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Article

Design, Synthesis and Antitubercular Activity of Novel Isoniazid-Cyclic-Amine-Azachalcones Hybrids

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In this work, it is described the design of twenty-four heterocyclic amine-azachalcones compounds through molecular hybridization of chalcone scaffold and fragments of isoniazid, fluoroquinolones, and linezolid with antituberculosis potential. The new compounds were synthesized via Claisen-Schmidt condensation, providing yields of 36-95%. Fifteen compounds showed antituberculosis activity against *Mycobacterium tuberculosis* H37Rv strain. Two amine-azachalcones **15** and **17** showed relevant biological activity with minimum inhibitory concentration (MIC) values of 6.62 and 4.85 μM, respectively. Compound **12** showed the best profile of antitubercular activity with MIC = 9.54 μM and selectivity index (SI) = 9.33. It was found that morpholine group is important to increase potency of antimycobacterial activity but also to add some toxicity to the chalcone molecular framework. The results described herein would be a guide in the designing of novel and optimized antitubercular derivatives based on the chalcone scaffold.

Keywords: isoniazid, heterocyclic amines, molecular hybridization, antitubercular activity, aldol condensation

Introduction

Tuberculosis (TB) disease is classified as one of the top ten leading causes of death in the world, due to its aggressive infection promoted by a unique agent. Moreover, one-fourth of the global population was detected with latent tuberculosis, occurring without any disease symptoms.[1] In 2017, ten million people manifested TB and the situation aggravated even more with 1.3 million deaths.[1]

The first-line treatment for TB involves the use of five different drugs such as isoniazid, pyrazinamide, streptomycin, ethambutol, and rifampicin, which can be used in association.[2] When the first-line drugs cannot be used, it is recommended the second-line treatment with levofloxacin or moxifloxacin, bedaquiline, and linezolid.[3] Failures in chemotherapy are related to several aspects such as high complexity (association of three or more drugs); long period of treatment (6 to 9 months or up to 24 months);[3,4] countless adverse effects (nausea, vomiting, and epigastric pain),[1,5] and bacterial resistance.

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Bacterial resistance is a cause of alarm for the health systems throughout the world.[3,6] Bacterial strains of TB have been classified into two types, TB multidrug-resistant (resistance to the first-line drugs), and TB extremely resistant (resistance to first and second-line drugs).[6] In this context, it is reinforced the relevance to search into new antituberculosis drug candidates.[7]

Natural products remain a fundamental source of inspiration in the discovery and development of new bioactive compounds.[8] Through secondary metabolism, natural products are able to create several types of substances, with multiple biological activities.[9]

Among the secondary metabolites, our research group is especially interested in chalcone scaffold, as a synthetic target. Chalcones are considered a privileged structure and have been widely used as a template in medicinal chemistry studies. Due to its structural diversity, chalcones have shown great potential in the molecular modification process, leading to the synthesis of compounds containing different pharmacophore groups, which can enhance its pharmacological effect.[10]

Recent reports[10] aimed at chalcones as compounds with a wide range of biological activities such as anticancer, cancer-preventative effects, anti-inflammatory, antibacterial, antidiabetic, antioxidant, antimicrobial, antiviral, antimalarial and neuroprotective effects. It is noteworthy that chalcone compounds are emerging as potential candidates for new antitubercular drugs (Figure 1).[10-15]

Structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) information has demonstrated that the nitrofuran scaffold as a substituent in chalcones **1**-**2** improved its antitubercular activities.[16,17] However, many studies showed other structural combinations, aiming for new antitubercular agents, as seen in chalcones **3**-**4**,[18,19] by the use of creative and intuitive molecular hybridization approach (Figure 1).[20]

In order to expand the chemical space of chalcones, this study aimed to synthesize and evaluate the antitubercular activity of twenty-four heterocyclic amine-azachalcone derivatives designed by molecular hybridization of chalcone scaffold **5** with structural fragments of isoniazid **6**, fluoroquinolones **7** and linezolid **8** (Scheme 1). Isoniazid **6** is the best and the oldest drug employed to treat TB, fluoroquinolone **7** and linezolid **8** are modern broad-spectrum antibiotics, which have shown antitubercular activity.[21-23]

Results and Discussion

Chemistry

Firstly, it was necessary to obtain *p*-aminated acetophenones with the appropriate substitution patterns (Table 1). The synthesis of acetophenones **35a**-**35h** occurred via aromatic nucleophilic substitution of 4-fluoro-acetophenone **33** in the presence of cyclic amines **34a**-**34h**, using K_2CO_3 and dimethyl sulfoxide (DMSO) as a solvent. Products were obtained in 71 to 99% yields; the reaction times were observed for 24 or 48 h until the complete consumption of starting materials (Scheme 2).[24]

Amine-azachalcones **9**-**32** were obtained via Claisen-Schmidt condensation of *p*-aminated acetophenones **35a**-**35h** and carbaldehydes **36a**-**36c** using a solution of NaOH in ethanol as solvent, which was added dropwise, providing the compounds **9**-**32** with yields ranging from 36 to 95% (Table 1).[25]

Structural modifications intended to increase the complexity of cyclic amines bonded to the aromatic ring, in order to generate a variation in the molecular volume in R'. Thus, compounds were obtained from simple *N*-heterocyclic amine templates, as pyrrolidine, to more complex molecules like 1-(pyrimidin-2-yl)piperazine.

When positional isomers were compared, pyridine compounds substituted with nitrogen at position 4 showed

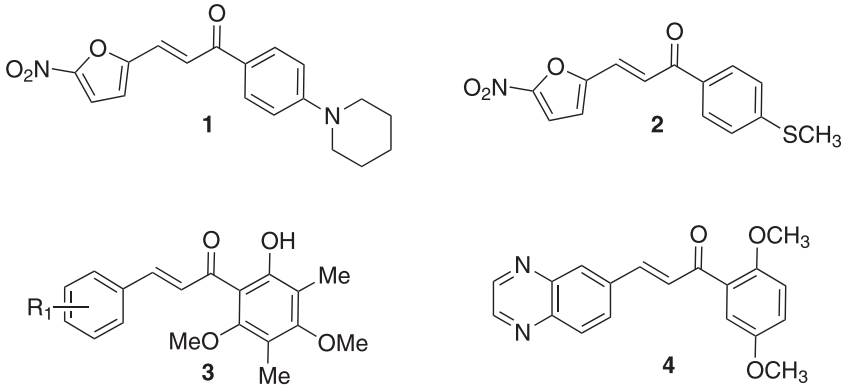
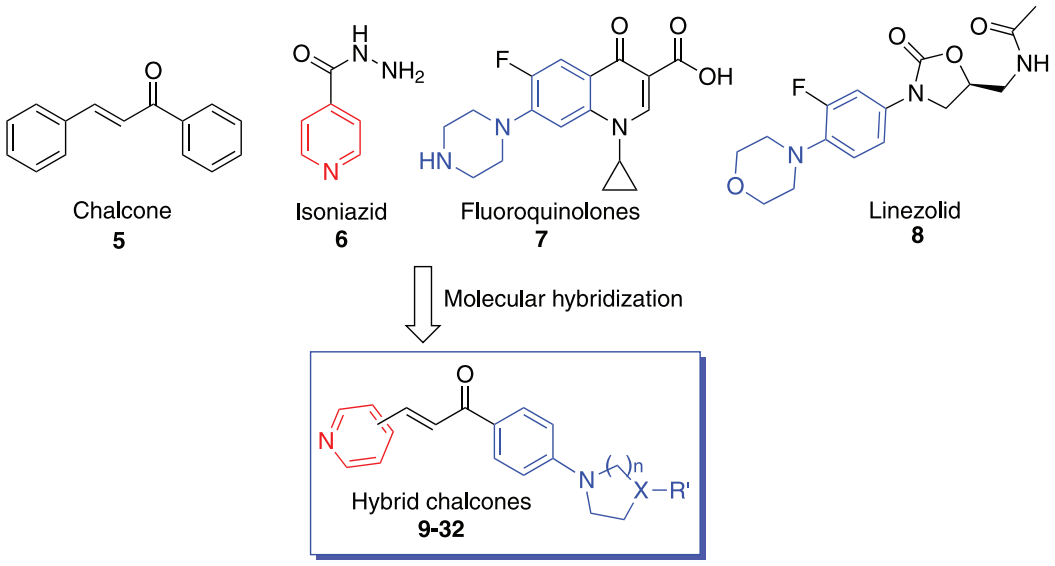
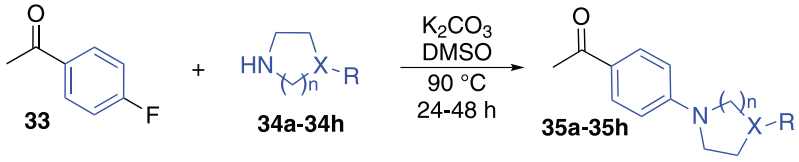


Figure 1. Structures of new chalcone antitubercular agents **1**-**4**.



Scheme 1. Design of novel heterocyclic amines azachalcones analogues **9**-**32** by molecular hybridization of chalcone scaffold with fragments of isoniazid, fluoroquinolones and/or linezolid.



Scheme 2. Synthesis of *p*-aminated acetophenones **35a**-**35h**. Reagents and reaction conditions: **33** (10 mmol), **34a**-**34h** (17 mmol), K_2CO_3 (17 mmol), DMSO (25 mL), 90 °C, 24 h (**35a**-**35c**, **35e**, **35f**) or 48 h (**35d**, **35g**, **35h**).

the worst yields (**11**, **14**, **17**, **20**, **23**, **26**, **32**) except for the compound **29**.

Unknown synthetic compounds **9**-**32** were characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS).

Antitubercular activity

The antitubercular activity of compounds **9**-**32** was evaluated against *Mycobacterium tuberculosis* H37Rv strain (Table 2). Minimum inhibitory concentrations (MICs) were defined as the lowest concentration that resulted in 90% of *M. tuberculosis* growth inhibition. Fifteen compounds of the series demonstrated some level of activity against *M. tuberculosis*.

The presence of a six-membered hetero-aromatic ring bonding directly to the cyclic amine group did not contribute to the antituberculosis activity (MIC > 250 μM) since the bulkiest azachalcones **27**-**32** were inactive.

Hybrid pyrrolidine substituted derivatives **9** and **10** were also inactive against *M. tuberculosis* (MIC > 250 μM). However, compound **11** showed good activity (MIC = 20.22 μM).

Compounds **12**-**14**, for instance, presented different biological activities. The isomers **12** and **14** exhibited values of MIC = 9.54 and 24.52 μM, respectively. On the other hand, isomer **13** was inactive (MIC > 250 μM).

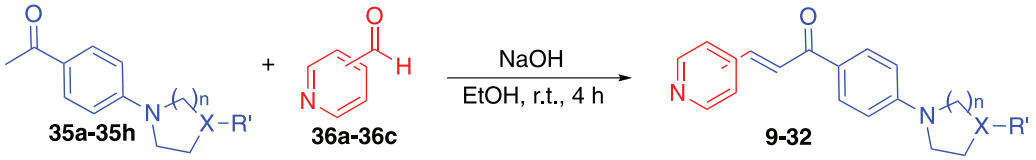
Thiomorpholine derivatives **18**-**20** showed MIC values ranging from 34.79 to 111.13 μM. Bioisosteric substitution of sulfur atom (in thiomorpholine derivatives) by oxygen (morpholine nucleus) improved the biological activity. Compounds **15**, **16** and **17** were considered the most active positional isomers in the series, exhibiting an MIC of 6.62, 14.03, and 4.85 μM, respectively.

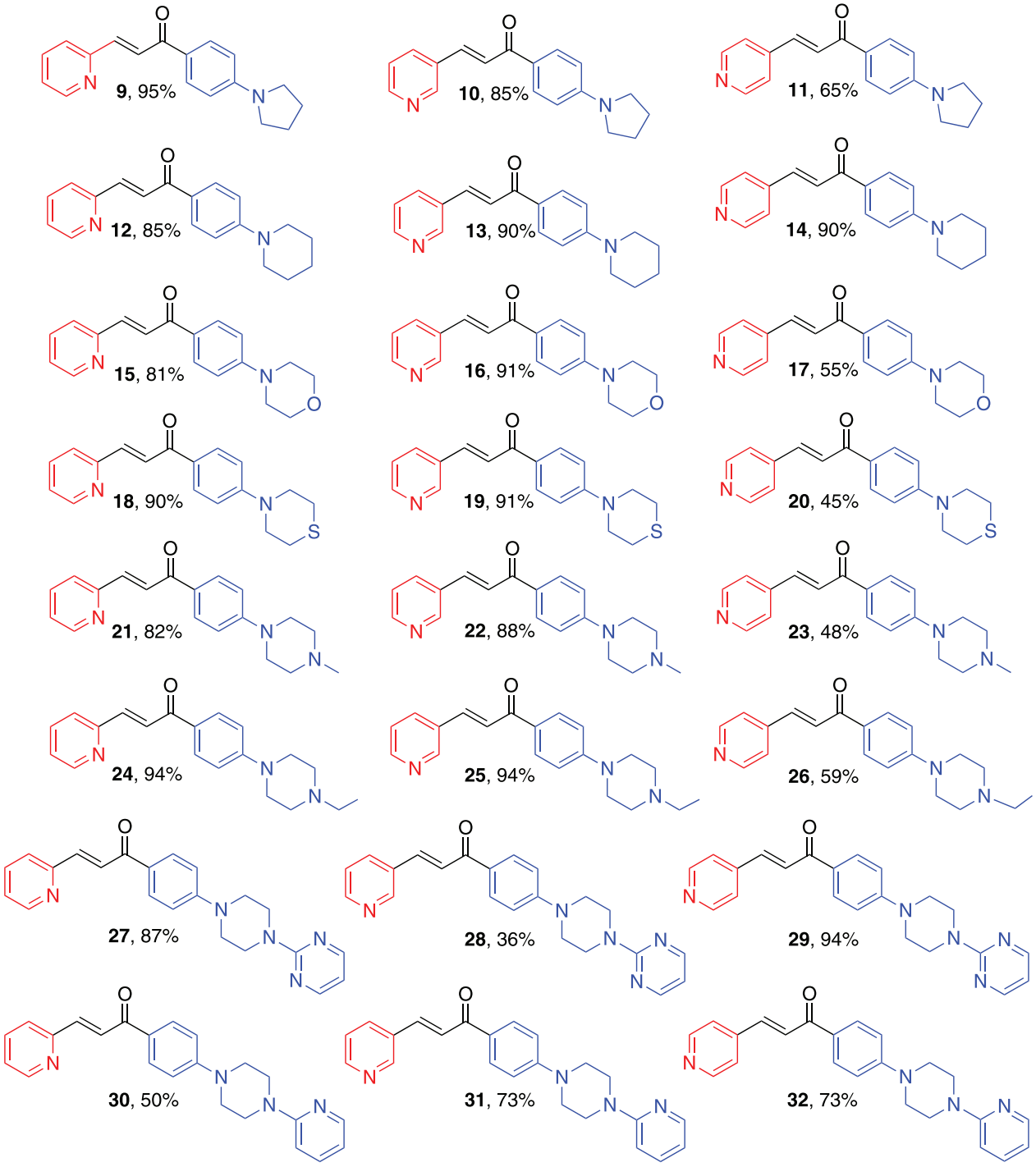
Additionally, *N*-alkyl piperazine derivatives **21**-**23** (MIC = 14.15, 26.64 and 16.72 μM, respectively) displayed good biological activities when compared to pyrazinamide (MIC = 26.80 μM).

Regarding SAR of positional isomers (2-pyridine, 3-pyridine, 4-pyridine), it was observed different MICs in the same structural group, indicating the importance of this factor in the biological activity. Derivatives containing 4-pyridines were more active than their 2- and 3- positional congeners (Table 2, compounds **11**, **17**, **20**, **26**). Positional isomers with 2-pyridine were the second most active (Table 2, compounds **12** and **21**). Compounds with a 3-pyridine nucleus were the least active (Table 2, compounds **10**, **13**, **16**, **22**, **25**).

In summary, the compounds with MIC < 10 μM were considered good hits in a series of antituberculosis drug

Table 1. Synthesis of the heterocyclic amine-azachalcones **9**-**32**





Reaction conditions: **35a**-**35h** (2 mmol), **36a**-**36c** (4 mmol), ethanol (10 mL) and NaOH (30 mmol solubilized in 25 mL of ethanol added dropwise), room temperature, 4 h, inert atmosphere.

Table 2. *In vitro* antituberculosis activity of azachalcones **9-32** against *M. tuberculosis* H37Rv strain

Compound	H37Rv MIC[a] / (μg mL^{-1})	H37Rv MIC[a] / μM	NIH/3T3 IC_{50}[b] / (μg mL^{-1})	SI[c]
9	> 250	> 250	17.39	0.07
10	> 250	> 250	32.21	0.13
11	5.63	20.22	64.78	11.51
12	2.79	9.54	26.04	9.33
13	> 250	> 250	32.87	0.13
14	7.17	24.52	2.87	0.40
15	1.95	6.62	2.71	1.39
16	4.13	14.03	2.94	0.71
17	1.43	4.85	4.99	3.49
18	34.50	111.13	79.16	2.29
19	15.30	49.28	5.28	0.34
20	10.80	34.79	2.78	0.26
21	4.35	14.15	2.88	0.66
22	8.19	26.64	20.73	2.53
23	5.14	16.72	2.77	0.54
24	3.62	11.26	2.71	0.75
25	7.76	24.14	23.39	3.01
26	3.38	10.51	2.60	0.77
27	> 250	> 250	32.43	0.13
28	> 250	> 250	361.58	1.44
29	> 250	> 250	32.78	0.13
30	> 250	> 250	23.21	0.09
31	> 250	> 250	26.94	0.10
32	> 250	> 250	29.42	0.11
INH[d]	0.015	0.10	NC[e]	NC[e]
RMP[f]	0.059	0.065	NC[e]	NC[e]
PYR[g]	3.3	26.80	NC	NC[e]
DOX[h]	NC[e]	NC[e]	2.50	NC[e]

[a]MIC: minimum inhibitory concentration (lowest concentration that results in 90% inhibition of growth of *M. tuberculosis*); [b]IC_{50}: half maximal inhibitory concentration on fibroblast cells (NIH/3T3); [c]SI: selectivity index (IC_{50} on fibroblast cells / MIC on *M. tuberculosis* H37Rv strain); [d]INH: isoniazid, positive control for *M. tuberculosis*; [e]NC: values not calculated; [f]RMP: rifampicin, positive control for *M. tuberculosis*; [g]PYR: pyrazinamide, positive control for *M. tuberculosis*; [h]DOX: doxorubicin, positive control for fibroblast cells.

candidates. Azachalcones **12**, **15** and **17** with MIC = 9.54 (selectivity index (SI) = 9.33), 6.62 (SI = 1.39) and 4.85 μM (SI = 3.49) showed higher activity than the reference drug tested, pyrazinamide (MIC = 26.80 μM). These derivatives also showed relevant antituberculosis profile when compared to ciprofloxacin (MIC = 9.43 μM).[18] Figure 2 shows the SAR highlights of azachalcones.

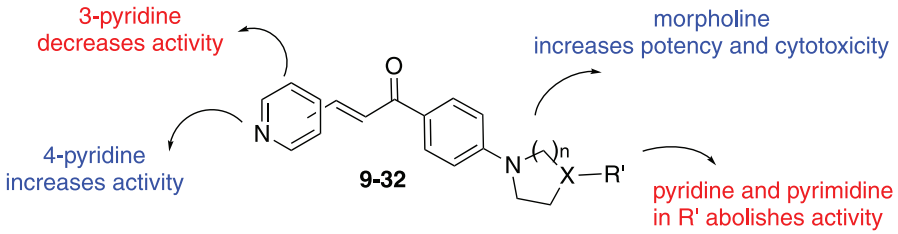


Figure 2. SAR of the hybrids azachalcones **9**-**32**.

Conclusions

In conclusion, twenty-four heterocyclic amine-azachalcones **9**-**32** were obtained from moderate to good yields. Antituberculosis activity was evaluated against the *M. tuberculosis* H37Rv strain. Although the higher potency of compounds **15** and **17** (MIC = 6.62 and 4.85 μM, respectively) towards antituberculosis activity, both analogues were considered more cytotoxic (SI = 1.39 and 3.49, respectively). However, azachalcone **12** showed the best profile of activity and selectivity index (MIC = 9.54 μM; SI = 9.33), which provide important information about the SAR.

Morpholine group as a substituent in molecular template was related to higher potency and relevant biological activity, but on the other hand it also increased the cytotoxicity of azachalcones. Further research might explore new structural modifications, in order to provide more information about SAR of azachalcones, and also, optimize the hit-like compounds of this series.

Experimental

General remarks

All solvents were purchased from Synth®, São Paulo, Brazil, and distilled before use according to the standard procedure. All reactions were performed under an atmosphere of dry nitrogen and monitored by thin-layer chromatography (TLC) using prepared plates (silica gel 60 F254 on aluminum purchased from Sigma-Aldrich®, St. Louis, MO, USA). The chromatograms were examined under both 254 and 360 nm UV light or with the developing agent ethanolic vanillin and heat. Flash column chromatography was performed on silica gel 60 (particle size 200-400 mesh ASTM, purchased from Sigma-Aldrich®, St. Louis, MO, USA) and eluted with hexane or hexane/ethyl acetate in different ratios. Melting points were determined using Fisatom 430D equipment. The ^{13}C and ^{1}H NMR spectra were recorded in $CDCl_3$ solutions using a Bruker 75 or 300 MHz spectrometer. Chemical shifts (δ) were expressed as parts *per* million (ppm) downfield from tetramethylsilane as the internal standard. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) measurements were carried out on a quadrupole time-of-flight instrument (UltrOTOF-Q, BrukerDaltonics, Billerica, MA, USA). General reagents as 4-fluoro-acetophenone **33**, compound **35b**, cyclic amines **34a**, **34c**-**34h**, carbaldehydes **36a**-**36c**, bases, were purchased from Sigma-Aldrich®, St. Louis, MO, USA.

General procedure for the preparation of *p*-aminated acetophenone (**35a**-**35h**)

To a solution of compounds **33** (10 mmol), **34a**-**34h** (17 mmol) in dimethyl sulfoxide (25 mL), K_2CO_3 (17 mmol) was added. The reaction was stirred at 90 °C for 24 h (**35a**, **35c**, **35e**, **35f**) or 48 h (**35d**, **35g**, **35h**). After that, a saturated aqueous NaCl solution (50 mL) was added. Then, the reaction was extracted with ethyl acetate (8 × 25 mL), the organic phase was washed with saturated aqueous NaCl solution (10 × 50 mL). The aqueous phase was retro-extracted with ethyl acetate (2 × 25 mL). Organic phases were dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. Compounds **35a**, **35c**, **35e**, **35f** did not require further purification. Compounds **35d**, **35g**, **35h** were purified by column chromatography on silica gel using hexane/ethyl acetate 8:2 as eluent.

1-(4-(Pyrrolidin-1-yl)phenyl)ethanone (**34a**)

Yield: 99%; light brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98-2.03 (m, 4H, $2CH_2$), 2.47 (s, 3H, CH_3), 3.33 (m, 4H, $2CH_2$), 6.48 (d, 2H, *J* 9.0 Hz, Ar-H), 7.83 (d, 2H, *J* 9.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4, 25.9, 29.7, 110.6, 124.9, 130.7, 150.9, 196.3.

1-(4-Morpholinophenyl)ethanone (**34c**)

Yield: 79%; light brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.51 (s, 3H, CH_3), 3.27-3.32 (m, 4H, $2CH_2$), 3.81-3.86 (m, 4H, $2CH_2$), 6.79 (d, 2H, *J* 9.0 Hz, Ar-H), 7.85 (d, 2H, *J* 9.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 47.5, 66.6, 113.3, 128.1, 130.3, 154.2, 196.4.

1-(4-Thiomorpholinophenyl)ethanone (**34d**)

Yield: 85%; light yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (s, 3H, CH_3), 2.65-2.70 (m, 4H, $2CH_2$), 3.73-3.79 (m, 4H, $2CH_2$), 6.85 (d, 2H, *J* 9.0 Hz, Ar-H), 7.88 (d, 2H, *J* 9.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 26.1, 50.3, 113.6, 127.2, 130.6, 152.9, 196.4.

1-(4-(4-Methylpiperazin-1-yl)phenyl)ethanone (**34e**)

Yield: 90%; light brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.58-2.63 (m, 4H, $2CH_2$), 3.37-3.42 (m, 4H, $2CH_2$), 6.86 (d, 2H, *J* 9.0 Hz, Ar-H), 7.86 (d, 2H, *J* 9.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 45.9, 47.1, 54.6, 113.5, 127.8, 130.4, 153.9, 196.5.

1-(4-(4-Ethylpiperazin-1-yl)phenyl)ethanone (**34f**)

Yield: 94%; beige solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.09 (t, 3H, *J* 6.0 Hz, CH_3), 2.43 (q, 2H, *J* 6.0 Hz, CH_2), 2.48 (s, 3H, CH_3), 2.52-2.57 (m, 4H, $2CH_2$), 3.32-3.37

(m, 4H, $2CH_2$), 6.83 (d, 2H, *J* 9.0 Hz, Ar-H), 7.84 (d, 2H, *J* 9.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.9, 26.1, 47.2, 52.3, 52.4, 113.3, 127.5, 130.3, 154.1, 196.5.

1-(4-(4-(Pyrimidin-2-yl)piperazin-1-yl)phenyl)ethanone (**34g**)

Yield: 92%; light brown solid; ^1H NMR (300 MHz, $CDCl_3$) δ 2.49 (s, 3H, CH_3), 3.39-3.44 (m, 4H, $2CH_2$), 3.95 (m, 4H, $2CH_2$), 6.50 (t, 1H, *J* 4.8 Hz, Ar-H), 6.87 (d, 2H, *J* 9.0 Hz, Ar-H), 7.87 (d, 2H, *J* 9.0 Hz, Ar-H), 8.31 (d, 2H, *J* 4.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 43.3, 47.2, 110.3, 113.5, 127.8, 130.4, 154.0, 157.8, 161.4, 196.5.

1-(4-(4-(Pyridin-2-yl)piperazin-1-yl)phenyl)ethanone (**34h**)

Yield: 99%; light brown solid; ^1H NMR (300 MHz, $CDCl_3$) δ 2.51 (s, 3H, CH_3), 3.54-3.59 (m, 4H, $2CH_2$), 3.70 (m, 4H), 6.63-6.68 (m, 2H, Ar-H), 6.88 (d, 2H, *J* 9.0 Hz, Ar-H), 7.44-7.49 (m, 1H, Ar-H), 7.87 (d, 2H, *J* 9.0 Hz, Ar-H), 8.19 (d, 1H, *J* 4.2 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 44.8, 45.1, 46.6, 47.0, 107.1, 107.3, 113.4, 113.7, 127.8, 130.4, 137.6, 148.0, 153.9, 159.1, 196.5.

General procedure for the preparation of chalcones **9**-**32**

To a solution of *p*-aminated acetophenone **35a**-**35h** (2 mmol), carbaldehyde **36a**-**36c** (4 mmol) in anhydrous ethanol (10 mL) at room temperature, under nitrogen atmosphere, and vigorously stirred, NaOH (30 mmol) solubilized in anhydrous ethanol (25 mL) was added dropwise. After the complete addition of NaOH (2 h), the reaction was kept at room temperature for two hours. Then, it was added 50 mL of a saturated solution of NaCl and the product was extracted with *tert*-butanol (4 × 20 mL), the organic phase was washed with saturated aqueous NaCl solution (3 × 50 mL). The aqueous phase was retro-extracted with *tert*-butanol (2 × 20 mL). Organic phases were dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate (7:3, 6:4, 1:1) as eluent and/or by recrystallization.

(*E*)-3-(Pyridin-2-yl)-1-(4-(pyrrolidin-1-yl)phenyl)prop-2-en-1-one (**9**)

Yield: 95%; yellow solid; mp 167 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.99-2.04 (m, 4H, $2CH_2$), 3.34-3.39 (m, 4H, $2CH_2$), 6.53 (d, 2H, *J* 8.8 Hz, Ar-H), 7.22-7.26 (m, 1H, Ar-H), 7.43 (d, 1H, *J* 7.7 Hz, Ar-H), 7.67-7.74 (m, 2H, Ar-H and CH), 8.05 (d, 2H, *J* 8.9 Hz, Ar-H), 8.15 (d, 1H, *J* 15.2 Hz, CH), 8.65 (d, 1H, *J* 3.9 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4, 47.6, 110.9, 123.9, 125.2, 125.3, 126.1, 131.3, 136.9, 140.3, 149.4, 151.2, 153.8, 187.5; HRMS (Fourier transform mass spectrometry (FTMS) + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2O$ $[M]^+$: 279.1497, found: 279.1516.

(*E*)-3-(Pyridin-3-yl)-1-(4-(pyrrolidin-1-yl)phenyl)prop-2-en-1-one (**10**)

Yield: 85%; yellow solid; mp 185 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.01-2.06 (m, 4H, $2CH_2$), 3.36-3.40 (m, 4H, $2CH_2$), 6.56 (d, 2H, *J* 9.0 Hz, Ar-H), 7.32 (dd, 1H, *J* 4.7, 2.7 Hz, Ar-H), 7.63 (d, 1H, *J* 15.7 Hz, CH), 7.73 (d, 1H, *J* 15.7 Hz, CH), 7.91 (dl, 1H, *J* 8.0 Hz, Ar-H), 7.98 (d, 2H, *J* 9.0 Hz, Ar-H), 8.57 (dd, 1H, *J* 4.7, 1.3 Hz, Ar-H), 8.84 (d, 1H, *J* 1.5 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4, 47.6, 111.0, 123.8, 124.3, 125.0, 131.1, 131.5, 134.7, 138.2, 149.4, 150.1, 151.2, 186.8; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2O$ $[M]^+$: 279.1497, found: 279.1528.

(*E*)-3-(Pyridin-4-yl)-1-(4-(pyrrolidin-1-yl)phenyl)prop-2-en-1-one (**11**)

Yield: 65%; yellow solid; mp 171-173 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.03-2.07 (m, 4H, $2CH_2$), 3.38-3.42 (m, 4H, $2CH_2$), 6.57 (d, 2H, *J* 9.0 Hz, Ar-H), 7.46 (d, 2H, *J* 9.0 Hz, Ar-H), 7.64 (d, 1H, *J* 15.7 Hz, CH), 7.73 (d, 1H, *J* 15.7 Hz, CH), 7.99 (d, 2H, *J* 9.0 Hz, Ar-H), 8.65 (d, 2H, *J* 5.5 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4, 47.6, 111.0, 122.0, 124.9, 126.5, 131.2, 139.0, 142.9, 150.5, 151.3, 186.7; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2O$ $[M]^+$: 279.1497, found: 279.1516.

(*E*)-1-(4-(Piperidin-1-yl)phenyl)-3-(pyridin-2-yl)prop-2-en-1-one (**12**)

Yield: 85%; yellow solid; mp 156-158 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 6H, $3CH_2$), 3.38 (s, 4H, $2CH_2$), 6.86 (d, 2H, *J* 9.0 Hz, Ar-H), 7.23-7.27 (m, 1H, Ar-H), 7.44 (d, 1H, *J* 8.0 Hz, Ar-H),7.68-7.74 (m, 2H, Ar-H and CH), 8.04 (d, 2H, *J* 9.0 Hz, Ar-H), 8.13 (d, 1H, *J* 15.2 Hz, CH), 8.66 (d, 1H, *J* 3.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 25.4, 48.5, 113.2, 124.0, 125.3, 125.8, 126.9, 131.1, 136.8, 140.8, 150.0, 153.7, 154.5, 187.69; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{20}N_2O$ $[M]^+$: 293.1654, found: 293.1655.

(*E*)-1-(4-(Piperidin-1-yl)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one (**13**)

Yield: 90%; yellow solid; mp 179-181 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.66 (s, 6H, $3CH_2$), 3.39 (s, 4H, $2CH_2$), 6.88 (d, 2H, *J* 9.0 Hz, Ar-H), 7.32 (dd, 1H, *J* 3.6, 8.0 Hz, Ar-H), 7.61 (d, 1H, *J* 15.7 Hz, CH), 7.74 (d, 1H, *J* 15.7 Hz, CH), 7.92 (d, 1H, *J* 8.0 Hz, Ar-H), 7.96 (d, 2H, *J* 9.0 Hz,

Ar-H), 8.57 (d, 1H, *J* 3.6 Hz, Ar-H), 8.83 (s, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 25.3, 48.4, 123.7, 124.0, 126.6, 130.9, 131.2, 134.5, 138.8, 149.6, 150.5, 154.4, 187.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{20}N_2O$ $[M]^+$: 293.1654, found: 293.1653.

(*E*)-1-(4-(Piperidin-1-yl)phenyl)-3-(pyridin-4-yl)prop-2-en-1-one (**14**)

Yield: 90%; yellow solid; mp 149-150 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (s, 6H, $3CH_2$), 3.38 (s, 4H, $2CH_2$), 6.55 (d, 2H, *J* 8.0 Hz, Ar-H), 7.46 (s, 2H, *J* 15.8 Hz, CH), 7.62 (d, 1H, *J* 15.8 Hz, CH), 7.72 (d, 1H, *J* 15.8 Hz, CH), 7.97 (d, 2H, *J* 8.0 Hz, Ar-H), 8.63 (s, 2H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 25.3, 48.4, 113.2, 122.6, 126.3, 127.8, 131.1, 138.5, 145.0, 148.3, 154.5, 186.4; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{20}N_2O$ $[M]^+$: 293.1654, found: 293.1740.

(*E*)-1-(4-Morpholinophenyl)-3-(pyridin-2-yl)prop-2-en-1-one (**15**)

Yield: 81%; yellow solid; mp 138-140 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.30-3.33 (m, 4H, $2CH_2$), 3.82-3.85 (m, 4H, $2CH_2$), 6.88 (d, *J* 9.0 Hz, 2H, Ar-H), 7.23-7.28 (m, 1H, Ar-H), 7.44 (d, *J* 7.7 Hz, 1H, Ar-H), 7.72 (m, 2H, Ar-H and CH), 8.06 (d, *J* 9.0 Hz, 2H, Ar-H), 8.13 (d, 1H, *J* 15.2 Hz, CH), 8.66 (d, 1H, *J* 3.9 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 47.4, 66.5, 113.3, 124.1, 125.3, 125.7, 128.5, 130.9, 136.9, 141.2, 150.0, 153.6, 154.3, 188.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2O_2$ $[M]^+$: 295.1446, found: 295.1455.

(*E*)-1-(4-Morpholinophenyl)-3-(pyridin-3-yl)prop-2-en-1-one (**16**)

Yield: 91%; yellow solid; mp 156-158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.31-3.34 (m, 4H, $2CH_2$), 3.83-3.86 (m, 4H, $2CH_2$), 6.90 (d, 2H, *J* 9.0 Hz, Ar-H), 7.33 (dd, *J* 7.4, 4.5 Hz, 1H, Ar-H), 7.60 (d, 1H, *J* 15.7 Hz, CH), 7.75 (d, 1H, *J* 15.7 Hz, CH), 7.91 (d, 1H, *J* 9.0 Hz, Ar-H), 7.99 (d, 2H, *J* 9.0 Hz, Ar-H), 8.59 (s, 1H, Ar-H), 8.84 (s, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 47.4, 66.5, 113.4, 123.7, 123.9, 128.3, 130.8, 134.6, 135.0, 139.4, 149.7, 150.6, 154.4, 187.4; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2O_2$ $[M]^+$: 295.1446, found: 295.1462.

(*E*)-1-(4-Morpholinophenyl)-3-(pyridin-4-yl)prop-2-en-1-one (**17**)

Yield: 55%; yellow solid; mp 122-124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.32-3.35 (m, 4H, $2CH_2$), 3.83-3.87 (m, 4H, $2CH_2$), 6.90 (d, 2H, *J* 9.0 Hz, Ar-H), 7.45 (dd, 2H, *J* 4.5, 1.6 Hz, Ar-H), 7.63 (d, 1H, *J* 15.8 Hz, CH), 7.69 (d, 1H, *J* 15.8 Hz, CH), 7.99 (d, 2H, *J* 9.0 Hz, Ar-H), 8.65 (dd, 2H, *J* 4.5, 1.5 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 47.3, 66.5, 113.3, 122.0, 126.1, 128.0, 130.8, 140.0, 142.6, 150.5, 154.4, 187.2; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2O_2$ $[M]^+$: 295.1446, found: 295.1610.

(*E*)-3-(Pyridin-2-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one (**18**)

Yield: 90%; yellow solid; mp 154-156 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67-2.71 (m, 4H, $2CH_2$), 3.78-3.81 (m, 4H, $2CH_2$), 6.83 (d, 2H, *J* 9.0 Hz, Ar-H), 7.25-7.28 (m, 1H, Ar-H), 7.44 (d, 1H, *J* 7.7 Hz, Ar-H), 7.68-7.75 (m, 2H, Ar-H and CH), 8.05 (d, 2H, *J* 9.0 Hz, Ar-H), 8.11 (d, 1H, *J* 15.2 Hz, CH), 8.66 (d, 1H, *J* 3.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 50.3, 113.7, 124.1, 125.3, 125.6, 127.7, 131.3, 136.8, 141.2, 150.1, 153.1, 153.6, 187.8; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2OS$ $[M]^+$: 311.1218, found: 311.1219.

(*E*)-3-(Pyridin-3-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one (**19**)

Yield: 91%; yellow solid; mp 120-122 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68-2.71 (m, 4H, $2CH_2$), 3.79-3.83 (m, 4H, $2CH_2$), 6.85 (d, 2H, *J* 8.7 Hz, Ar-H), 7.31-7.35 (m, 1H, Ar-H), 7.60 (d, 1H, *J* 15.6 Hz, CH), 7.75 (d, 1H, *J* 15.6 Hz, CH), 7.91 (d, 1H, *J* 6.0 Hz, Ar-H), 7.97 (d, 2H, *J* 9.0 Hz, Ar-H), 8.59 (d, 1H, *J* 3.7 Hz, Ar-H), 8.84 (s, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 50.3, 113.5, 113.7, 123.7, 123.8, 127.3, 131.1, 134.6, 139.3, 149.7, 150.7, 153.0, 187.1; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2OS$ $[M]^+$: 311.1218, found: 311.1216.

(*E*)-3-(Pyridin-4-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one (**20**)

Yield: 55%; yellow solid; mp 142-144 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68-2.71 (m, 4H, $2CH_2$), 3.80-3.84 (m, 4H, $2CH_2$), 6.85 (d, 2H, *J* 9.0 Hz, Ar-H), 7.45 (d, 2H, *J* 6.0 Hz, Ar-H), 7.60-7.71 (m, 2H, 2CH), 7.97 (d, 2H, *J* 9.0 Hz, Ar-H), 8.65 (d, 2H, *J* 6.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 50.2, 113.6, 122.0, 126.1, 127.1, 131.2, 139.8, 142.6, 150.5, 153.1, 187.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{18}N_2OS$ $[M]^+$: 311.1218, found: 311.1222.

(*E*)-1-(4-(4-Methylpiperazin-1-yl)phenyl)-3-(pyridin-2-yl)prop-2-en-1-one (**21**)

Yield: 82%; yellow solid; mp 143-145 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 2.53-2.56 (m, 4H, $2CH_2$), 3.37-3.40 (m, 4H, $2CH_2$), 6.88 (d, 2H, *J* 9.0 Hz, Ar-H), 7.22-7.27 (m, 1H, Ar-H), 7.43 (d, 1H, *J* 7.7 Hz, Ar-H), 7.67-7.76 (m, 2H, Ar-H and CH), 8.05 (d, 2H, *J* 8.8 Hz, Ar-H), 8.11 (d, 1H, *J* 15.2 Hz, CH), 8.62 (d, 1H,

J 4.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.0, 47.0, 54.6, 113.5, 124.1, 125.3, 125.6, 128.0, 131.0, 136.8, 141.2, 150.1, 153.6, 154.1, 187.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{21}N_3O$ $[M]^+$: 308.1763, found: 308.1769.

(*E*)-1-(4-(4-Methylpiperazin-1-yl)phenyl)-3-(pyridin-3-yl) prop-2-en-1-one (**22**)

Yield: 88%; yellow solid; mp 143 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 2.50-2.58 (m, 4H, $2CH_2$), 3.34-3.43 (m, 4H, $2CH_2$), 6.90 (d, 2H, *J* 9.0 Hz, Ar-H), 7.32 (dd, 1H, *J* 8.0, 4.8 Hz, Ar-H), 7.60 (d, 1H, *J* 15.7 Hz, CH), 7.74 (d, 1H, *J* 15.7 Hz, CH), 7.89-7.93 (m, 1H, Ar-H), 7.98 (d, 2H, *J* 9.0 Hz, Ar-H), 8.58 (dd, 1H, *J* 4.8, 1.6 Hz, Ar-H), 8.83 (d, 1H, *J* 2.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.1, 47.1, 54.6, 113.5, 123.5, 123.9, 127.7, 130.8, 134.7, 134.9, 148.7, 149.6, 150.5, 154.2, 187.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{21}N_3O$ $[M]^+$: 308.1763, found: 308.1758.

(*E*)-1-(4-(4-Methylpiperazin-1-yl)phenyl)-3-(pyridin-4-yl) prop-2-en-1-one (**23**)

Yield: 48%; yellow solid; mp 97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 2.53-2.56 (m, 4H, $2CH_2$), 3.37-3.40 (m, 4H, $2CH_2$), 6.88 (d, *J* 9.0 Hz, 2H, Ar-H), 7.44 (d, *J* 4.69 Hz, 2H, Ar-H), 7.61 (d, *J* 15.8 Hz, 1H, CH), 7.68 (d, 1H, *J* 15.7 Hz, CH), 7.96 (d, 2H, *J* 9.0 Hz, Ar-H), 8.63 (s, 2H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 46.9, 54.5, 113.5, 121.3, 122.0, 126.1, 127.4, 130.9, 139.8, 142.6, 150.4, 154.3, 187.1; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{21}N_3O$ $[M]^+$: 308.1763, found: 308.1759.

(*E*)-1-(4-(4-Ethylpiperazin-1-yl)phenyl)-3-(pyridin-2-yl)prop-2-en-1-one (**24**)

Yield: 94%; yellow solid; mp 150 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, 3H, *J* 6.0 Hz, CH_3), 2.48 (q, 2H, *J* 6.0 Hz, CH_2), 2.51-2.61 (m, 4H, $2CH_2$), 3.40-3.43 (m, 4H, $2CH_2$), 6.90 (d, 2H, *J* 9.0 Hz, Ar-H), 7.27-7.29 (m, 1H, Ar-H), 7.45 (d, 1H, *J* 7.7 Hz, Ar-H), 7.69-7.77 (m, 2H, Ar-H and CH), 8.06 (d, 2H, *J* 9.0 Hz, Ar-H), 8.13 (d, 1H, *J* 15.2 Hz, CH), 8.67 (d, 1H, *J* 4.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 47.2, 52.3, 52.5, 113.3, 124.1, 125.3, 127.9, 131.0, 136.8, 141.1, 150.1, 153.6, 154.2, 187.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{20}H_{23}N_3O$ $[M]^+$: 322.1919, found: 322.1916.

(*E*)-1-(4-(4-Ethylpiperazin-1-yl)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one (**25**)

Yield: 94%; yellow solid; mp 171-173 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, 3H, *J* 6.0 Hz, CH_3), 2.60 (q, 2H, *J* 6.0 Hz, CH_2), 2.53-2.67 (m, 4H, $2CH_2$), 3.36-3.54 (m, 4H, $2CH_2$), 6.91 (d, 2H, *J* 9.0 Hz, Ar-H), 7.29 (dd, 1H, *J* 8.0, 3.0 Hz, Ar-H), 7.60 (d, 1H, *J* 15.7 Hz, CH), 7.75 (d, 1H, *J* 15.8 Hz, CH), 7.92 (d, 2H, *J* 8.0 Hz, Ar-H), 7.98 (d, 1H, *J* 9.0 Hz, Ar-H), 8.59 (dd, 1H, *J* 4.8, 1.4 Hz, Ar-H), 8.84 (d, 1H, *J* 1.7 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.7, 46.8, 47.0, 52.3, 113.4, 113.5, 123.7, 123.9, 127.8, 130.8, 134.5, 139.3, 149.8, 150.7, 154.1, 187.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{20}H_{23}N_3O$ $[M]^+$: 322.1919, found: 322.1916.

(*E*)-1-(4-(4-Ethylpiperazin-1-yl)phenyl)-3-(pyridin-4-yl)prop-2-en-1-one (**26**)

Yield: 59%; yellow solid; mp 144-146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11 (t, 3H, *J* 6.0 Hz, CH_3), 2.47 (q, 2H, *J* 6.0 Hz, CH_2), 2.56-2.59 (m, 4H, $2CH_2$), 3.39-3.42 (m, 4H, $2CH_2$), 6.89 (d, 2H, *J* 9.0 Hz, Ar-H), 7.44 (dd, 2H, *J* 4.5, 1.6 Hz, Ar-H), 7.63 (d, 1H, *J* 15.7 Hz, CH), 7.68 (d, 1H, *J* 15.7 Hz, CH), 7.96 (d, 2H, *J* 9.0 Hz, Ar-H), 8.63 (dd, 2H, *J* 4.5, 1.6 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.9, 47.1, 52.3, 54.4, 113.4, 120.0, 122.0, 123.2, 129.5, 139.8, 142.6, 149.9, 154.3, 187.1; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{20}H_{23}N_3O$ $[M]^+$: 322.1919, found: 322.1901.

(*E*)-3-(Pyridin-2-yl)-1-(4-(4-(pyrimidin-2-yl)piperazin-1-yl) phenyl)prop-2-en-1-one (**27**)

Yield: 87%; yellow solid; mp 180-182 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.46-3.50 (m, 4H, $2CH_2$), 3.96-4.00 (m, 4H, $2CH_2$), 6.53 (t, 1H, *J* 4.7 Hz, Ar-H), 6.93 (d, 2H, *J* 8.9 Hz, Ar-H), 7.23-7.26 (m, 1H, Ar-H), 7.45 (d, 1H, *J* 7.7 Hz, Ar-H), 7.69-7.76 (m, 2H, Ar-H and CH), 8.06-8.15 (m, 3H, Ar-H and CH), 8.33 (d, 2H, *J* 4.7 Hz, Ar-H), 8.67 (d, 1H, *J* 4.1 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 43.2, 47.0, 110.4, 113.5, 124.2, 125.5, 125.8, 128.1, 131.1, 140.9, 149.8, 153.4, 154.1, 157.8, 165.8, 187.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{22}H_{21}N_5O$ $[M]^+$: 372.1824, found: 372.1844.

(*E*)-3-(Pyridin-3-yl)-1-(4-(4-(pyrimidin-2-yl)piperazin-1-yl) phenyl)prop-2-en-1-one (**28**)

Yield: 36%; yellow solid; mp 208-210 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.42-3.44 (m, 4H, $2CH_2$), 3.93-3.96 (m, 4H, $2CH_2$), 6.49 (t, 1H, *J* 4.7 Hz, Ar-H), 6.89 (d, 2H, *J* 9.0 Hz, Ar-H), 7.29 (dd, 1H, *J* 7.9, 4.9 Hz, Ar-H), 7.56 (d, 1H, *J* 15.7 Hz, CH), 7.71 (d, 1H, *J* 15.7 Hz, CH), 7.88 (dd, 1H, *J* 6.1, 1.9 Hz, Ar-H), 7.96 (d, 2H, *J* 9.0 Hz, Ar-H), 8.29 (d, 2H, *J* 4.7 Hz, Ar-H), 8.55 (d, 1H, *J* 3.7 Hz, Ar-H), 8.80 (s, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 43.2, 47.0, 110.4, 113.3, 113.5, 123.7, 123.9, 127.9, 130.8, 131.2, 134.5, 139.3, 149.8, 150.7, 154.1, 157.8, 187.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{22}H_{21}N_5O$ $[M]^+$: 372.1824, found: 372.1834.

(*E*)-3-(Pyridin-4-yl)-1-(4-(4-(pyrimidin-2-yl)piperazin-1-yl) phenyl)prop-2-en-1-one (**29**)

Yield: 94%; yellow solid; mp 207-208 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48-3.52 (m, 4H, $2CH_2$), 3.97-4.01 (m, 4H, $2CH_2$), 6.54 (m, 1H, *J* 4.7 Hz, Ar-H), 6.93 (d, 2H, *J* 9.0 Hz, Ar-H), 7.46 (dd, 2H, *J* 6.0, 1.5 Hz, Ar-H), 7.64 (d, 1H, *J* 15.7 Hz, CH), 7.70 (d, 1H, *J* 15.7 Hz, CH), 8.00 (d, 2H, *J* 9.0 Hz, Ar-H), 8.33 (d, 2H, *J* 4.7 Hz, Ar-H), 8.65 (d, 2H, *J* 5.6 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 43.2, 46.9, 110.4, 113.4, 122.0, 126.1, 127.6, 130.9, 139.9, 142.6, 150.5, 154.2, 157.8, 161.5, 187.2; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{22}H_{21}N_5O$ $[M]^+$: 372.1824, found: 372.1826.

(*E*)-3-(Pyridin-2-yl)-1-(4-(4-(pyridin-2-yl)piperazin-1-yl) phenyl)prop-2-en-1-one (**30**)

Yield: 50%; yellow solid; mp 152-154 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51-3.55 (m, 4H, CH_2), 3.70-3.74 (m, 4H, CH_2), 6.63-6.68 (m, 2H, Ar-H), 6.92 (d, 2H, *J* 9.0 Hz, Ar-H), 7.25-7.28 (m, 1H, Ar-H), 7.44 (d, 1H, *J* 7.7 Hz, Ar-H), 7.47-7.53 (m, 1H, Ar-H), 7.68-7.76 (m, 2H, Ar-H and CH), 8.08 (d, 2H, *J* 9.0 Hz, Ar-H), 8.13 (d, 1H, *J* 15.2 Hz, CH), 8.20 (dd, 1H, *J* 4.2, 1.1 Hz, Ar-H), 8.67 (d, 1H, *J* 3.3 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.7, 46.9, 107.1, 113.4, 113.7, 123.7, 123.9, 127.8, 130.8, 131.1, 134.5, 137.6, 139.3, 148.0, 149.8, 150.7, 154.0, 159.0, 187.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{23}H_{22}N_4O$ $[M]^+$: 371.1872, found: 371.1872.

(*E*)-1-(4-(4-(Pyridin-2-yl)piperazin-1-yl)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one (**31**)

Yield: 73%; yellow solid; mp 192-194 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.52-3.55 (m, 4H, $2CH_2$), 3.71-3.74 (m, 4H, $2CH_2$), 6.65-6.68 (m, 2H, Ar-H), 6.93 (d, 2H, *J* 9.0 Hz, Ar-H), 7.31-7.35 (m, 1H, Ar-H), 7.48-7.53 (m, 1H, Ar-H), 7.61 (d, 1H, *J* 15.9 Hz, CH), 7.75 (d, 1H, *J* 15.9 Hz, CH), 7.92 (d, 1H, *J* 9.0 Hz, Ar-H), 8.01 (d, 2H, *J* 9.0 Hz, Ar-H), 8.19 (m, 1H, Ar-H), 8.59 (m, 1H, Ar-H), 8.84 (d, 1H, *J* 1.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.7, 76.9, 107.1, 113.4, 113.7, 123.7, 123.9, 127.8, 130.8, 131.1, 134.5, 137.6, 139.3, 148.0, 149.8, 150.7, 154.0, 159.0, 187.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{23}H_{22}N_4O$ $[M]^+$: 371.1872, found: 371.1876.

(*E*)-1-(4-(4-(Pyridin-2-yl)piperazin-1-yl)phenyl)-3-(pyridin-4-yl)prop-2-en-1-one (**32**)

Yield: 73%; yellow solid; mp 214-216 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.53-3.57 (m, 4H, $2CH_2$), 3.72-3.75 (m, 4H, $2CH_2$), 6.64-6.68 (m, 2H, Ar-H), 6.94 (d, 2H, *J* 9.0 Hz, Ar-H), 7.46 (dd, 2H, *J* 6.0, 1.6 Hz, Ar-H), 7.48-7.54 (m, 1H, Ar-H), 7.64 (d, 1H, *J* 15.7 Hz, CH), 7.70 (d, 1H, *J* 15.7 Hz, CH), 8.00 (d, 2H, *J* 9.0 Hz, Ar-H), 8.19-8.23 (m, Ar-H), 8.66 (dd, 2H, *J* 6.0, 1.6 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.7, 46.8, 107.1, 113.3, 113.8, 122.0, 126.1, 127.5, 130.9, 137.7, 139.9, 142.6, 148.0, 150.5, 154.1, 159.0, 187.2; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{23}H_{22}N_4O$ $[M]^+$: 371.1872, found: 371.1872.

Antitubercular assay

The strains of *M. tuberculosis* H37Rv (ATCC27294) were grown in the Ogawa-Kudoh medium for 10 days at 37 °C. For testing, a colony was removed and cultured in Middlebrook 7H9 broth (Difco®, Franklin Lakes, NJ, United States) supplemented with oleic acid, bovine serum albumin, dextrose and catalase (OADC enrichment BBL/Becton-Dickinson®, Franklin Lakes, NJ, United States), and it was also added 0.5% glycerol as carbon source and 0.5% Tween 80 (Sigma-Aldrich®, St. Louis, MO, USA) to prevent the appearance of lumps. The broth was maintained for 15 days at 37 °C. The bacterial suspensions were prepared and adjusted to the No. 1 McFarland scale. The stock solutions of tested extract, fractions, and isolated compounds were solubilized in DMSO (Sigma-Aldrich®, St. Louis, MO, USA) and diluted in Middlebrook 7H9 broth (Difco®, Franklin Lakes, NJ, United States) supplemented with bovine serum and OADC enrichment (oleic albumin dextrose catalase) (BBL/Becton Dickinson®, Franklin Lakes, NJ, United States). Rifampicin and isoniazid were solubilized according to the manufacturer's recommendations (Sigma-Aldrich®, St. Louis, MO, USA) and used as positive control drugs. The determination of antimycobacterial activity was performed using the resazurin microtiter assay.[26] Briefly, 100 µL of Middlebrook 7H9 broth (Difco®, Franklin Lakes, NJ, United States) supplemented was dispensed in each well of a sterile flat-bottom 96-well plate, then serial dilutions were made of solutions in order to obtain different concentrations of tested compounds (0.98 to 250 µg mL^{-1}) and reference drug (0.004 to 1 µg mL^{-1}). After dilutions, 100 µL of bacterial suspension (5×10^5 CFU mL^{-1}) was added to each well. Plates were incubated for 7 days at 37 °C, after this period, 30 µL of resazurin solution (Sigma-Aldrich®, St. Louis, MO, USA) diluted in 0.01% sterile water was added to each well and the samples were incubated for 24 h at 37 °C. The reading was performed based on the color change and the absorbance on a microplate reader TP-Reader (Thermo Plate®) at a wavelength of 492 nm. Each compound was analyzed in triplicate on alternate days. The MIC was defined as the lowest concentration that results in 90% inhibition of growth of *M. tuberculosis*.[27] Compounds with an MIC value < 100 µg mL^{-1} were defined as active against *M. tuberculosis*. Several authors[28] consider MIC values of

plant samples less than 200 μg mL^{-1} as being active against *M. tuberculosis*. However, for this to work, we suggest the following MIC values: inactive, ≥ 100 μg mL^{-1}; moderate, ≥ 10 and ≤ 100 μg mL^{-1}; and high, ≤ 10 μg mL^{-1}.

Cytotoxicity assay

Fibroblasts (NIH/3T3) obtained from Rio de Janeiro Cell Bank (Brazil) were seeded in 96-well plates (1×10^4 cells mL^{-1}) and incubated with synthetic compounds at 37 °C, 5% CO_2 for 48 h at the concentrations of 0.25-250 μg mL^{-1}.[29,30] Doxorubicin (Sigma-Aldrich®, St. Louis, MO, USA) was used as the reference drug at concentrations of 0.025-25 μg mL^{-1}. Cell growth was estimated by the sulforhodamine B colorimetric method (SRB).[31] DMSO (Vetec®, Rio de Janeiro, RJ, Brazil) was used as a negative control at the concentration necessary to solubilize the highest concentration of the test samples and did not interfere with cell viability. The percentage of growth was calculated as described by Monks *et al*.[32] IC_{50} (half maximal inhibitory concentration on fibroblast cells) was determined by nonlinear regression analysis (Microcal Origin 6.0®[33] and Microsoft Office Excel 2007®). Selectivity index (SI) was calculated according to de Medeiros *et al*.[34]

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Adriano C. M. Baroni was responsible for the conceptualization, resources, writing original draft, review and editing; Júlio Croda for the resources and antimycobacterial experiments; Renata T. Perdomo for the resources and cytotoxicity experiments; Jefferson R. S. Oliveira for the synthesis of chalcones and writing original draft; Flora M. F. Moreira for the antimycobacterial experiments; Giovana B. Gomes for the cytotoxicity experiments; Cristiane Y. K. Shiguemoto for the writing of the original draft; Amarith R. das Neves for the writing of original draft, review and editing; Sandro L. Barbosa and Palimécio G. Guerrero Jr. for the writing review and editing.

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