, 1H, H-7 of isatin, *J* = 7.80), 7.04 (t, 1H ,H-5 of isatin, *J* = 7.80 Hz), 7.36 (t, 1H, H-6 of isatin, *J*=7.50 Hz), 8.81 (br s, 1H, H-4 of isatin), 11.13 (s, 1H, NH of isatin exchanged with D_2O), 13.03 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 12.01, 12.04, 64.66, 112.81, 113.32, 115.62, 116.13, 119.433, 124.69, 125.49, 130.07, 133.25, 135.79, 142.99, 147.45, 166.37, 168.72, 170.93; MS

m/z [%]: 414 [M^+, 11.55], 175 [100]; Anal. Calcd. for $C_{18}H_{14}N_4O_4S_2$ (414.45): C, 52.16; H, 3.40; N, 13.52; found C, 52.40; H, 3.68; N, 13.74.

4.1.8.8. Ethyl 2-{[-5-(-5-fluoro-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**14h**).

Red powder (yield 0.65 g, 50%), m.p. >300 °C; IR (KBr, *v* cm^{-1}): 3120, 3159 (2 NH) and 1720, 1716, 1693 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.28 (t, 3H, $\underline{CH_3}$-CH_2-, *J* = 6.40 Hz), 2.61 (s, 3H, CH_3), 4.24 (q, 2H, CH_3-$C\underline{H_2}$-, *J* = 6.40 Hz), 6.87-6.91 (m, 1H, H-6 of fluoro isatin), 7.20 (br s, 1H, H-7 of flouroisatin), 8.60 (br s, 1H, H-4 of fluoroisatin), 11.14 (s, 1H, NH of isatin exchanged with D_2O), 13.03 (s, 1H, NH exchanged with D_2O); MS *m/z* [%]:432 [M^+, 100]; Anal. Calcd. for $C_{18}H_{13}FN_4O_4S_2$ (432.44): C, 49.99; H, 3.03; N, 12.96; found C, 50.15; H, 3.11; N, 13.23.

4.1.8.9. Ethyl 2-{[-5-(-5-chloro-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**14i**).

Red powder (yield 0.54 g, 40%), m.p. >300 °C; IR (KBr, *v* cm^{-1}): 3155, 3109 (2 NH) and 1708, 1685, 1670 (3 C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ *ppm*: 1.29 (br s, 3H, $\underline{CH_3}$-CH_2-), 2.55 (s, 3H, CH_3), 4.24 (br s, 2H, CH_3-$C\underline{H_2}$-), 6.92 (br s, 1H, H-6 of chloroisatin), 7.33 (br s, 1H, H-7 of chloroisatin), 9.03 (br s, 1H, H-4 of chloroisatin), 11.07 (s, 1H, NH of chloroisatin exchanged with D_2O), 11.94 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 17.96, 24.39, 70.57, 118.55, 125.62, 126.21, 133.69, 135.23, 136.38, 137.26, 140.75, 147.11, 153.44, 168.23, 171.74, 174.54, 175.25, 176.20; MS *m/z* [%]: 450 [M^++2, 8.29], 448 [M^+, 23.70], 209 [100]; Anal. Calcd. for $C_{18}H_{13}ClN_4O_4S_2$ (448.9): C, 48.16; H, 2.92; N, 12.48; found C, 48.02; H, 3.08; N, 12.65.

4.1.8.10. Ethyl 2-{[-5-(-5-bromo-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**14j**).

Red powder (yield 1.0 g, 70%), m.p. > 300 °C; IR (KBr, *v* cm^{-1}): 3155, 3101 (2 NH) and 1708, 1685, 1670 (3 C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ *ppm*: 1.29 (br s, 3H, $C\underline{H_3}$-CH_2-), 2.55 (s, 3H, $C\underline{H_3}$), 4.24 (br s, 2H, CH_3-$C\underline{H_2}$-), 6.92 (br s, 1H, 4,$\underline{H\text{-}6}$ of bromoisatin), 7.34 (br s, 1H ,H-7 of bromoisatin), 9.02 (s, 1H, H-4 of bromoisatin), 11.07 (s, 1H, NH of bromoisatin exchanged

with D_2O), 11.92 (s, 1H, NH exchanged with D_2O); Anal. Calcd. for $C_{18}H_{13}BrN_4O_4S_2$ (493.35): C, 43.82; H, 2.66; N, 11.36; found C, 43.58; H, 2.72; N, 11.52.

4.1.8.11. Ethyl 2-{[-5-(-5-methoxy-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**14k**).

Reddish black powder (yield 0.8 g, 60%), m.p. > 300 °C; IR (KBr, ν cm^{-1}): 3155, 3132 (2 NH) and 1708, 1701, 1693 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.27 (t, 3H, C$\underline{H_3}$-CH_2-, *J* = 6.4 Hz), 2.59 (s, 3H, C$\underline{H_3}$), 3.73 (s, 3H, OC$\underline{H_3}$), 4.22 (q, 2H, CH_3-C$\underline{H_2}$-, *J*=6.8 Hz), 6.78 (d, 1H, H-6 of methoxy isatin, *J*=8.40 Hz), 6.92 (d, 1H, H-7 of methoxy isatin, *J*= 8.0 Hz), 8.48 (s, 1H,H-4of methoxy isatin), 11.42 (s, 1H, NH of methoxy isatin exchanged with D_2O), 13.18 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 17.71, 24.39, 61.87, 70.34, 118.48, 121.88, 125.22, 125.92, 127.88, 132.57, 138.44, 143.82, 153.17, 160.92, 168.23, 171.77, 174.38, 175.33, 176.36; MS *m/z* [%]: 444 [M^+, 35.58], 72 [100]; Anal. Calcd. for $C_{19}H_{16}N_4O_5S_2$ (444.48): C, 51.34; H, 3.63; N, 12.61; found C, 51.58; H, 3.71; N, 12.49.

4.1.8.12. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-1-methyl-2-oxoindolin-3-ylidene)thiazolidin-4-one (**15a**).

Red powder (yield 0.65 g, 55%), m.p. >300 °C; IR (KBr, ν cm^{-1}): 3113 (NH) and 1689, 1662, 1658 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.47 (s, 3H, CH_3C=O), 2.61 (s, 3H, CH_3), 3.22 (s, 3H, *N*-CH3), 7.04-7.12 (m, 2H, H-5, H-7 of isatin), 7.43 (t, 1H, H-6 of isatin, *J* = 7.6 Hz), 8.85 (s, 1H, H-4 of isatin), 12.98 (s, 1H, NH exchanged with D_2O); MS *m/z* [%]: 398 [M^+, 17.55], 71 [100]; Anal. Calcd. for $C_{18}H_{14}N_4O_3S_2$ (398.46): C, 54.26; H, 3.54; N, 14.06; found C, 54.18; H, 3.60; N, 14.23.

4.1.8.13. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-5-chloro-1-methyl-2-oxoindolin-3-ylidene)thiazolidin-4-one (**15b**).

Red powder (yield 0.78 g, 60%), m.p. >300 °C; IR (KBr, ν cm^{-1}): 3136 (NH) and 1716, 1689 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.48 (s, 3H, CH_3C=O), 2.64 (s, 3H, CH_3), 3.23 (s, 3H, *N*-CH_3), 7.13 (d, 1H, H-6 of isatin, *J* = 8.4 Hz), 7.51 (d, 1H, H-7 of isatin, *J* = 8.8 Hz), 8.90 (s, 1H, H-4 of isatin), 13.04 (s, 1H, NH , exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 13.07, 25.85, 27.87, 110.40, 119.99, 127.50, 128.12, 129.56, 130.89, 131.41, 134.71,

143.64, 147.22, 165.86, 167.57, 168.96, 171.25, 193.70; MS *m/z* [%]: 434 [M^++2, 29.18], 432 [M^+, 65.73], 223 [100]; Anal. Calcd. for $C_{18}H_{13}ClN_4O_3S_2$ (432.9): C, 49.94; H, 3.03; N, 12.94; found C, 49.87; H, 3.14; N, 13.20.

4.1.8.14.. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-1-benzyl-2-oxoindolin-3-ylidene)thiazolidin-4-one (**15c**).

Red powder (yield 0.92 g, 65%), m.p. 285-287°C; IR (KBr, *v* cm^{-1}): 3109 (NH) and 1708, 1693, 1651 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.48 (s, 3H, CH_3C=O), 2.65 (s, 3H, CH_3), 5.02 (s, C$\underline{H_2}$-Ar), 7.04 (d, 1H, H-7 of isatin, *J* = 8.0 Hz), 7.10 (t, 1H, H-5 of isatin, *J* = 8 Hz), 7.21-7.37 (m, 6H, H-6 of isatin, Ar-H of benzyl ring), 8.88 (br s, 1H, H-4 of isatin), 13.08 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 19.06, 33.83, 50.50, 116.75, 121.11, 122.15, 124.46, 125.32, 130.88, 132.36, 134.17, 134.75, 136.18, 139.04, 139.14, 141.51, 150.85, 153.14, 172.26, 174.09, 177.62, 199.73; MS *m/z* [%]: 474 [M^+, 19.65], 265 [100]; Anal. Calcd. for $C_{24}H_{18}N_4O_3S_2$ (474.55): C, 60.74; H, 3.82; N, 11.81; found C, 60.89; H, 3.89; N, 12.07.

4.1.8.15. 2-[(5-Acetyl-4-methylthiazol-2-yl)imino]-5-(-1-benzyl-5-chloro-2-oxoindolin-3-ylidene)thiazolidin-4-one (**15d**).

Red powder (yield 1.07 g, 70%), m.p. > 300°C; IR (KBr, *v* cm^{-1}): 3109 (NH) and 1693, 1665, 1655 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.48 (s, 3H, CH_3C=O), 2.63 (s, 3H, CH_3), 5.02 (s, C$\underline{H_2}$-Ar), 7.04 (d, 1H, H-6 of chloroisatin, *J* = 8.8 Hz), 7.26-7.34 (m, 5H, Ar-H of benzyl ring), 7.43 (d, 1H, H-7 of chloroisatin, *J* = 8.00 Hz), 8.90 (s, 1H, H-4 of chloroisatin), 13.04 (s, 1H, NH exchanged with D_2O); MS *m/z* [%]: 511 [M^++2, 1.52], 509 [M^+, 5.85], 466 [100]; Anal. Calcd. for $C_{24}H_{17}ClN_4O_3S_2$ (509.0): C, 56.63; H, 3.37; N, 11.01; found C, 56.48; H, 3.31; N, 11.28.

4.1.8.16. Ethyl 4-methyl-2-{[-5-(-1-methyl-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}thiazole-5-carboxylate (**15e**).

Red powder (yield 0.83 g, 65%), m.p. > 300 °C; IR (KBr, *v* cm^{-1}): 3109 (NH) and 1720, 1710, 1655 (3 C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.27 (t, 3H, $\underline{CH_3}$-CH_2-, *J* = 7.2 Hz), 2.62 (s, 3H, CH_3) , 3.22 (s, 3H, *N*-CH_3), 4.24 (q, 2H, CH_3-C$\underline{H_2}$-, *J*=7.20 Hz), 7.07-7.13 (m, 2H, H-5, H-7 of isatin), 7.43 (t, 1H, H-6 of isatin, *J* = 7.8 Hz), 8.84 (br s, 1H, H-4 of isatin), 13.05 (s, 1H,

NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz) δ *ppm*: 12.20, 25.82, 64.74, 65.73, 110.00, 112.91, 113.41, 119.20, 125.00, 125.56, 130.12, 133.12, 135.62, 145.43, 147.55, 166.51, 168.04, 168.84, 171.02; MS *m/z* [%]: 428 [M^+, 33.42], 189 [100]; Anal. Calcd. for $C_{19}H_{16}N_4O_4S_2$ (428.48): C, 53.26; H, 3.76; N, 13.08; found C, 53.40; H, 3.88; N, 13.29.

4.1.8.17. Ethyl 2-{[-5-(-5-chloro-1-methyl-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**15f**).

Red powder (yield 0.73 g, 53%), m.p. > 300 °C; IR (KBr, ν cm^{-1}): 3117 (NH) and 1708, 1701, 1693 (3C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.28 (t, 3H, $C\underline{H_3}$-CH_2-, J = 6.8 Hz), 2.64 (s, 3H, CH3), 3.23 (s, 3H, *N*-CH_3 of chloroisatin), 4.25 (q, 2H, CH_3-$C\underline{H_2}$-, J = 7.2 Hz), 7.13 (d, 1H, H-6 of chloroisatin, J = 8.4 Hz), 7.51 (d, H1, H-7 of chloroisatin, J = 8.4 Hz), 8.88 (s, 1H, H-4 of chloroisatin), 13.05 (s, 1H, NH exchanged with D_2O); MS *m/z* [%]: 464 [M^++2, 25.08], 462 [M^+, 55.88], 223 [100]. Anal. Calcd. for $C_{19}H_{15}ClN_4O_4S_2$ (462.92): C, 49.30; H, 3.27; N, 12.10; found C, 49.64; H, 3.08; N, 12.43.

4.1.8.18. Ethyl 2-{[-5-(-1-benzyl-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**15g**).

Red powder (yield 0.6 g, 40%), m.p. 260-261°C; IR (KBr, ν cm^{-1}): 3186 (NH) and 1716, 1693, 1670 (3 C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.27 (br s, 3H, $\underline{CH_3}$-CH_2-), 2.61 (s, 3H, CH_3) , 4.23 (q, 2H, CH_3-$C\underline{H_2}$-, J = 6.4 Hz) , 5.00 (s, 2H, $\underline{CH_2}$-Ar) , 7.01 (d, 1H, H-7 of isatin, J = 8.0 Hz) , 7.08 (t, 1H, H-5 of isatin, J = 8.0 Hz), 7.23-7.37 (m, 6H, H-4 of isatin, 5H of benzyl ring), 8.86 (br s, 1H, H-4 of isatin), 13.05 (s, 1H, NH exchanged with D_2O); MS *m/z* [%]: 504 [M^+, 1.76], 164 [100]; Anal. Calcd. for $C_{25}H_{20}N_4O_4S_2$ (504.58): C, 59.51; H, 4.00; N, 11.10 found C, 59.32; H, 4.13; N, 11.43.

4.1.8.19. Ethyl 2-{[-5-(-1-benzyl-5-chloro-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene]amino}-4-methylthiazole-5-carboxylate (**15h**).

Red powder (yield 1.0 g, 67%), m.p. > 300 °C; IR (KBr, ν cm^{-1}): 3109 (NH) and 1693, 1666, 1655 (3 C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 1.27 (br s, 3H, $\underline{CH_3}$-CH_2-), 2.61 (s, 3H, $\underline{CH_3}$), 4.23 (q, 2H, CH_3-$C\underline{H_2}$- , J=6.8 Hz), 5.01 (s, 2H, $C\underline{H_2}$-Ar), 7.05 (d, 1H, H-6 of chloroisatin J = 8.2 Hz), 7.24-7.34 (m, 5H, of benzyl ring), 7.42 (d, 1H, H-7 of chloroisatin J = 8.2), 8.89 (s, 1H, H-4 of chloroisatin), 13.21 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (TFA-d_1, 100 MHz)

δ ppm: 12.17, 24.39, 44.67, 64.73, 110.00, 111.61, 112.89, 120.25, 126.41, 127.76, 128.33, 128.86, 129.62, 130.86, 131.38, 132.84, 134.57, 143.05, 147.64, 166.05, 167.84, 168.75, 170.48; Anal. Calcd. for $C_{25}H_{19}ClN_4O_4S_2$ (539.02): C, 55.71; H, 3.55; N, 10.39; found C, 56.02; H, 3.67; N, 10.18.

4.2. Biological Evaluation

4.2.1. Anti-tubercular Activity

M. tuberculosis (RCMB 010126) strain was obtained from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Isoniazid was used as reference drug. Anti-tubercular activity of the newly synthesized molecules (**8a,b**, **11a-d**, **14a-k** and **15a-h**) was evaluated using the Microplate Alamar Blue Assay (MABA) as reported earlier [12, 16]. For *ex vivo* SPOTi assay, *M. aurum* [Optical Density (OD)600≈1] were first checked for quality control using cold Ziehl–Neelsen (ZN) staining (also called 'acid fast staining'; TB-colour staining kit, BDH/Merck) according to the manufacturer's protocol. Wells with DMSO or H_2O were used as negative controls and isoniazid as a positive control.

4.2.2. Intracellular assay using a mycobacteria-infected macrophage model

For the intracellular killing assay, RAW 264.7 macrophages (5×10^5 cells per well) were infected with *M. aurum* at 1:10 MOI (multiplicity of infectivity) for 1 h at 37°C in a 24-well plate. The culture was washed with RPMI-1640 thrice and incubated with different concentration of inhibitors (0, 12.5, 25, 50, 100 and 200 µg/mL) in RPMI-1640 complete medium. Inhibitors were incubated along with infected macrophages for 48 h at 37°C CO_2 incubator. Macrophages were then washed twice with RPMI-1640 and lysed in 500 µL of distilled water at room temperature for 10 min. The lysed cells were centrifuged and suspended in 50 µL of distilled water. Then, 5 µL of suspended cells was spotted onto wells of a 24-well plate containing MB7H10 OADC agar and incubated at 37°C for 4 days to determine intracellular survival and *ex vivo* MIC.

4.2.3. Antimicrobial activity

All tested strains were provided from culture collection of the RCMB. Antibacterial and antifungal activities were expressed as the diameter of inhibition zones; agar well diffusion

method was used as reported earlier [13, 14]. While, MIC was performed by a serial dilution technique described by Irobi*et al*. [31]. Amphotericin B and ciprofloxacin were used as antifungal and antibacterial positive control; respectively. The results reported are means of at least three separate experiments.

4.2.4. In vitro cytotoxic activity by NCI-USA.

The anticancer assays were performed in accordance with the protocol of the Drug Evaluation Branch, NCI, Bethesda [20]. A 48 h drug exposure protocol was used and sulforhodamine B (SRB) protein assay [23] was applied to estimate the cell viability and growth, as reported earlier [32]. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. After cell inoculation, the microtiter plates were incubated at 37 ^{o}C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs, then two plates of each cell line were fixed *in situ* with trichloroacetic acid, to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Aliquot of 100 µl of the drug dilution was added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentration (10 µM). Triplicate wells were prepared for each individual dose. Following drug addition, the plates were incubated for an additional 48 h at 37 ^{o}C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 ^{o}C. The supernatant was discarded, then the plates were washed 5 times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero (*Tz*), control growth (*C*), and test growth in the presence of drug (T_i)], the percentage growth

was calculated at the drug concentration level. Percentage growth inhibition was calculated as:

$[(T_i - T_z) / (C - T_z)]$ x 100 for concentrations for which $T_i \geq T_z$

$[(T_i - T_z) / T_z]$ x 100 for concentrations for which $T_i < T_z$

4.2.5. In vitro cytotoxic activity WI-38 cells (human lung fibroblast normal cell line)

WI-38 cells (normal breast cells), were obtained from American Type Culture Collection. The cells were propagated in DMEM supplemented with 10% heat-inactivated FBS (Hyclone), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin. All of the other chemicals and reagents were from Sigma, or Invitrogen. Cytotoxicity was determined using MTT assay [19] as reported earlier [33]. The 50% inhibitory concentration (IC_{50}) was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA). The data presented are the mean of at least three separate experiments.

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FIGURE CAPTIONS

Figure 1. Structures of agents with reported anti-tubercular activity (**I-V**)and the design of the target compounds (**8a,b**, **11a-d**, **14a-k** and **15a-h**).

Figure 2. Growth inhibition of *M. aurum* inside RAW 264.7 cells for different concentrations of selected conjugates (**14g, 14i, 14k, 15a, 15b, 15e, 15g, 15h)** along with negative control only DMSO.

SCHEME CAPTIONS

Scheme 1. **Synthesis of the key intermediates (5a, b)**. Reagents and conditions: (**i**) SO_2Cl_2, dry toluene, RT, 72 hrs; (**ii**) Thiourea, absolute ethanol, reflux, 3 hrs; (**iii**) $ClCH_2COCl$, dry DMF, RT, 6 hrs; (**iv**) NH_4SCN, absolute ethanol, reflux, 6 hrs.

Scheme 2. Synthesis of the target compounds (8a,b and **11a-d)**. Reagents and conditions: (**i**) Dry DMF, $POCl_3$, 2 hrs; (**ii**) Glacial CH_3COOH, anhydrous CH_3COONa, reflux, 3 hrs; (**iii**) Bromomethane **9a** or benzyl bromide **9b**, dry DMF, sodium hydride, RT; 24 hrs (yields: 75-83%).

Scheme 3. Synthesis of the target compounds (14a-k and **15a-h)**. Reagents and conditions: (**i**) Bromomethane **9a** or benzylbromide **9b**, dry DMF, anhydrous potassium carbonate, RT, 24 hrs; (**ii**) Glacial CH_3COOH, anhydrous CH_3COONa, reflux, 3 hrs (yields: 64-75%).

TABLE CAPTIONS

Table 1. Anti-tubercular activities of the target compounds (**8a,b**, **11a-d**, **14a-k** and **15a-h**) against the clinically isolated strain *of M. tuberculosis* RCMB 010126.

Table 2. Antibacterial activity of the target conjugates (**8a,b**, **11a-d**, **14a-k** and **15a-h**); expressed as inhibition diameter zones (I.Z.) in mm, and minimum inhibitory concentrations (MIC) in µg/mL.

Table 3. Antifungal activity of the target conjugates (**8a,b**, **11a-d**, **14a-k** and **15a-h**); expressed as (I.Z.) in mm, and (MIC) in µg/mL.

Table 4. Activity of the selected compounds on *M. aurum* (expressed as MIC in µg/mL) as well as their cytotoxicity against macrophage RAW 264.7 (expressed as GIC_{50} in µg/mL) and selectivity index expressed as absolute number.

Table 5. The minimum inhibitory concentration (MIC in µg/mL) of compounds **15a** and **15b** against methicillin-resistant *Staphylococcus aureus* (MRSA ATCC 33592) and vancomycin- resistant *Enterococcus faecium* (ATCC 700221).

Table 6. *In vitro* cytotoxic activity of compounds **15a, 15b** (NSC: 791602) **and 15h** (NSC: 791608) against NCI-USA 60 cancer cell lines and normal lung cells WI-38 and their selectivity index.