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GRAPHICAL ABSTRACT

New chalcone-sulfonamide hybrids exhibiting anticancer and antituberculosis activity

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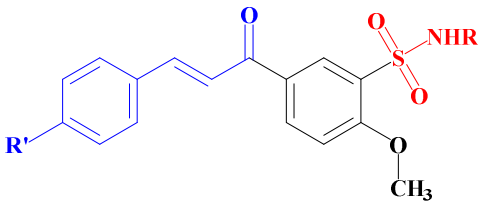
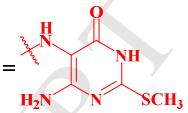
Antiproliferative Hybrids

R'= -H (a), -Cl (b), $-CH_3$ (c), $-OCH_3$ (d), $-3,4,5-(OCH_3)_3$ (e), $3,4-O-CH_2-O-$ (f)



8: R = H,

9: R =



Anticancer activity

8b = GI_{50} 0.57 µM, K-562 (*Leukemia*)

9b = GI_{50} 1.58 µM, U251 (*CNS Cancer*)

Antitubercular activity

8a = MIC_{H37Rv} 16 µM

8b = MIC_{H37Rv} 14 µM

9b = MIC_{H37Rv} 20 µM

New chalcone-sulfonamide hybrids exhibiting anticancer and antituberculosis activity

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Abstract

New sulfonamides **5/6** derived from 4-methoxyacetophenone **1** were synthesized by *N*-sulfonation reaction of ammonia (**3**) and aminopyrimidinone (**4**) with its sulfonyl chloride derivative **2**. Sulfonamides **5** and **6** were used as precursors of two new series of chalcones **8a-f** and **9a-f**, which were obtained through Claisen-Schmidt condensation with aromatic aldehydes **7a-f**. Compounds **5/6**, **8a-d**, **8f**, **9a-d**, and **9f** were screened by the US National Cancer Institute (NCI) at 10 µM against sixty different human cancer cell lines (one-dose trial). Chalcones **8b** and **9b** satisfied the pre-determined threshold inhibition criteria and were selected for screening at five different concentrations (100, 10, 1.0, 0.1, and 0.01 µM). Compound **8b** exhibited remarkable GI_{50} values ranging from 0.57-12.4 µM, with cytotoxic effects being observed in almost all cases, especially against the cell lines K-562 of *Leukemia* and LOX IMVI of *Melanoma* with GI_{50} = 0.57 and 1.28

μM, respectively. Moreover, all compounds were screened against *Mycobacterium tuberculosis* H37Rv, chalcones **8a-c** and **9a-c** were the most active showing MIC values between 14 and 42 μM, and interestingly they were devoid of antibacterial activity against *Mycobacterium smegmatis* and *Staphylococcus aureus*. These antituberculosis hits showed however low selectivity, being equally inhibitory to *M. tuberculosis* and mammalian T3T cells. The chalcone-sulfonamide hybrids **8a-f** and **9a-f** resulted to be appealing cytotoxic agents with significant antituberculosis activity.

Keywords: Antibacterial, anticancer, antituberculosis, chalcones, sulfonamides.

Highlights

- New chalcone-sulfonamide hybrids were synthesized from 4-methoxyacetophenone.
- The 4-chloro-substituted compounds **8b** and **9b** showed important anticancer activity with GI_{50} 0.57-40.1 μM.
- Hybrids **8b** and **9b** showed significant growth inhibition (MIC ≤ 20 μM) of *M. tuberculosis* H37Rv.

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1 Introduction

It is well known that cancer and tuberculosis (TB) are major global health challenges. Cancer is the second cause of human mortality (below cardiovascular disease) and the number of cases is currently increasing with an estimation of 22 million new cases by 2030 [1]. Cancer diagnosis and treatment are a great challenge to physicians [2], and the development of new cancer therapeutic entities is still a major focus of medicinal chemistry efforts worldwide. TB is currently the leading cause of death from a single infectious agent. The *Mycobacterium tuberculosis* bacilli are able to remain latent in one-third of human population [3], and there are multidrug-resistant bacteria isolates circulating in all the continents [4]. Early phases of drug discovery for TB are quite important in medicinal chemistry research, and it is not clear if there are enough lead compounds entering the pipeline to assure novel therapeutics in the following decade.

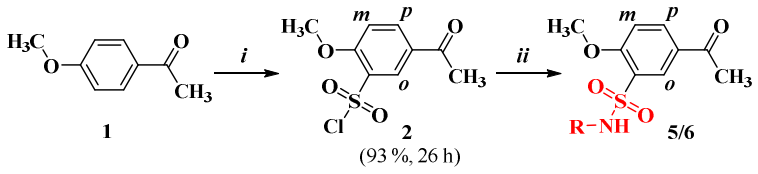
Sulfonamides are important scaffolds for the development of biologically relevant molecules because they display a wide variety of pharmacological properties including anti-inflammatory [5], antimicrobial [6–8], anticancer [9,10], antiviral [11], diuretic [12] and hypoglycemic [13]. The target versatility of sulfonamides allows the properties tuning, for example by assembling hybrids with other relevant pharmacophores [14]. The hybrids by combination of the sulfonamide moiety with heterocyclic rings [15] or chalcones have shown anticancer activity [16,17]. Sulfonamides, such as sulfamethoxazole, have widely been used in antibacterial chemotherapy because they interfere with folic acid biosynthesis [18]. Recent studies have also employed the sulfonamide group in the development of antituberculosis agents [19,20]. Their biochemical advantages are the coordination to metal ions in diverse metalloenzymes by the S=O and NH_2 polar groups, the establishment of particular hydrogen bonds reproducing the tetrahedral transition states of many enzymatic reactions and the possibility of forming lipophilic interactions via the aryl groups [10,21]. These chemical characteristics grant sulfonamides their ability to inhibit a large number of

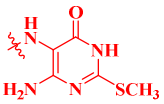
metalloenzymes including carbonic anhydrases [9] and peptidases [22], among others. We report here the synthesis of new chalcone-sulfonamide hybrids derived from 4-methoxyacetophenone and the study of their anticancer and antituberculosis activities.

2 Results and Discussion

2.1 Chemistry

The synthesis was started with the preparation of the intermediate benzenesulfonyl chloride **2**, wich was carried out by the chlorosulfonation reaction of compound **1**, using chlorosulfonic acid and thionyl chloride (Scheme 1). The use of an equimolar amount of the chlorinating agent resulted in a low yield (20%). In order to enhance the conversion, an excess 2:1 of the chlorinating agent was employed reaching 93% yield after 26 h. Compound **2** showed to be stable and it was possible to confirm its structure by spectroscopic techniques such as FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The IR spectrum of chloride **2** shows absorption bands at 3108, 2871, 1684, 1254 and 1167 cm^{-1} corresponding to stretching vibration of C-H Ar, C-H, C=O, C-O-C and S=O bonds, respectively. The ^{1}H NMR spectrum showed two downfield signals at 8.52 ppm as a doublet with J = 2.0 Hz and 8.31 ppm as a double doublet with J = 8.8 and 2.0 Hz corresponding to H_o and H_p protons, respectively. At 7.21 ppm a doublet with J = 8.8 Hz was assigned to the signal of H_m. The ^{13}C NMR spectrum showed the expected nine signals for carbons in compound **2**. The mass spectrum revealed characteristic isotopic profile for the molecular peak *m/z* 248/250 for the presence of one chlorine atom in the molecule.



Compound	R	Reaction time (h)	Yield (%)
5	-H (**3**)	4	82
6	 (**4**)	2	81

Scheme 1. General procedure for the synthesis of sulfonamides **5/6**. Reagents and conditions: *i*) $HOSO_2Cl$, $SOCl_2$ at 0 °C, **1** and then rt, *ii*) EtOH, ammonia, rt (for **5**) and TEA, R-NH_2, rt (for **6**).

The synthesis of the sulfonamides intermediates (**5/6**) was performed through *N*-sulfonation reactions of compound **2** with ammonia **3** and aminopyrimidinone **4** (Scheme 1). Compound **5** was synthetized using a mixture 1:1 ammonia:ethanol in 82% yield. However, several synthetic conditions were tested ranging diverse solvents and bases for the synthesis of sulphonamide **6** (Table 1). In low polar solvents (e.g. dichlorometane or chloroform) the reactions were unfavored. In contrast, the use of a protic solvent such as ethanol increased the yield and reduced the reaction time even at room temperature. Finally, the reaction was optimized by adding triethylamine as acid scavenger, to afford the desired product **6** in 81% yield.

Table 1. Screening conditions for the synthesis of sulfonamide **6**.

Entry	Solvent (1.5 mL)	Base (3 drops)	Concentration (w/v %)	Temperature	Time (h)	Yield (%)
1	DCM	Et_3N	---	rt	170	Traces
2	$CHCl_3$	Et_3N	---	rt	170	Traces
3	EtOH	Et_3N	---	rt	2	81
4	EtOH	Et_3N	---	reflux	0.25	Complex mixture
5	EtOH	NaOH	50	rt	2.5	30

The structures of compounds **5/6** were confirmed by spectroscopic techniques. The most relevant features in the IR spectrum of sulfonamide **5** correspond to the presence of two stretching vibration bands at 3329 and 3208 cm^{-1} belonging to the N-H bond in a primary amino group, confirming the substitution. In the ^{1}H NMR spectrum, the product **5** showed similar chemical shift values for aromatic protons to those of compound **2** but slightly shifted upfield, additionally, at 7.25 ppm is observed a singlet integrating for two protons, corresponding to the sulfonamidic amino group. The structure of sulfonamide **5** was also confirmed from single-crystal X-ray data, Figure 1 (a).

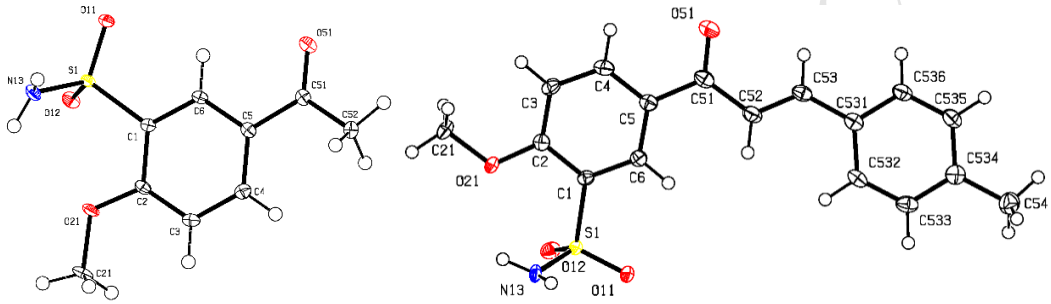
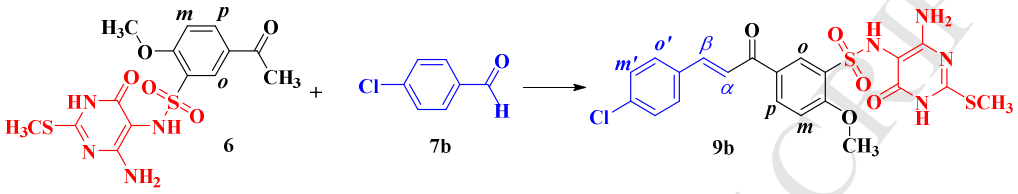


Figure 1. Molecular structure of the compounds **5** (a) and **8c** (b) determined from single-crystal X-ray data, showing the atom-labelling scheme.

Sulfonamides **5/6** reacted with several substituted aromatic aldehydes **7a-f** through a Claisen-Schmidt condensation to obtain two series of chalcone-sulfonamide hybrids **8a-f** and **9a-f**. In all cases, ethanol was used as solvent and an aliquot of aqueous NaOH solution as the base (Table 2). Chalcones derived from sulfonamide **6** (i.e. **9a-f**, Figure 3) turned out to be more sensitive to base concentration and some by-products were observed under the same reaction conditions used for preparation of chalcones derived from sulfonamide **5** (i.e. **8a-f**, Figure 2) as shown in Entry 1. For this reason, Entry 2 was considered as the best reaction conditions to provide products **9a-g**. By using the methodologies of Entry 1 and 2, products **8a-g** and **9a-g** were initially obtained in their

sodium salt forms, respectively. Therefore, a subsequent treatment with aqueous HCl solution was necessary.

Table 2. Screening conditions for the synthesis of chalcone-sulfonamide hybrid **9b** as model reaction.



Entry	Solvent (1.5 mL)	Base (2 drops)	Concentration (w/v %)	Temperature	Time (h)	Yield (%)
1	EtOH	NaOH	50	rt	6	Complex mixture
2	EtOH	NaOH	30	rt	2.5	56
3	EtOH	NaOH	30	reflux	3	Complex mixture

Regarding to the NMR spectra, the twelve compounds synthetized (**8a-f** and **9a-f**) showed similar chemical shifts, although in compounds **9a-f** the presence of the corresponding signals for the pyrimidine moiety are also present, and therefore we discuss only the spectroscopic data for **8b** as the representative of this series. The ^{1}H NMR spectrum revealed the C=C double bond formation due to the presence of a multiplet between 7.98 and 7.92 ppm integrating for three protons assigned to H_{α} and $H_{o'}$ protons, a doublet at 7.74 ppm with J = 15.5 Hz corresponding to H_{β} and a doublet for two hydrogens at 7.53 ppm with J = 7.6 Hz assigned to $H_{m'}$. The coupling constant for H_{β} disclosed the *E* configuration on the enone moiety. All proton signals from the aryl ring bound to sulfonamide were observed, including those corresponding to the amino group. In the ^{13}C NMR spectrum was observed the two signals for α and β carbon atoms at 122.2 and 142.6 ppm, respectively. The structure of hybrid **8c** was confirmed by single-crystal X-ray data. The ORTEP representation is shown in Figure 1 (b).

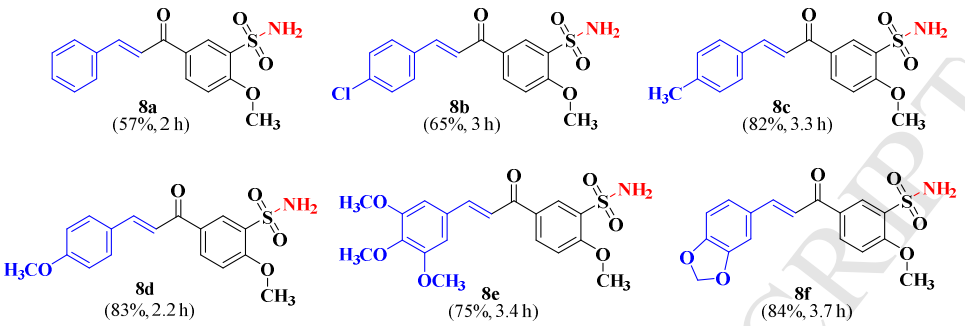


Figure 2. Chalcone-sulfonamide hybrids derived from sulfonamide **5**.

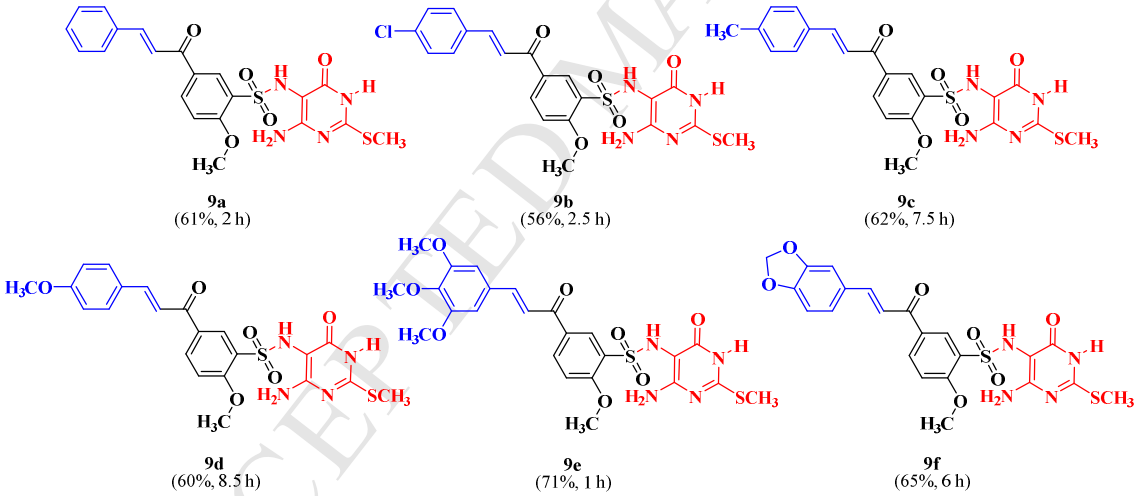


Figure 3. Chalcone-sulfonamide hybrids derived from compound **6**.

2.2 Anticancer activity

A preliminary screening of the structures of compounds **5/6**, **8a-f** and **9a-f** was carried out by COMPARE algorithm, all of them excepting chalcones **8e** and **9e** were selected by the Developmental Therapeutics Program (DTP) at National Cancer Institute (NCI) for single

concentration trials. The assay involves a primary *in vitro* evaluation of submitted compounds against 60 human cancer cell lines at 10 µM concentration. The results for each compound are reported as a mean value of the growth percent (GP) of the treated cells (Figure 4a). Table 3 summarizes the results obtained for compounds **5/6**, **8a-d**, **8f**, **9a-d** and **9f**.

Table 3. Range and mean growth percent of NCI human cancer cell lines treated with selected compounds at one dose (10 μM).

Compound	Mean growth	Most sensitive cell lines	Growth percent of most sensitive cell line (%)
5	98.28	HL-60(TB) *(Leukemia)*	74.74
6	99.84	M14 *(Melanoma)*	94.85
8a	68.07	K-562 *(Leukemia)*	7.90
		RPMI-8226 *(Leukemia)*	-31.74*
		K-562 *(Leukemia)*	3.05
		HOP-92 *(Non-Small Cell Lung Cancer)*	-26.57*
8b	44.42	HCT-116 *(Colon Cancer)*	-78.33*
		M14 *(Melanoma)*	-57.32*
		786-0 *(Renal Cancer)*	-30.67*
		PC-3 *(Prostate Cancer)*	-55.63*
8c	56.09	K-562 *(Leukemia)*	3.04
8d	78.45	SR *(Leukemia)*	28.10
		K-562 *(Leukemia)*	38.78
8f	82.97	HCT-116 *(Colon Cancer)*	19.22
		K-562 *(Leukemia)*	44.59
9a	80.00	HCT-116 *(Colon Cancer)*	35.18
		K-562 *(Leukemia)*	35.63
		SF-539 *(CNS Cancer)*	-5.49*
9b	52.87	K-562 *(Leukemia)*	3.84
		U251*(CNS Cancer)*	-4.20*
9c	84.26	HCT-116 *(Colon Cancer)*	10.78

		K-562 *(Leukemia)*	38.79
9d	90.59	HCT-116 *(Colon Cancer)*	24.01
		K-562 *(Leukemia)*	54.26
9f	89.03	HCT-116 *(Colon Cancer)*	18.36
		K-562 *(Leukemia)*	53.79

* Negative value means lethality. This percentage indicates there is no net growth of tumor cells over the course of the experiment, instead, compound causes the death of the respective cancer cell.

Sulfonamides **5/6** exhibited low activity against the cancer cell lines analyzed. Nevertheless, their α,β-unsaturated derivatives showed a significant increase in the antitumor activity. In general, both series of chalcones displayed significant activity against *Leukemia* panel, on cancer cell lines K-562 (GP = 3.04-54.26 %). Specifically, the compounds **8a**, **8b**, **8c** and **9b** at 10 μM showed the strongest effect, decreasing the viability of this cell line to less than 10% (Figure 4a). The K-562 cell line are known to be positive for the BCR-ABL fusion gene, as well as to show similarity to primary granulocytes and erythrocytes [23]. K-562 cells contain mutations on the genes cyclin-dependent kinase (CDKN2A) and tumor protein (TP53) [24], which induce a cell phenotype with an active proliferative capacity driven by inhibition of apoptosis. As apoptosis is an important mechanism providing targets for antitumor drugs, that has been shown to inhibit K-562 cells grow [25-28], we hypothesised, that these chalcones activate apoptosis causing the effect that we see in table 3. All compounds also showed activity against *Colon Cancer* in sub-panel HCT-116 (GP = -78.33-44.62%) and *CNS Cancer* on cell line U251 (GP = -4.20-35.40%). Compounds **8b** and **9b** were the most active of both series against the majority of cancer cell lines, especially HCT-116 (*Colon Cancer*) for **8b** and SF-539 (*CNS Cancer*) for **9b** with lethality values of 78.33% and 5.49%, respectively. Both compounds satisfied the pre-determined threshold inhibition criteria of NCI, therefore they were selected for a Five-Dose screening, involving the evaluation against 60 cancer cell lines at five different concentrations (100, 10, 1.0, 0.1, and 0.01 μM) to determine GI_{50} (growth inhibitory activity) and LC_{50} (cytotoxic activity) values. Compounds **8b** and **9b** exhibited potent

anticancer activity against several cancer cell lines with GI_{50} values between 0.57-12.4 μM and 1.56-40.1 μM, respectively (Table 4). Chalcone **8b** showed the best results against *Leukemia* panel, especially in K-562 and SR cell lines with GI_{50} values of 0.57 and 0.86 μM. This compound also displayed important activity against HCT-116 (*Colon Cancer*) with GI_{50} = 1.36 μM, LOX IMVI (*Melanoma*) with GI_{50} = 1.28 μM and MCF7 (*Breast Cancer*) with GI_{50} = 1.30 μM. Cytotoxic activity of **8b** was also remarkable, most relevant results were obtained against HCT-116 (*Colon Cancer*), SF-539 (*CNS Cancer*) and LOX IMVI (*Melanoma*) with LC_{50} values of 5.36, 6.82 and 5.47 μM (Table 4). Compound **9b** demonstrated to be a good cytotoxic agent against HCT-116 (*Colon Cancer*) with LC_{50} = 5.55 μM, U251 (*CNS Cancer*) with LC_{50} = 6.16 μM and BT-549 (*Breast Cancer*) LC_{50} = 6.17 μM. Ras proteins are essential components of signalling networks that control cell proliferation [29], and the HCT-116 cells have a mutation in codon 13 of the KRAS proto-oncogene [30]. Studies in HCT-116 cells have shown that a number of anticancer drugs target the cell cycle molecular pathways [31], and thus, we hypothesize that compound **8b** could be targeting similar biochemical mechanisms. CDKN2A mutation in HCT-116, modifies the cell cycle cellular control, and as such, many CDK inhibitors have been shown anticancer effect in these cells. For example, roscovitine, purvalanol, and 9-nitropaullone inhibit CDC2, CDK2 and CDK5 [32]. Similar to the K-562 *Leukemia* cells, the SF-539 *CNS cancer* cells also contained mutations on the TP53 gene, which encodes for a tumor suppressor protein that in case of DNA damage, either activates DNA repair or directs the cell to apoptosis. We propose that the synthetic chalcone-sulfonamide hybrids could either inhibit CDKs and thus arrest cell cycle, or induce apoptosis or both, on the cells that lack a functional TP53 protein, as there is strong evidence of apoptosis induction by different types of chalcones [33-35]. As summary, both 4-chloro-substituted chalcones **8b** and **9b** showed strong cytotoxic effects against several cancer cell lines, achieving LC_{50} values in the low micromolar range (Figure 4b). Both **8b** and **9b** constitute hit structures for the development of further anticancer agents.

Table 4. Cytotoxic activity of compounds **8b** and **9b** in Five-Dose test expressed as GI_{50} and LC_{50} of cancer cell lines.[a]

Panel/Cell line	Compounds			
	8b		9b	
	GI_{50}[b]	LC_{50}[c]	GI_{50}	LC_{50}
		Leukemia		
CCRF-CEM	3.34	>100	2.66	>100
HL-60(TB)	3.08	>100	3.20	>100
K-562	0.57	>100	2.77	>100
MOLT-4	2.93	>100	3.71	>100
RPMI-8226	2.61	>100	2.17	>100
SR	0.86	>100	3.57	>100
		Non-Small Cell Lung Cancer		
A549/ATCC	2.69	30.8	2.57	30.0
EKVX	4.56	50.5	3.94	80.3
HOP-62	2.51	31.2	1.81	8.70
HOP-92	2.14	21.4	2.59	32.6
NCI-H226	3.66	95.7	2.34	52.9
NCI-H23	2.54	40.9	2.53	40.4
NCI-H322M	2.04	28.8	2.38	28.0
NCI-H460	2.58	30.0	2.49	29.1
NCI-H522	1.93	12.1	1.88	8.83
		Colon Cancer		
COLO 205	2.03	17.4	1.76	6.68
HCC-2998	1.78	7.48	1.92	6.50
HCT-116	1.36	5.36	1.56	5.55
HCT-15	2.14	59.3	3.24	83.9
HT29	3.26	84.0	2.93	45.3
KM12	1.73	8.26	2.09	9.76
SW-620	1.76	8.45	1.58	28.3

		CNS Cancer		
SF-268	3.13	38.3	2.69	36.1
SF-295	3.04	31.9	2.72	34.7
SF-539	1.60	6.82	2.10	15.1
SNB-19	3.10	33.4	2.52	32.2
U251	1.96	9.33	1.58	6.16
		Melanoma		
LOX IMVI	1.28	5.47	1.63	6.34
MALME-3M	5.89	54.8	3.02	41.8
M14	2.13	21.2	2.00	15.7
MDA-MB-435	4.34	41.6	2.95	48.0
SK-MEL-2	12.4	53.2	12.8	61.1
SK-MEL-28	3.24	37.2	2.10	17.0
SK-MEL-5	5.69	42.7	2.72	32.0
UACC-257	12.2	50.8	8.27	50.1
UACC-62	4.88	44.8	2.33	27.4
		Ovarian Cancer		
IGROV1	2.67	48.1	3.25	80.2
OVCAR-3	2.04	9.70	1.94	8.00
OVCAR-4	3.47	40.4	4.50	43.6
OVCAR-5	3.08	44.9	2.83	38.2
OVCAR-8	3.55	32.6	3.07	37.1
NCI/ADR-RES	3.64	>100	40.1	>100
SK-OV-3	5.96	43.1	3.65	36.6
		Renal Cancer		
786-0	1.74	7.82	1.95	8.74
A498	10.4	47.1	10.6	47.6
ACHN	1.94	8.75	3.23	36.5
CAKI-1	2.72	32.5	3.83	79.5
RXF 393	1.98	7.96	1.60	6.67
SN12C	2.72	31.5	3.45	73.4
TK-10	2.47	27.5	2.98	32.3
UO-31	1.89	22.0	1.89	12.9

		Prostate Cancer		
PC-3	2.24	17.0	2.53	32.8
DU-145	3.42	36.3	3.58	33.9
		Breast Cancer		
MCF7	1.30	>100	1.97	>100
MDA-MB-231/ATCC	3.48	40.1	3.42	40.3
HS 578T	3.28	>100	3.56	>100
BT-549	2.29	27.3	1.63	6.17
T-47D	3.20	59.0	2.45	38.8

[a] Data obtained from NCI's *in vitro* disease–oriented human cancer cell lines screen in μM. [b] GI_{50} was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation, determined at five concentration levels (100, 10, 1.0, 0.1, and 0.01 μM). [c] LC_{50} is a parameter of cytotoxicity that reflects the concentration needed to kill 50% of the cells.

2.3 Antibacterial activity and mammalian cytotoxicity

Among the synthesized compounds, the chalcone-sulfonamide hybrids **8a**, **8b** and **9b** were the most potent growth inhibitors of *Mycobacterium tuberculosis* H37Rv, with minimum inhibitory concentration (MIC) values of 16, 14 and 20 μM respectively (Table 5). The compounds **8c**, **9a** and **9c** also showed significant activity with MIC values between 30 and 42 μM. The structure-activity analysis revealed that the absence of substitution (**8a** and **9a**), the 4-chloro- (**8b** and **9b**) or 4-methyl-substitution (**8c** and **9c**) on ring B, consistently increased the anti-TB effect. However, the 4-methoxy- (**8d** and **9d**), 3,4,5-trimethoxy- (**8e** and **9e**) and 3,4-methylenedioxy-substitution (**8f** and **9f**) eliminated the antituberculosis effect. Sulfonamides **5** and **6** without the chalcone moiety, were also devoid of any anti-TB activity (MIC >130 μM). All the evaluated compounds **5/6**, **8a-f** and **9a-f**, showed little inhibitory effect against *Mycobacterium smegmatis* mc^2155 and *Staphylococcus aureus* strains including drug-resistant clinical isolates, with MIC values >175 μM and >57 μM,

respectively. This result suggested antibacterial specificity against *M. tuberculosis*, and opens the door to explore in detail their mechanism of action. However, evaluation of mammalian cytotoxicity on normal (non-cancer) mouse fibroblasts showed that most of the synthetic compounds were highly citotoxic (Table 5) with LC_{50} values <20 μM for most of them, with the exception of the sulfonamides **5** and **6** and the hybrids **8d** and **8f** with respective LC_{50} values >2.7 mM, >1.6 mM, and 96 μM and 132 μM (Figure 4c). The chalcone-sulphonamide hybrids **8e** and **9e** were highly cytotoxic to fibroblasts with LC_{50} values lower around 2 μM. Surprisingly, the tri-methoxylated chalcone-sulphonamide hybrids **8e** and **9e** were highly cytotoxic to fibroblasts with LC_{50} values around 2 μM and were devoid of anti-tuberculosis activity (Figure 4d), suggesting a mammalian-specific mechanism of action. The selectivity index (SI) is calculated as the ratio between LC_{50} and MIC values against *M. tuberculosis*. In order to progress to further *in vitro* and *in vivo* anti-TB assays, an experimental compound must be more selective to kill mycobacteria than mammalian cells and the SI value should be higher than 10 [36]. None of the synthetic compounds showed SI higher than 1, indicating that the compounds were equally toxic to *M. tuberculosis* and mammalian cells.

Table 5. Anti-TB and cytotoxic activities of the synthesized compounds **5/6**, **8a-f** and **9a-f**.

Compound	Minimum inhibitory concentration against *M. tuberculosis* H37Rv (μM)	Cytotoxicity LC_{50} on 3T3 fibroblast cells (μM)	Selectivity index ($SI = \frac{LC_{50}}{MIC_{H37Rv}}$)
5	>218	>2.7 mM	nd
6	>130	>1.6 mM	nd
8a	16	7.7 ±1.5	0.48
8b	14	4.1 ± 1.8	0.29
8c	30	4.3 ± 0.2	0.14
8d	>144	96 ± 37	nd
8e	>123	2.4 ± 0.7	nd

8f	>138	132 ± 58	nd
9a	42	17 ± 3	0.40
9b	20	13 ± 5	0.65
9c	41	7.2 ± 0.8	0.18
9d	>100	6.5 ± 4.6	nd
9e	>89	2.1 ± 1.1	nd
9f	>97	7.1 ± 1.1	nd
Isoniazid	0.36	nd	nd
Cycloheximide	nd	< 2.2	nd

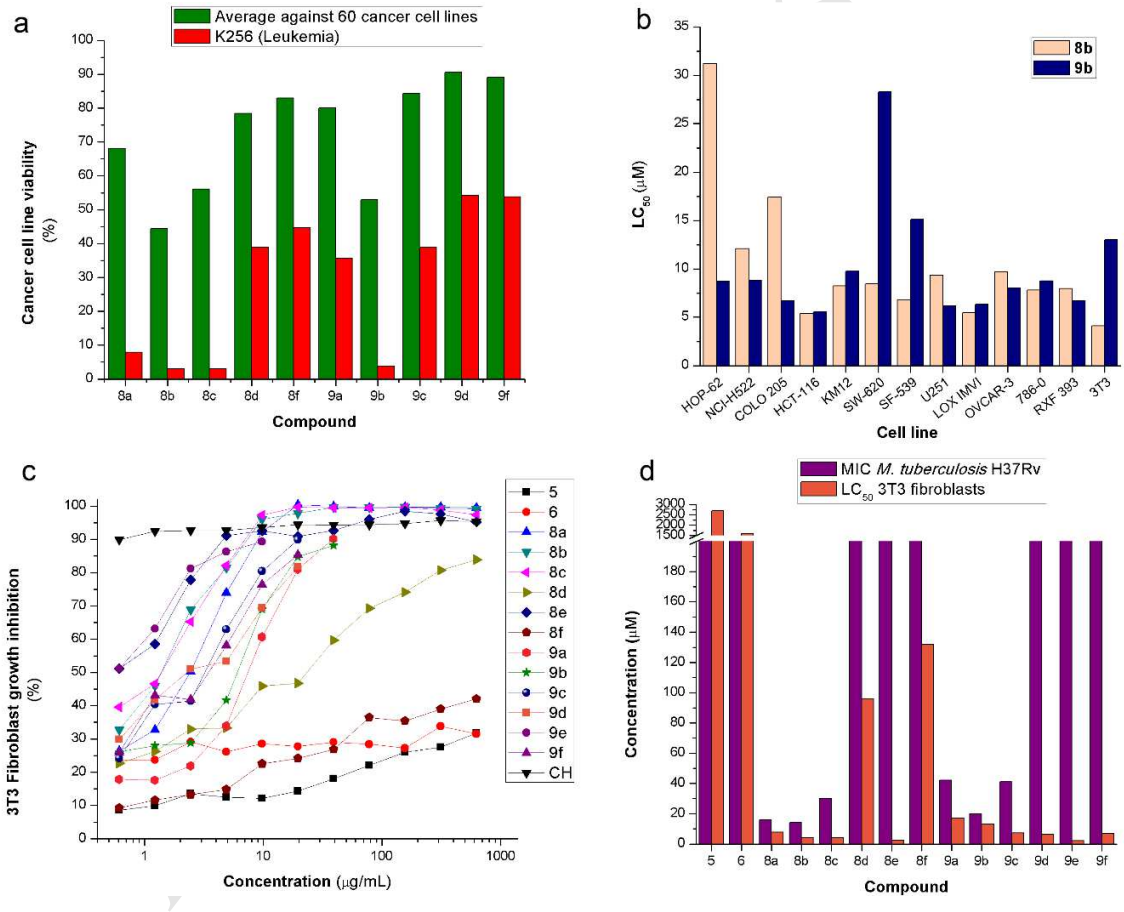


Figure 4. Anti-cancer and anti-tuberculosis activities of the synthetized compounds. **a.** Average viability of the cancer cell lines and the leukemia K256 line, evaluated at a single concentration (10 µM) of the compounds. **b.** Cytotoxicity of the most active **8b** and **9b** compounds on selected cell lines. **c.** Dose-response

3T3 fibroblast inhibitory plot of the synthetized compounds and cycloheximide (CH). **d.** Comparison of growth inhibition of *M. tuberculosis* H37Rv and cytotoxicity on 3T3 fibroblasts.

3 Conclusions

New sulfonamides **5/6** derived from the starting 4-methoxyacetophenone were synthesized by *N*-sulfonation reactions of ammonia **3** and aminopyrimidinone **4** with sulfonyl chloride **2**. Two series of chalcones **8a-f** and **9a-f** were obtained through Claisen-Schmidt condensation of sulfonamides **5** and **6** with aromatic aldehydes **7a-f** in basic media, with short reaction times and good yields. Compounds **5/6**, **8a-d**, **8f**, **9a-d** and **9f** were selected by NCI for single concentration assay. Sulfonamides **5/6** showed low anticancer activity against the cancer cell lines evaluated, however, their chalcone hybrids exhibited significant activity against *Leukemia*, C*olon* and *CNS* cancer cells. The 4-chloro-substituted compounds **8b** and **9b** were the most active and passed to five-dose screen. In this trial, compound **8b** demonstrated remarkable growth inhibition and cytotoxic values, and thus constitute a promising hit in the development of anticancer agents. The hybrids **8b** and **9b** also showed potent growth inhibition (MIC $\leq$ 20 μM) of *M. tuberculosis* H37Rv, with no activity against rapid-growing bacteria *M. smegmatis* or *S. aureus*. The evaluation of cytotoxicity against 3T3 mouse fibroblasts showed low selectivity, and thus these agents were considered equally toxic to *M. tuberculosis* and mammalian cells. The chalcone-sulfonamide hybrids are appealing cytotoxic agents with antituberculosis activity. They may be further optimized to target distinctively cancer or mycobacterial cells.

4 Experimental

4.1 General

All chemicals and solvents were purchased from Sigma-Aldrich unless stated otherwise. Melting points were measured using a Stuart SMP10 melting point device and are uncorrected. ATR-FTIR spectra were recorded on a Shimadzu IRAffinity–1. The ^{1}H and ^{13}C NMR spectra were run on a BRUKER DPX 400 spectrometer operating at 400 and 100 MHz respectively, using DMSO–d_6 and $CDCl_3$ as solvents and TMS as internal standard. The mass spectra were recorded on a SHIMADZU–GCMS–QP2010 spectrometer operating at 70 eV. Elemental analyses were performed on a Thermo Finnigan Flash EA1112 CHN elemental analyzer and the values are within ±0.4% of the theoretical values. The single cristal x-ray data were collected in a Diffractometer Bruker D8 Venture at "Centro de Instrumentación Científico y Técnico", (CICT) in "Universidad de Jaén" (UJA). Thin layer chromatography (TLC) was performed on a 0.2 mm pre–coated aluminum plates of silica gel (Merck 60 F_{254}) and spots visualized with ultraviolet irradiation.

4.2 Chemistry

4.2.1 General procedure for the synthesis of benzenesulfonyl chloride 2

A mixture of chlorosulfonic acid (60 mmol) and thionyl chloride (20 mmol) was stirred in an ice bath for 30 min. Subsequently, 4-methoxyacetophenone **1** (10 mmol) was added portionwise and the reaction mixture was stirred at room temperature for 26 h. Then, it was quenched in ice-water and the precipitate obtained was filtered and washed with water affording compound **2**. The product did not require further purification.

***5-Acetyl-2-methoxybenzenesulfonyl chloride* (2)**

Orange solid; 93 % yield; m.p. 104-105 °C. FTIR (ATR) ν(cm^{-1}): 3108 (C-H), 1684 (C=O), 1254 (C-O-C), 1167 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.52 (d, *J* = 2.0 Hz, 1H, H_o), 8.31 (dd, *J* = 8.8, 2.0 Hz, 1H, H_p), 7.21 (d, *J* = 8.8 Hz, 1H, H_m), 4.14 (s, *J* = 7.8 Hz, 3H, O-CH_3), 2.61 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 194.7 (C), 160.6 (C), 137.1 (CH), 131.8 (C), 130.7 (CH), 129.7 (C), 113.4 (CH), 57.3 (CH_3), 26.5 (CH_3). MS (EI, *m/z* (%)): 248/250 (M^+/$M+2^+$, 22/8), 233 (100), 119 (22), 69 (23), 43 (43). Anal. calcd. for $C_9H_9ClO_4S$: C, 43.47; H, 3.65; S, 12.89. Found: C, 43.50; H, 3.75; S, 13.00.

4.2.2 General procedure for the synthesis of sulfonamide 5

A mixture of chloride **2** (0.31 mmol) and ammonia **3** (1 mL) in ethanol (1 mL) was stirred at room temperature and the progress was monitored by TLC. After completion, the solid formed was filtered and washed with ethanol and water, compound **5** did not required further purification.

***5-Acetyl-2-methoxybenzenesulfonamide* (5)**

Pale pink solid; 82 % yield; m.p. 195-198 °C. FTIR (ATR) ν(cm^{-1}): 3329 and 3208 (N-H), 3098 (C-H), 1657 (C=O), 1159 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.29 (d, *J* = 2.3 Hz, 1H, H_o), 8.19 (dd, *J* = 8.7, 2.3 Hz, 1H, H_p), 7.32 (d, *J* = 8.7 Hz, 1H, H_m), 7.24 (s, 2H, NH_2), 4.00 (s, 3H, O-CH_3), 2.56 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 195.7 (C), 159.6 (C), 134.4 (CH), 131.4 (C), 128.8 (C), 127.7 (CH), 112.6 (CH), 56.7 (CH_3), 26.4 (CH_3). MS (EI, *m/z* (%)): 229 (M^+, 22), 214 (100), 119 (25), 76 (57), 43 (87). Anal. calcd. for $C_9H_{11}NO_4S$: C, 47.15; H, 4.84; N, 6.11; S, 13.98. Found: C, 47.09; H, 4.93; N, 6.15; S, 14.01. Crystal data for **5** were deposited at CCDC

with reference CCDC 1865855: Chemical formula $C_9H_{11}NO_4S$, Mr 229.25, Triclinic, P-1, 100 K, cell dimensions a, b, c (Å) 8.1560 (7), 8.4673 (7), 8.5234 (7) Å α, β, γ (º) 80.690 (3), 74.603 (3), 61.443 (3). V (Å^3) 498.03 (7), Z = 2, F(000)= 240, Dx (Mg m-3) = 1.53, Mo Kα, μ (mm-1)= 0.32, pale pink crystal, size (mm) = 0.23 × 0.14 × 0.1. Data collection: Diffractometer Bruker D8 Venture (APEX 3), Monochromator multilayer mirror, CCD rotation images, thick slices φ & θ scans, Mo INCOATEC high brilliance microfocus sealed tube (λ= 0.71073 Å), multiscan absorption correction (SADABS-2016/2), Tmin, Tmax 0.717, 0.746. No. of measured, independent and observed [I > 2σ(I)] reflections 25127, 2277, 2108, Rint= 0.028, θ values (°): θmax = 27.5, θmin = 2.5; Range h = -10→10, k = -10→10, l = -11→11, Refinement on F^2:R[F^2 > 2σ(F^2)]= 0.026, wR(F^2)= 0.072, S=1.104. No. of reflections 2277, No. of parameters 138, No. of restraints 0. Weighting scheme: w = 1/σ^2(Fo^2) + (0.0324P)^2 + 0.3110P where P = (Fo^2 + 2Fc^2)/3. (Δ/σ) < 0.002, Δρmax, Δρmin (e Å^{-3}) 0.32, −0.5.

4.2.3 General procedure for the synthesis of sulfonamide 6

To a mixture of chloride **2** (0.31 mmol) and aminopyrimidinone **4** (0.26 mmol) in ethanol (1.5 mL), TEA (0.15 mL) was added. The reaction mixture was stirred at room temperature and the progress was monitored by TLC. After completion, a further amount of TEA was added until pH = 7 and the solid thus formed was filtered and washed with ethanol to provide compound **6**.

***5-Acetyl-N-(4-amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-2 methoxybenzenesulfonamide* (6)**

Pale pink solid; 81 % yield; m.p. 235-238 °C. FTIR (ATR) ν(cm^{-1}): 3470 and 3343 (N-H), 3109 (C-H), 1676 and 1643 (C=O), 1151 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.68 (s, 1H, NH-

CO), 8.39 (s, 1H, NH-C_5), 8.17 – 8.11 (m, 2H, H_o and H_p), 7.25 (d, J = 8.4 Hz, 1H, H_m), 6.39 (s, 2H, NH_2-C_6), 3.99 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3), 2.41 (s, 3H, SCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 195.7 (C), 161.3 (C), 161.0 (C), 159.8 (C), 159.8 (C), 134.9 (C), 129.5 (CH), 128.9 (CH), 128.1 (C), 112.7 (CH), 92.6 (C), 56.8 (CH_3), 26.4 (CH_3), 12.8 (CH_3). MS (EI, *m/z* (%)): 384 (M^+, 32), 199 (38), 171 (100), 135 (100), 70 (61). Anal. calcd. for $C_{14}H_{16}N_4O_5S_2$: C, 43.74; H, 4.20; N, 14.57; S, 16.68. Found: C, 43.65; H, 4.15; N, 14.60; S, 16.45.

4.2.4 General procedure for the synthesis of chalcone-hybrids 8a-f

To a mixture of sulfonamide **5** (0.20 mmol) and the corresponding aldehyde **7a-f** (0.24 mmol) in ethanol (1.5 mL), it was added 2 drops of 50% (w/v) NaOH solution. The reaction mixture was stirred at room temperature for 2-3.7 h. The sodium salt formed was filtered and washed with ethanol. Then, it was poured into water and neutralized by adding 10% (w/v) HCl aqueous solution. The precipitate was filtered and washed with water to afford the corresponding compounds **8a-f**.

5-Cinnamoyl-2-methoxybenzenesulfonamide **(8a)**

Beige solid; 57 % yield; m.p. 191-193 °C. FTIR (ATR) ν(cm^{-1}): 3352 and 3260 (N-H), 2985 (C-H), 1656 (C=O), 1593 (C=C), 1155 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.48 (d, J = 8.7, 1.9 Hz, 1H, H_p), 8.45 (s, J = 1.9 Hz, 1H, H_o), 7.97–7.87 (m, 3H, H_α and $H_{o'}$), 7.76 (d, J = 15.6 Hz, 1H, H_β), 7.50–7.44 (m, 3H, $H_{m'}$ and $H_{p'}$), 7.37 (d, J = 8.7 Hz, 1H, H_m), 7.28 (s, 2H, NH_2), 4.03 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 187.0 (C), 159.7 (C), 144.1 (CH), 134.8 (CH), 134.6 (C), 131.8 (C), 130.7 (CH), 129.4 (C), 129.0 (CH), 129.0 (CH), 128.2 (CH), 121.6 (CH), 112.7 (CH), 56.8 (CH_3). MS (EI, *m/z* (%)): 317 (M^+, 14), 316 (23), 81 (45), 69 (100), 55 (42), 41 (42). Anal. calcd. for $C_{16}H_{15}NO_4S$: C, 60.55; H, 4.76; N, 4.41; S, 10.10. Found: C, 60.50; H, 4.80; N, 4.52; S, 10.23.

(E)-5-(3-(4-Chlorophenyl)acryloyl)-2-methoxybenzenesulfonamide **(8b)**

White solid; 65 % yield; m.p. 230-232 °C. FTIR (ATR) ν(cm^{-1}): 3364 and 3219 (N-H), 3090 (C-H), 1655 (C=O), 1591 (C=C), 1155 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.58–8.33 (m, 2H, H_o and H_p), 7.98-7.92 (m, 3H, H_α and $H_{o'}$), 7.74 (d, J = 15.5 Hz, 1H, H_β), 7.53 (d, J = 7.6 Hz, 2H, $H_{m'}$), 7.36 (d, J = 8.4 Hz, 1H, H_m), 7.28 (s, 2H, NH_2), 4.03 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.8 (C), 159.7 (C), 142.6 (CH), 135.1 (C), 134.8 (CH), 133.6 (C), 131.8 (C), 130.6 (CH), 129.3 (C), 128.9 (CH), 128.2 (CH), 122.2 (CH), 112.3 (CH), 56.8 (CH_3). MS (EI, *m/z* (%)): 351/353 (M^+/$M+2^+$, 100/41), 316 (49), 271 (63), 214 (36), 165 (19). Anal. calcd. for $C_{16}H_{14}ClNO_4S$: C, 54.63; H, 4.01; N, 3.98; S, 9.11. Found: C, 54.59; H, 3.99; N, 4.02; S, 9.04.

(E)-2-Methoxy-5-(3-(p-tolyl)acryloyl)benzenesulfonamide **(8c)**

Beige solid; 82 % yield; m.p. 205-207 °C. FTIR (ATR) ν(cm^{-1}): 3381 and 3265 (N-H), 2955 (C-H), 1653 (C=O), 1595 (C=C), 1153 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.47 (dd, J = 8.6, 2.2 Hz, 1H, H_p), 8.44 (d, J = 2.2 Hz, 1H, H_o), 7.87 (d, J = 15.6 Hz, 1H, H_α), 7.78 (d, J = 8.0 Hz, 2H, $H_{o'}$), 7.73 (d, J = 15.6 Hz, 1H, H_β), 7.35 (d, J = 8.6 Hz, 1H, H_m), 7.29-7.27 (m, 4H, $H_{m'}$ and NH_2), 4.03 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.8 (C), 159.6 (C), 144.1 (CH), 140.8 (C), 134.6 (CH), 131.9 (C), 131.7 (C), 129.5 (C), 129.5 (CH), 128.9 (CH), 128.1 (CH), 120.4 (CH), 112.6 (CH), 56.7 (CH_3), 21.1 (CH_3). MS (EI, *m/z* (%)): 331 (M^+, 36), 330 (43), 316 (100), 251 (14), 214 (8). Anal. calcd. for $C_{17}H_{17}NO_4S$: C, 61.62; H, 5.17; N, 4.23; S, 9.67. Found: C, 61.60; H, 5.22; N, 4.23; S, 9.46. Crystal data for **8c** were deposited at CCDC with reference CCDC 1865863: Chemical formula $C_{17}H_{17}NO_4S$, Mr 331.38, Orthorhombic, $P2_12_12_1$, 100 K, cell dimensions a, b, c (Å) 10.4960 (8), 12.2051 (8), 12.5300 (8), α, β, γ (º) 90, 90, 90. V (Å^3) 1605.15 (19), Z = 4, F(000)= 696.0, Dx (Mg m-3) = 1.371, Mo Kα, μ (mm-1)= 0.221, colorless block crystal, size (mm) = 0.26 × 0.23 × 0.1. Data collection: Diffractometer Bruker D8 Venture (APEX 3), Monochromator multilayer mirror, CCD rotation images, thick slices φ & θ scans, Mo INCOATEC high brilliance microfocus sealed tube (λ= 0.71073 Å), multiscan absorption

correction (SADABS-2016/2), Tmin, Tmax 0.657, 0.746. No. of measured, independent and observed [I > 2σ(I)] reflections 24961, 3698, 3213, Rint= 0.059, θ values (°): θmax = 27.5, θmin = 2.3; Range h = -13→13, k = -15→15, l = -16→16, Refinement on F^2:R[F^2 > 2σ(F^2)]= 0.035, wR(F^2)= 0.071, S=1.073. No. of reflections 3698, No. of parameters 210, No. of restraints 0. Weighting scheme: w = $1/\sigma^2(Fo^2)$ + (0. 0358 P)^2 + 0.2642 P where P = (Fo^2 + 2Fc^2)/3. (Δ/σ) < 0.003, Δρmax, Δρmin (e Å^{-3}) 0.18, −0.38. Flack parameter x -0.01(3).

(E)-2-Methoxy-5-(3-(4-methoxyphenyl)acryloyl)benzenesulfonamide **(8d)**

Pale yellow solid; 83 % yield; m.p. 214-216 °C. FTIR (ATR) ν(cm^{-1}): 3375 and 3265 (N-H), 3073 (C-H), 1655 (C=O), 1597 (C=C), 1153 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.47-8.43 (m, 2H, H_o and H_p), 7.85 (d, *J* = 8.8 Hz, 2H, $H_{o'}$), 7.80 (d, *J* = 15.5 Hz, 1H, H_α), 7.73 (d, *J* = 15.5 Hz, 1H, H_β), 7.35 (d, *J* = 8.6 Hz, 1H, H_m), 7.27 (s, 2H, NH_2), 7.03 (d, *J* = 8.8 Hz, 2H, $H_{m'}$), 4.02 (s, 3H, OCH_3), 3.82 (s, 3H, *p'*-OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.7 (C), 161.4 (C), 159.5 (C), 144.1 (CH), 134.6 (CH), 131.7 (C), 130.9 (CH), 129.6 (C), 128.1 (CH), 127.3 (C), 119.0 (CH), 114.4 (CH), 112.6 (CH), 56.7 (CH_3), 55.4 (CH_3). MS (EI, *m/z* (%)): 347 (M^+, 100), 316 (23), 267 (18), 161 (25), 133 (18). Anal. calcd. for $C_{17}H_{17}NO_5S$: C, 58.78; H, 4.93; N, 4.03; S, 9.23. Found: C, 58.88; H, 4.90; N, 3.98; S, 8.96.

(E)-2-Methoxy-5-(3-(3,4,5-trimethoxyphenyl)acryloyl)benzenesulfonamide **(8e)**

Pale yellow solid. 75 % yield; m.p. 204-208 °C. FTIR (ATR) ν(cm^{-1}): 3366 and 3260 (N-H), 3086 (C-H), 1659 (C=O), 1595 (C=C), 1150 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.49 (dd, *J* = 8.7, 2.2 Hz, 1H, H_p), 8.43 (d, *J* = 2.2 Hz, 1H, H_o), 7.87 (d, *J* = 15.5 Hz, 1H, H_α), 7.71 (d, *J* = 15.5 Hz, 1H, H_β), 7.36 (d, *J* = 8.7 Hz, 1H, H_m), 7.27 (s, 2H, NH_2), 7.23 (s, 2H, $H_{o'}$), 4.03 (s, 3H, OCH_3), 3.87 (s, 6H, *m'*-OCH_3), 3.72 (s, 3H, *p'*-OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.9 (C), 159.6 (C), 153.1 (C), 144.6 (CH), 139.9 (C), 134.8 (CH), 131.8 (C), 130.2 (C), 129.5 (C), 128.2 (CH), 120.8 (CH), 112.5 (CH), 106.7 (CH), 60.2 (CH_3), 56.8 (CH_3), 56.2 (CH_3). MS (EI, *m/z* (%)):

407 (M^+, 100), 376 (48), 364 (11), 214 (14). Anal. calcd. for $C_{19}H_{21}NO_7S$: C, 56.01; H, 5.20; N, 3.44; S, 7.87. Found: C, 56.00; H, 5.23; N, 3.51; S, 7.79.

***(E)-5-(3-(Benzo[d][1,3]dioxol-5-yl)acryloyl)-2-methoxybenzenesulfonamide* (8f)**

Yellow solid. 84 % yield; m.p. 272-275 °C. FTIR (ATR) ν(cm^{-1}): 3289 and 3182 (N-H), 3113 (C-H), 1641 (C=O), 1595 (C=C), 1250 (C-O-C), 1157 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.48 (d, J = 8.7 Hz, 1H, H_p), 8.44 (s, 1H, H_o), 7.81 (d, J = 15.4 Hz, 1H, H_α), 7.69 (d, J = 15.4 Hz, 1H, H_β), 7.66 (s, 1H, $H_{o''}$), 7.36-7.32 (m, 2H, $H_{o'}$ and $H_{m'}$), 7.26 (s, 2H, NH_2), 6.99 (d, J = 8.7 Hz, 1H, H_m), 6.11 (s, 2H, OCH_2O), 4.02 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.7 (C), 159.5 (C), 149.6 (C), 148.1 (C), 144.1 (CH), 134.6 (CH), 131.7 (C), 129.6 (C), 129.2 (C), 128.1 (CH), 126.0 (CH), 119.4 (CH), 112.5 (CH), 108.6 (CH), 107.0 (CH), 101.7 (CH_2), 56.7 (CH_3). MS (EI, *m/z* (%)): 361 (M^+, 76), 236 (38), 83 (60), 57 (100), 43 (76). Anal. calcd. for $C_{17}H_{15}NO_6S$: C, 56.50; H, 4.18; N, 3.88; S, 8.87. Found: C, 56.45; H, 4.18; N, 3.91; S, 8.65.

4.2.5 General procedure for the synthesis of chalcone-hybrids 9a-f

To a mixture of sulfonamide **6** (0.20 mmol) and the corresponding aldehyde **7a-f** (0.24 mmol) in ethanol (1.5 mL), it was added 2 drops of 30% (w/v) NaOH solution. The reaction mixture was stirred at room temperature until the reaction was finished by TLC. Then, 10% (w/v) HCl was added to reach neutral pH (7.0) and the precipitate was filtered and washed with water and ethanol. The purification of compounds **9a**, **9b**, **9e** and **9f** was carried out by recrystallization from ethanol, while compounds **9c** and **9d** were purified by CC using silica gel as the stationary phase and a mixture AcOEt: EtOH (15:1) as mobile phase**.**

N-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-5-cinnamoyl-2-methoxybenzenesulfonamide **(9a)**

Beige solid; 61 % yield; m.p. 239-240 °C. FTIR (ATR) ν(cm^{-1}): 3474, 3362 and 3298 (N-H), 3057 (C-H), 1661 and 1645 (C=O), 1616 (C=C), 1148 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.73 (s, 1H, NH-CO), 8.49 – 8.39 (m, 2H, H_p and NH-C_5), 8.34 (d, J = 2.3 Hz, 1H, H_o), 7.97 – 7.83 (m, 3H, H_α and $H_{o'}$), 7.72 (d, J = 15.6 Hz, 1H, H_β), 7.51 – 7.42 (m, 3H, $H_{m'}$ and $H_{p'}$), 7.30 (d, J = 8.8 Hz, 1H, H_m), 6.41 (s, 2H, NH_2-C_6), 4.03 (s, 3H, OCH_3), 2.41 (s, 3H, SCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.9 (C), 161.4 (C), 161.1 (C), 144.0 (CH), 135.1 (CH), 134.7 (C), 130.6 (CH), 129.8 (C), 129.5 (CH), 128.9 (CH, CH), 128.7 (C), 127.7 (C), 123.8 (C), 121.6 (CH), 112.8 (CH), 92.6 (C), 56.9 (CH_3), 12.8 (CH_3). MS (EI, *m/z* (%)): 472 (M^+, 0.6), 238 (100), 135 (79), 86 (56), 77 (70). Anal. calcd. for $C_{21}H_{20}N_4O_5S_2$: C, 53.38; H, 4.27; N, 11.86; S, 13.57. Found: C, 53.40; H, 4.27; N, 11.89; S, 13.36.

(E)-N-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-5-(3-(4-chlorophenyl)acryloyl)-2-methoxybenzenesulfonamide **(9b)**

Pale yellow solid; 56 % yield; m.p. 242-244 °C. FTIR (ATR) ν(cm^{-1}): 3478 and 3364 (N-H), 3053 (C-H), 1643 (C=O), 1595 (C=C), 1155 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.73 (s, 1H, NH-CO), 8.43 (dd, J = 8.6, 2.2 Hz, 1H, H_p), 8.35 (d, J = 2.2 Hz, 1H, H_o), 7.97-7.92 (m, 3H, H_α and $H_{m'}$), 7.71 (d, J = 15.6 Hz, 1H, H_β), 7.52 (d, J = 8.5 Hz, 2H, $H_{o'}$), 7.30 (d, J = 8.6 Hz, 1H, H_m), 6.42 (s, 2H, NH_2-C_6), 4.03 (s, 3H, OCH_3), 2.41 (s, 3H, SCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.7 (C), 161.5 (C), 159.3 (C), 158.2 (C), 155.5 (C), 142.4 (CH), 135.1 (CH), 135.0 (C), 133.6 (C), 130.6 (CH), 129.8 (C), 129.5 (CH), 128.9 (CH), 128.6 (C), 122.3 (CH), 112.7 (CH), 92.5 (C), 56.9 (CH_3), 12.7 (CH_3). MS (EI, *m/z* (%)): 507 (M^+, 2), 236 (28), 83 (68), 57 (100), 43 (89). Anal. calcd. for $C_{21}H_{19}ClN_4O_5S_2$: C, 49.75; H, 3.78; N, 11.05; S, 12.65. Found: C, 49.82; H, 3.70; N, 10.99; S, 12.43.

***(E)-N-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-2-methoxy-5-(3-(p-tolyl)acryloyl)benzenesulfonamide* (9c)**

White solid; 62 % yield; m.p. 237-239 °C. FTIR (ATR) ν(cm^{-1}): 3478 and 3362 (N-H), 2851 (C-H), 1649 (C=O), 1597 (C=C), 1153 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.74 (s, 1H, NH-CO), 8.45 – 8.37 (m, 2H, H_p and NH-C_5), 8.32 (d, *J* = 2.2 Hz, 1H, H_o), 7.85 (d, *J* = 15.5 Hz, 1H, H_α), 7.77 (d, *J* = 8.1 Hz, 2H, $H_{o'}$), 7.69 (d, *J* = 15.5 Hz, 1H, H_β), 7.30-7.26 (m, 3H, H_m and $H_{m'}$), 6.40 (s, 2H, NH_2-C_6), 4.02 (s, 3H, OCH_3), 2.41 (s, 3H, SCH_3), 2.35 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 187.0 (C), 161.4 (C), 161.2 (C), 144.2 (CH), 140.9 (C), 136.0 (C), 135.2 (CH), 132.0 (C), 129.8 (C), 129.7 (CH), 129.5 (CH), 129.3 (CH), 129.0 (C), 128.9 (C), 120.6 (CH), 112.8 (CH), 92.7 (C), 56.9 (CH_3), 21.2 (CH_3), 12.8 (CH_3). MS (EI, *m/z* (%)): 486 (M^+, 0.5), 252 (85), 237 (100), 135 (72), 43 (59). Anal. calcd. for $C_{22}H_{22}N_4O_5S_2$: C, 54.31; H, 4.56; N, 11.52; S, 13.18. Found: C, 54.29; H, 4.55; N, 11.61; S, 12.98.

***(E)-N-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-2-methoxy-5-(3-(4-methoxyphenyl)acryloyl)benzenesulfonamide* (9d)**

Pale yellow solid; 60 % yield; m.p. 230-232 °C. FTIR (ATR) ν(cm^{-1}): 3462 and 3335 (N-H), 3055 (C-H), 1645 (C=O), 1591 (C=C), 1153 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.73 (s, 1H, NH-CO), 8.47 – 8.37 (m, 2H, H_p and NH-C_5), 8.32 (d, *J* = 2.0 Hz, 1H, H_o), 7.84 (d, *J* = 8.6 Hz, 2H, $H_{o'}$), 7.77 (d, *J* = 15.5 Hz, 1H, H_α), 7.69 (d, *J* = 15.5 Hz, 1H, H_β), 7.29 (d, *J* = 8.8 Hz, 1H, H_m), 7.01 (d, *J* = 8.6 Hz, 2H, $H_{m'}$), 6.41 (s, 2H, NH_2-C_6), 4.02 (s, 3H, OCH_3), 3.82 (s, 3H, *p'*-OCH_3), 2.41 (s, 3H, SCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 187.5 (C), 161.9 (C), 161.6 (C), 161.5 (C), 160.3 (C), 144.6 (CH), 136.4 (C), 135.5 (CH), 131.2 (CH), 129.8 (CH), 129.3 (C), 127.6 (C), 119.3 (CH), 114.9 (CH), 113.2 (CH), 93.0 (C), 63.6 (C), 57.2 (CH_3), 55.8 (CH_3), 13.2 (CH_3). MS (EI, *m/z* (%)): 268 (80), 135 (50), 71 (63), 57 (99), 43 (100). Anal. calcd. for $C_{22}H_{22}N_4O_6S_2$: C, 52.58; H, 4.41; N, 11.15; S, 12.76. Found: C, 52.65; H, 4.41; N, 11.04; S, 12.90.

(E)-N-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-2-methoxy-5-(3-(3,4,5-trimethoxyphenyl))acryloyl)benzenesulfonamide **(9e)**

Yellow solid; 71 % yield; m.p. 241-243 °C. FTIR (ATR) ν(cm^{-1}): 3472, 3362 and 3310 (N-H), 2947 (C-H), 1653 (C=O), 1603 (C=C), 1273 (C-O-C), 1128 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.73 (s, 1H, NH-CO), 8.40 (d, *J* = 31.3 Hz, 3H, H_p, H_o and NH-C_5), 7.89 (d, *J* = 15.2 Hz, 1H, H_α), 7.69 (d, *J* = 15.2 Hz, 1H, H_β), 7.30 (d, *J* = 7.9 Hz, 1H, H_m), 7.23 (s, 2H, $H_{o'}$), 6.41 (s, 2H, NH_2-C_6), 4.03 (s, 3H, OCH_3), 3.86 (s, 6H, *m'*-OCH_3), 3.72 (s, 3H, *p'*-OCH_3), 2.42 (s, 3H, SCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 187.3 (C), 161.6 (C), 161.4 (C), 153.4 (C), 144.8 (CH), 140.1 (C), 136.3 (C), 135.6 (CH), 130.5 (C), 130.0 (C), 129.7 (CH), 129.1 (C), 127.4 (C), 121.1 (CH), 113.0 (CH), 106.8 (CH), 92.9 (C), 60.5 (CH_3), 57.2 (CH_3), 56.5 (CH_3), 13.1 (CH_3). MS (EI, *m/z* (%)): 563 (M^+, 1), 368 (10), 83 (75), 57 (100), 43 (82). Anal. calcd. for $C_{24}H_{26}N_4O_8S_2$: C, 51.24; H, 4.66; N, 9.96; S, 11.40. Found: C, 51.11; H, 4.72; N, 9.98; S, 11.53.

(E)-N-(4-Amino-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-5-yl)-5-(3-(benzo[d][1,3]dioxol-5)-yl)acryloyl)-2-methoxybenzenesulfonamide **(9f)**

Yellow solid; 65 % yield; m.p. 198-200 °C. FTIR (ATR) ν(cm^{-1}): 3607, 3520, 3456 and 3308 (N-H), 3152 (C-H), 1665 and 1635 (C=O), 1595 (C=C), 1227 (C-O-C), 1157 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.73 (s, 1H, NH-CO), 8.43 (dd, *J* = 8.8, 2.3 Hz, 1H, H_p), 8.40 (s, 1H, NH-C_5), 8.32 (d, *J* = 2.3 Hz, 1H, H_o), 7.80 (d, *J* = 15.5 Hz, 1H, H_α), 7.68-7.64 (m, 2H, H_β and $H_{o''}$), 7.33 (dd, *J* = 8.0, 1.5 Hz, 1H, $H_{o'}$), 7.28 (d, *J* = 8.8 Hz, 1H, H_m), 6.99 (d, *J* = 8.0 Hz, 1H, $H_{m'}$), 6.40 (s, 2H, NH_2-C_6), 6.10 (s, 2H, OCH_2O), 4.02 (s, 3H, OCH_3), 2.41 (s, 3H, SCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 186.6 (C), 161.6 (C), 161.3 (C), 159.8 (C), 149.6 (C), 149.0 (C), 148.4 (C), 148.1 (C), 144.0 (CH), 135.9 (CH), 129.4 (CH), 129.2 (C), 128.9 (C), 126.0 (CH), 119.5 (CH), 112.6 (CH), 108.5 (CH), 107.1 (CH), 101.6 (CH_2), 92.6 (C), 56.8 (CH_3), 12.7 (CH_3). MS (EI, *m/z* (%)):

516 (M^+, 0.1), 368 (15), 236 (26), 97 (70), 57 (100). Anal. calcd. for $C_{22}H_{20}N_4O_7S_2$: C, 51.16; H, 3.90; N, 10.85; S, 12.41. Found: C, 51.23; H, 3.98; N, 10.81; S, 12.36.

4.3 Anticancer activity

All compounds were screened by the US National Cancer Institute (NCI) initially at a single high dose (10^{-5} M) in the full NCI 60 cell panel representing *Leukemia, Melanoma* and *Lung, Colon, CNS, Ovary, Kidney, Prostate* and *Breast cancers*. Only compounds which satisfied pre-determined threshold inhibition criteria in a minimum number of cell lines went through to the full five-dose assay with evaluation at 100, 10, 1.0, 0.1 and 0.01 μM. The human cancer cell lines were cultivated on RPMI–1640 medium containing 5% fetal bovine serum and 2 mM L–glutamine. In a typical screening experiment, the cells were inoculated into 96–well plates and incubated at 37°C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to the addition of the test compounds. After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of sample addition (Tz). The compounds were dissolved in dimethyl sulfoxide (DMSO) at 400–fold the desired maximum test concentration and diluted to twice the desired maximum test concentration with complete medium containing 50 μg/mL gentamicin. An additional four 10–fold serial dilutions were made to provide a total of five drug concentrations plus the control. Aliquots of 100 μL of these dilutions were added to the 96 well plates already containing 100 μL of medium, resulting in the required final sample concentrations. The plates were further incubated for 48 h at the same conditions. 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 °C. The supernatant was discarded, and plates were washed five times with tap water and air dried.

Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, the 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. Using the absorbance measurements [time zero (Tz), control growth in the absence of drug, and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as: [(Ti – Tz)/(C – Tz)] × 100 for concentrations for which Ti > Tz, and [(Ti – Tz)/Tz] × 100 for concentrations for which Ti < Tz. Two dose–response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from [(Ti – Tz)/(C – Tz)] × 100 = 50, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells and the LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning), indicating a net loss of cells; calculated from [(Ti – Tz)/Tz] × 100 = –50). The values were calculated for each of these two parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested [37].

4.4 Antituberculosis and antibacterial activity

The agar dilution spot culture growth inhibition (SPOTi) assay [38] was performed to evaluate the minimum inhibitory concentration (MIC) values of the synthetic compounds against the laboratory strain *Mycobacterium tuberculosis* H37Rv in the biosafety level 3 laboratory of the National Health

Institute in Bogota. A stock solution of the compounds was prepared in DMSO at a concentration of 50 mg/mL. Dilutions of the compounds were prepared in DMSO at 20, 10, 5 and 2 mg/mL. In 24 well plates, 2 μL of each dilution was dispensed in each well and 2 mL of molten Middlebrook 7H10 medium (HiMedia, Mumbai, India) supplemented with 0.5% glycerol and 10% oleic acid, albumin, dextrose and catalase (OADC, BD, USA) were added to the wells, so that the evaluated concentrations of the compounds were 50, 20, 10, 5 and 2 mg/L. An inoculum having a cell density of 10^6 CFU/mL was prepared from a 4 weeks culture of *Mycobacterium tuberculosis* H37Rv (ATCC 27294) grown in Löwenstein-Jensen slants at 37°C. Two microliters of the diluted inoculum were dispensed in the middle of the agar from each well, and the plates were incubated for 2-3 weeks at 37°C. Isoniazid was included as a positive control at 10, 1, 0.1, 0.05, and 0.01 mg/L concentrations. After the incubation period, the plates were observed and the MIC was determined as the minimum concentration on which growth was not observed. The experiment was repeated on a different day observing exactly the same results. The SPOTi agar dilution method was also employed for MIC determination against *Mycobacterium smegmatis* mc^2155. The cells were passaged first in Middlebrook 7H9 and then spotted into Middlebrook 7H10 medium without OADC. The plates were incubated for 48 h at 37°C. The concentrations tested were 100, 50, 20, 10 and 5 mg/L and kanamycin was used as a positive control. The evaluation of the compounds against a panel of 12 *Staphylococcus aureus* laboratory strains and clinical isolates with a varied pattern of drug-susceptibility was performed on LB broth by two-fold serial dilution with a maximum concentration of the synthetic compounds of 32 mg/L. Growth was determined by optical density measurements at 600 nm every 120 min for 36 h. Vancomycin was used as positive control and the experiments were performed in duplicate.

4.5 Cytotoxicity on mouse fibroblast cells

The 3T3 mouse fibroblast cell line (ATCC® CCL-163™) was cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% bovine fetal serum and 1% streptomycin-penicillin and passaged twice before the assay in 21 cm^2 cell culture Petri dishes at 37°C in 5% CO_2 incubator and 100% humidity. The cells were then cultured in 96 well plates for 24 h before the assay to a cell density of 10^4 cells per well. A 96 well master plate was used for the dilution of the compounds in DMEM medium, by diluting 5 μL of the 100 mg/mL DMSO stock of the compounds into 195 μL of the DMEM medium and performing two-fold serial dilution with 100 μL. One hundred microliters were transferred to each plate containing the 3T3 cells and incubated for 48 h. Cycloheximide was used as a positive control and DMSO as negative control under the same dilution conditions. After 48 h of incubation, 10 μL of a freshly prepared MTT solution in PBS at 5 mg/mL were added to each well, and the plates were further incubated for 1.5 h. The media was then removed and 130 μL of DMSO was added to each well. After 30 min incubation, the absorbance was read at 530 nm on a microplate reader. The experiment was performed in duplicate on different days with different cell cultures and different stock of the compounds. The IC_{50} values were determined by interpolation from the mean absorbance data of 100% viability (negative control) and 0% viability (positive control).

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Highlights

- New chalcone-sulfonamide hybrids were synthesized from 4-methoxyacetophenone.
- The 4-chloro-substituted compounds **8b** and **9b** showed important anticancer activity with GI_{50} 0.57-40.1 μM.
- Hybrids **8b** and **9b** showed significant growth inhibition (MIC ≤ 20 μM) of *M. tuberculosis* H37Rv.