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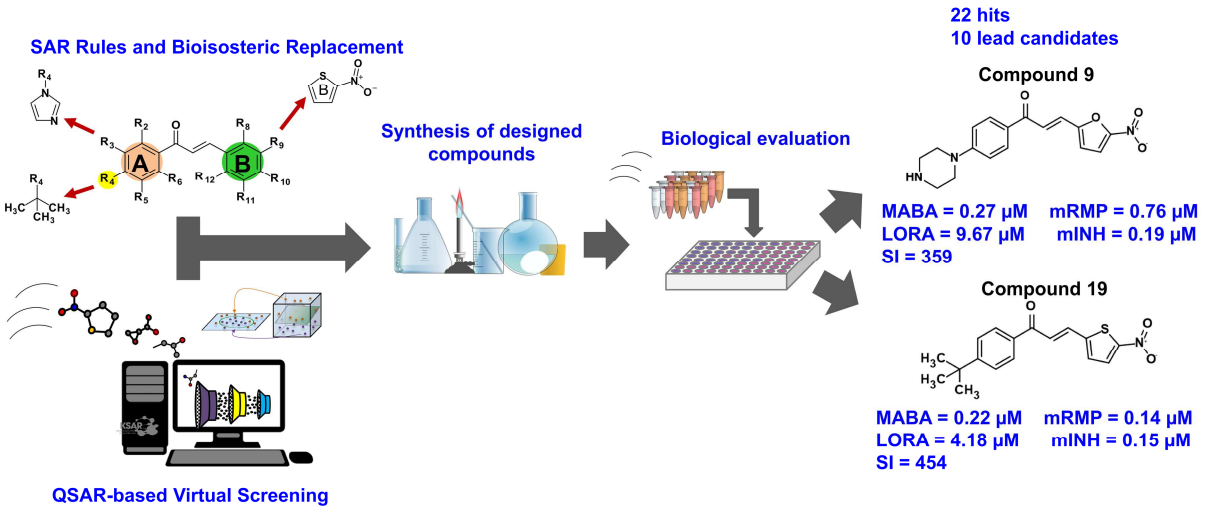

QSAR-driven Design, Synthesis and Discovery of Potent Chalcone Derivatives with Antitubercular Activity

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ABSTRACT

New anti-tuberculosis (anti-TB) drugs are urgently needed to battle drug-resistant *Mycobacterium tuberculosis* strains and to shorten the current 6-12-month treatment regimen. In this work, we have continued the efforts to develop chalcone-based anti-TB compounds by using an *in silico* design and QSAR-driven approach. Initially, we developed SAR rules and binary QSAR models using literature data for targeted design of new heteroaryl chalcone compounds with anti-TB activity. Using these models, we prioritized 33 compounds for synthesis and biological evaluation. As a result, 10 heteroaryl chalcone compounds (**4**, **8**, **9**, **11**, **13**, **17-20**, and **23**) were found to exhibit nanomolar activity against replicating mycobacteria, low micromolar activity against nonreplicating bacteria, and nanomolar and micromolar against rifampin (RMP) and isoniazid (INH) monoresistant strains (rRMP and rINH) (<1 μM and <10 μM, respectively). The series also show low activity against commensal bacteria and generally show good selectivity toward *M. tuberculosis*, with very low cytotoxicity against Vero cells (SI = 11-545). Our results suggest that our designed heteroaryl chalcone compounds, due to their high potency and selectivity, are promising anti-TB agents.

KEYWORDS: Tuberculosis, *in silico* design, QSAR, nitroaromatic compounds, chalcone, anti-TB agents.

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused predominantly by *Mycobacterium tuberculosis* (*M. tb.*). Tuberculosis is reported in every country around the globe and the World Health Organization (WHO) estimates that about a third of the world's population is infected with *M. tb.* [1–3]. According to the WHO, in 2014 there were registered almost 10 million of new TB cases and 1.5 million deaths; 400,000 of which were HIV-positive. As a frequent co-infection, TB is aggravated by the spread of HIV and is a major cause of death among HIV/AIDS patients [3–5].

Drug-sensitive TB can be cured by a combination of isoniazid (INH), rifampin (RMP), pyrazinamide (PZA), and ethambutol (EMB) taken under supervision for 4 months, and 2 months of treatment with only two drugs RMP and INH, consisting the basis of the DOTS program (*D*irectly *O*bserved *T*herapy *S*hort-course). The emergence of multidrug-resistance (MDR-TB) and extensively drug-resistant (XDR-TB) has created substantial new challenges for TB treatment [6,7]. The treatment of resistant strains requires a prolongation of the therapy with drugs that are more toxic, less effective, and more costly [8]. Over the past 16 years, significant investment by academia, funding agencies, and initiatives such as WHO Stop TB Partnership [9] and The Global Alliance for TB Drug Development [10], has led to a renaissance of research in the field of TB and led to the discovery of bedaquiline and delamanid, two new anti-TB drugs approved in 2012 and 2013 respectively for treatment of adults with MDR-TB [11,12].

The development of computer science has found broad application in the drug discovery area [13]. Computer-aided drug design (CADD) has become an integral part of the drug discovery process in both academia and pharma companies [13,14]. Elucidation of quantitative structure-activity relationships (QSAR) is one of the main approaches of CADD

[15–18]. QSAR modeling has been widely used for identification of novel anti-TB agents. In many studies, QSAR was used to design new anti-TB agents [2,19–32]. However, in most the cases, QSAR has been used to modify previously discovered congeneric series of chemicals (Table S1, supplementary material).

Chalcones or 1,3-diaryl-2-propen-1-ones represent one class of natural products and essential intermediates in the biosynthesis of flavonoids. Chalcones are low molecular weight compounds possessing a broad spectrum of biological activities [33–46] including antibacterial [47,48] and anti-TB [38,49,50] activities.

The goal of this work was the design, synthesis and discovery of new chalcone and heteroaryl chalcones with potent anti-TB activity. To achieve this goal, we performed the following steps: (i) collection of available data and rigorous data curation; (ii) generation of structure-activity relationships (SAR) using matched molecular pair analysis (MMPA) to design new chalcones with potential anti-TB activity by bioisosteric replacement; (iii) development of rigorously validated binary QSAR models; (iv) perform virtual screening of designed compounds; (v), organic synthesis and structure identification (NMR, MS, and IR) of selected VS hits; and (vi) *in vitro* experimental evaluation of designed hits under normoxic (MABA) and hypoxic (LORA) conditions.

RESULTS AND DISCUSSION

Design of chalcone and heteroaryl chalcone compounds

For the initial design of new chalcone derivatives with anti-TB activity, we retrieved 604 chalcones compounds with inhibition data against *M. tb.* H37Rv from PubChem Bioassay [51], ChEMBL [52], SciFinder database [53], and from literature. After collecting and integrating all the data, chemical structures and activity values were rigorously curated

following the protocols established by Fourches et al [54–56]. Briefly, structural normalization of specific chemotypes, such as aromatic and nitro groups, was performed using ChemAxon Standardizer (v. 15.10.12.0, ChemAxon, Budapest, Hungary, http://www.chemaxon.com). Inorganic salts, organometallic compounds, and mixtures were also removed. After structural standardization, the duplicates were identified using ISIDA Duplicates[57] and HiT QSAR[58]. Analysis of duplicates also allowed to estimate inter- and intra-lab variability. No suspicious data sources were found.

The curated dataset consisted of 571 chalcones, which were the subject for SAR analysis using matched molecular pairs analysis (MMPA, Figure 1). Matched Molecular Pairs Analysis (MMPA) is a useful approach in drug discovery to identify and compare matched molecular pairs from a set of compounds and determining the property change associated [59]. Matched pairs are molecules that differ only by a particular, well defined, structural transformation and are used to study changes in biological properties [60]. Therefore, the MMPA can reveals changes in biological properties between structures with high similarity. In our work, we used the MACCS keys descriptor [61] and Tanimoto coefficient [62] to evaluate structural similarity related with the changes in biological activity (Figure 1).

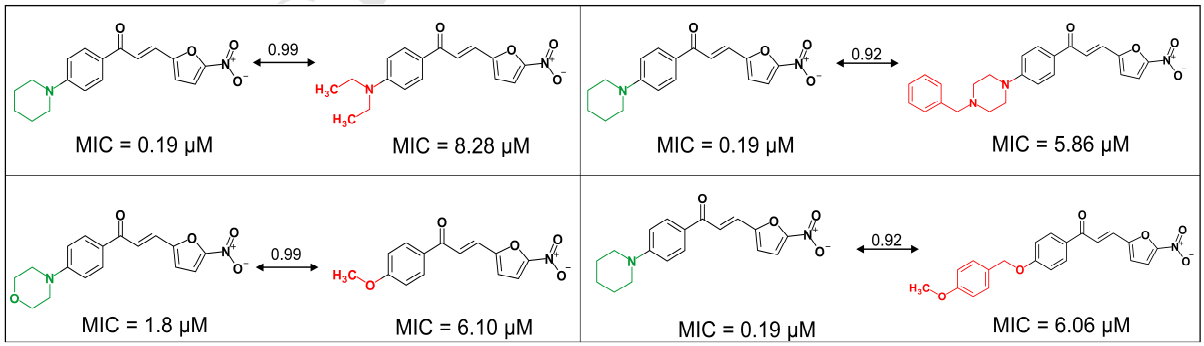


Figure 1. MMPA of selected molecular pairs of chalcones and heteroaryl chalcones with anti-TB activity reported in the literature. The number above the arrows indicate the Tanimoto coefficient between the molecular pair (high structural similarity, Tanimoto

coefficient > 0.90). The groups in green and in red represent the structural differences between the molecules.

The information revealed by the matched molecular pairs allowed us to derive the following SAR rules (Figure 2): (i) hydrophobic and hydrogen bond acceptor groups, e.g., halogens, phenyl, and heterocyclic amines, in *p*-position of ring A are favorable to anti-TB activity; (ii) substitution of benzene ring B by nitrofuran, increases the activity; (iii) any substituent in any position of ring B decreases the activity; and (iv) halogen in *ortho*- or *meta*-position of the ring A decreases the activity. These SAR rules were used to design new compounds using the bioisosteric replacement in the BROOD v.2.0 software [63] and SwissBioisosteres server [64].

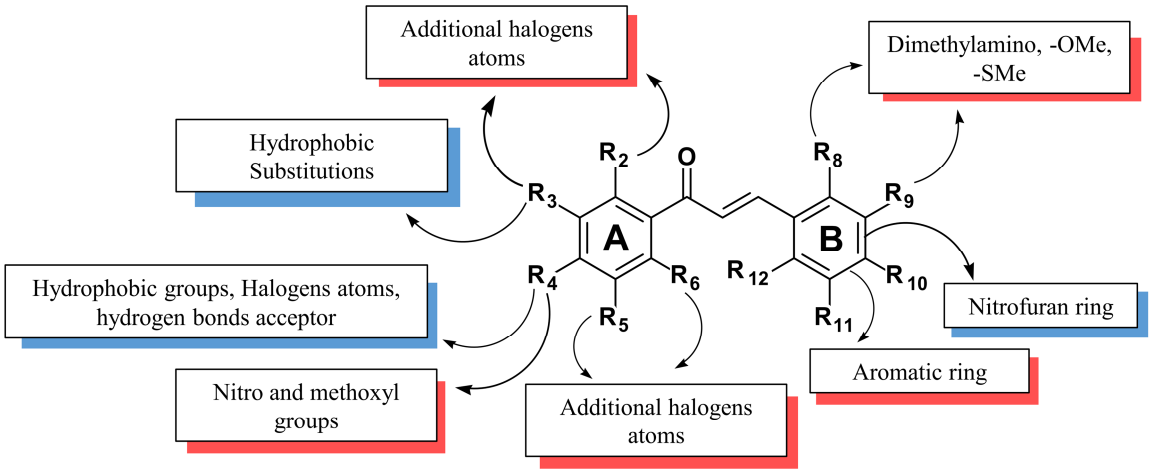


Figure 2. Derived SAR rules for chalcones with anti-TB activity. Modifications in blue shading increase the activity; with red – decrease the activity.

QSAR-DRIVEN design

QSAR modeling. MACCS [65], AtomPairs [66,67], Morgan [67,68], FeatMorgan [69], and Avalon fingerprints [70] combined with support vector machine (SVM) [71], gradient boosting machine (GBM) [72], and random forest (RF) [73] machine learning methods were used for the development of 15 different binary QSAR models. These models

were united in a consensus ensemble model (Table 1). The dataset was balanced prior to the modeling to keep the ratio of active to inactive compounds as 1:1. The results of 5-fold external cross-validation demonstrated high predictive power of the developed consensus model (Table 1). Ten rounds of Y-randomization were performed (CCR≈0.5, see Table S2, supplementary material) and indicated that developed models were not obtained due to chance correlations.

Table 1. Statistical characteristics of developed QSAR models estimated by 5-fold external CV.

Models	CCR	Kappa	Se	Sp	Coverage
MACCS-GBM	0.73	0.46	0.76	0.70	0.71
AtomPairs-GBM	0.71	0.41	0.71	0.70	0.71
Morgan-GBM	0.76	0.51	0.77	0.74	0.68
FeatMorgan-GBM	0.74	0.47	0.76	0.71	0.66
Avalon-GBM	0.74	0.47	0.79	0.68	0.77
MACCS-RF	0.75	0.51	0.79	0.72	0.71
AtomPairs-RF	0.75	0.50	0.73	0.77	0.71
Morgan-RF	0.76	0.52	0.79	0.73	0.68
FeatMorgan-RF	0.75	0.50	0.70	0.80	0.66
Avalon-RF	0.74	0.49	0.76	0.73	0.77
MACCS-SVM	0.77	0.53	0.77	0.76	0.71
AtomPairs-SVM	0.74	0.48	0.74	0.74	0.71
Morgan-SVM	0.76	0.53	0.80	0.73	0.68
FeatMorgan-SVM	0.76	0.51	0.75	0.76	0.66
Avalon-SVM	0.73	0.46	0.72	0.74	0.77
Consensus*	***0.77***	***0.53***	***0.79***	***0.74***	***1.00***

GBM: Gradient Boosting Machine; SVM: Support Vector Machine; RF: Random Forest; CCR: correct classification rate; Kappa: Cohen's kappa coefficient; Se: sensitivity; Sp: specificity. *Consensus model was developed by averaging the predictions of all 15 single models.

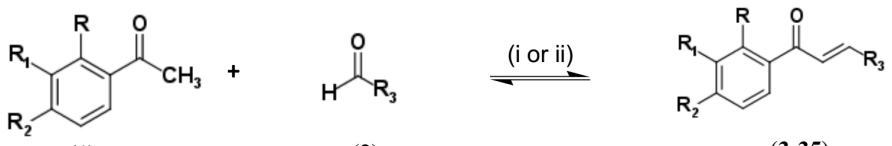
Then, the developed consensus model was used for virtual screening of the chalcones designed by bioisosteric replacement aiming at prioritizing the compounds for chemical synthesis. The chalcones obtained by bioisosteric replacement (Table S3) are drug-like

compounds and satisfy Veber [74] and Lipinski [75] rules. In addition, the designed compounds contained no PAINs substructures [76,77].

Chemistry

Based on the results of the *in silico* design, we synthesized the selected nitrofuran- **3-17**, nitrothiophene- **18-24** and chlorothiophene **25** containing chalcones (Scheme 1). The standard Claisen-Schmidt condensation [78] under basic condition could not be used because the starting materials (aldehydes, nitrofurans, nitrothiophenes, and chlorothiophenes) are alkali-sensitive. Thus, the modified Claisen-Schmidt condensation was performed using acetic acid as solvent and sulfuric acid as catalyst [79,80]. Compounds **26-35** were synthesized following standard Claisen-Schmidt condensation using 20% NaOH as catalyst [78] (see Experimental Section of the Supplementary material for details of spectra and purity data).

Scheme 1. Synthesis of aryl chalcones and heteroaryl chalcone derivatives.



(**1**) (**2**) (**3-35**)

(**3**) R = H, R_1 = H, R_2 = Br, R_3 = 5-nitrofuran
(**4**) R = H, R_1 = H, R_2 = Morpholine, R_3 = 5-nitrofuran
(**5**) R = H, R_1 = H, R_2 = Piperidine, R_3 = 5-nitrofuran
(**6**) R = H, R_1 = H, R_2 = Imidazole, R_3 = 5-nitrofuran
(**7**) R = H, R_1 = H, R_2 = *tert*-butyl, R_3 = 5-nitrofuran
(**8**) R = H, R_1 = H, R_2 = cyclohexyl, R_3 = 5-nitrofuran
(**9**) R = H, R_1 = H, R_2 = piperazine, R_3 = 5-nitrofuran
(**10**) R = H, R_1 = H, R_2 = phenyl, R_3 = 5-nitrofuran
(**11**) R = CH_3, R_1 = H, R_2 = H, R_3 = 5-nitrofuran
(**12**) R = H, R_1 = H, R_2 = *N*-butyl, R_3 = 5-nitrofuran
(**13**) R = H, R_1 = H, R_2 = I, R_3 = 5-nitrofuran
(**14**) R = H, R_1 = CH_3, R_2 = H, R_3 = 5-nitrofuran
(**15**) R = H, R_1 = H, R_2 = pyrrolidine, R_3 = 5-nitrofuran
(**16**) R = H, R_1 = Br, R_2 = H, R_3 = 5-nitrofuran
(**17**) R = H, R_1 = H, R_2 = CH_3, R_3 = 5-nitrofuran
(**18**) R = H, R_1 = H, R_2 = imidazole, R_3 = 5-nitrothiophene
(**19**) R = H, R_1 = H, R_2 = *tert*-butyl, R_3 = 5-nitrothiophene
(**20**) R = H, R_1 = H, R_2 = *N*-butyl, R_3 = 5-nitrothiophene
(**21**) R = H, R_1 = H, R_2 = cyclohexyl, R_3 = 5-nitrothiophene
(**22**) R = H, R_1 = H, R_2 = morpholine, R_3 = 5-nitrothiophene
(**23**) R = H, R_1 = H, R_2 = SCH_3, R_3 = 5-nitrothiophene
(**24**) R = H, R_1 = H, R_2 = CH3, R_3 = 5-nitrothiophene
(**25**) R = H, R_1 = H, R_2 = Imidazole, R_3 = chlorothiophene
(**26**) R = H, R_1 = H, R_2 = piperidine, R_3 = 3-nitrophenyl
(**27**) R = H, R_1 = H, R_2 = phenyl, R_3 = *p*-dimethylaminophenyl
(**28**) R = H, R_1 = H, R_2 = phenyl, R_3 = *p*-methoxyphenyl
(**29**) R = H, R_1 = H, R_2 = CH_3, R_3 = furan
(**30**) R = H, R_1 = H, R_2 = I, R_3 = *p*-methoxyphenyl
(**31**) R = H, R_1 = H, R_2 = piperidine, R_3 = furan
(**32**) R = H, R_1 = H, R_2 = Br, R_3 = *p*-methoxyphenyl
(**33**) R = H, R_1 = H, R_2 = *tert*-butyl, R_3 = pyrrole
(**34**) R = CH_3, R_1 = H, R_2 = piperidine, R_3 = *p*-nitrophenyl
(**35**) R = H, R_1 = H, R_2 = *tert*-butyl, R_3 = furan

Reagents and conditions: (i) H_2SO_4 conc., AcOH, reflux, 100 °C, 4 – 24 h; (**1**) acetophenones, (**2**) nitrofuraldehyde or nitrothiophenecarboxaldehyde, (**3-25**) analogs nitrofurans or nitrotiophenes. (ii) 20% NaOH, EtOH, room temperature, 10 h; (**1**) acetophenones; (**2**) aromatics aldehydes; (**26-35**) phenyl analogs, furan or pyrrole.

Among designed and synthesized compounds, 17 compounds are new and were not published previously (**6-9**, **11**, **14**, **15**, **17-23**, **25**, **31**, and **33**), and thirty compounds (**6-35**) were not tested against tuberculosis before.

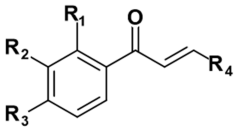
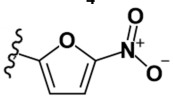
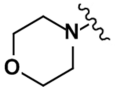
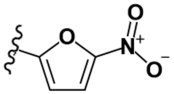
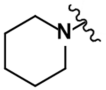
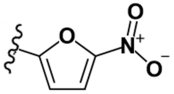
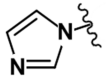
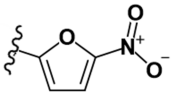
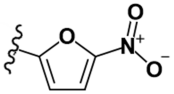
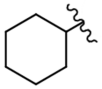
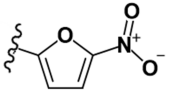
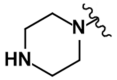
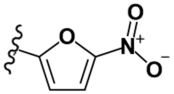
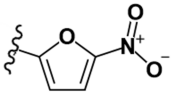
Antituberculosis activity

The compounds were submitted to biological assays against *M. tb.* H37Rv, under both *aerobic* (replicating) and *anaerobic* (non-replicating) conditions using MABA and LORA assays, respectively [81,82]. Minimum inhibitory concentrations (MICs) were defined as the lowest compound concentration effecting ≥90% inhibition of fluorescence or luminescence, respectively. We evaluated 33 chalcones including three known compounds (Table 2) [79]. Twenty-two compounds had low MICs in both the MABA and LORA assays. Compounds containing substituents in the *para*-position of ring A, and containing nitrofurans and nitrothiophenes as ring B (Figure 2) were the most potent. *Ortho*- and *meta*-substituted compounds were somewhat less active. Ten designed compounds **4**, **8**, **9**, **11**, **13**, **17-20**, and **23** had MABA MICs of < 1 µM and LORA MICs of < 10 µM (Table 2). And four this compounds 9, 18, and 19 were more potent than (MIC = 0.27, 0.19, and 0.22 µM respectively) of standard drug INH (MIC = 0.41 µM) used on treatment of TB. Already the compound 23 exhibited MIC similar (0.45 µM) to INH.

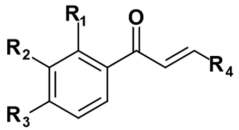
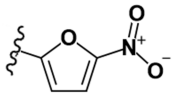
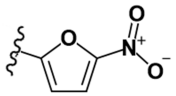
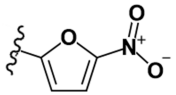
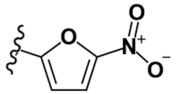
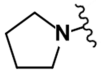
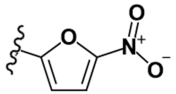
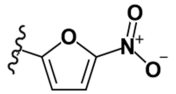
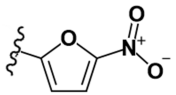
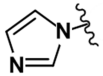
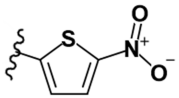
The most potent compound was the nitrothiophene analogue **18** with MABA MIC = 0.19 µM and LORA MIC = 1.73 µM. The substitution of furan ring by thiophene or nitro-substituted thiophene (e.g., **6** and **18)** led to 5.5-*fold* increase of the activity in the MABA (1.05 µM to 0.19 µM) and 4-fold for LORA (6.94 µM to 1.73 µM). The compounds **19** and

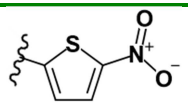
20 were the most active in MABA, however **20** lost activity in the LORA in comparison to its nitrofuran analogue **12**. Nitrothiophenes **21** and **22,** unlike their nitrofuran analogues **8** and **4**, were inactive (MIC>10 μM) in both MABA and LORA assays.

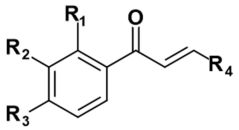
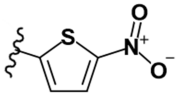
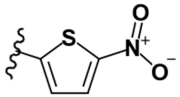
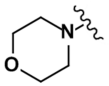
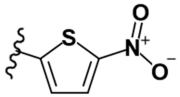
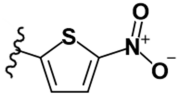
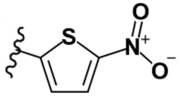
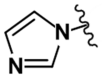
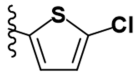
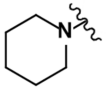
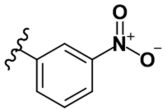
Table 2. *In vitro* antituberculosis activity reported in minimum inhibitory concentration (MIC, µM) (MABA and LORA), MABA MIC of selected compounds against isogenic monodrug-resistant *M. tb*., rRMP and rINH, spectrum of activity and selectivity index of designed chalcones.

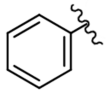
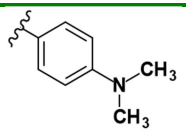
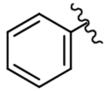
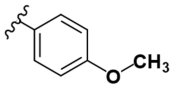
Code	Compounds				Minimum Inhibitory Concentration (µM)								SI
					MABA	LORA	rRMP	rINH	*C. alb.*	*E. coli*	*S. aureus*	*M. smeg.*	
	R_1	R_2	R_3	R_4									
3	H	H	Br		2.50±0.62	6.76±0.08	0.76±0.10	0.58±0.02	4.93±2.10	>10	0.36±0.11	7.12±0.06	ND
4	H	H			0.81±0.02	9.85±2.93	0.55±0.02	0.11±0.07	>10	>10	1.19±0.46	>10	123
5	H	H			3.42±0.43	10.91±3.27	0.07±0.04	<0.03	>10	>10	>10	>10	ND
6	H	H			1.05±0.29	6.94±0.05	1.19±1.02	1.44±1.05	>10	3.18±1.10	0.34±0.10	>10	94
7	H	H	$-C(CH_3)_3$		0.35±0.11	>10	0.29±0.16	0.22±0.14	>10	>10	>10	>10	284
8	H	H			0.81±0.04	3.33±0.72	1.12±0.03	1.23±0.06	>10	>10	>10	>10	122
9	H	H			0.27±0.06	9.67±2.63	0.76±0.08	0.19±0.04	>10	>10	>10	>10	359
10	H	H			3.91±1.95	3.18±0.04	3.45±1.05	0.29±0.08	>10	>10	>10	>10	25

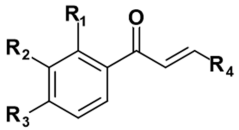
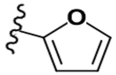
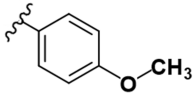
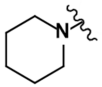
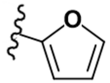
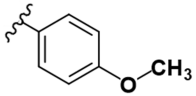
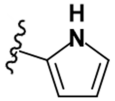
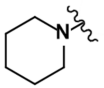
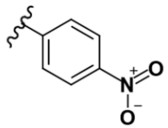
MABA: Microplate Alamar Blue Assay; LORA: Low Oxygen Recovery Assay; rRMP: monoresistant to rifampin; rINH: monoresistant to isoniazid. SI: Selectivity Index (Vero Cell IC_{50}/MABA MIC). *C. alb*: *Candida albicans*; *M. smeg.*: *Mycobacterium smegmatis.*

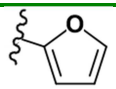
Code	Compounds				Minimum Inhibitory Concentration (µM)								SI
					MABA	LORA	rRMP	rINH	*C. alb.*	*E. coli*	*S. aureus*	*M. smeg.*	
	R_1	R_2	R_3	R_4									
11	$-CH_3$	H	H		0.57±0.01	4.59±0.01	1.08±0.04	1.07±0.03	6.36±0.09	>10	1.09±0.08	9.50±0.01	41
12	H	H	$(CH_2)_3CH_3$		1.31±1.01	4.21±0.22	4.70±0.45	2.23±1.02	>10	>10	2.23±0.00	>10	57
13	H	H	-I		0.60±0.34	4.12±0.14	1.18±0.25	1.08±0.09	>10	>10	>10	4.62	166
14	H	$-CH_3$	H		1.27±0.04	4.75±0.18	3.78±1.31	1.93±0.08	>10	>10	2.23±0.00	>10	43
15	H	H			>10	4.30±0.00	-	-	>10	>10	>10	>10	ND
16	H	-Br	H		1.32±0.45	5.65±2.22	1.88±0.30	1.12±1.98	>10	>10	1.68±0.29	4.84±0.10	52
17	H	H	$-SCH_3$		0.66±0.06	5.07±1.03	0.24±0.07	0.80±0.06	>10	>10	0.58±0.08	4.81±0.01	61
18	H	H			0.19±0.15	1.73±0.21	0.60±0.10	0.30±0.06	>10	>10	0.28±0.30	4.39±0.05	225

19	H	H	$-C(CH_3)_3$		0.22±0.13	4.18±0.55	0.14±0.01	0.15±0.05	>10	>10	>10	>10	454

Code	Compounds				Minimum Inhibitory Concentration (µM)								SI
					**MABA**	**LORA**	**rRMP**	**rINH**	***C. alb.***	***E. coli***	***S. aureus***	***M. smeg.***	
	R_1	**R_2**	**R_3**	**R_4**									
20	H	H	$-(CH_2)_3CH_3$		0.54±0.31	5.56±0.66	0.48±0.00	0.31±0.02	>10	>10	>10	>10	81
21	H	H			>10	5.75±0.01	-	-	>10	>10	>10	>10	ND
22	H	H			>10	>10	-	-	>10	>10	>10	>10	ND
23	H	H	$-SCH_3$		0.45±0.20	5.96±2.47	0.14±0.03	0.22±0.01	>10	>10	>10	>10	222
24	H	H	$-CH_3$		1.87±0.31	2.05±0.37	1.21±0.01	0.96±0.04	>10	>10	>10	>10	ND
25	H	H			9.29±0.45	9.75±0.63	8.89±2.03	9.03±1.80	>10	>10	>10	>10	ND
26	H	H			>10	>10	-	-	>10	>10	>10	>10	ND

27	H	H			>10	>10	-	-	>10	>10	>10	>10	ND
28	H	H			>10	>10	-	-	>10	>10	1.18±0.40	>10	ND

Code	Compounds				Minimum Inhibitory Concentration (µM)								SI
					**MABA**	**LORA**	**rRMP**	**rINH**	***C. alb.***	***E. coli***	***S. aureus***	***M. smeg.***	
	R_1	**R_2**	**R_3**	**R_4**									
29	H	H	$-SCH_3$		>10	>10	-	-	>10	>10	>10	>10	ND
30	H	-I	H		4.90±1.83	>10	8.31±1.01	7.75±0.08	>10	>10	>10	>10	20
31	H	H			>10	>10	-	-	>10	>10	>10	>10	ND
32	H	-Br	H		8.54±2.07	8.20±1.60	9.28±2.01	4.94±1.92	>10	>10	>10	>10	11
33	H	H	$-C(CH_3)_3$		>10	>10	-	-	>10	>10	>10	>10	ND
34	H	H			>10	>10	-	-	>10	>10	>10	>10	ND

35	H	H	$-C(CH_3)_3$		>10	>10	-	-	>10	>10	>10	>10	ND
RMP					0.05	0.19	>1	-	-	-	-	>10	2000
INH					0.41	>256	-	>5	-	-	-	>10	
Amph.B					-	-	-	-	<0.004	-	-	-	
Amp					-	-	-	-	-	1.18	0.25	-	

MABA: Microplate Alamar Blue Assay; LORA: Low Oxygen Recovery Assay; rRMP: monoresistant to rifampin; rINH: monoresistant to isoniazid. SI: Selectivity Index (Vero Cell IC_{50}/MABA MIC). RMP: rifampin, INH: isoniazid, Amph. B: amphotericin B, Amp.: ampicillin

Cytotoxicity assay

To verify the possibility that the anti-TB activity of the designed compounds arises from general toxicity, Vero cells were used to estimate the *in vitro* cytotoxicity of the 18 most potent compounds in MABA and LORA assays. These compounds demonstrated modest to high selectivity on this assay, with selectivity indices (SI) ranging between 11 and 454 (Table 2).

Spectrum of activity

We also investigated selectivity of compounds with respect to activity against *Candida albicans*, *Escherichia coli*, *Staphylococus aureus*, and *M. smegmatis* (Table 2). Most of the tested compounds had MIC >10 µM, except **3**, **4**, **6**, **11-14**, **16-18**, **20**, and **28** that exhibited MICs against *S. aureus* of 0.28-2.23 µM.

Conversely, these compounds demonstrated broad-spectrum activity against non-tuberculosis mycobacterias (NTMs), i.e., *M. abscessus*, *M. chelonae*, *M. marinum*, *M. avium*, *M. kansasii*, and *M. bovis* (Table S3). Compounds **3**, **8-13**, **15-25**, **30**, **and 32** had MICs <10 µM against *M. avium*, *M. kansasii*, and *M. bovis*, and compound **10** demonstrated MICs of 0.14 µM and 0.08 µM against *M. kansasii* and *M. bovis*, respectively.

Evaluation in *M. tb.* resistant strains

We evaluated the subset of most potent compounds (**3-14**, **16-21, 24** and **25**) against rifampin- and isoniazid-resistant strains of *M. tb.* H37Rv (Table 2). All tested compounds were potent against resistant strains (MIC < 10 µM), and compounds **3-5**, **7**, **9**, and **17-21** exhibit MIC < 1 µM against resistant strains. Compound **5** was the most potent compound with MIC of 0.07 µM against rRMP and < 0.03 µM against rINH strains, indicating that this compound is promising for RMP and INH resistant strains.

CONCLUSIONS

The integration of *in silico* design, QSAR-driven virtual screening, synthesis, and experimental evaluation in a single pipeline led to discovery of new and promising anti-TB compounds. After the compilation of the initial dataset and its rigorous curation, the specific SAR rules were developed and used for designing of new chalcones by bioisosteric replacement. For instance, hydrophobic groups and H-bond acceptors are preferred in the *para*-position of ring A combined with nitrofuran or nitrothiophene serving as ring B. Then, the developed consensus QSAR model of antimicrobial activity and applied it for virtual screening and prioritization of designed compounds. Thirty-three chalcone derivatives were synthesized, structures were confirmed by spectroscopic methods and tested against normoxic, replicating (MABA), and hypoxic, non-replicating (LORA) cultures of *M. tb.*

We identified 20 compounds with MIC <10 µM in MABA including 10 compounds (**4**, **8**, **9**, **11**, **13**, **17-20**, and **23**) with MIC < 1 µM in MABA and < 10 µM in LORA. All tested compounds were also active against *M. tb.* resistant strains to isoniazid or rifampicin. The compounds were mostly inactive against all other tested bacteria strains and against mammalian (VERO) cells. Therefore, those compounds are selective for the genus *Mycobacteria* with moderate activity against *S. aureus*. Our compounds satisfy the criteria for developing new anti-TB hits published by Katsuno and coauthors[83] and due to their high potency and activity against resistant strains. Therefore, the designed and synthetized chalcones and heterochalcones could serve as perspective starting points for the development of anti-tubercular agents.

EXPERIMENTAL SECTION

Computational Design

Dataset. We retrieved 604 chalcone and heteroaryl chalcone compounds with experimental data tested against *M. tb.* H37Rv from the PubChem (AID: 1626 and AID: 1949) [51], ChEMBL [52], SciFinder [53], and from the literature. Compounds that had inconclusive IC_{50} values were considered unreliable and were not included in the modeling.

Data curation. The compiled dataset of 604 compounds was carefully curated following the protocols proposed by Fourches et al.[54–56] Briefly, explicit hydrogens were added, whereas specifics chemotypes such as aromatic and nitro groups were normalized using ChemAxon Standardizer (v.15.1.26.0, ChemAxon, Budapest, Hungary, http://www.chemaxon.com). Polymers, inorganic salts, organometallic compounds, mixtures, and duplicates were removed. Modeling-ready curated dataset contained 571 compounds.

SAR analysis. SAR analysis was performed using the MMP (Matched Molecular Pairs) approach [84], Structural similarity was calculated using Tanimoto coefficient obtained on MACCS keys.

SAR analysis and bioisosteric replacement. SAR analysis was performed using the MMP (Matched Molecular Pairs) approach [84]. Structural similarity was calculated using Tanimoto coefficient [85] obtained on MACCS keys. Bioisosteric replacement was performed in the *p*-substituents on the ring A (Figure 1), i.e., piperidine of the most active chalcone (MIC = 0.19 µM), (2E)-3-(5-nitrofuran-2-yl)-1-[4-(piperidin-1-yl)phenyl]prop-2-en-1-one, described in literature [79]. Design of these bioisosters were performed using BROOD v.2.0 software [63] and SwissBioisosteres webserver (http://www.swissbioisostere.ch) [64].

Molecular fingerprints. Five different types of fingerprints were used: molecular access system (MACCS) structural key fingerprints [65], AtomPair [66,67], Morgan, [67,68]

FeatMorgan, [69] and Avalon.[70] All fingerprints were calculated using the open-source cheminformatics toolkit RDKit v.2.4.0 [86].

Dataset analysis and under-sampling. The curated dataset was unbalanced (148 active and 423 inactive compounds), which is not recommended to build binary QSAR models. Therefore, we decided to balance the dataset using linear under-sampling strategy developed by Braga, R.C. (Neves et al, 2016) [87]. Unlike the traditional under-sampling methods which randomly balance the dataset, this strategy retains the most representative inactive compounds in the balanced dataset, thus assuring as high as possible coverage of original chemical space. As a result, balanced dataset containing 148 active and 148 inactive compounds was used for the modeling.

Machine learning techniques. SVM[71], GBM [72], and Random Forest (RF) [73], approaches implemented in R v.3.0.3 [88] were used for the building and optimization of statistically acceptable QSAR models. All machine learning classifiers were implemented using the R v.3.0.3 [88]. More details about these machine modeling techniques are given in Supplementary material.

External validation of developed QSAR models. 5-fold external cross-validation is the standard approach for the estimation of predictive power of QSAR models [89]. In this procedure, the dataset is randomly divided in five subsets of equal size (20% of compounds each). One of these subsets serve as an external validation fold and the other four subsets are used building of the model. The same procedure is repeated five times to place each compound once in the corresponding external fold. Then, the predictivity of the models is estimated based on these external folds. Description of statistical characteristics used for estimation of robustness and external predictivity of developed models is provided in supplementary material.

Consensus modeling. The underlying idea of consensus predictions is that an implicit SAR for a given dataset can be formally manifested by a variety of QSAR models built with different types of molecular descriptors and diverse machine learning approaches. Rigorously built individual models form an ensemble that allows for consensus bioactivity prediction using all models at once. The development of consensus models is generally recommended because usually they result in better predictivity and better coverage of chemical space during virtual screening [90]. To obtain consensus prediction, we have averaged the predictions of all individual models.

Chemical synthesis

All the chemicals and solvents were purchased from Sigma Aldrich®. The progress of all reactions was monitored on Merck KGaA precoated silica gel plates 0.25 mm (with fluorescence indicator UV_{254}) using ethyl acetate/n-hexane as solvent system. Spots were visualized by irradiation with ultraviolet light (254 nm). Melting points (mp) were determined using open capillary method on Melting Point III Marte® apparatus. Proton (^{1}H) and (^{13}C) NMR spectra were recorded on Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C using DMSO-d_6 and $CDCl_3$ as solvents referenced. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H and ^{13}C). Spectra Mass was performed on a LCMS-2020 Liquid Chromatograph Mass Spectrometer Shimadzu, the column was Agilent XDB-C18, 35µM, 21x20 nm. IR spectra were recorded on a PerkinElmer model Spectrum 400 (medium, sweep of 4000 to 400 cm^{-1}). Synthesized compounds were ≥96% pure as determined by high performance liquid chromatography (HPLC) Shimadzu with PDA detector, Nucleodur 100-5 CN-RP column 205x4.6mm, mobile phase water/0.1% TFA and acetonitrile with flow of 1 mL/min.

For the synthesis of **3-25**, substituted acetophenones (0.5 equiv, 0.5 mmol) and nitroaromatics (0.5 equiv., 0.5 mmol) were dissolved in acetic acid (1 mL) and concentrated

sulfuric acid (0.05 mL) and were stirred at 100° C until completion of the reaction (4-24 h). The cooled mixture and the solid was washed with iced methanol (200 mL) for purification. For the synthesis of **26-35**, 0.4 mL of aqueous NaOH (20% w/v) was added to the solution of the acetophenones substituted in 4' position (1 mmol) in EtOH. The resulting mixture was stirred at the room temperature for 10 hours. The formed precipitate was filtered and washed with cold water. If no precipitation occurred, the resulting mixture was neutralized with 5% HCl filtered and dried. The crude was then subjected to chromatography column with EtOAc/Hexane (7:3, v/v) as eluent.

(2E)-1-(4-bromophenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-63) 3. Yellow solid; yield 33% (107 mg, 0.33 mmol); mp 182°C; HPLC purity 98.13%. ^{1}H NMR ($CDCl_3$): δ = 7.95 (d, 2H, J = 8.0 Hz), 7.70 (d, 2H J = 8.0 Hz), 7.72 (d, 1H, J = 15.0 Hz), 7.58 (d, 1H, J = 15.0 Hz), 7.39 (d, 1H, J = 4.0 Hz), 6.87 (d, 1H, J = 4.0 Hz). ^{13}C NMR ($CDCl_3$): δ = 187.1, 152.4, 135.4, 131.8 (2 C), 130.4, 129.7 (2 C), 128.5, 128.1, 123.9, 116.4, 112.7. IR (KBr): ν = 1663 (s; ν(C=O)), 1607 (s; ν(C=C$_{\alpha\beta}$)), 1475, 1301 (s; ν(Ar-NO$_2$).

(2*E*)-1-[4-(morpholin-4-yl)phenyl]-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-64) 4. Red solid, yield 13% (42 mg, 0.12 mmol); mp 86°C; HPLC purity 98.19%. ^{1}H NMR ([D$_6$] DMSO): δ = 8.02 (d, 2H, J = 8.0 Hz), 7.88 (d, 1H, J = 15.0 Hz), 7.80 (d, 1H, J = 3.6 Hz), 7.51 (d, 1H, J = 15.0 Hz), 7.42 (d, 1H, J = 3.6 Hz), 7.04 (d, 2H, J = 9.2 Hz), 3.73 (m, 4H), 3.41 (m, 4H). ^{13}C NMR ([D$_6$] DMSO): δ = 185.2, 154.1, 153.8, 130.8 (2 C), 127.1, 126.6, 125.6, 116.9, 114.9, 113.1 (2 C), 65.8 (2 C), 46.6 (2 C). IR (KBr): ν = 1660 (s; ν(C=O)), 1601 (s; ν(C=C$_{\alpha\beta}$)), 1515, 1355 (s; ν(Ar-NO$_2$)), 1238 (s; ν(C-N)), 1119 (s; ν(C-O)). ESI (+)-MS (MeOH): m/z = 329 [M+H]$^+$

(2*E*)-3-(5-nitrofuran-2-yl)-1-[4-(piperidin-1-yl)phenyl]prop-2-en-1-one (LabMol-65) 5. Red solid, yield 17% (56 mg, 0.17 mmol); mp 220°C; HPLC purity 98.41%. ^{1}H NMR ([D$_6$]

DMSO): δ = 7.97 (d, 2H, J = 9.2 Hz), 7.86 (d, 1H, J = 15.0 Hz), 7.80 (d, 1H, J = 4.0 Hz), 7.44 (d, 1H, J = 15.0 Hz), 7.40 (d, 1H, J = 4,0 Hz), 6.99 (d, 2H, J = 9.2 Hz), 4.43 (s, 4H), 1.60 (s, 6H). ^{13}C NMR ([D_6] DMSO): δ = 184.9, 154.1, 153.9, 131.0 (2 C), 126.7, 125.8, 125.3, 116.8, 115.0, 112.9 (2 C), 47.6 (2 C), 24.9 (2 C), 24.0. IR (KBr): ν = 1642 (s, ν(C=O)), 1607 (s; ν(C=$C_{\alpha\beta}$)), 1578, 1354 (s, ν(Ar-NO_2)), 1235 (s; ν(C-N)). ESI (+)-MS (MeOH): m/z = 327 $[M+H]^+$

(2*E*)-1-[4-(1*H*-imidazol-1-yl)phenyl]-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-66) 6. Brown solid; yield 12% (36 mg, 0.12 mmol); mp 232°C; HPLC purity 99.08%. ^{1}H NMR ([D_6] DMSO): δ = 9.07 (s, 1H); 8.32 (d, 2H, J = 8.0 Hz), 8.15 (s, 1H), 7.97 (d, 2H, J = 8.0 Hz), 7.94 (d, 1H, J = 16.0 Hz), 7.83 (d, 1H, J = 4.0 Hz), 7.63 (d, 1H, J = 16.0 Hz), 7.52 (s, 1H), 7.48 (d, 1H, J = 4.0 Hz). ^{13}C NMR ([D_6] DMSO): δ = 187.2, 153.2, 152.1, 139.6, 135.9, 135.8, 130.7 (2 C), 129.0, 126.3, 121.0 (2 C), 119.2, 118.0, 114.9. IR (KBr): ν = 1662 (s; ν(C=O)), 1609 (s; ν(C=$C_{\alpha\beta}$)), 1566, 1352 (s, ν(Ar-NO_2)). ESI (+)-MS (MeOH): m/z = 310 $[M+H]^+$

(2*E*)-1-(4-tert-butylphenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-68) 7. Yellow solid; yield 22% (67 mg, 0.22 mmol); mp 180°C; HPLC purity 99.89%. ^{1}H NMR ($CDCl_3$): δ = 1.37 (s, 9H), 6.84 (d, 1H, J = 4.0 Hz), 7.39 (d, 1H, J = 4.0 Hz), 7.55 (d, 1H, J = 15.0 Hz), 7.55 (d, 2H, J = 8.4Hz), 7.77 (d, 1H J = 15.0Hz), 8.01 (d, 2H, J = 8.4Hz). ^{13}C NMR ($CDCl_3$): δ = 188.3, 157.2, 152.8, 151.8, 134.2, 128.2 (2 C), 127.3, 125.4 (2 C), 124.8, 115.8, 112.8, 34.8, 30.6 (3 C). IR (KBr): ν = 1651 (s; ν(C=O)), 1596 (s; ν(C=$C_{\alpha\beta}$)), 1527, 1354 (s, ν(Ar-NO_2)), 1391 (m, ν(CH_3)). ESI (+)-MS (MeOH): m/z = 300 $[M+H]^+$

(2*E*)-1-(4-cyclohexylphenyl)-3-(5-nitrofuran-2-yl)prop-en-2-one (LabMol-72) 8. Yellow solid; yield 44% (72 mg, 0.22 mmol); mp 162° C; HPLC purity 98.59%. ^{1}H NMR ($CDCl_3$): δ = 8.00 (d, 2H, J = 8.0 Hz), 7.77 (d, 1H, J = 15.6 Hz), 7.54 (d, 1H, J = 15.6 Hz), 7.38 (d, 1H, J

= 3.6 Hz), 7.37 (d, 2H, *J* = 8.0 Hz), 6.84 (d, 1H, *J* = 3.6 Hz), 2.61 (s, 1H); 2.18 (s, 2H); 1.89 (s, 2H), 1.78 (s, 1H), 1.39 (m, 6H). ^{13}C NMR ($CDCl_3$): 187.7, 154.1 (2 C), 152.9, 134.6, 128.5 (2 C), 127.3, 127.0 (2 C), 124.8, 115.8, 112.8, 44.3, 33.6 (2 C), 26.3 (2 C), 25.6. IR (KBr): *v* = 2925 (s; *v*(C-H)) 1651 (s; *v*(C=O)), 1593 (s; *v*(C=$C_{\alpha\beta}$)), 1526, 1353 (s, *v*(Ar-NO_2)), 1481 (m, *v*(CH_2)). ESI (+)-MS (MeOH): m/z = 326 $[M+H]^+$.

(2*E*)-3-(5-nitrofuran-2-yl)-1-[4-(piperazin-1-yl)phenyl]prop-2-en-1-one (LabMol-73) 9. Red solid; yield 42% (59 mg, 0.12 mmol); mp 221° C; HPLC purity 98.07%. ^{1}H NMR ($CDCl_3$): δ = 8.00 (d, 2H, *J* = 8.8 Hz), 7.79 (d, 1H, *J* = 15.2 Hz), 7.51 (d, 1H, *J* = 15.2 Hz), 7.38 (d, 1H, *J* = 4.0 Hz), 6.90 (d, 2H, *J* = 8.8 Hz), 6.78 (d, 1H *J* = 4.0 Hz), 3.44 (s, 4H), 1.69 (s, 5H). ^{13}C NMR ($CDCl_3$): δ 185.2, 154.2 (2 C), 153.5, 130.8 (2 C), 125.9, 125.7, 125.3, 115.1, 112.9, 112.7 (2 C), 47.9 (2 C), 24.9 (2 C), 23.9. IR (KBr) *v* = 1618 (s; *v*(C=O)), 1609 (s; *v*(C=$C_{\alpha\beta}$)), 1580, 1354 (s, *v*(Ar-NO_2)), 1513 (m, *v*(N-H)), HR-MS (m/z) (ESI): calcd for C17H18N3O4 $[M + H^+]$: 328.1291; found: 328.1289.

(2*E*)-3-(5-nitrofuran-2-yl)-1-(4-phenylphenyl)prop-2-en-1-one (LabMol-74) 10. Yellow solid; yield 62% (100 mg, 0.31 mmol); mp 200°C; HPLC purity 99.29%. ^{1}H NMR ($CDCl_3$): *δ* = 8.16 (d, 2H, *J* = 8.4 Hz), 7.83 (d, 1H, *J* = 15.5 Hz), 7.77 (d, 2H, *J* = 8.4 Hz), 7.67 (d, 2H, *J* = 7.2 Hz), 7.59 (d, *J* = 15.5 Hz, C*H* α, 1H), 7.50 (s, 2H); 7.44 (d, 1H *J* = 7.2 Hz), 7.40 (s, 1H), 6.86 (s, 1H). ^{13}C NMR ($CDCl_3$): *δ* = 187.6, 152.7 (2 C), 145.9, 139.2, 135.4, 128.0 (2 C), 128.6 (2 C), 128.0, 127.6, 127.1 (2 C), 126.9 (2 C), 124.6, 116.1, 112.8. IR (KBr): *v* = 1660 (s; *v*(C=O)), 1598 (s; *v*(C=$C_{\alpha\beta}$)), 1597, 1352 (s, *v*(Ar-NO_2)), 1513, 1474 (s, *v*(ArC=C)). ESI (+)-MS (MeOH): m/z = 320 $[M+H]^+$.

(2*E*)-1-(2-methylphenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-ona (LabMol-75) 11. Yellow solid; yield 9% (12 mg, 0.04 mmol); mp 114° C; HPLC purity 99.20%. ^{1}H NMR ($CDCl_3$): *δ* = 7.61 (s, 1H), 7.44 (s, 1H), 7.41 (s, 1H), 7.35 (s, 1H), 7.37 (d, 1H, *J* = 3.6 Hz), 7.31 (s, 2H),

6.83 (d, 1H, J = 3.6 Hz), 2.50 (s, 3H). ^{13}C NMR ($CDCl_3$): δ = 193.0, 152.5, 137.6, 137.3, 131.4, 131.1, 128.7, 128.2, 127.9, 125.3, 115.7, 112.7 (2 C), 20.2. IR (KBr): ν = 1663 (s; ν(C=O)), 1608 (s; ν(C=C$_{\alpha\beta}$)), 1483, 1348 (s, ν(Ar-NO_2)), 1476 (s, ν(CH_3)). ESI (+)-MS (MeOH): m/z = 258 $[M+H]^+$, HR-MS (m/z) (ESI): calcd for $C_{14}H_{12}NO_4$ $[M + H^+]$: 258.0760; found: 258.0770.

(2*E*)-1-(4-butylphenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-77) 12. Yellow solid, yield 10% (16 mg, 0.05 mmol); mp 100°C; HPLC purity 97.93%. ^{1}H NMR (MHz $CDCl_3$): δ = 8.00 (d, 2H, J = 8.4 Hz), 7.78 (d, 1H, J = 15.6 Hz), 7.55 (d, 1H, J = 15.6 Hz), 7.39 (d, 1H, J = 4.0 Hz), 7.35 (d, 2H, J = 8.4 Hz), 6.84 (d, 1H, J = 4.0 Hz), 2.71 (t, 2H, J = 8.0 Hz), 1.65 (q, 2H, J = 8.0 Hz), 1.39 (s, 2H J = 8.0 Hz), 0.95 (t, 3H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ = 188.5, 161.5, 145.1, 140.8, 139.9, 136.9, 130.0 (2 C), 129.8, 127.1, 126.9, 118.6, 114.1 (2 C), 94.0, 55.0. IR (KBr): ν = 2934 (s; ν(C-H), 1652 (s; ν(C=O)), 1607 (s; ν(C=C$_{\alpha\beta}$)), 1594, 1351 (s; ν(Ar-NO_2)), 1481 (s; ν(CH_3)), 810 (s; ν(CH_2)). ESI (+)-MS (MeOH): m/z = 300 $[M+H]^+$, HR-MS (m/z) (ESI): calcd for $C_{17}H_{18}NO_4$ $[M + H^+]$: 300.1230; found: 300.1235.

(2*E*)-1-(4-iodophenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-78) 13. Brown solid; yield 33% (61 mg, 0.16 mmol); mp 194°C; HPLC purity 99.49%. ^{1}H NMR ($CDCl_3$): δ = 7.91 (d, 2H, J = 8.4 Hz), 7.78 (d, 2H, J = 8.4 Hz), 7.70 (d, 1H, J = 15.6 Hz), 7.57 (d, 1H, J = 15.6 Hz), 7.39 (d, 1H, J = 4.0 Hz), 6.87 (d, 1H, J = 4.0 Hz). ^{13}C NMR ($CDCl_3$): δ = 187.4, 152.4 (2 C), 137.8 (2 C), 136.0, 129.5 (2 C), 128.1, 123.9, 116.4, 112.7, 101.4. IR (KBr): ν = 1658 (s; ν(C=O)), 1606 (s; ν(C=C$_{\alpha\beta}$)), 1579, 1351 (s; ν(Ar-NO_2)).

(2*E*)-1-(3-methylphenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-79) 14. Yellow solid; yield 17% (22 mg, 0.08 mmol); mp 141° C; HPLC purity 97.92%. ^{1}H NMR ($CDCl_3$): δ = 7.86 (s, 2H); 7.76 (d, 1H, J = 15.6 Hz), 7.55 (d, 1H, J = 15.6 Hz), 7.45 (s, 2H), 7.39 (d,

1H, *J* = 4.0 Hz), 6.85 (d, 1H, *J* = 4,0 Hz), 2,48 (s, 3H). ^{13}C NMR ($CDCl_3$): δ = 188.3, 152.8, 138.4, 138.8, 134.0, 128.7, 128.3, 127.5, 125.5, 124.8, 116.0, 112.8 (2 C), 20.2. IR (KBr): v = 3131 (s; v(C-H)) 1661 (s; v(C=O)), 1606 (s; v(C=$C_{\alpha\beta}$)), 1579, 1349 (s; v(Ar-NO_2)). ESI (+)-MS (MeOH): m/z = 258 $[M+H]^+$, HR-MS (m/z) (ESI): calcd for $C_{14}H_{12}NO_4$ [M + H^+]: 258.0760; found: 258.0764.

(2*E*)-3-(5-nitrofuran-2-yl)-1-[4-(pyrrolidin-1-yl)phenyl]prop-2-en-1-one (LabMol-81) 15. Red solid; yield 36% (57 mg, 0.18 mmol); mp 242° C; HPLC purity 96.87%. ^{1}H NMR ($CDCl_3$): δ = 8.03 (d, 2H, *J* = 8.8 Hz), 7.82 (d, 1H, *J* = 15.6 Hz), 7.52 (d, 1H, J = 15.6 Hz), 7.38 (d, 1H, *J* = 4.0 Hz), 6.77 (d, 1H, *J* = 3.6 Hz), 6.59 (d, 2H, *J* = 8.8 Hz), 3.42 (s, 4H); 2.07 (s, 4H). ^{13}C NMR ($CDCl_3$): δ = 185.0, 153.6, 151.1, 130.9 (2 C), 125.6, 125.5, 124.3, 114.9, 113.0, 110.7 (2 C), 42.2 (2 C), 24.0 (2 C). IR (KBr): v = 2854 (s; v(C-H)), 1643 (s; v(C=O)), 1610 (s; v(C=$C_{\alpha\beta}$)), 1578, 1354 (s; v(Ar-NO_2)) 1199 (s; v(C-N)). ESI (+)-MS (MeOH): m/z = 313 $[M+H]^+$

(2*E*)-1-(3-bromophenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-82) 16. Brown solid, yield 32% (62 mg, 0.19 mmol); mp 158°C; HPLC purity 99.98%. ^{1}H NMR ($CDCl_3$): δ = 8.18 (s, 1H); 7.98 (d, 1H, *J* = 7.6 Hz), 7.76 (d, 1H, *J* = 8.0 Hz), 7.69 (d, 1H, *J* = 15.6 Hz), 7.57 (d, 1H, *J* = 15.6 Hz), 7.44 (t, 1H, J_1 = 7.6Hz, J_2 = 8.0 Hz), 7.39 (d, 1H, *J* = 4.0 Hz), 6.88 (d, 1H, *J* = 4.0 Hz). ^{13}C NMR ($CDCl_3$): δ = 186.9, 152.3, 151.9, 138.5, 136.0, 131.2, 130.0, 128.4, 126.7, 123.9, 122.8. 116.5, 112.7. IR (KBr): v = 1664 (s; v(C=O)), 1607 (s; v(C=$C_{\alpha\beta}$)), 1566, 1354 (s; v(Ar-NO_2)). ESI (+)-MS (MeOH): m/z = 321 $[M+H]^+$; HR-MS (m/z) (ESI): calcd for C13H9BrNO4 [M + H^+]: 321.9709; found: 321.9701

(2*E*)-1-[4-(methylsulfanyl)phenyl]-3-(5-nitrofuran-2-yl)prop-2-en-1-one (LabMol-92) 17. Brown solid; yield 24% (35 mg, 0.12 mmol); mp 160° C; HPLC purity 99.15%. ^{1}H NMR ($CDCl_3$): δ = 8.00 (d, 2H, *J* = 8.0 Hz), 7.76 (d, 1H, *J* = 16.0 Hz), 7.56 (d, 1H, *J* = 16.0 Hz),

7.39 (d, 1H, *J* = 4.0 Hz), 7.34 (d, 2H, *J* = 8.0 Hz), 6.84 (d, 1H, *J* = 4.0 Hz), 2.56 (s, 3H). ^{13}C NMR ($CDCl_3$): δ = 186.6, 152.8 (2 C), 146.6, 132.9, 128.7 (2 C), 127.4, 124.7 (2 C), 124.5, 115.9, 112.8, 14.3. IR (KBr): ν = 3117 (m, ν(C-H)), 1658 (s; ν(C=O)), 1607 (s; ν(C=C$_{\alpha\beta}$)), 1589, 1354 (s; ν(Ar-NO_2)), 1394 (s; ν(CH_3)). ESI (+)-MS (MeOH): m/z = 290 $[M+H]^+$, HR-MS (m/z) (ESI): calcd for C14H12NO4 [M + H^+]: 258.0760; found: 258.0770

(2*E*)-1-[4-(1*H*-imidazol-1-yl)phenyl]-3-(5-nitrothiofen-2-yl)prop-2-en-1-one (LabMol-84) 18. Green solid; yield 55% (180 mg, 0.55 mmol); mp 223° C; HPLC purity 98.99%. ^{1}H NMR ([D_6] DMSO): δ = 9.80 (s, 1H), 8.42 (d, 3H, *J* = 8.0 Hz), 8.17 (d, 1H, *J* = 4.0 Hz), 8.05 (d, 1H, *J* = 16.0 Hz), 8.05 (d, 2H, *J* = 8.0 Hz), 7.95 (d, 1H, *J* = 8.0 Hz), 7.94 (d, 1H, *J* = 16.0 Hz), 7.84 (d, 1H, *J* = 4.0 Hz). ^{13}C NMR ([D_6] DMSO): δ = 187.2, 151.9, 146.4, 138.5, 137.1, 135.5, 135.2, 131.5, 130.6 (2 C), 130.6, 125.1, 122.0 (2 C), 121.7, 120.6. IR (KBr): ν = 1663 (s; ν(C=O)), 1605 (s; ν(C=C$_{\alpha\beta}$)), 1595, 1339 (s; ν(Ar-NO_2)), 1284 (m; ν(C-NAr)). ESI (+)-MS (MeOH): m/z = 326 $[M+H]^+$. HR-MS (m/z) (ESI): calcd for $C_{16}H_{12}N_3O_3S$ [M+H^+]: 326.0593; found: 326.0607.

(2*E*)-1-(4-tert-butylphenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (LabMol-86) 19. Green solid; yield 63% (99 mg, 0.31 mmol); mp 192° C; HPLC purity 99.55%. ^{1}H NMR ($CDCl_3$): δ = 7.96 (d, 2H, *J* = 8.4 Hz), 7.88 (d, 1H, *J* = 4.4 Hz), 7.80 (d, 1H, *J* = 15.6 Hz), 7.55 (d, 2H, *J* = 8.4 Hz), 7.52 (d, 1H, *J* = 15.6 Hz), 7.27 (d, 1H, *J* = 4.4 Hz), 1.37 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 187.8, 157.1, 151.6, 146.1, 134.2, 134.09, 129.1, 128.7, 128.1 (2 C), 125.4 (2 C), 124.6, 34.8, 30.6 (3 C). IR (KBr): ν = 2962 (s; ν(C-H)), 1657 (s; ν(C=O)), 1691 (s; ν(C=C$_{\alpha\beta}$)), 1586, 1334 (s; ν(Ar-NO_2)), 1366 (m; ν(CH_3)). ESI (+)-MS (MeOH): m/z = 316 $[M+H]^+$, HR-MS (m/z) (ESI): calcd for $C_{17}H_{18}NO_3S$ [M + H^+]: 316.1001; found: 316.1006.

(2*E*)-1-(4-butylphenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (LabMol-87) 20. Green solid; yield 38% (61 mg, 0.19 mmol); mp 145° C; HPLC purity 99.76%. ^{1}H NMR ($CDCl_3$): δ = 7.95 (d, 2H, J = 8.0 Hz), 7.89 (d, 1H, J = 4.4 Hz), 7.80 (d, 1H, J = 15.6 Hz), 7.51 (d, 1H, J = 15.6 Hz), 7.34 (d, 2H, J = 8.0 Hz), 7.27 (d, 1H, J = 4.0 Hz), 2.71 (t, 2H, J = 8.0 Hz), 1.65 (q, 2H, J = 8.0 Hz), 1.38 (s, 2H, J = 8.0 Hz), 0.95 (t, 3H, J = 8.0 Hz) 3H). ^{13}C NMR ($CDCl_3$): δ = 187.7, 151.5, 149.1, 146.1, 134.5, 134.0, 129.1, 128.7, 128.5 (2 C), 128.3 (2 C), 124.6, 35.3, 32.8, 21.9, 13.4. IR (KBr): ν = 2928 (s; ν(C-H)), 1657 (s; ν(C=O)), 1598 (s; ν(C=C$_{\alpha\beta}$)), 1593, 1330 (s; ν(Ar-NO$_2$)), 1366 (m; ν(CH$_3$)), 816 (m; ν(CH$_2$)). ESI (+)-MS (MeOH): m/z = 316 $[M+H]^+$, HR-MS (m/z) (ESI): calcd for $C_{17}H_{18}NO_3S$ $[M + H^+]$: 316.1001; found: 316.1005

(2*E*)-1-(4-cyclohexylphenyl)-3-(5-nitrothiofen-2-yl)prop-2-en-1-one (LabMol-88) 21. Green solid; yield 64% (110 mg, 0.32 mmol); mp 182° C; HPLC purity 99.79%. ^{1}H NMR ($CDCl_3$): δ = 7.95 (d, 2H, J = 8.0 Hz), 7.88 (d, 1H, J = 4.0 Hz), 7.79 (d, 1H, J = 15.2 Hz), 7.52 (d, 1H, J = 15.2 Hz), 7.37 (d, 2H, J = 8.0 Hz), 7.27 (d, 1H, J = 4.0 Hz), 2.61 (s, 1H), 1.89 (s, 4H), 1.79 (s, 1H), 1.47 (s, 4H), 1.30 (s, 1H). ^{13}C NMR ($CDCl_3$): δ = 187.7, 154.0, 151.5, 146.1, 134.6, 134.0, 129.1, 128.7, 128.4 (2 C), 126.9 (2 C), 124.7, 44.3, 33.6 (2 C), 26.3 (2 C), 25.6. IR (KBr): ν = 2926 (s; ν(C-H)), 1656 (s; ν(C=O)), 1606 (s; ν(C=C$_{\alpha\beta}$)), 1589, 1334 (s; ν(Ar-NO$_2$)), 1427 (m; ν(CH$_2$)).

(2*E*)-1-[4-morpholin-4-yl)phenyl]-3-(nitrothiofen-2-yl)prop-2-en-1-one (LabMol-89) 22. Yellow solid; yield 9% (17 mg, 0.04 mmol); mp 240° C; HPLC purity 98.62%. ^{1}H NMR ($CDCl_3$): δ = 7.99 (d, 2H, J = 9.2 Hz), 7.89 (d, 1H, J = 4.0 Hz), 7.79 (d, 1H, J = 15.2 Hz), 7.53 (d, 1H, J = 15.6 Hz), 7.25 (d, 1H, J = 4.4 Hz), 6.93 (d, 2H, J = 9.2 Hz), 3.88 (t, 4H, J = 4.0 Hz), 3.38 (t, 4H, J = 4.0 Hz). ^{13}C NMR ($CDCl_3$): δ = 185.6, 154.1, 146.6 (2 C), 133.1, 130.4 (2 C), 128.7, 127.3, 124.8, 112.9 (2 C), 66.1 (2 C), 46.8 (2 C). IR (KBr): ν = 1648 (s;

ν(C=O)), 1604 (s; ν(C=C$_{\alpha\beta}$)), 1584, 1335 (s; ν(Ar-NO$_2$)), 1428 (m; ν(CH$_2$)), 1119 (w; ν(C-O)). ESI (+)-MS (MeOH): m/z = 345 $[M+H]^+$.

(2*E*)-1-[4-(methylsulfanyl)phenyl]-3-(5-nitrotiophen-2-yl)prop-2-en-1-one (LabMol-93) 23. Green solid; yield 61% (189 mg, 0.61 mmol); mp 204° C; HPLC purity 99.21%. ^{1}H NMR (CDCl$_3$): δ = 7.95 (d, 2H, *J* = 8.0 Hz), 7.89 (d, 1H, *J* = 4.0 Hz), 7.81 (d, 1H, *J* = 16.0 Hz), 7.50 (d, 1H, *J* = 16.0 Hz), 7.34 (d, 2H, *J* = 8.0 Hz), 7.29 (d, 1H, *J* = 4.0 Hz), 2.57 (s, 3H). ^{13}C NMR (CDCl$_3$): δ = 186.9, 146.6, 146.0, 134.2, 133.0, 129.2, 128.7, 128.5 (2 C), 124.7 (2 C), 124.3, 14.3. IR (KBr): ν = 1654 (s; ν(C=O)), 1604 (s; ν(C=C$_{\alpha\beta}$)), 1589, 1331 (s; ν(Ar-NO$_2$)), 1428 (m; ν(CH$_3$)).

(2E)-1-(4-methylphenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (LabMol-95) 24. Green solid; yield 68% (186 mg, 0.68 mmol); mp 194° C; HPLC purity 99.4%. ^{1}H NMR (CDCl$_3$): 7.94 (d, 2H, *J* = 8.0 Hz), 7.90 (d, 1H, *J* = 4.0 Hz), 7.81 (d, 1H, *J* = 16.0 Hz), 7.53 (d, 1H, *J* = 16.0 Hz), 7.35 (d, 2H, *J* = 8.0 Hz), 7.28 (d, 1H, *J* = 4.0 Hz), 2.47 (s, 3H). ^{13}C NMR (CDCl$_3$): 188.1, 146.4, 144.6, 134.7, 134.5, 129.6 (2C), 129.5, 129.1, 128.7 (2C), 125.0, 21.7. IR (KBr): ν = 3077 (m; ν(CH$_3$)), 1659 (s; ν(C=O)), 1609 (s; ν(C=C$_{\alpha\beta}$)), 1594, 1336 (s; ν(Ar-NO$_2$)). ESI (+)-MS (MeOH): m/z = 274 $[M+H]^+$.

(2*E*)-3-(5-chlorothiophen-2-yl)-1-[4-(1*H*-imidazol-1-yl)phenyl]prop-2-en-1-one (LabMol-94) 25. Green solid; yield 17% (54 mg, 0.17 mmol); mp 178° C; HPLC purity 99.2%. ^{1}H NMR (CDCl$_3$): δ = 8.13 (d, 2H, *J* = 8.0 Hz), 7.98 (s, 1H), 7.84 (d, 1H, *J* = 16.0 Hz), 7.54 (d, 2H, *J* = 8.0 Hz), 7.38 (s, 1H), 7.24 (d, 2H, J = 16.0 Hz), 7.19 (s, 1H), 7.17 (d, 1H, *J* = 4.0 Hz), 6.94 (d, 1H, *J* = 4.0 Hz). ^{13}C NMR (CDCl$_3$): δ = 188.6, 140.5, 138.8, 137.1, 136.5, 135.3, 134.0, 132.1, 131.1, 130.3 (2C), 127.7, 120.8 (2C), 119.9, 117.7. IR (KBr): ν = 1645 (s; ν(C=O)), 1608 (s; ν(C=C$_{\alpha\beta}$)), 810 (s; ν(C-Cl)). ESI (+)-MS (MeOH): m/z = 315 $[M+H]^+$.

(2*E*)-3-(3-nitrophenyl)-1-[4-(piperidin-1-yl)phenyl]prop-2-en-1-one (LabMol-67) 26. Yellow solid, yield 84% (84 mg, 0.25 mmol); mp 181°C; HPLC purity 99.97%. ^{1}H NMR ($CDCl_3$): 8.51 (s, 1H), 8.23 (d, 2H, *J* = 8.0 Hz), 8.01 (d, 2H, *J* = 8.0 Hz), 7.91 (d, 1H, *J* = 8.0 Hz), 7.80 (d, 1H, *J* = 16.0 Hz), 7.69 (d, 1H, *J* = 16.0 Hz), 7.60 (t, 1H, *J* = 8.0 Hz), 6.91 (d, 2H, *J* = 8.0 Hz), 3.43 (s, 4H), 1.69 (s, 6H). ^{13}C NMR ($CDCl_3$): 186.5, 154.1, 148.3, 139.1, 136.8, 133.8, 130.6 (2 C), 129.5, 126.1, 124.4, 123.7, 121.6, 112.6 (2 C), 48.0 (2 C), 25.0 (2 C), 24.2. IR (KBr): *v* = 2933 (m, *v*(C-H)), 1651 (s; *v*(C=O)), 1610 (s; *v*(C=$C_{\alpha\beta}$)), 1588, 1349 (s; *v*(Ar-NO_2)), 1227 (s; *v*(C-N)). ESI (+)-MS (MeOH): m/z = 337 $[M+H]^+$

(2*E*)-3-[4-(dimethylamino)phenyl]-1-(4-phenylphenyl)prop-2-en-1-one (LabMol-69) 27. Yellow solid; yield 53% (143 mg, 0.43 mmol); mp 164°C; HPLC purity 99.36%. ^{1}H NMR ($CDCl_3$): 8.07 (d, 2H, *J* = 4.0 Hz), 7.81 (s, 1H), 7.69 (d, 2H, *J* = 4.0 Hz), 7.63 (d, 2H, *J* = 4.0 Hz), 7.56 (d, 2H, *J* = 8.0 Hz), 7.45 (d, 2H, *J* = 8.0 Hz), 7.38 (d, 2H, *J* = 4.0 Hz), 6.68 (d, 2H, J = 4.0 Hz), 3.02 (s, 6H). ^{13}C NMR ($CDCl_3$): 190.3, 152.3, 146.0, 145.1, 140.4, 138.0, 130.7 (2 C), 129.1 (3 C), 128.3 (2 C), 127.5 (2 C), 127.3 (2 C), 122.9, 112.1 (2 C), 40.3 (2 C). IR (KBr): *v* = 1647 (s; *v*(C=O)), 1603 (s; *v*(C=$C_{\alpha\beta}$)), 1228 (s; *v*(C-N)) ESI (+)-MS (MeOH): m/z = 328 $[M+H]^+$

(2*E*)-3-(4-methoxyphenyl)-1-(4-phenylphenyl)prop-2-en-1-one (LabMol-70) 28. Yellow solid; yield 25% (79 mg, 0.25 mmol); mp 152°C; HPLC purity 100.00%. ^{1}H NMR ($CDCl_3$): 7.81 (d, 1H, *J* = 15.0Hz), 8.09 (d, 2H, *J* = 10 Hz), 7.71 (d, 2H, *J* = 5.0 Hz), 7,64 (d, 2H, *J* = 10 Hz), 7.61 (d, 2H, *J* = 10 Hz); 7,45 (d, 1H, *J* = 15.0 Hz), 6.93 (d, 2H, *J* = 5.0 Hz). 3.84 (s, 3H). ^{13}C NMR ($CDCl_3$): 190.2, 161.9, 145.5, 144.8, 140.2, 137.4, 130.5 (2 C), 129.3 (3 C), 128.4 (2 C), 127.9, 127.5 (2 C), 127.4 (2 C), 120.0, 114.7 (2 C), 55.6. IR (KBr): *v* = 1647 (s; *v*(C=O)), 1597 (s; *v*(C=$C_{\alpha\beta}$)), 1303, 1037 (s; *v*(C-O)). ESI (+)-MS (MeOH): m/z = 315 $[M+H]^+$

(2*E*)-3-(furan-2-yl)-1-[4-(methylsulfanyl)phenyl]prop-2-en-1-one (LabMol-71) 29. Yellow solid; yield 19% (49 mg, 0.20 mmol); mp 114°C; HPLC purity 99.05%. ^{1}H NMR ($CDCl_3$): 7.95 (d, 2H, *J* = 6.8 Hz), 7.58 (d, 1H, *J* = 12.4 Hz), 7.51 (s, 1H), 7.43 (d, 1H, *J* = 12.4 Hz), 7.29 (d, 2H, *J* = 6.8 Hz), 6.70 (d, 1H, *J* = 2.4 Hz), 6.50 (q, 1H, *J* = 2.4 Hz), 2.52 (s, 3H). ^{13}C NMR ($CDCl_3$): 188.6, 151.9, 145.7, 145.0, 134.6, 130.5, 129.0 (2 C), 125.2 (2 C), 119.2, 116.2, 112.8, 14.9. IR (KBr): ν = 1656 (s; ν(C=O)), 1596 (s; ν(C=C$_{\alpha\beta}$)), 1549, 1476 (ArC=C), 1336 (s; ν(CH_3)), 1297, 1094 (s; ν(C-O)). ESI (+)-MS (MeOH): m/z = 245 $[M+H]^+$.

(2*E*)-1-(3-iodophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (LabMol-76) 30. White solid, yield 6% (22 mg, 0.06 mmol); mp 110°C; HPLC purity 99.64%. ^{1}H NMR ($CDCl_3$): δ = 8.32 (s, 1H), 7.96 (d, 1H, *J* = 8.0 Hz), 7.89 (d, 1H, *J* = 8.0 Hz), 7.79 (d, 1H, *J* = 15.6 Hz), 7.61 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 1H, *J* = 15.6 Hz), 7.24 (t, 1H, *J* = 8.0 Hz), 6.95 (d, 2H, *J* = 8.0 Hz), 3.86 (s, 3H). ^{13}C NMR ($CDCl_3$): δ = 188.5, 161.5, 145.1, 140.8, 139.9, 136.9, 130.0 (2 C), 129.8, 127.1, 126.9, 118.6, 114.1 (2 C), 94.0, 55.0. IR (KBr): ν = 1657 (s; ν(C=O)), 1600 (s; ν(C=C$_{\alpha\beta}$)), 1559, 1510 (s, ν((ArC=C)), 1323 (s; ν(CH_3)). ESI (+)-MS (MeOH): m/z = 365 $[M+H]^+$.

(2*E*)-3-(furan-2-yl)-1-[4-(piperidin-1-yl)phenyl]prop-2-en-1-one (LabMol-80) 31. Yellow solid; yield 30% (86 mg, 0.30 mmol); mp 182° C; HPLC purity 99.32%. ^{1}H NMR ($CDCl_3$): δ = 8.00 (d, 2H, *J* = 8.8 Hz), 7.58 (d, 1H, *J* = 15.2 Hz), 7.54 (m, 1H), 7.50 (d, 1H, *J* = 15.2 Hz), 6.90 (d, 2H, *J* = 8,8 Hz), 6.67 (d, 1H, *J* = 3.2 Hz), 6.50 (dd, 1H, *J* = 3.2 Hz), 3.40 (s, 4H), 1.68 (s, 6H). ^{13}C NMR ($CDCl_3$): δ = 186.8, 153.9, 151.7, 143.9, 130.3 (2 C), 128.6, 126.6, 119.2, 114.6, 112.9 (2 C), 112.0, 48.1 (2 C), 24.9 (2 C), 23.9. IR (KBr): ν = 2941 (m; ν(C-H), 1648 (s; ν(C=O)), 1604 (s; ν(C=C$_{\alpha\beta}$)), 1597, 1559 (s, ν(ArC=C)), 1390 (s; ν(C-N)). ESI (+)-MS (MeOH): m/z = 282 $[M+H]^+$.

(2*E*)-1-(3-bromophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (LabMol-83) 32. Brown solid; yield 41% (130 mg, 0.41 mmol); mp 90°C; HPLC purity 99.66%. ^{1}H NMR ($CDCl_3$): δ = 8.13 (s, 1H), 7.93 (d, 1H, *J* = 8.0 Hz), 7.80 (d, 1H, *J* = 15.6 Hz), 7.70 (d, 1H, *J* = 8.0 Hz), 7.62 (d, 2H, *J* = 8.0 Hz), 7.39 (d, 1H, *J* = 8.0 Hz), 7.35 (d, 1H, *J* = 15.6 Hz), 6.95 (d, 2H *J* = 8.0 Hz), 3.87 (s, 3H). ^{13}C NMR ($CDCl_3$): δ = 188.6, 161.5, 145.1, 139.9, 134.9, 131.0, 130.0 (2 C), 129.7, 126.9, 126.5, 122.5, 118.6, 114.1 (2 C), 55.0. IR (KBr): ν = 1662 (s; ν(C=O)), 1594 (s; ν(C=C$_{\alpha\beta}$)), 1570, 1513 (s, ν(ArC=C)), 1260, 1042 (s; ν(C-O)), 556 (m, ν(C-Br)).

(2*E*)-1-(4-tert-butylphenyl)-3-(1H-pyrrol-2-yl)prop-2-en-1-one (LabMol-85) 33. White solid; yield 7% (20 mg, 0.07 mmol); mp 158° C; HPLC purity 99.81%. ^{1}H NMR ($CDCl_3$): δ = 9.07 (s, 1H), 7.95 (d, 2H, *J* = 8.4 Hz), 7.77 (d, 1H, *J* = 15.6 Hz), 7.50 (d, 2H, *J* = 8.4 Hz), 7.19 (d, 1H, *J* = 15.6 Hz), 7.00 (s, 1H), 6.72 (s, 1H), 6.34 (s, 1H), 1.36 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 189.6, 155.7, 135.5, 133.9, 128.9, 127.8 (2 C), 125.1 (2 C), 122.6, 115.4, 114.6, 111.0, 34.6, 30.7 (3 C). ESI (+)-MS (MeOH): m/z = 254 $[M+H]^+$.

(2*E*)-3-(4-nitrophenyl)-1-[4-piperidin-1-yl)phenyl]prop-2-en-1-one (LabMol-90) 34. Yellow solid, yield 89% (300 mg, 0.89 mmol); mp 198°C; HPLC purity 99.50%. ^{1}H NMR ($CDCl_3$): δ = 8.25 (d, 2H, *J* = 8.0 Hz), 7.99 (d, 2H, *J* = 8.0 Hz), 7.78 (d, 1H, *J* = 16.0 Hz), 7.77 (d, 2H, *J* = 8.0 Hz), 7.68 (d, J = 16.0 Hz), 6.90 (d, 2H, *J* = 8.0 Hz), 3.41 (s, 4H), 1.69 (s, 6H). ^{13}C NMR ($CDCl_3$): δ = 186.2, 154.1, 147.8, 141.3, 139.0, 130.6 (2 C), 130.0, 128.2 (2 C), 126.0, 125.6, 123.7 (2 C), 112.7 (2 C), 48.0 (2 C), 24.9 (2 C), 23.9. IR (KBr): ν = 2942 (m, ν(C-H)), 1655 (s; ν(C=O)), 1609 (s; ν(C=C$_{\alpha\beta}$)), 1595, 1514 (s, ν(ArC=C)), 1593, 1336 (s, ν(Ar-NO$_2$)), 1196 (s; ν(C-N)), 556 (m, ν(C-Br)). ESI (+)-MS (MeOH): m/z = 337 $[M+H]^+$.

(2*E*)-1-(4-tert-butylphenyl)-3-(furan-2-yl)prop-2-en-1-one (LabMol-91) 35. Yellow solid; yield 7.8% (20 mg, 257 mmol); mp 84°C; HPLC purity 99.8%. ^{1}H NMR ($CDCl_3$): δ = 7.99 (d, 2H, *J* = 8.4 Hz), 7.60 (d, 1H, *J* = 15.2 Hz), 7.52 (d, 3H, *J* = 8.4 Hz), 7.48 (d, 1H, *J* = 15.2

Hz), 6.72 (s, 1H), 6.52 (s, 1H), 1.37 (s, 9H). ^{13}C NMR ($CDCl_3$): δ = 188.7, 156.1, 151.3, 144.3, 135.1, 129.9, 128.0 (2 C), 125.1 (2 C), 119.0, 115.5, 112.2, 34.7, 30.7 (3 C). IR (KBr): ν = 2962 (m, ν(C-H)), 1655 (s; ν(C=O)), 1605 (s; ν(C=$C_{\alpha\beta}$)), 1285 (s, ν(C-O)). ESI (+)-MS (MeOH): m/z = 255 $[M+H]^+$.

Biological Evaluation

Anti-TB activity. MICs against *M. tb.* H37Rv (ATCC 27294) as well as the rifampin (rRMP, ATCC 35838) and isoniazid (rINH, ATCC 35822) mono-resistant strains under normoxic, replicating conditions were determined using the Microplate Assay Blue Alamar (MABA) as previously described [91–93]. Briefly, cultures were incubated in 200 µL Middlebrook 7H12 medium together with test compound in 96-well plates for 7d and 37° C. Resazurin and Tween 80 were added and incubation continued for 24h at 37° C. Fluorescence was determined at excitation/emission wavelengths of 530/590 nm, respectively. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.[61] MICs against *M. tb.* H37Rv under hypoxic, non-replicating conditions were determined using the Low Oxygen Recovery Assay as previously described [82,93] except that the luxABCDE reporter[95] was used instead of the luxAB reporter gene. The MIC was defined as the lowest concentration of compound which reduced luminescence by 90% after 10 days exposure to compound under hypoxic conditions followed by 28 hours of normoxic recovery and comparison to untreated controls.

Cytotoxicity in mammalian cells. Vero cells (ATCC CRL-1586) were cultured in 10% Fetal Bovine Serum (FBS) in Eagle minimum essential medium plus penicillin and streptomycin. Cells were prepared and washed in HBSS (1x pH = 7.4) and Trypsin-EDTA 0.25%, and then morphology was verified by microscopy. After adjusting the density to 3-5x10^5 cells/mL in MEM media, 100 µL of the cell suspension were incubated with test compounds at 37° C for

72 hours; visual inspection was performed each 24 hours. Then, 20 µL of 0.6 mM resazurin were added into each well and incubated for 4 hours. The fluorescence was determined by excitation/emission wavelengths of 530/590 nm. The concentration of test compound effecting a reduction in fluorescence of 50% relative to untreated cells was calculated as the IC_{50}.

Spectrum of activity. *Mycobacterium abscessus* (ATCC 19977), *M. chelonae* (ATCC 35752), *M. marinum* (ATCC 927), *M. avium* (ATCC 15769), *M. kansasii* (ATCC 12478), and *M. bovis* (ATCC 35734) were cultured in Middlebrook 7H9 Broth with 0.2% (v/v) glycerol, 0.05% Tween 80, and 10% (v/v) albumin-dextrose-catalase (BBL™ OADC Enrichment, Cat. N°. 212352). *M. smegmatis* (ATCC MC2155) was cultured in 7H12 medium. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) were cultured in cation-adjusted Mueller Hinton (CAMH) broth and *Candida albicans* (ATCC 90028) in RPMI media until an absorbance at 570 nm of 0.2-0.5 was achieved. Cultures were diluted 1:5000 to 1:10,000 into fresh media in 96-well plates and incubated at 37° C with test compounds. Incubation times were 3 days for *M. smegmatis*, 3-7 days for other mycobacteria, 36-48 hours for *C. albicans* and 16-20 hours for *S. aureus* and *E. coli*. The MIC for *C. albicans*, *S. aureus* and *E. coli* was defined as the lowest concentration effecting a reduction of ≥90% in A_{570} relative to untreated cultures. The MABA MICs for mycobacteria are defined as described above.

ASSOCIATED CONTENT

Supporting Information. More computational details regarding molecular fingerprints calculation and QSAR model development are available in the Supporting Information, as well as additional tables of results and structural characterization for all synthetized compounds. This material is available free of charge via the Internet at http://ejmedch.

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ABBREVIATIONS

TB, tuberculosis; *M. tb.*, *Mycobacterium tuberculosis*; WHO, World Health Organization; DOTS, Directly Observed Therapy Short-course; RMP, rifampin; INH, isoniazid; PZA, pyrazinamide; EMB, ethambutol; DS-TB, drug sensitive TB; MDR-TB, multidrug-resistance; XDR-TB, extensively drug-resistance; STOP-TB, STOP tuberculosis strategy; CADD, computer assisted drug design; QSAR, Quantitative Structure Activity Relationship; MMPA, Matched Molecular Pairs of Analysis; SAR, Structure Activity Relationship; MACCS, Molecular ACCess System keys; SVM, Support Vector Machine; RF, Random Forest; CCR, correct classification rate; Se, sensitivity; Sp, Specificity; NMR, Nuclear Magnetic Resonance; MS, Mass Spectrometry; HPLC, High-Performance Liquid Chromatography; MABA, microplate alamar blue assay; LORA, low oxygen recovery assay; MIC, minimum inhibitory concentration; SI, selectivity index; NTM, non-tuberculosis mycobacterias; AD, applicability domain; DMSO, dimethylsulfoxide; ATCC, American Type Culture Collection; CAMH, Mueller Hinton Media; RPMI, Roswell Park Memorial Institute; HBSS, Hank's Balanced Salt Solution; rRMP, resistant isogenic strain rifampin; rINH, resistant isogenic isoniazid.

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Highlights

- Predictive QSAR models aided design and synthesis of chalcone derivatives anti-TB
- Ten chalcone-like derivatives exhibited high anti-TB activity and selectivity
- Compounds also showed broad spectrum against others mycobacterium strains
- Compounds' high activity against monoresistant strains implies new mode of action