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FULL PAPER

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New thiazolyl-pyrazoline derivatives bearing nitrogen mustard as potential antimicrobial and antiprotozoal agents

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Abstract

A new series of *N*-substituted pyrazoline derivatives **6a–g**, **7a–g**, **8a–g**, and **9a–g** was synthetized by reaction of hydrazine derivatives and chalcone–thiazole hybrids bearing nitrogen mustard **5a–g**. The chalcones **5a–g** were obtained by Claisen–Schmidt condensation of thiazole-2-nitrogen mustard **3** and selected acetophenones **4a–g**. These new compounds **6/7/8/9a–g** were screened for their antifungal activity against *Cryptococcus neoformans*, with IC_{50} values of 3.9–7.8 μg/ml for the *N*-3, 5-dichlorophenyl pyrazolines **9e–g**. Interestingly, those compounds show low cytotoxic effects toward erythrocytes (RBC). In addition, *N*-acetyl (**6a,b**) and *N*-formyl pyrazolines (**7a**, **7b**, **7c**, and **7g**) showed inhibitory activity against methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and vancomycin-intermediate *S. aureus*, with the most important minimum inhibitory concentration values ranging from 31.25 to 125 μg/ml. Regarding the antiprotozoal activity, thiazolyl-pyrazolines **9g**, **8f**, and **7c** display high activity against *Plasmodium falciparum*, *Leishmania (V) panamensis*, and *Trypanosoma cruzi*, with EC_{50} values of 11.80, 6.46, and 4.98 μM, respectively, and with **7c** being approximately 2.6-fold more potent than benznidazole with a selectivity index of 1.61 on U-937 human cells, showing promising potential as a novel antitrypanosomal agent.

KEYWORDS

antibacterial activity, antifungal activity, antiprotozoal activity, chalcone, pyrazoline

1 | INTRODUCTION

Antimicrobial infections are a major concern for global public health due to the emergence of multidrug-resistant strains.[1–3] These pathogens are known as "superbugs" and they exhibit different mechanisms to avoid antibiotic actions such as reduced permeability, mutations in the target site, and enzymatic modifications.[4] Neglected tropical diseases are an important cause of mortality in tropical and subtropical regions from 149 countries,[5,6] affecting more than one billion people[6] with malaria, leishmaniasis, and Chagas

disease, among others, which require toxic and expensive chemotherapy agents, such as pentavalent antimonials, miltefosine, amphotericin B, benznidazole, and nifurtimox.[5,7] Unfortunately, these treatments are contraindicated and in some cases are not effective in the chronic phase due to parasite resistance.[7] However, these emerging resistances could be overcome by the synthesis and modification of new biologically active molecules. Several researchers have demonstrated a broad spectrum of biological activities of the 2-aminothiazole (2-AT) derivatives as antibacterial,[8] antifungal,[9] antitumoral,[10] antimalarial,[11–13] antidiabetic,[14] anti-inflammatory,[15] and antiviral[16] agents (Figure 1). Many authors have suggested that the 2-AT moiety may be used for fragment-based drug discovery strategy, because it plays an important role in the treatment of different diseases.[17,18]

Additionally, nitrogen mustards are an important class of alkylating agents, because they show interesting biological properties due to their high chemical reactivity with nucleophiles such as nitrogen atoms of nucleic acids and sulfhydryl groups in proteins.[19] Moreover, they lead to ADN/ARN alkylation, protein cross-linking, and depletion of glutathione, thus promoting apoptotic cell death.[19–21] However, *N*,*N*-bis(2-chloroethyl)amine pharmacophore requires adequate carriers to reduce the toxicity against normal cells[19,22] and hydrolysis rate[20,21] by increasing its specificity during drug delivery. In the last few years, a number of modifications have been made, including the binging of the nitrogen mustard moiety to a conjugated linker (carbonyl[22,23]/aryl[24]/heterocycle[21]) to reduce the basicity of nitrogen mustard and enhance drug delivery.[21,22] Although nitrogen mustards are widely used in the design of compounds with antitumor activity such as melphalan, chlorambucil, and cyclophosphamide,[19,25–27] there are few reports that explore the potential of this moiety in the inhibition of microorganisms.[22,28]

2-Pyrazolines are important anti-inflammatory and analgesic agents, and some of them are potent drugs such as metamizol, phenylbutazone, and morazone.[29] Also, pyrazoline derivatives exhibit pharmacological activities such as antitumor,[30] antibacterial,[31] anti-inflammatory,[32] antifungal,[33] and antidepressant activities[15] (Figure 2). Recent studies have revealed that thiazolyl-pyrazoline derivatives show potent antifungal,[34] antibacterial,[29] and antioxidant[35] activities, in addition to inhibitory effects on the epidermal growth factor receptor tyrosine kinase as a target for anticancer therapy,[36–39] among others.[18] For example, Altintop et al.[34] reported that novel thiazolyl-pyrazolines bearing thiophenes showed antifungal activity against different *Candida* spp. and a notable antiproliferative effect on A549 human lung adenocarcinoma cell line with low cytotoxicity against NIH/3T3 cells.

Given the global public health problems that generate antimicrobial and parasitic infections and the wide medical application of thiazoles, nitrogen mustard, and 2-pyrazoline derivatives, we describe here the synthesis of novel thiazole-2-nitrogen mustards for the design of new series of *N*-substituted thiazolyl-pyrazolines as potential antimicrobial and antiprotozoal agents.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthetic routes employed for the synthesis of the target derivatives **5/6/7/8/9a–g** are illustrated in Schemes 1–4. In Scheme 1, the intermediate 2,4-dichlorothiazole-5-carbaldehyde **2** was prepared by Vilsmeier–Haack reaction of 1,3-thiazolidine-2,4-dione **1** with dimethylformamide and $POCl_3$ according to the methodology reported in the literature.[40] The preparation of the thiazole-2-nitrogen mustard **3** was initially conducted in dichloromethane and triethylamine, the reaction mixture was stirred at room temperature for 7 days, and the residue was purified by column chromatography using $CHCl_3$ as eluent to provide compound **3** in 45% yield. In an attempt to develop an efficient procedure to obtain compound **3**, the reaction was conducted under basic reaction conditions using NaOH or K_2CO_3 as reported for the synthesis of 2-substituted 4-chloro-1, 3-thiazole-5-carbaldehydes with amines and thiophenols.[40–43] However, when the reaction mixture was refluxed in the presence of NaOH in acetonitrile for 3 hr, the thin-layer chromatography (TLC) analysis showed the formation of by-products without consumption

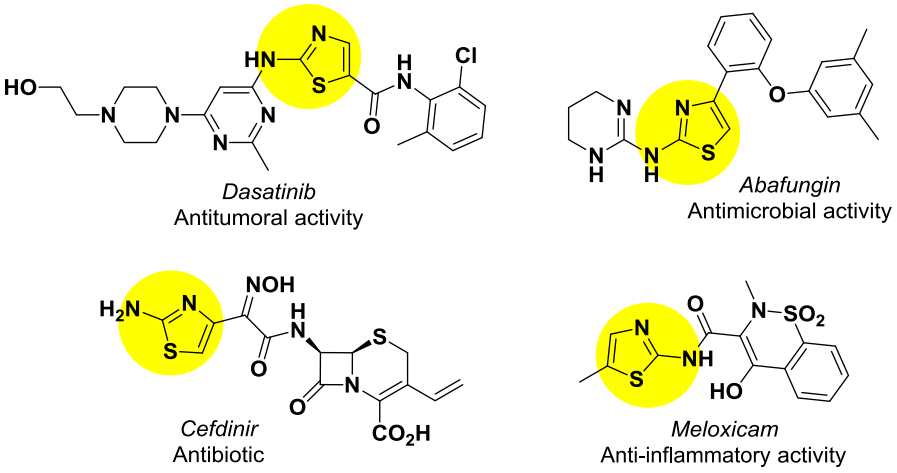


FIGURE 1 Structures of drugs containing the 2-aminothiazole scaffold

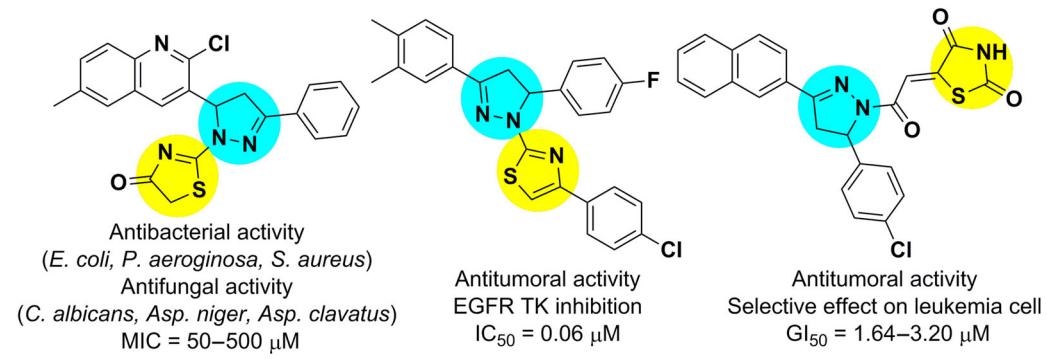
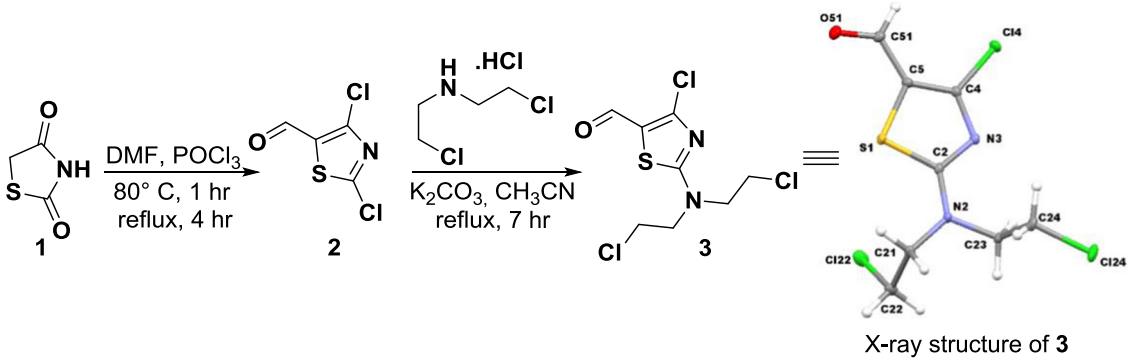


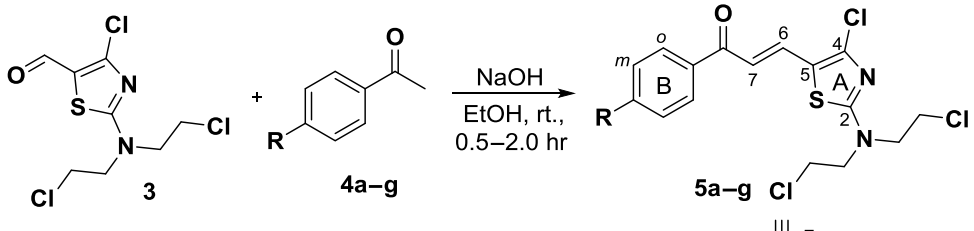
FIGURE 2 Structures of thiazolyl-pyrazolines derivatives with notable biological activity. EGFR, epidermal growth factor receptor; MIC, minimal inhibitory concentration; TK, tyrosine kinase



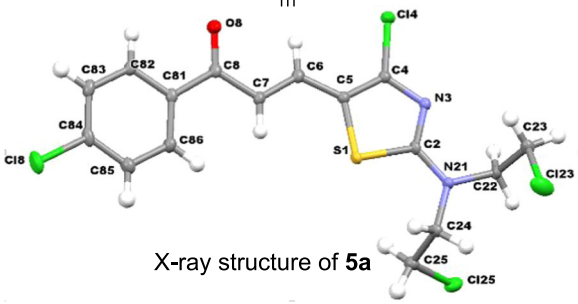
SCHEME 1 Synthesis of aldehyde **3** from 1,3-thiazolidine-2,4-dione **1**

of aldehyde **2**. Better results were obtained with K_2CO_3 in acetonitrile under reflux, reducing the reaction time to 7 hr and affording the thiazole-2-nitrogen mustard **3** in 44% yield. The structure of compound **3** was unambiguously determined by single-crystal X-ray diffraction. The Oak Ridge Thermal Ellipsoid Plot (ORTEP) drawing of the structure for compound **3** is shown in Scheme 1.

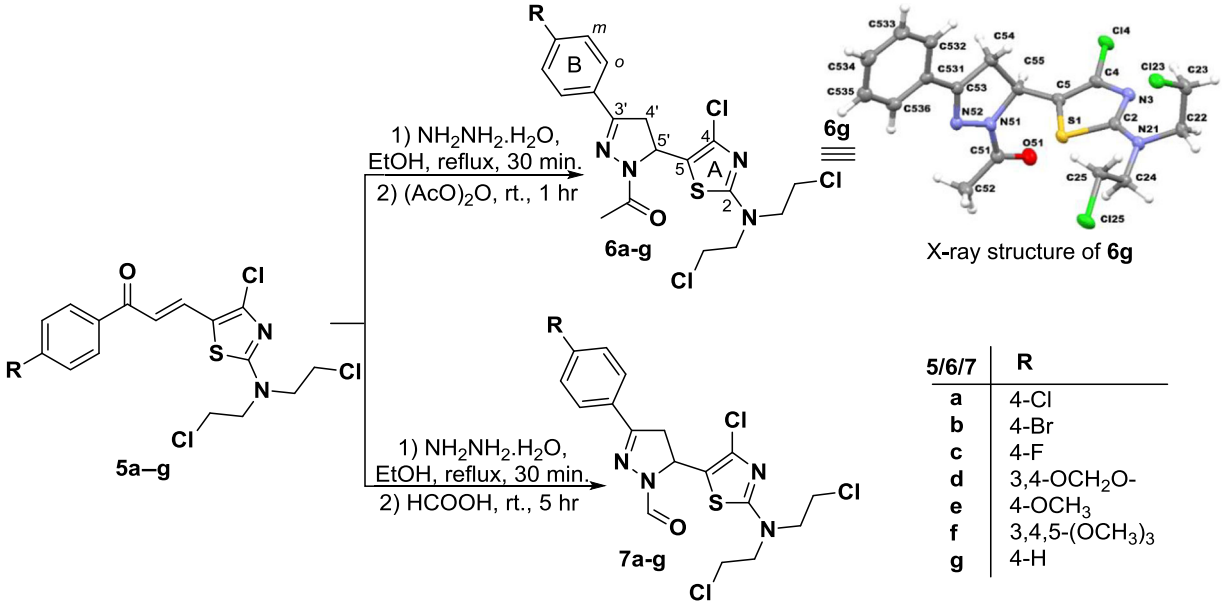
Prompted by the above-mentioned biological activity, thiazole-2-nitrogen mustard **3** was used as a core scaffold to prepare new chalcone–thiazole hybrids **5a–g**. Thus, compound **3** was subjected to a Claisen–Schmidt condensation with acetophenone **4a** using NaOH in ethanol at room temperature for 0.5 hr (Scheme 2). Upon consumption of compound **3** (TLC analysis), the reaction mixture was



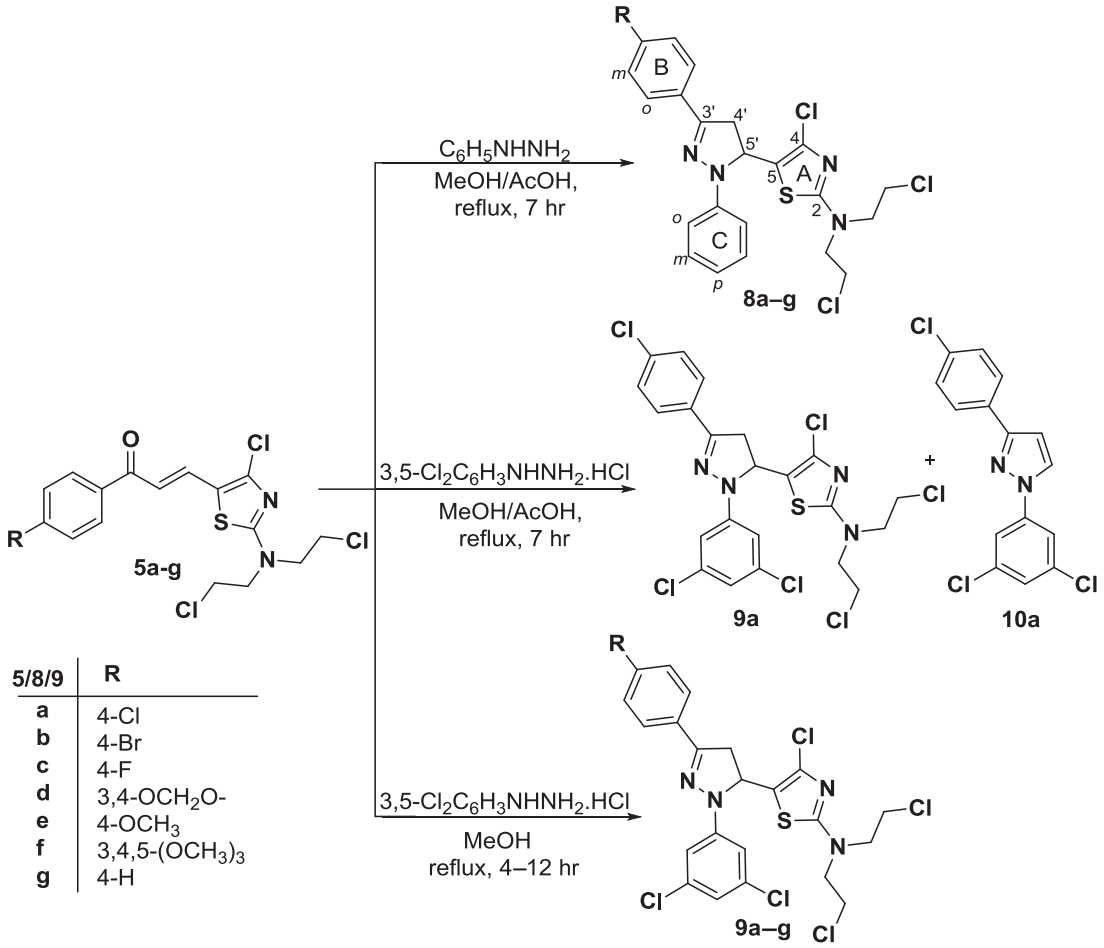
4/5	R
a	4-Cl
b	4-Br
c	4-F
d	3,4-OCH_2O-
e	4-OCH_3
f	3,4,5-$(OCH_3)_3$
g	4-H



SCHEME 2 Synthesis of substituted chalcones **5a–g** by Claisen–Schmidt condensation of compound **3** and acetophenones **4a–g**



SCHEME 3 Synthesis of *N*-acetyl and *N*-formyl pyrazolines **6/7a–g**



SCHEME 4 Synthesis of compounds **8a–g** and **9a–g**

filtered and washed with ethanol and water to afford **5a** in a 90% yield. It should be mentioned that longer reaction times led to the formation of some by-products. By using this methodology and appropriate acetophenones **4a–g**, we synthesized the set of hybrids **5a–g** in good yields (90–50%).

The structural elucidation of compounds **5a–g** was performed by spectroscopic techniques, Fourier-transform infrared spectroscopy (FTIR), ^{1}H NMR (nuclear magnetic resonance), ^{13}C NMR, and mass spectrometry. Compounds **5a–g** showed similar spectroscopic data; therefore, compound **5a** is discussed here as the representative of this series. Infrared (IR) spectrum of compound **5a** showed mainly absorption bands at 1,645, 1,586, and 1,529 cm^{-1}, assigned to the stretching vibration of C═O, C═N, and C═C bonds. In the ^{1}H NMR spectrum, a multiplet between 7.96 and 7.87 ppm integrating for three protons corresponding to protons H_6 and H_{Bo}, a doublet at 7.45 ppm with ^{3}J = 8.3 Hz assigned to two protons H_{Bm}, and a doublet at 6.82 ppm with coupling constant ^{3}J = 15.0 Hz for proton H_7 were observed, indicating the *E* configuration of H_6 and H_7 in the α,β-unsaturated fragment. The ^{13}C NMR spectrum showed 12 signals for carbons of compound **5a**. In addition, the molecular structure and conformation were confirmed for X-ray analysis, and its ORTEP drawing is shown in Scheme 2.

Once we obtained the chalcone–thiazole hybrids bearing nitrogen mustard **5a–g**, we performed the synthesis of diverse *N*-acetyl and *N*-formyl pyrazolines **6/7** by using reported procedures.[44,45] For the synthesis of *N*-acetyl derivatives **6a–g**, chalcones **5a–g** were treated with hydrazine hydrate in EtOH under reflux, resulting in the formation of the corresponding *N*-H pyrazolines, followed by addition of acetic anhydride via "one-pot" reaction, affording the pyrazoline derivatives **6a–g** in good yields (70–52%). On the basis of these results, the reaction was extended to the synthesis of *N*-formyl pyrazolines (Scheme 3) in the presence of formic acid in place of acetic anhydride and the desired products **7a–g** were successfully obtained in good yields (76–52%).

Compounds **6/7a–g** were fully characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The IR spectra of the series **6/7a–g** show a sharp band at 1653–1678 cm^{-1}, assigned to the stretching vibration of the C═O bond. In the ^{1}H NMR spectra for *N*-acetyl pyrazolines **6a–g**, a singlet for methyl protons of acetyl group was observed at 2.24–2.27 ppm, whereas the ^{1}H NMR spectra for *N*-formyl pyrazolines **7a–g** showed a singlet for formyl proton at 8.86–8.90 ppm. In all cases, the methylene diastereotopic protons $H_{4a'}$ and $H_{4b'}$ (H_A and H_M), and the stereogenic proton $H_{5'}$ (H_X) appear as three double-doublets, which form AMX spin systems. The ^{13}C NMR spectra showed all the expected signals for carbons of compounds **6/7a–g**. Compound **6g** gave suitable crystals for X-ray analysis by slow evaporation technique from EtOH, confirming the proposed structure and its conformation, which is shown in Scheme 3.

According to recent papers on *N*-aryl pyrazolines, the introduction of the aryl group at the *N*-1 position of the dihydropyrazole ring leads to biological activity improvement.[33,45–48] However, chlorophenyl derivatives may enhance potency and binding efficiency compared to their unsubstituted phenyl derivatives.[2,49,50] In addition, these derivatives show an improvement in metabolic stability and pharmacokinetic profile, as recently demonstrated by Eggert et al.,[49] who identified that the 3,4-dichloro derivative (IC_{50} = 0.08 μM) is more active than the corresponding 4-chloro (IC_{50} = 0.8 μM) and unsubstituted phenyl derivatives (IC_{50} > 20 μM) against SMYD2 for the treatment of cancer. To test this hypothesis, we decided to synthesize the novel *N*-phenyl and *N*-3,5-dichlorophenyl derivatives to evaluate the effect of the aryl and the chlorine substituents on biological activities.

Therefore, we carried out the reaction of chalcone–thiazole hybrids **5a–g** with phenylhydrazine in the presence of acetic acid in MeOH under reflux, which afforded *N*-phenylpyrazoline **8a–g** (Scheme 4) in good yields (66–50%). A similar reaction with 3,5-dichlorophenylhydrazine showed the formation of two products corresponding to the analogous *N*-3,5-dichlorophenyl pyrazoline **9a** and pyrazole **10a** (Scheme 4). Then, we attempted the reaction in glacial acetic acid as a solvent, and after 8 hr, 3-(4-chlorophenyl)-1-(3,5-dichlorophenyl)-1*H*-pyrazole **10a** was obtained with the absence of *N*-3,5-dichlorophenyl pyrazoline **9a**. However, when the reaction was performed in MeOH, compound **9a** was obtained in 88% yield (Scheme 4). Therefore, compounds **9a–g** were prepared from chalcones **5a–g** and 3,5-dichlorophenylhydrazine hydrochloride in MeOH (Scheme 4). Compounds with electron-withdrawing groups **5a** (4-Cl), **5b** (4-Br), and **5c** (4-F) had to be kept 12 hr under reflux to allow the cyclization of the hydrazine intermediate to the desired products **9a**, **9b**, and **9c**, respectively. However, the reactions of 3,5-dichlorophenylhydrazine hydrochloride and chalcones with electron-donating groups **5d** (3,4-OCH_2O), **5e** (4-OCH_3), and **5f** (3,4,5-$(OCH_3)_3$), unsubstituted on phenyl ring **5g** (4-H), occurred more easily under reflux for 4 hr because their corresponding hydrazones were more soluble in the reaction medium, allowing their cyclization in shorter reaction times. Under these conditions, the *N*-3,5-dichlorophenyl pyrazolines **9a–g** were obtained in good to excellent yields (90–59%).

The structural assignment of compound **10a** was performed on the basis of NMR data and mass spectrometry. In the ^{1}H NMR spectrum, two doublets for the protons of pyrazole ring $H_{5'}$ and $H_{4'}$ at 8.73 and 7.15 ppm, with coupling constant ^{2}J = 2.6 Hz, and two doublets at 8.05 and 7.56 ppm, with coupling constant J = 1.8 Hz, corresponding to H_{Co} and H_{Cp}, respectively, were observed. At 7.99 and 7.52 ppm, two doublets with coupling constant J = 8.5 Hz, which were assigned to aromatic protons H_{Bo} and H_{Bp}, were observed. Furthermore, the ^{13}C NMR spectrum of compound **10a** showed all the expected signals.

Regarding the structural elucidation of *N*-aryl pyrazolines **8a–g** and **9a–g**, we will discuss compound **8a** as the representative of these series (**8/9a–g**). The IR spectrum of **8a** showed absorption bands between 1,522 and 1,597 cm^{-1}, associated to the stretching vibrations of the C═N and C═C bonds. In the ^{1}H NMR spectrum, the signals of $H_{4a'}$ (H_A) appeared as a double-doublet at 3.17 ppm with coupling constant $^{2}J_{AM}$ = 17.1 Hz and $^{3}J_{AX}$ = 6.6 Hz; the signals of $H_{4b'}$ (H_M) and CH_2–N appeared as a multiplet between 3.78 and 3.70 ppm integrating for five protons, and the corresponding signal of $H_{5'}$ (H_X)

appeared as a double-doublet at 5.51 ppm with coupling constant $^3J_{MX}$ = 11.8 Hz and $^3J_{AX}$ = 6.6 Hz. The ^{13}C NMR spectrum showed 16 signals for carbons of compound **8a**.

2.2 | Biology

2.2.1 | Antifungal activity

According to the literature, the 2-pyrazoline moiety confers interesting biological properties to compounds, including the antifungal activity.[18,33,34] Compounds **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** were tested for antifungal properties against two clinically important fungal species, *Candida albicans* and *Cryptococcus neoformans*, which are involved in invasive fungal infections in immunocompromised patients, leading to high risk of morbidity and mortality.[51–53]

The antifungal activity was evaluated by the *Broth Microdilution Method*, *M27* (4th ed.) for yeasts of Clinical and Laboratory Standards Institute (CLSI).[54] The percentages of growth inhibition for each fungus were determined for all compounds at the concentration range of 3.9–250 μg/ml, which allowed to establish IC_{50} values of each compound. IC_{50} represents the minimum inhibitory concentration of each compound that inhibits 50% of fungal growth. Compounds with $IC_{50} > 250$ μg/ml were considered inactive; with $250 \geq IC_{50} > 125$ μg/ml, marginally active; with $125 \geq IC_{50} > 31.2$ μg/ml, moderately active, and with $IC_{50} \leq 31.2$ μg/ml, highly active. Amphotericin B was used as drug control and displayed 100% inhibition at all the concentrations tested.

Among the synthesized compounds, aldehyde **3** and chalcone–thiazole hybrids **5a–g** were inactive ($IC_{50} > 250$ μg/ml) against *C. albicans* and *C. neoformans*, suggesting that thiazole-2-nitrogen mustard and prop-2-en-1-one moieties do not confer an antifungal effect. Table 1 shows the IC_{50} values of compounds **6/7/8/9a–g** against *C. albicans* and *C. neoformans*.

Regarding the behavior of **6/7/8/9a–g** against *C. albicans*, almost all the compounds were inactive ($IC_{50} > 250$ μg/ml), and only compound **8e** (4-OCH_3) was marginally active (IC_{50} = 125 μg/ml). Instead, *C. neoformans* were highly sensitive to compounds **8a–g** and **9d–g** but not to *N*-acetyl **6a–g** and *N*-formyl pyrazolines **7a–g**. From these results, it is clear that cyclization of the chalcones–thiazole hybrids **5a–g** into their pyrazoline derivatives increases the antifungal effects of the compounds.

For a better analysis of the results, the percentages of inhibition of compounds **8a–g** and **9a–g** against *C. neoformans* were recorded in dose–responses curves (Figure 3).

Figure 3 clearly shows that five compounds (**8a,b,d,e,g**), over the seven compounds, **8a–g**, display high *C. neoformans* inhibition with IC_{50} = 15.6–31.2 μg/ml. The remaining two compounds, **8c** (4-F) and **8f** (3,4,5-$(OCH_3)_3$), were moderately active with IC_{50} = 62.5 and 125 μg/ml, respectively. Interestingly enough, three compounds, **9e–g** (4-OCH_3, 3,4,5-$(OCH_3)_3$, and 4-H), over the seven compounds, **9a–g**, showed the highest activity (IC_{50} = 3.9–7.8 μg/ml) of all series. Compound **9d** (3,4-OCH_2O–) showed marginal activity (IC_{50} = 250 μg/ml) and compounds **9a–c** (4-Cl, 4-Br, 4-F) are inactive ($IC_{50} > 250$ μg/ml).

These results clearly show that all thiazolyl-pyrazolines, **8a–g**, containing an unsubstituted phenyl ring as the azole *N*-substituent R_1 display high activities against *C. neoformans* with IC_{50} in the range 15.6–125 μg/ml. The substituent R does not appear to have a key role in the anticryptococcal activity of compound **8**, as the IC_{50} values of **8a** (4-Cl) and **8b** (4-Br) derivatives (IC_{50} = 15.6 μg/ml) differ in only one dilution with the IC_{50} of **8g** (4-H), **8d** (3,4-OCH_2O–), or **8e** (4-OCH_3) derivatives (IC_{50} = 31.25 μg/ml), which is considered the error of the method.[55] So, almost all compounds **8** can be considered with similar anticryptococcal activity.

However, it is important to note that among *N*-3, 5-dichlorophenyl pyrazolines, **9a–g**, compounds **9e** (4-OCH_3), **9f** (3,4,5-$(OCH_3)_3$), and **9g** (4-H) possess the highest activities of all studied compounds against *C. neoformans*, with IC_{50} values of 7.8, 3.9, and 7.8 μg/ml, respectively. These results suggest that the occurrence of 3,5-dichlorophenyl as the substituent R_1 along with 4-OCH_3 (**9e**) or 3,4,5-$(OCH_3)_3$ (**9f**) or 4-H (**9g**) in the same molecule leads to an increase in the anticryptococcal activity of these compounds, thus opening an avenue for further research. Table 2 shows the percentages of inhibition of these three compounds at different concentrations, which are also reflected in the dose–response curves of Figure 3.

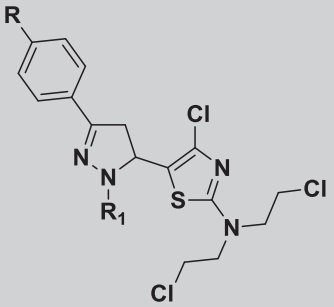
2.2.2 | Antibacterial activity

All synthesized compounds (**3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, **9a–g**) were tested for antibacterial activity against wild-type and multidrug-resistant Gram-negative and -positive bacteria including methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *K. pneumoniae* BAA 1705, and *Neisseria gonorrhoeae*. Minimum inhibitory concentration (MIC) was determined by broth microdilution in those compounds with reproducible antimicrobial effects in the screening, as reported in Table 3. The agar microdilution test was performed for *N. gonorrhoeae*.

Compounds *N*-acetyl **6** and *N*-formyl pyrazoline **7** were more active than their *N*-aryl derivatives **8/9**, particularly against MSSA, MRSA, VISA, and *N. gonorrhoeae*, where **8a** and **8b** showed discrete inhibition against VISA with MIC values of 250 and 500 μg/ml, and inhibited *N. gonorrhoeae* with MIC values of 250 and 500 μg/ml, respectively. However, the introduction of chlorine atoms in the *N*-aryl group abolishes the antibacterial effects of compounds **9a–g**, which were inactive against all bacterial strains tested, which is in contrast to those results obtained for antifungal activity.

Compounds **6a** (4-Cl, *N*-acetyl), **6b** (4-Br, *N*-acetyl), **7a** (4-Cl, *N*-formyl), **7b** (4-Br, *N*-formyl), **7c** (4-F, *N*-formyl), and **7g** (4-H, *N*-formyl) evidenced good or discrete activity against MSSA, MRSA, VISA, and *N. gonorrhoeae*, ranging from 31.5 to 250 μg/ml, indicating that the presence of halogens in the structure improves the antibacterial effect. The best antibacterial activity was displayed by compound **7a** (4-Cl), which showed inhibitory activity against MSSA ATCC 25923 (MIC = 62.5 μg/ml), as well as two multidrug-resistant

TABLE 1 Antifungal activity of thiazolyl-pyrazoline derivatives **6/9a–g** against *Candida albicans* ATCC 10231 and *Cryptococcus neoformans* ATCC 32264 expressed in IC_{50} (μg/ml)



Compound	R	R_1	*C. albicans* ATCC 10231	*C. neoformans* ATCC 32264
6a	4-Cl	$-COCH_3$	250	250
6b	4-Br	$-COCH_3$	>250	250
6c	4-F	$-COCH_3$	>250	>250
6d	3,4-OCH_2O–	$-COCH_3$	>250	>250
6e	4-OCH_3	$-COCH_3$	>250	>250
6f	3,4,5-$(OCH_3)_3$	$-COCH_3$	>250	>250
6g	4-H	$-COCH_3$	>250	>250
7a	4-Cl	–CHO	>250	250
7b	4-Br	–CHO	>250	250
7c	4-F	–CHO	>250	250
7d	3,4-OCH_2O–	–CHO	>250	>250
7e	4-OCH_3	–CHO	>250	250
7f	3,4,5-$(OCH_3)_3$	–CHO	>250	250
7g	4-H	–CHO	>250	250
8a	4-Cl	$-C_6H_5$	250	**15.6**
8b	4-Br	$-C_6H_5$	250	**15.6**
8c	4-F	$-C_6H_5$	>250	**62.5**
8d	3,4-OCH_2O–	$-C_6H_5$	250	**31.25**
8e	4-OCH_3	$-C_6H_5$	**125**	**31.25**
8f	3,4,5-$(OCH_3)_3$	$-C_6H_5$	250	**125**
8g	4-H	$-C_6H_5$	250	**31.25**
9a	4-Cl	3,5-$Cl_2C_6H_3$	>250	>250
9b	4-Br	3,5-$Cl_2C_6H_3$	>250	>250
9c	4-F	3,5-$Cl_2C_6H_3$	>250	>250
9d	3,4-OCH_2O–	3,5-$Cl_2C_6H_3$	250	250
9e	4-OCH_3	3,5-$Cl_2C_6H_3$	250	**7.8**
9f	3,4,5-$(OCH_3)_3$	3,5-$Cl_2C_6H_3$	250	**3.9**
9g	4-H	3,5-$Cl_2C_6H_3$	250	**7.8**
Amphotericin B			0.5	0.5

Note: Active compounds are highlighted.

Abbreviations: ATCC, American Type Culture Collection. IC_{50}, inhibitory concentration of each compound that inhibits 50% of fungal growth.

strains, MRSA ATCC 43300 (MIC = 125 μg/ml) and VISA (MIC = 31.5 μg/ml), and showed discrete inhibition against *N. gonorrhoeae* (MIC = 500 μg/ml). Compound **7b** (4-Br) was also active against the multidrug-resistant bacterium, VISA (MIC = 31.5 μg/ml), and showed discrete inhibition against *N. gonorrhoeae* (MIC = 250 μg/ml).

For *N. gonorrhoeae*, compounds **7g** and **8a** exhibited MIC values of 125 μg/ml, being the most active against this bacterium, which is of great interest due to the emergence of multidrug-resistant strains for almost all antibiotics.[56] None of the tested compounds were active against *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. However, it should be noted that compounds **6a** (4-Cl), **6b** (4-Br), **7a** (4-Cl), **7b** (4-Br), **7c** (4-F), and **7g** (4-H) inhibited the multidrug-resistant strain VISA. Therefore, those compounds have good potential as a starting point to develop novel anti-VISA agents.

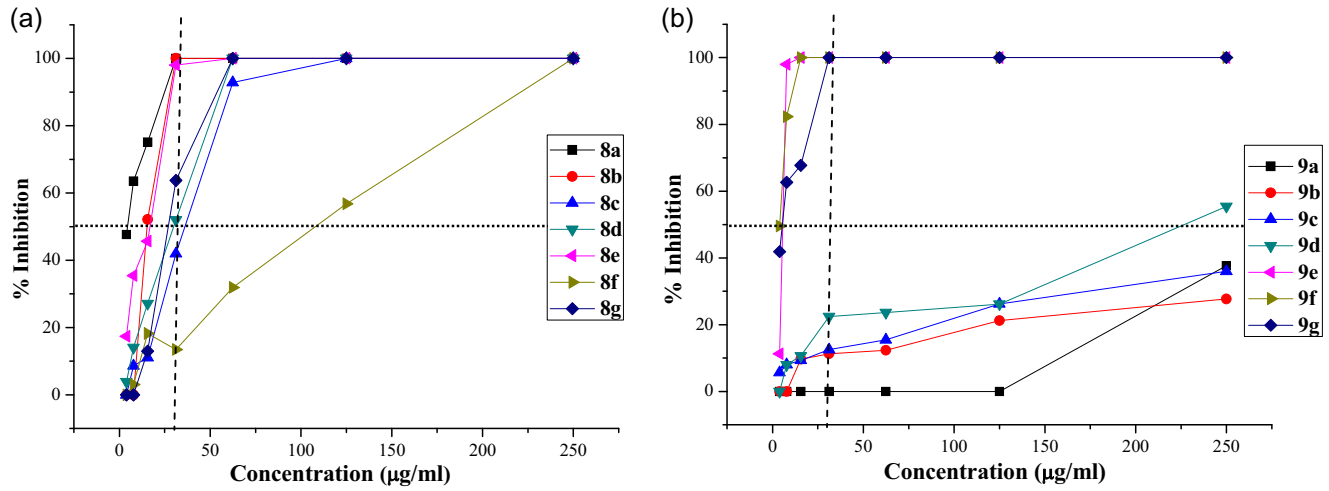


FIGURE 3 Comparative antifungal activities of (a) *N*-phenyl **8a–g** and (b) *N*-3,5-dichlorophenyl **9a–g** pyrazolines against *Cryptococcus neoformans* ATCC 10231. The horizontal dotted lines show 50% inhibition. The vertical dotted lines show the concentration 31.25 µg/ml. Compounds with $IC_{50} \leq 31.2$ µg/ml are considered highly active

2.2.3 | Hemolytic activity

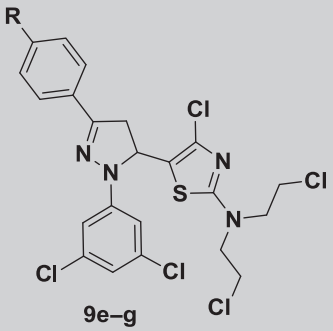
All compounds were tested on human red blood cells (RBCs) to evaluate the toxicity of each compound to induce irreversible damages in the membrane of erythrocytes by the release of hemoglobin and intracellular components.[57,58] Results show that compounds **3**, **5a–d**, **5f–g**, **6a–c**, **6e–g**, **7a–b**, **7d–g**, **8c–g**, and **9a–g** lead to 100% hemolysis at a concentration of ≥ 200 µM, indicating that these compounds would be nontoxic. Compounds **5e**, **6d**, **7c**, and **8a–b** were considered moderately toxic. However, no hemolytic toxicity was observed below 100 µM, as shown in Table 4. Interestingly, compounds **9e–g** were highly active against *C. neoformans*, with IC_{50} values between 3.9 and 7.8 µM and low cytotoxic effects on erythrocytes, which suggests that these compounds can be considered as important structural scaffolds for the development of anticryptococcal agents. However, compounds **6a**, **6b**, **7a**, **7b**, and **7g** that showed antibacterial activity against MSSA, MRSA, VISA, and *N. gonorrhoeae* exhibited little or no toxicity on red blood cells and membranes at concentrations used in therapeutics. These low hemolytic activities offer a promising potential to the development of biological agents.

2.2.4 | Antiprotozoal activity

Cytotoxic activity in human macrophages U-937

All synthesized compounds were screened for their cytotoxicity on U-937 human cells at four serial dilution concentrations (200, 50, 12.5, and 3.125 µM) to determine the 50% lethal concentration

TABLE 2 Percentages of inhibition of *N*-3,5-dichlorophenyl pyrazolines **9e–g** against *Cryptococcus neoformans* ATCC 32264



9e–g

		Concentrations (µg/ml)						
	R	250	125	62.5	31.25	15.62	7.81	3.9
9e	4-OCH_3	100	100	100	100	100	97.99 ± 0.81	11.31 ± 0.39
9f	3,4,5-$(OCH_3)_3$	100	100	100	100	100	82.3 ± 1.06	49.55 ± 0.45
9g	4-H	100	100	100	100	67.73 ± 0.26	62.7 ± 0.31	41.86 ± 0.19
Amphotericin B		100	100	100	100	100	100	100

TABLE 3 Antibacterial activity of novel thiazole derivatives **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g** and **9a–g** expressed in MIC (μg/ml)[a,b]

Compound	*S. aureus* ATCC 25923 (MSSA)	*S. aureus* ATCC 43300 (MRSA)	VISA	*Neisseria gonorrhoeae* ATCC 31426
6a	250	250	**125**	250
6b	250	250	**125**	–
6c	–	–	–	–
6d	500	500	250	–
6e–g	–	–	–	–
7a	**62.5**	**125**	**31.25**	500
7b	**125**	**125**	**31.25**	250
7c	250	250	**125**	250
7d–f	–	–	–	–
7g	250	–	**62.5**	**125**
8a	500	500	250	**125**
8b	–	–	500	250
8c–g	–	–	–	–
9a–g	–	–	–	–

Abbreviations: ATCC, American Type Culture Collection; MIC, minimal inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; VISA, vancomycin-intermediate *Staphylococcus aureus*.

[a]Minimal inhibitory concentration of each compound that inhibits visible bacterial growth. Active compounds are highlighted.

[b]Compounds **3** and **5a–g** were inactive against wild-type and multidrug-resistant Gram-negative and -positive bacteria-inactive compound (MIC > 1,000 μg/ml). None of the tested compounds were active against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *K. pneumoniae* BAA 1705.

TABLE 4 Hemolytic activity for compounds **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** expressed in LC_{50} (μM)[a]

Compound	LC_{50}[a]	Compound	LC_{50}
3	**>200**	**7f**	**242.77 ± 50.82**
5a	**>200**	**7g**	**>200**
5b	**>200**	**8a**	100.00
5c	**>200**	**8b**	**134.64 ± 8.32**
5d	**>200**	**8c**	**796.97 ± 230.92**
5e	198.74 ± 37.28	**8d**	**352.23 ± 76.89**
5f	**867.03 ± 265.41**	**8e**	**338.63 ± 76.13**
5g	**>200**	**8f**	**730.56 ± 253.93**
6a	**293.31 ± 36.77**	**8g**	**419.68 ± 143.67**
6b	**526.00 ± 26.24**	**9a**	**>200**
6c	**>200**	**9b**	**>200**
6d	100.00	**9c**	**>200**
6e	**269.28 ± 37.76**	**9d**	**278.80 ± 4.18**
6f	**>200**	**9e**	**>200**
6g	**>200**	**9f**	**867.03 ± 187.03**
7a	**464.12 ± 94.89**	**9g**	**300.30 ± 76.02**
7b	**225.43 ± 12.75**		
7c	161.02 ± 16.48		
7d	**>200**		
7e	**>200**		

Note: Data represent mean value ± standard deviation. Bold values are the nontoxic compounds.

[a]Lethal concentration on human red blood cells.

(LC_{50}). Compounds with LC_{50} < 100 μM were considered as potentially cytotoxic, with 100 ≤ LC_{50} < 200 μM as mildly cytotoxic, and with LC_{50} ≥ 200 μM as noncytotoxic. As can be seen in Table 5, almost all the thiazole derivatives, **3**, **5a–g**, **6a–g**, **7a–c**, **7e–g**, **8a–g**, and **9a–e**, displayed high cytotoxic activity on human macrophages U-937. However, compounds **7d** (3,4-OCH_2O–), **9f** (3,4,5-$(OCH_3)_3$), and **9g** (4-H) were mildly cytotoxic (LC_{50} > 100 μM). Also, it is relevant to highlight the potentially cytotoxic activity of amphotericin B (LC_{50} = 49.78 μM) and doxorubicin (LC_{50} = 0.90 μM), which are important commercial drugs.

Antiplasmodial activity

On the basis of the large number of biological agents containing thiazole ring and the increasingly numerous examples of thiazole derivatives that are active as antimalarial agents,[11–13] it was envisaged that the antiplasmodial activity of these novel thiazole derivatives would be worthwhile. The antiplasmodial activity was tested on asynchronic cultures of *Plasmodium falciparum* (3D7 strain, sensitive to chloroquine [CQ]) at serial dilution for all compounds, which allowed to determine the EC_{50} values of each compound (Table 6). Compounds with EC_{50} ≤ 25 μM were considered highly active. From these results, it is found that compounds **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** were highly active against *P. falciparum*, being more active than aldehyde **3**, suggesting that thiazole-2-nitrogen mustard moiety does not confer antiplasmodial activity, but the activity can be tuned with the introduction of the prop-2-en-1-one and pyrazoline moieties. The substituents R and R_1 do not seem to have a role in the activity. Compound **8g** (4-H) was the most active among the thiazole derivatives, with EC_{50} value of 10.28 μM. However, compounds **7d** (3,4-OCH_2O–) and **9g** (4-H) showed a selective index (SI) higher than 10, being more active against *P. falciparum* than U-937 human cells, with EC_{50} values of 12.24 μM (LC_{50} = 142.82 μM, SI > 11.66) and 11.80 μM (LC_{50} = 182.98 μM, SI = 15.50), respectively, indicating that both compounds (**7d** and **9g**) have potential for the development of antimalarial agents.[59]

Antileishmanial activity

Antileishmanial activity of **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** were evaluated in intracellular amastigotes of *Leishmania (V) panamensis* (MHOM/CO/87/UA140–EpiR–GFP strain), and the results are summarized in Table 7. Most compounds exhibited high activity against *Leishmania* amastigotes (EC_{50} ≤ 25 μM), showing an SI ≥ 1. Interestingly, chalcone–thiazole hybrids (**5**) showed higher antileishmanial activity than *N*-aryl pyrazolines (**8/9**). As can be seen in Table 7, compounds **8b** (4-Br), **8f** (3,4,5-$(OCH_3)_3$), and **9d** (3,4-OCH_2O–) also showed remarkable activity, with EC_{50} values of 10.62, 6.46, and 10.56 μM, respectively. The results obtained with aldehyde **3** indicate that thiazole-2-nitrogen mustard moiety does not confer antileishmanial activity, but its derivatives are highly active against

TABLE 5 In vitro cytotoxicity on U-937 human cells of novel thiazole derivatives **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** expressed in LC_{50} (μM)[a]

Compound	LC_{50}[a]	Compound	LC_{50}
3	17.68 ± 6.25	**7f**	21.45 ± 2.52
5a	8.90 ± 0.24	**7g**	10.64 ± 0.89
5b	13.49 ± 2.27	**8a**	24.07 ± 10.72
5c	25.30 ± 7.48	**8b**	17.49 ± 0.43
5d	62.61 ± 22.35	**8c**	15.81 ± 1.93
5e	10.85 ± 0.55	**8d**	17.44 ± 3.02
5f	19.06 ± 3.47	**8e**	50.18 ± 9.50
5g	8.51 ± 0.88	**8f**	20.64 ± 3.04
6a	7.80 ± 0.20	**8g**	22.96 ± 5.18
6b	13.48 ± 2.19	**9a**	19.18 ± 2.08
6c	6.93 ± 0.40	**9b**	19.17 ± 4.85
6d	14.21 ± 1.70	**9c**	23.40 ± 9.71
6e	11.07 ± 1.50	**9d**	34.69 ± 6.87
6f	25.84 ± 3.91	**9e**	40.25 ± 6.81
6g	9.73 ± 1.77	**9f**	**101.30 ± 9.67**
7a	15.69 ± 3.47	**9g**	**182.98 ± 1 8.17**
7b	35.15 ± 8.57	Doxorubicin	0.90 ± 0.07
7c	8.00 ± 0.86	Amphotericin B[b]	49.78 ± 6.8
7d	**142.82 ± 16.32**	Benznidazole[c]	>200
7e	21.81 ± 3.02	CQ[d]	178.51 ± 5.98

Note: Data represent mean value ± standard deviation. Bold values are the nontoxic compounds.
[a]Lethal concentration on human cell U-937.
[b]Antileishmanial drug control.
[c]Antitrypanosomal drug control.
[d]Chloroquine diphosphate salt (CQ): Antimalarial drug control.

Leishmania amastigotes. These findings suggest that the prop-2-en-1-one and pyrazoline fragments significantly improve the antileishmanial activity. This could be due to the presence of the α,β-unsaturated moiety of the chalcone that easily reacts with thiol and amino groups.[7] Also, 2-pyrazolines (nitrogen-containing heterocyclic compound) could form hydrogen bonding[60] in the active site of the enzymes of *Leishmania*.

Antitrypanosomal activity

All compounds were screened on intracellular amastigotes of *Trypanosoma cruzi*, Tulahuen strain transfected with β-galactosidase gene. The activity was determined according to the ability of the compound to reduce the infection of U-937 cells by *T. cruzi*. Results are summarized in Table 8. The new 21 compounds evidence good activity against *T. cruzi*, with EC_{50} ($EC_{50} \leq 25$ μM). Compounds **5a** (4-Cl), **5g** (4-H), **6c** (4-F), and **7c** (4-F) were highly active against *T. cruzi*, with EC_{50} value between 4.98 and 10.37 μM, showing better activity than the benznidazole (EC_{50} = 13.34 μM), which was used as drug control. Compound **7c** is a particularly interesting compound, because it exhibits the highest trypanosomicidal activity (EC_{50} = 4.98 μM), which is approximately 2.6-fold more potent than benznidazole with an SI of 1.61 on U-937 human cells and has potential as a novel antitrypanosomal agent.

Finally, if we compare the synthesized series, the presence of carbonyl group in chalcone–thiazole hybrids (**5a–g**) and *N*-acetyl and *N*-formyl pyrazolines (**6/7a–g**) increases or maintains the trypanosomicidal activity as compared to the *N*-phenyl and *N*-3,5-dichlorophenyl pyrazolines (**8/9a–g**). This behavior could be related to the number of hydrogen-bond acceptors of these compounds, as shown in Figure 4, which was generated using PyMOL.[61] Moreover, the substitution of the halogen atoms (4-Cl, 4-Br, and 4-F) by methylenedioxy, methoxy, and trimethoxy groups slightly diminishes the activity. An exception to this behavior is **6d** versus **6c**, where the methylenedioxy derivative was more active.

3 | CONCLUSIONS

A new series of nitrogen mustard class of thiazolyl-pyrazoline derivatives **6/9a–g** was obtained in good yields by reacting chalcone–thiazole hybrids **5a–g** with hydrazine derivatives under mild conditions. Compounds **3**, **5a**, and **6g** give good-quality single crystals, which confirm the molecular structure and conformation of the different derivatives by X-ray analysis. The antifungal activity studies revealed that all the *N*-aryl pyrazolines **8a–g**, possessing an unsubstituted phenyl ring as R1, display high anticryptococcal activities, with IC_{50} values between 15.6 and 125 μg/ml. However, the highest antifungal activity was displayed by the *N*-3,5-dichlorophenyl pyrazolines **9e**, **9f**, and **9g**, with IC_{50} values of 3.9–7.8 μg/ml against *C. neoformans* and **9f** and **9g** being important scaffolds with LC_{50} > 200 μM against human red blood cells. Moreover, the results on antibacterial activity showed that the *N*-formyl pyrazoline **7a** was the

TABLE 6 In vitro antimalarial screening of novel thiazole derivatives **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** expressed in EC_{50} (μM)[a]

Compound	EC_{50}[a]	IS[b]	Compound	EC_{50}	IS
3	25.35 ± 0.56	0.69	**7f**	**11.01 ± 0.36**	1.94
5a	**17.46 ± 1.59**	0.50	**7g**	**11.00 ± 0.34**	0.96
5b	**14.95 ± 0.92**	0.90	**8a**	**10.62 ± 0.15**	2.27
5c	**10.93 ± 0.61**	2.31	**8b**	**10.92 ± 0.13**	1.60
5d	**11.95 ± 0.06**	**5.23**	**8c**	**11.23 ± 0.49**	1.41
5e	**11.36 ± 0.75**	0.95	**8d**	**10.70 ± 0.12**	1.63
5f	**11.27 ± 0.25**	1.69	**8e**	**10.77 ± 0.29**	4.66
5g	**11.26 ± 0.63**	0.75	**8f**	**13.93 ± 0.85**	1.48
6a	**12.33 ± 0.47**	0.63	**8g**	**10.28 ± 0.36**	2.23
6b	**13.54 ± 0.25**	0.99	**9a**	**23.78 ± 1.02**	0.80
6c	**10.61 ± 0.11**	0.65	**9b**	**19.50 ± 1.18**	0.98
6d	**10.59 ± 0.36**	1.34	**9c**	**10.81 ± 0.33**	2.16
6e	**10.60 ± 0.20**	1.04	**9d**	**19.66 ± 2.49**	1.76
6f	**10.56 ± 0.35**	2.44	**9e**	**19.68 ± 1.90**	2.04
6g	**10.52 ± 0.22**	0.92	**9f**	**16.29 ± 1.05**	**6.21**
7a	**10.60 ± 0.27**	1.48	**9g**	**11.80 ± 0.35**	**15.50**
7b	**11.25 ± 0.72**	**3.12**	CQ[c]	4.25 ± 0.40	38.2
7c	**10.88 ± 0.41**	0.73			
7d	**12.24 ± 0.94**	**11.66**			
7e	**10.89 ± 0.40**	2.00			

Note: Data represent mean value ± standard deviation. Bold values are the most active compounds.
[a]Effective concentration (EC_{50}): Concentration of compounds that decrease 50% of intracellular parasite.
[b]Index of selectivity (IS) = LC_{50}/EC_{50}; LC_{50}: Lethal concentration on human cell U-937.
[c]Chloroquine diphosphate salt (CQ): Antimalarial drug control.

most active against MSSA, MRSA, and VISA, with MIC values of 62.5, 125, and 31.25 μg/ml, respectively. Compounds **7d** and **9g** showed remarkable activity, with EC_{50} values of 12.24 and 11.80 μM against *P. falciparum* and SI higher than 10. The best antileishmanial activity against *L. panamensis* was displayed by **8f**, with EC_{50} = 6.46 μM. Moreover, compound **7c** (4-F) was highly active against *T. cruzi*, with an EC_{50} value of 4.98 μM, being more active than the drug control (benznidazole). These compounds represent novel hits for the development of antimicrobial and antiprotozoal agents.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Reagents and solvents used were purchased from Sigma-Aldrich. Reactions were monitored by TLC on Merck silica gel 60 F_{254} aluminum plates. Melting points were measured in open glass capillaries using a Stuart SMP10 melting point apparatus. Attenuated total reflection (ATR)–FTIR spectra were obtained on a Shimadzu IRAffinity-1. ^1H and ^{13}C NMR spectra were run on a Bruker DPX 400 spectrometer operating at 400 and 100 MHz, respectively, using $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 as solvent and tetramethylsilane as an internal standard. Mass spectra were obtained on a Shimadzu–GCMS–QP2010 spectrometer operating at 70 eV. The elemental analyses were performed using a Thermo Finnigan Flash EA1112 CHN (STIUJA) elemental analyzer. The single-crystal X-ray data were collected in a diffractometer Bruker D8 Venture at "Centro de Instrumentación Científico y Técnico," (CICT) in "Universidad de Jaén" (UJA).

The original NMR spectra of the investigated compounds are provided as Supporting Information, as are their InChI codes together with some biological activity data.

4.1.2 | Preparation of 2-[bis(2-chloroethyl)amino]-4-chlorothiazole-5-carbaldehyde (3)

A mixture of 2,4-dichlorothiazole-5-carbaldehyde (300 mg, 1.65 mmol), bis(2-chloroethyl)amine hydrochloride (588 mg, 3.30 mmol), and K_2CO_3 (1,140 mg, 8.25 mmol) in acetonitrile (5.0 ml) was heated under reflux for 7 hr. After cooling, the reaction was quenched by the addition of water (20 ml) and extracted with dichloromethane (3 × 20 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexane/dichloromethane (4:10) to yield the desired product **3** as a pale yellow solid.

TABLE 7 Antileishmanial activity of thiazole derivatives **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** expressed in EC_{50} (μM)[a]

Compound	EC_{50}[a]	IS[b]	Compound	EC_{50}	IS
3	53.37 ± 45.51	0.33	**7f**	n.d.	–
5a	**>4**	<2.25	**7g**	n.d.	–
5b	**>7**	<1.92	**8a**	31.49 ± 6.43	0.76
5c	**>13**	<1.94	**8b**	**10.62 ± 1.06**	1.65
5d	>30	<2.09	**8c**	**16.95 ± 4.63**	0.93
5e	**>5**	<2.17	**8d**	**15.76 ± 3.07**	1.11
5f	**>10**	<1.91	**8e**	**20.20 ± 2.13**	2.48
5g	**>4**	<2.12	**8f**	**6.46 ± 0.05**	3.19
6a	**>4**	<1.95	**8g**	**10.14 ± 0.81**	2.26
6b	**>7**	<1.92	**9a**	41.81 ± 23.56	0.46
6c	**>3**	<2.31	**9b**	**17.78 ± 2.30**	1.08
6d	**>7**	<2.03	**9c**	**20.09 ± 3.54**	1.16
6e	n.d.	–	**9d**	**10.56 ± 0.28**	3.28
6f	n.d.	–	**9e**	**14.42**	2.77
6g	n.d.	–	**9f**	41.64 ± 6.63	2.43
7a	n.d.	–	**9g**	54.12 ± 10.21	3.38
7b	n.d.	–	Amphotericin B[c]	0.7 ± 0.07	51.7
7c	n.d.	–			
7d	n.d.	–			
7e	n.d.	–			

Note: Data represent mean value ± standard deviation. Bold values are the most active compounds.
>Maximum concentration evaluated because the host cell dies at higher concentrations.
Abbreviation: n.d., not determined.
[a]Effective concentration 50 (EC_{50}): Concentration of compounds that decrease 50% of intracellular parasite.
[b]Index of selectivity (IS) = LC_{50}/EC_{50}. LC_{50}: Lethal concentration on human cell U-937.
[c]Antileishmanial drug control.

*2-[Bis(2-chloroethyl)amino]-4-chlorothiazole-5-carbaldehyde (**3**)*

Pale yellow crystals; 42% yield; m.p. 115–116°C. FTIR (ATR) ν (cm^{-1}): 1,634 (C═O), 1,543 and 1,535 (C═N and C═C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.80 (s, 1H, CHO), 3.95 (t, J = 6.1 Hz, 4H, 2×CH_2–N), and 3.81 (t, J = 6.1 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 180.4 (CHO), 171.4 (C), 147.7 (C), 120.6 (C), 54.7 (CH_2), and 40.4 (CH_2). Mass spectrometry (electron impact) (MS [EI]): *m/z* 286/288/290/292 [M^+/M + 2^+/M + 4^+/M + 6^+] (28/28/9/1), 237/239/241 (100/68/13), 175/177 (93/33), 63 (83), 41 (18). Anal. calcd. for $C_8H_9Cl_3N_2OS$: C, 34.41; H, 3.15; N, 9.74; S, 11.15. Found: C, 34.42; H, 3.13; N, 9.73; S, 11.20. Crystals suitable for single-crystal X-ray diffractions analysis were obtained from slow evaporation in dichloromethane/ethanol and deposited in Cambridge Crystallographic Data Centre (CCDC) with deposition number 1919158.

4.1.3 | General procedure for the synthesis of [bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl-chalcones 5a–g

A mixture of 2-[bis(2-chloroethyl)amino]-4-chlorothiazole-5-carbaldehyde **3** (1.74 mmol), the selected acetophenone **4a–g** (1.95 mmol), and a pellet of NaOH in ethanol (20.0 ml) was stirred at room temperature for 0.5 to 2.0 hr. The precipitate formed was filtered and washed with ethanol and water to obtain compounds **5a–g** as pure yellow solids.

*(E)-3-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-1-(4-chlorophenyl)prop-2-en-1-one (**5a**)*

Yellow solid; 90% yield; m.p. 148–149°C. FTIR (ATR) ν (cm^{-1}): 3,044 (═C–H), 2,894 (C–H), 1,645 (C═O), 1,586 and 1,529 (C═N and C═C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.96–7.87 (m, 3H, H_6, H_{Bo}), 7.45 (d, J = 8.3 Hz, 2H, H_{Bm}), 6.82 (d, J = 15.0 Hz, 1H, H_7), 3.93 (t, J = 6.0 Hz, 4H, 2×CH_2–N), and 3.82 (t, J = 6.0 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 187.9 (C═O), 167.0 (C), 143.3 (C), 139.2 (C), 136.6 (C), 133.8 (CH), 129.8 (CH), 129.0 (CH), 119.0 (CH), 117.1 (C), 54.5 (CH_2), and 40.6 (CH_2). MS (EI): *m/z* 422/424/426/428/430 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (1.04/2.41/0.10/0.07/0.10), 237/239/241/243 (16/16/3/1), 97 (50), 83 (66), 57 (97), 43 (100), and 41 (69). Anal. calcd. for $C_{16}H_{14}Cl_4N_2OS$: C, 45.31; H, 3.33; N, 6.60; S, 7.56. Found: C, 45.29; H, 3.34; N, 6.59; S, 7.58. Crystals suitable for single-crystal X-ray diffraction analysis were obtained from slow evaporation in dichloromethane/ethanol and deposited in CCDC with deposition number 1919157.

*(E)-3-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-1-(4-bromophenyl)prop-2-en-1-one (**5b**)*

Yellow solid; 86% yield; m.p. 150–152°C. FTIR (ATR) ν (cm^{-1}): 3,045 (═C–H), 2,876 (C–H), 1,647 (C═O), and 1,585 and 1,528 (C═N and

TABLE 8 Antitrypanosomal activity of thiazole derivatives **3**, **5a–g**, **6a–g**, **7a–g**, **8a–g**, and **9a–g** expressed in EC_{50} (μM)[a]

Compound	EC_{50}[a]	IS[b]	Compound	EC_{50}	IS
3	**>10**	<1.76	**7f**	**>10**	<2
5a	**10.37 ± 1.50**	0.86	**7g**	**17.73 ± 3.46**	0.6
5b	**>6**	<2.24	**8a**	**16.86 ± 1.92**	1.43
5c	**21.98 ± 4.06**	1.15	**8b**	**19.53 ± 0.73**	0.90
5d	>30	<2.09	**8c**	34.64 ± 3.90	0.46
5e	**14.67 ± 2.08**	0.74	**8d**	**19.02 ± 2.2**	0.92
5f	27.76 ± 4.31	0.69	**8e**	75.30 ± 6.22	0.67
5g	**9.44 ± 0.35**	0.90	**8f**	50.19 ± 8.32	0.41
6a	**>4**	<1.8	**8g**	37.05 ± 2.67	0.62
6b	145 ± 5	0.09	**9a**	**>10**	<1.92
6c	**7.87 ± 0.45**	0.88	**9b**	36.97 ± 0.43	0.52
6d	**13.38 ± 0.84**	1.06	**9c**	34.85 ± 4.81	0.67
6e	**21.24 ± 3.47**	0.52	**9d**	>25	<1.73
6f	114.01 ± 18.60	0.23	**9e**	>25	<2.01
6g	**21.29 ± 03.10**	0.46	**9f**	>50	<2.03
7a	**18.02 ± 1.67**	0.87	**9g**	118.01 ± 2.61	1.55
7b	**23.05 ± 2.54**	1.52	Benznidazole[c]	13.34 ± 0.10	>14.99
7c	**4.98 ± 0.99**	**1.61**			
7d	150.33 ± 20.62	0.95			
7e	**18.71 ± 2.65**	1.17			

Note: Data represent mean value ± standard deviation. Bold values are the most active compounds. >Maximum concentration evaluated because the host cell dies at higher concentrations.

[a]Effective concentration 50 (EC_{50}): Concentration of compounds that decrease 50% of intracellular parasite.

[b]Index of selectivity (IS) = LC_{50}/EC_{50}. LC_{50}: Lethal concentration on human cell U-937.

[c]Antitrypanosomal drug control.

C═C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.92 (d, *J* = 15.0 Hz, 1H, H_6), 7.83 (d, *J* = 8.3 Hz, 2H, H_{Bo}), 7.62 (d, *J* = 8.3 Hz, 2H, H_{Bm}), 6.81 (d, *J* = 15.0 Hz, 1H, H_7), 3.93 (t, *J* = 6.0 Hz, 4H, 2×CH_2–N), and 3.82 (t, *J* = 6.0 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 188.1 (C═O), 167.1 (C), 143.4 (C), 137.1 (C), 133.9 (CH), 132.0 (CH), 130.0 (CH), 127.9 (C), 118.9 (CH), 117.1 (C), 54.6 (CH_2), and 40.6 (CH_2). MS (EI): *m/z* 466/468/470/472/474 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (0.68/1.62/0.93/0.30/0.04), 431/433/435 (57/100/49), 183/185 (9/9), 155/157 (9/10), 57 (35), and 43 (39). Anal. calcd. for $C_{16}H_{14}BrCl_3N_2OS$: C, 41.01; H, 3.01; N, 5.98; S, 6.84. Found: C, 41.04; H, 2.99; N, 5.96; S, 6.86.

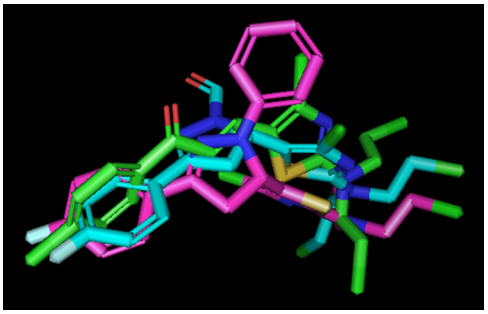


FIGURE 4 Three-dimensional structures of compounds **5c** (green), **7c** (cyan), and **8c** (purple)

*(E)-3-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-1-(4-fluorophenyl)prop-2-en-1-one (**5c**)*

Yellow solid; 66% yield; m.p. 153–154°C. FTIR (ATR) ν (cm^{-1}): 3,017 (═C–H), 2,940 (C–H), 1,641 (C═O), and 1,595 and 1,525 (C═N and C═C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.99 (dd, *J* = 8.6, $^4J_{H-F}$ = 5.5 Hz, 2H, H_{Bo}), 7.91 (d, *J* = 15.0 Hz, 1H, H_6), 7.15 (t, *J* = 8.6 Hz, 2H, H_{Bm}), 6.84 (d, *J* = 15.0 Hz, 1H, H_7), 3.92 (t, *J* = 6.0 Hz, 4H, 2×CH_2–N), and 3.82 (t, *J* = 5.9 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 187.6 (C = O), 167.0 (C), 165.6 (d, $^1J_{C-F}$ = 254.1 Hz, C), 143.1 (C), 134.6 (d, $^4J_{C-F}$ = 2.9 Hz, C), 133.5 (CH), 131.0 (d, $^3J_{C-F}$ = 9.1 Hz, CH), 119.1 (CH), 117.1 (C), 115.8 (d, $^2J_{C-F}$ = 21.8 Hz, CH), 54.5 (CH_2), and 40.6 (CH_2). MS (EI): *m/z* 406/408/410/412 [M^+/M + 2^+/M + 4^+/M + 6^+] (1.19/1.37/0.79/0.03), 371/373/375 (63/46/9), 81 (49), 69 (100), and 43 (74). Anal. calcd. for $C_{16}H_{14}Cl_3FN_2OS$: C, 47.13; H, 3.46; N, 6.87; S, 7.86. Found: C, 47.11; H, 3.44; N, 6.89; S, 7.89.

*(E)-1-(Benzo[d][1,3]dioxol-5-yl)-3-{2-[bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}prop-2-en-1-one (**5d**)*

Yellow solid; 64% yield; m.p. 149–150°C. FTIR (ATR) ν (cm^{-1}): 3,011 (═C–H), 2,907 (C–H), 1,610 (C═O), and 1,572 and 1,525 (C═N and C═C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.88 (d, *J* = 15.0 Hz, 1H, H_6), 7.57 (dd, *J* = 8.2, 1.7 Hz, 1H, H_{Bo}), 7.47 (d, *J* = 1.7 Hz, 1H, $H_{Bo'}$), 6.87 (d, *J* = 8.2 Hz, 1H, H_{Bm}), 6.83 (d, *J* = 15.0 Hz, 1H, H_7), 6.05 (s, 2H, –OCH_2O–), 3.92 (t, *J* = 6.3 Hz, 4H, 2×CH_2–N), and 3.81 (t, *J* = 6.3 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 187.1 (C═O), 166.7

(C), 151.7 (C), 148.4 (C), 142.5 (C), 133.1 (C), 132.8 (CH), 124.5 (CH), 119.4 (CH), 117.3 (C), 108.4 (CH), 108.0 (CH), 102.0 (–OCH_2O), 54.5 (CH_2), and 40.6 (CH_2). MS (EI): *m/z* 432/434/436/438 [M^+/M + 2^+/M + 4^+/M + 6^+] (0.82/1.15/0.34/0.29), 397/399/401 (40/28/6), 81 (51), 69 (100), and 43 (84). Anal. calcd. for $C_{17}H_{15}Cl_3N_2O_3S$: C, 47.08; H, 3.49; N, 6.46; S, 7.39. Found: C, 47.10; H, 3.47; N, 6.44; S, 7.43.

*(E)-3-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-1-(4-methoxyphenyl)prop-2-en-1-one (**5e**)*

Yellow solid; 60% yield; m.p. 124–125°C. FTIR (ATR) ν (cm^{-1}): 3,010 (=C–H), 2,845 (C–H), 1,641 (C=O), and 1,599 and 1,582 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.97 (d, *J* = 8.7 Hz, 2H, H_{Bo}), 7.89 (d, *J* = 15.1 Hz, 1H, H_6), 6.96 (d, *J* = 8.7 Hz, 2H, H_{Bm}), 6.89 (d, *J* = 15.1 Hz, 1H, H_7), 3.92 (t, *J* = 6.2 Hz, 4H, 2×CH_2–N), 3.88 (s, 3H, OCH_3), and 3.81 (t, *J* = 6.2 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 187.6 (C=O), 166.7 (C), 163.5 (C), 142.3 (C), 132.5 (CH), 131.2 (C), 130.7 (CH), 119.7 (CH), 117.4 (C), 114.0 (CH), 55.6 (OCH_3), 54.5 (CH_2), and 40.7 (CH_2). MS (EI): *m/z* 418/420/422/424 [M^+/M + 2^+/M + 4^+/M + 6^+] (0.11/0.41/0.19/0.31), 97 (43), 83 (62), 57 (91), and 43 (100). Anal. calcd. for $C_{17}H_{17}Cl_3N_2O_2S$: C, 48.64; H, 4.08; N, 6.67; S, 7.64. Found: C, 48.61; H, 4.07; N, 6.64; S, 7.67.

*(E)-3-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**5f**)*

Yellow solid; 54% yield; m.p. 136–137°C. FTIR (ATR) ν (cm^{-1}): 3,005 (=C–H), 2,939 (C–H), 1,676 (C=O), and 1,584 and 1,568 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.89 (d, *J* = 15.1 Hz, 1H, H_6), 7.20 (s, 2H, H_{Bo}), 6.79 (d, *J* = 15.1 Hz, 1H, H_7), 3.94 (s, 6H, 2×OCH_3), 3.93–3.89 (m, 7H, OCH_3, 2×CH_2–N), and 3.81 (t, *J* = 6.1 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 188.2 (C=O), 166.9 (C), 153.3 (C), 142.9 (C), 142.6 (C), 133.6 (C), 133.3 (CH), 119.5 (CH), 117.1 (C), 106.2 (CH), 61.1 (OCH_3), 56.6 (OCH_3), 54.5 (CH_2), and 40.6 (CH_2). MS (EI): *m/z* 478/480/482/484 [M^+/M + 2^+/M + 4^+/M + 6^+] (1.07/1.88/0.58/0.11), 443/445/447 (86/63/15), 83 (62), 57 (88), and 43 (100). Anal. calcd. for $C_{19}H_{21}Cl_3N_2O_4S$: C, 47.56; H, 4.41; N, 5.84; S, 6.68. Found: C, 47.54; H, 4.39; N, 5.83; S, 6.70.

*(E)-3-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-1-phenylprop-2-en-1-one (**5g**)*

Yellow solid; 50% yield; m.p. 125–127°C. FTIR (ATR) ν (cm^{-1}): 3,091 (=C–H), 2,951 (C–H), 1,645 (C=O), and 1,585 and 1,520 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.97 (d, *J* = 7.1 Hz, 2H, H_{Bo}), 7.92 (d, *J* = 15.1 Hz, 1H, H_6), 7.57 (t, *J* = 7.3 Hz, 1H, H_{Bp}), 7.49 (t, *J* = 7.4 Hz, 2H, H_{Bm}), 6.89 (d, *J* = 15.1 Hz, 1H, H_7), 3.93 (t, *J* = 6.2 Hz, 4H, 2×CH_2–N), and 3.82 (t, *J* = 6.1 Hz, 4H, 2×CH_2–Cl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 189.3 (C=O), 166.9 (C), 142.9 (C), 138.3 (C), 133.3 (CH), 132.8 (CH), 128.7 (CH), 128.4 (CH), 119.7 (CH), 117.3 (C), 54.5 (CH_2), and 40.6 (CH_2). MS (EI): *m/z* 388/390/392/394 [M^+/M + 2^+/M + 4^+/M + 6^+] (2.30/2.72/1.69/0.94), 353/355/357 (100/66/21), 83 (0.66), and 57 (0.87). Anal. calcd. for $C_{16}H_{15}Cl_3N_2OS$: C, 49.31; H, 3.88; N, 7.19; S, 8.23. Found: C, 49.33; H, 3.85; N, 7.21; S, 8.28.

4.1.4 | General procedure for the synthesis of *N*-acetyl and *N*-formyl pyrazolines 6/7a–g

A mixture of the selected chalcone **5a–g** (0.24 mmol) and hydrazine hydrate (4.80 mmol) in ethanol (3.0 ml) was heated under reflux for 30 min. Then, acetic anhydride (2.0 ml) was added dropwise, after which the mixture was stirred at room temperature for another 1 hr. The reaction mixture was treated with H_2O and extracted with dichloromethane (3 × 30 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/ethanol (50:1) to yield compounds **6a–g**.

The synthesis of the *N*-formyl pyrazolines **7a–g** was similar to that of the *N*-acetyl derivatives, except that formic acid (2.0 ml) was used in place of acetic anhydride and the reaction mixture was stirred at room temperature for 5 hr. The residue was purified by silica gel column chromatography using dichloromethane/ethyl acetate (20:1) to obtain compounds **7a–g**.

*1-(5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (**6a**)*

White solid; 65% yield; m.p. 135–137°C. FTIR (ATR) ν (cm^{-1}): 3,028 (=C–H), 2,965 (C–H), 1,668 (C=O), and 1,595 and 1,555 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.79 (d, *J* = 8.6 Hz, 2H, H_{Bo}), 7.54 (d, *J* = 8.6 Hz, 2H, H_{Bm}), 5.69 (dd, *J* = 11.9, 4.8 Hz, 1H, $H_{5'}$), 3.88–3.77 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), 3.73 (t, *J* = 6.7 Hz, 4H, 2×CH_2–N), 3.27 (dd, *J* = 18.2, 4.8 Hz, 1H, $H_{4a'}$), and 2.26 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.6 (C=O), 165.6 (C), 153.2 (C), 135.1 (C), 130.8 (C), 129.7 (C), 128.9 (CH), 128.4 (CH), 118.1 (C), 52.9 ($C_{5'}$), 52.3 (CH_2), 40.7 (CH_2), 40.4 ($C_{4'}$), and 21.7 ($COCH_3$). MS (EI): *m/z* 478/480/482/484/486 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (14/18/9/2/1), 443/445/447/449 (25/25/9/1), 306/308/310 (100/66/16), and 43 (13). Anal. calcd. for $C_{18}H_{18}Cl_4N_4OS$: C, 45.02; H, 3.78; N, 11.67; S, 6.68. Found: C, 45.00; H, 3.77; N, 11.63; S, 6.70.

*1-(5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (**6b**)*

White solid; 64% yield; m.p. 81–83°C. FTIR (ATR) ν (cm^{-1}): 3,025 (=C–H), 2,962 (C–H), 1,665 (C=O), and 1,593 and 1,531 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.72 (d, *J* = 8.8 Hz, 2H, H_{Bo}), 7.68 (d, *J* = 8.8 Hz, 2H, H_{Bm}), 5.69 (dd, *J* = 11.9, 4.8 Hz, 1H, $H_{5'}$), 3.88–3.77 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), 3.73 (t, *J* = 6.8 Hz, 4H, 2×CH_2–N), 3.27 (dd, *J* = 18.2, 4.8 Hz, 1H, $H_{4a'}$), and 2.26 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.6 (C=O), 165.6 (C), 155.5 (C), 131.8 (CH), 130.7 (C), 130.0 (C), 128.5 (CH), 123.8 (C), 118.1 (C), 52.9 ($C_{5'}$), 52.3 (CH_2), 40.7 (CH_2), 40.5 ($C_{4'}$), and 22.2 ($COCH_3$). MS (EI): *m/z* 522/524/526/528/530 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (8.28/18.20/12.08/4.40/0.59), 487/489/491/493 (26), 306/308/310 (100/69/14), and 43 (2). Anal. calcd. for $C_{18}H_{18}BrCl_3N_4OS$: C, 41.20; H, 3.46; N, 10.68; S, 6.11. Found: C, 41.18; H, 3.47; N, 10.66; S, 6.14.

*1-(5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (**6c**)*

Pale yellow solid; 58% yield; m.p. 134–136°C. FTIR (ATR) ν (cm^{-1}): 3,016 (=C–H), 2,964 (C–H), 1,668 (C=O), and 1,523 (C=N and C=C). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.84 (dd, J = 8.8, $^4J_{H-F}$ = 5.5 Hz, 2H, H_{Bo}), 7.32 (t, J = 8.9 Hz, 2H, H_{Bm}), 5.71 (dd, J = 11.9, 4.7 Hz, 1H, $H_{5'}$), 3.89–3.77 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), 3.74 (t, J = 5.8 Hz, 4H, 2×CH_2–N), 3.28 (dd, J = 18.2, 4.7 Hz, 1H, $H_{4a'}$), and 2.26 (s, J = 9.7 Hz, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 168.0 (C=O), 166.1 (C), 163.8 (d, $^1J_{C-F}$ = 248.5 Hz, C), 153.8 (C), 131.2 (C), 129.5 (d, $^3J_{C-F}$ = 8.7 Hz, CH), 127.9 (d, $^4J_{C-F}$ = 3.1Hz, C), 118.7 (C), 116.4 (d, $^2J_{C-F}$ = 21.9 Hz, CH), 53.3 ($C_{5'}$), 52.8 (CH_2), 41.2 (CH_2), 41.1 ($C_{4'}$), and 22.2 ($COCH_3$). MS (EI): *m/z* 462/464/466/468 [M^+/M + 2^+/M + 4^+/M + 6^+] (22/22/8/1), 427/430/432 (30/22/5), 306/308/310 (100/67/14), 148 (23), and 43 (12). Anal. calcd. for $C_{18}H_{18}Cl_3FN_4OS$: C, 46.61; H, 3.91; N, 12.08; S, 6.91. Found: C, 46.59; H, 3.94; N, 12.06; S, 6.95.

*1-(3-(Benzo[d][1,3]dioxol-5-yl)-5-{2-[bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (**6d**)*

Pale yellow solid; 60% yield; m.p. 90–93°C. FTIR (ATR) ν (cm^{-1}): 3,027 (=C–H), 2,963 (C–H), 1,661 (C=O), and 1,558 and 1,531 (C=N and C=C). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.35 (d, J = 1.6 Hz, 1H, $H_{Bo'}$), 7.25 (dd, J = 8.1, 1.6 Hz, 1H, H_{Bo}), 7.00 (d, J = 8.1 Hz, 1H, H_{Bm}), 6.10 (s, 2H, –OCH_2O–), 5.66 (dd, J = 11.8, 4.6 Hz, 1H, $H_{5'}$), 3.83–3.77 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), 3.74 (t, J = 5.7 Hz, 4H, 2×CH_2–N), 3.22 (dd, J = 18.1, 4.6 Hz, 1H, $H_{4a'}$), and 2.24 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.3 (C=O), 165.5 (C), 153.8 (C), 149.3 (C), 147.8 (C), 130.6 (C), 124.9 (C), 121.9 (CH), 118.3 (C), 108.5 (CH), 105.9 (CH), 101.6 (–OCH_2O–), 52.6 ($C_{5'}$), 52.3 (CH_2), 40.7 (CH_2), 40.6 ($C_{4'}$), and 21.6 ($COCH_3$). MS (EI): *m/z* 488/490/492/494 [M^+/M + 2^+/M + 4^+/M + 6^+] (4.13/4.82/1.63/0.54), 453/455/457 (26), 306/308/310 (16/11/2), 57 (86), and 43 (100). Anal. calcd. for $C_{19}H_{19}Cl_3N_4O_3S$: C, 46.59; H, 3.91; N, 11.44; S, 6.55. Found: C, 46.62; H, 3.88; N, 11.42, S, 6.51.

*1-(5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (**6e**)*

Pale yellow solid; 52% yield; m.p. 147–149°C. FTIR (ATR) ν (cm^{-1}): 3,028 (=C–H), 2,965 (C–H), 1,662 (C=O), and 1,558 and 1,531 (C=N and C=C). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.72 (d, J = 8.8 Hz, 2H, H_{Bo}), 7.02 (d, J = 8.8 Hz, 2H, H_{Bm}), 5.66 (dd, J = 11.7, 4.4 Hz, 1H, $H_{5'}$), 3.86–3.76 (m, 8H, OCH_3, $H_{4b'}$, 2×CH_2–Cl), 3.73 (t, J = 5.5 Hz, 4H, 2×CH_2–N), 3.23 (dd, J = 18.1, 4.4 Hz, 1H, $H_{4a'}$), and 2.24 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.3 (C=O), 165.5 (C), 161.0 (C), 153.9 (C), 130.6 (C), 128.3 (CH), 123.3 (C), 118.4 (C), 114.3 (CH), 55.4 (OCH_3), 52.5 ($C_{5'}$), 52.3 (CH_2), 40.7 (CH_2), 40.6 ($C_{4'}$), and 21.7 ($COCH_3$). MS (EI): *m/z* 474/476/478/480 [M^+/M + 2^+/M + 4^+/M + 6^+] (40.12/40.44/15.00/2.46), 439/441/443 (72/49/10), 306/308/310 (100/72/15), 148/150 (43/15), and 43 (14). Anal. calcd. for $C_{19}H_{21}Cl_3N_4O_2S$: C, 47.96; H, 4.45; N, 11.78; S, 6.74. Found: C, 47.99; H, 4.42; N, 11.75; S, 6.78.

*1-(5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (**6f**)*

Pale yellow solid; 70% yield; m.p. 103–106°C. FTIR (ATR) ν (cm^{-1}): 3,028 (=C–H), 2,838 (C–H), 1,653 (C=O), and 1,570 and 1,531 (C=N and C=C). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.05 (s, 2H, H_{Bo}), 5.69 (dd, J = 11.8, 4.5 Hz, 1H, $H_{5'}$), 3.85–3.77 (m, 11H, 2×OCH_3, $H_{4b'}$, 2×CH_2–Cl), 3.76–3.70 (m, 7H, OCH_3, 2×CH_2–N), 3.35 (dd, J = 18.1, 4.5 Hz, 1H, H_{4a}), and 2.27 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.4 (C=O), 165.5 (C), 154.3 (C), 153.0 (C), 139.6 (C), 130.5 (C), 126.2 (C), 118.4 (C), 104.2 (CH), 60.1 (OCH_3), 56.0 (OCH_3), 52.8 ($C_{5'}$), 52.3 (CH_2), 40.7 (CH_2), 40.1 ($C_{4'}$), and 21.6 ($COCH_3$). MS (EI): *m/z* 534/536/538/540 [M^+/M + 2^+/M + 4^+/M + 6^+] (73/75/29/5), 499/501/503 (100/68/16), 306/308/310 (95/69/15), 148/150 (47/18), and 43 (12). Anal. calcd. for $C_{21}H_{25}Cl_3N_4O_4S$: C, 47.07; H, 4.70; N, 10.46; S, 5.98. Found: C, 47.05; H, 4.68; N, 10.49; S, 6.01.

*1-(5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (**6g**)*

Pale yellow solid; 53% yield; m.p. 125–127°C. FTIR (ATR) ν (cm^{-1}): 3,019 (=C–H), 2,967 (C–H), 1,668 (C=O), and 1,557 and 1,541 (C=N and C=C). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.78 (dd, J = 6.6, 3.1 Hz, 2H, H_{Bo}), 7.50–7.45 (m, 3H, H_{Bm}, H_{Bp}), 5.69 (dd, J = 11.9, 4.7 Hz, 1H, $H_{5'}$), 3.89–3.77 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), 3.74 (t, J = 5.8 Hz, 4H, 2×CH_2–N), 3.27 (dd, J = 18.2, 4.7 Hz, 1H, $H_{4a'}$), and 2.26 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.5 (C=O), 165.6 (C), 154.1 (C), 130.8 (C), 130.7 (C), 130.5 (CH), 128.8 (CH), 126.6 (CH), 118.2 (C), 52.7 ($C_{5'}$), 52.3 (CH_2), 40.7 (CH_2), 40.5 ($C_{4'}$), and 21.7 ($COCH_3$). MS (EI): *m/z* 444/446/448/450 [M^+/M + 2^+/M + 4^+/M + 6^+] (21.24/23.68/8.42/1.89), 409/411/413 (37/25/5), 306/308/310 (100/68/14), 83 (47), and 43 (18). Anal. calcd. for $C_{18}H_{19}Cl_3N_4OS$: C, 48.50; H, 4.30; N, 12.57; S, 7.19. Found: C, 48.48; H, 4.33; N, 12.59; S, 7.23. Crystals suitable for single-crystal X-ray diffraction analysis were obtained from slow evaporation in ethanol and deposited in CCDC with deposition number 1919156.

*5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**7a**)*

Beige solid; 68% yield; m.p. 75–78°C. FTIR (ATR) ν (cm^{-1}): 3,035 (=C–H), 2,914 and 2,893 (C–H), 1,676 (C=O), and 1,551 and 1,533 (C=N and C=C). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.89 (s, 1H, CHO), 7.67 (d, J = 7.7 Hz, 2H, H_{Bo}), 7.42 (d, J = 7.7 Hz, 2H, H_{Bm}), 5.74 (dd, J = 11.7, 4.5 Hz, 1H, $H_{5'}$), 3.77 (t, J = 5.5 Hz, 4H, 2×CH_2–N), 3.76–3.69 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), and 3.26 (dd, J = 17.9, 4.5 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 165.5 (C), 160.1 (CHO), 154.6 (C), 137.1 (C), 133.4 (C), 129.3 (CH), 129.2 (C), 128.1 (CH), 116.6 (C), 54.3 (CH_2), 52.4 ($C_{5'}$), 41.1 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 464/466/468/470/472 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (3.11/4.18/2.23/0.52/0.07), 429/431/433/435 (2.84/2.53/0.81/0.27), 83 (100), and 49 (16). Anal. calcd. for $C_{17}H_{16}Cl_4N_4OS$: C, 43.80; H, 3.46; N, 12.02, S, 6.88. Found: C, 43.78; H, 3.49; N, 12.04; S, 6.85.

*5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-bromophenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**7b**)*

White solid; 62% yield; m.p. 159–160°C. FTIR (ATR) ν (cm^{-1}): 3,034 (=C–H), 2,914 and 2,893 (C–H), 1,678 (C=O), and 1,551 and 1,535 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.89 (s, 1H, CHO), 7.63–7.56 (m, 4H, H_{Bo}, H_{Bm}), 5.74 (dd, J = 11.7, 5.0 Hz, 1H, $H_{5'}$), 3.78 (t, J = 5.7 Hz, 4H, 2×CH_2–N), 3.76–3.70 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), and 3.26 (dd, J = 17.8, 5.0 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 165.5 (C), 160.1 (CHO), 154.6 (C), 133.4 (C), 132.3 (CH), 129.6 (C), 128.3 (CH), 125.4 (C), 116.6 (C), 54.3 (CH_2), 52.4 ($C_{5'}$), 41.0 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 508/510/512/514/516 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (1.73/3.31/2.20/0.67/0.11), 473/475/477 (1.58/2.14/1.07), 167 (40), 149 (100), 71 (38), and 57 (34). Anal. calcd. for $C_{17}H_{16}BrCl_3N_4OS$: C, 39.98; H, 3.16; N, 10.97; S, 6.28. Found: C, 39.98; H, 3.15; N, 10.98; S, 6.26.

*5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**7c**)*

Reddish solid; 52% yield; m.p. 94–97°C. FTIR (ATR) ν (cm^{-1}): 3,038 (=C–H), 2,924 and 2,887 (C–H), and 1,668 (C=O), 1,530 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.89 (s, 1H, CHO), 7.73 (dd, J = 8.9, $^4J_{H-F}$ = 5.3 Hz, 2H, H_{Bo}), 7.14 (t, J = 8.6 Hz, 2H, H_{Bm}), 5.74 (dd, J = 11.7, 5.0 Hz, 1H, $H_{5'}$), 3.78 (t, J = 5.6 Hz, 4H, 2×CH_2–N), 3.76–3.70 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), and 3.26 (dd, J = 17.8, 5.0 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 165.5 (C), 164.4 (d, $^1J_{C-F}$ = 252.2 Hz, C), 160.1 (CHO), 154.6 (C), 133.4 (C), 128.9 (d, $^3J_{C-F}$ = 8.7 Hz, CH), 127.0 (d, $^4J_{C-F}$ = 3.2 Hz, C), 116.7 (C), 116.3 (d, $^2J_{C-F}$ = 22.0 Hz, CH), 54.3 (CH_2), 52.4 ($C_{5'}$), 41.2 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 448/450/452/454 [M^+/M + 2^+/M + 4^+/M + 6^+] (13.07/13.61/4.81/0.70), 413/415/417 (11/7/2), 83 (51), 69 (100), and 43 (82). Anal. calcd. for $C_{17}H_{16}Cl_3FN_4OS$: C, 45.40; H, 3.59; N, 12.46; S, 7.13. Found: C, 45.38; H, 3.57; N, 12.42; S, 7.17.

*3-(Benzo[d][1,3]dioxol-5-yl)-5-{2-[bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**7d**)*

Beige solid; 57% yield; m.p. 163–165°C. FTIR (ATR) ν (cm^{-1}): 3,027 (=C–H), 2,965 and 2,895 (C–H), 1,657 (C=O), and 1,553 and 1,541 (1-(5-{2-[bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-bromophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.86 (s, 1H, CHO), 7.33 (d, J = 1.3 Hz, 1H, $H_{Bo'}$), 7.10 (dd, J = 8.1, 1.3 Hz, 1H, H_{Bo}), 6.84 (d, J = 8.1 Hz, 1H, H_{Bm}), 6.03 (s, 2H, –OCH_2O–), 5.71 (dd, J = 11.6, 4.9 Hz, 1H, $H_{5'}$), 3.79 (t, J = 5.7 Hz, 4H, 2×CH_2–N), 3.75–3.67 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), and 3.22 (dd, J = 17.7, 4.9 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 165.5 (C), 160.0 (CHO), 155.3 (C), 150.2 (C), 148.5 (C), 133.1 (s), 124.9 (C), 122.1 (CH), 116.9 (C), 108.5 (CH), 106.4 (CH), 101.8 (–OCH_2O), 54.3 (CH_2), 52.2 ($C_{5'}$), 41.3 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 474/476/478/480 [M^+/M + 2^+/M + 4^+/M + 6^+] (25.75/29.54/10.28/1.97), 439/441/443 (33/21/4), 148/150 (54/20), 69 (83), 57 (100), and 43 (95). Anal. calcd. for $C_{18}H_{17}Cl_3N_4O_3S$: C, 45.44; H, 3.60; N, 11.78; S, 6.74. Found: C, 45.42; H, 3.58; N, 11.80; S, 6.78.

*5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**7e**)*

Beige solid; 55% yield; m.p. 134–136°C. FTIR (ATR) ν (cm^{-1}): 3,005 (=C–H), 2,873 and 2,839 (C–H), 1,670 (C=O), and 1,547 and 1,526 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.87 (s, 1H, CHO), 7.67 (d, J = 8.8 Hz, 2H, H_{Bo}), 6.95 (d, J = 8.8 Hz, 2H, H_{Bm}), 5.72 (dd, J = 11.6, 4.8 Hz, 1H, $H_{5'}$), 3.86 (s, 3H, OCH_3), 3.78 (t, J = 6.4 Hz, 4H, 2×CH_2–N), 3.75–3.69 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), and 3.25 (dd, J = 17.7, 4.8 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 165.4 (C), 161.9 (C), 160.0 (CHO), 155.5 (C), 133.1 (C), 128.5 (CH), 123.2 (C), 117.1 (C), 114.5 (CH), 55.6 (OCH_3), 54.3 (CH_2), 52.1 ($C_{5'}$), 41.3 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 460/462/464/466 [M^+/M + 2^+/M + 4^+/M + 6^+] (34.73/35.37/12.88/2.56), 425/427/429 (47/31/6), 97 (43), 69 (80), 57 (91), and 43 (100). Anal. calcd. for $C_{18}H_{19}Cl_3N_4O_2S$: C, 46.82; H, 4.15; N, 12.13; S, 6.94. Found: C, 46.79; H, 4.12; N, 12.10; S, 6.97.

*5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**7f**)*

Beige solid; 76% yield; m.p. 162–163°C. FTIR (ATR) ν (cm^{-1}): 3,026 (=C–H), 2,878 and 2,826 (C–H), 1,676 (C=O), and 1,566 and 1,553 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.90 (s, 1H, CHO), 6.96 (s, 2H, H_{Bo}), 5.74 (dd, J = 11.7, 4.9 Hz, 1H, $H_{5'}$), 3.91 (s, 6H, 2×OCH_3), 3.89 (s, 3H, OCH_3), 3.78 (t, J = 5.6 Hz, 4H, 2×CH_2–N), 3.76–3.70 (m, 5H, $H_{4b'}$, 2×CH_2–Cl), and 3.27 (dd, J = 17.7, 4.9 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 165.5 (C), 160.0 (CHO), 155.4 (C), 153.6 (C), 140.8 (C), 133.2 (C), 126.0 (C), 116.9 (C), 104.2 (CH), 61.1 (OCH_3), 56.5 (OCH_3), 54.4 (CH_2), 52.4 ($C_{5'}$), 41.3 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 520/522/524/526 [M^+/M + 2^+/M + 4^+/M + 6^+] (64.37/66.67/24.51/3.82), 485/487/489 (31/20/4), 63 (100), 43 (49), and 36 (90). Anal. calcd. for $C_{20}H_{23}Cl_3N_4O_4S$: C, 46.03; H, 4.44; N, 10.74; S, 6.14. Found: C, 46.06; H, 4.41; N, 10.70; S, 6.17.

*5-{2-[Bis(2-chloroethyl)amino]-4-chlorothiazol-5-yl}-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**7g**)*

Beige solid; 70% yield; m.p. 74–77°C. FTIR (ATR) ν (cm^{-1}): 3,039 (=C–H), 2,926 and 2,876 (C–H), and 1,670 (C=O), 1,527 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.90 (s, 1H, CHO), 7.74 (dd, J = 7.6, 2.0 Hz, 2H, H_{Bo}), 7.49–7.38 (m, 3H, H_{Bm}, H_{Bp}), 5.74 (dd, J = 11.7, 5.0 Hz, 1H, $H_{5'}$), 3.82–3.76 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.73 (s, J = 5.4 Hz, 4H, 2×CH_2–Cl), and 3.29 (dd, J = 17.8, 5.0 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 165.5 (C), 160.2 (CHO), 155.7 (C), 133.2 (C), 131.0 (CH), 130.7 (C), 129.0 (CH), 126.9 (CH), 116.9 (C), 54.4 (CH_2), 52.2 ($C_{5'}$), 41.2 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 430/432/434/436 [M^+/M + 2^+/M + 4^+/M + 6^+] (14.80/14.98/5.43/0.79), 395/397/399 (13/8/2), 81 (48), 69 (100), and 43 (42). Anal. calcd. for $C_{17}H_{17}Cl_3N_4OS$: C, 47.29; H, 3.97; N, 12.98; S, 7.43. Found: C, 47.30; H, 3.98; N, 12.99; S, 7.48.

4.1.5 | General procedure for the synthesis of *N*-phenyl pyrazolines (8a–g)

A total of 4 drops of glacial acetic acid were added into a mixture of the selected chalcone **5a–g** (0.24 mmol) and phenylhydrazine (1.20 mmol) in MeOH (5.0 ml) at room temperature. The reaction mixture was heated under reflux for 7 hr and then cooled to room temperature. The resulting solid was filtered and washed with

methanol and water to afford compounds **8a–g**, which did not require further purification.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[3-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl]thiazol-2-amine (**8a**)*

Pale yellow solid; 66% yield; m.p. 146–148°C. FTIR (ATR) ν (cm^{-1}): 3,036 (=C–H), 2,958 and 2,914 (C–H), and 1,595 and 1,527 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.64 (d, J = 8.6 Hz, 2H, H_{Bo}), 7.36 (d, J = 8.6 Hz, 2H, H_{Bm}), 7.26 (t, J = 7.5 Hz, 2H, H_{Cm}), 7.16 (d, J = 7.8 Hz, 2H, H_{Co}), 6.88 (t, J = 7.3 Hz, 1H, H_{Cp}), 5.51 (dd, J = 11.8, 6.6 Hz, 1H, $H_{5'}$), 3.78–3.70 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.70–3.65 (m, 4H, 2×CH_2–Cl), and 3.17 (dd, J = 17.1, 6.6 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.2 (C), 146.7 (C), 144.5 (C), 134.9 (C), 132.3 (C), 130.9 (C), 129.3 (CH), 129.0 (CH), 127.2 (CH), 120.5 (CH), 119.3 (C), 114.1 (CH), 58.0 ($C_{5'}$), 54.3 (CH_2), 41.7 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 512/514/516/518/520 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (86/100/50/12/3), 477/479/481/483 (89/82/48/4), 274/276 (49/21), and 173 (4). Anal. calcd. for $C_{22}H_{20}Cl_4N_4S$: C, 51.38; H, 3.92; N, 10.89; S, 6.23. Found: C, 51.41; H, 3.91; N, 10.84; S, 6.28.

*5-[3-(4-Bromophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl]-4-chloro*-N,N-*bis(2-chloroethyl)thiazol-2-amine (**8b**)*

Pale yellow solid; 60% yield; m.p. 132–133°C. FTIR (ATR) ν (cm^{-1}): 3,038 (=C–H), 2,972 and 2,903 (C–H), and 1,593 and 1,535 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.60 (d, J = 8.6 Hz, 2H, H_{Bo}), 7.53 (d, J = 8.6 Hz, 2H, H_{Bm}), 7.29 (t, J = 7.4 Hz, 2H, H_{Cm}), 7.17 (d, J = 8.1 Hz, 2H, H_{Co}), 6.90 (t, J = 7.2 Hz, 1H, H_{Cp}), 5.53 (dd, J = 11.7, 6.6 Hz, 1H, $H_{5'}$), 3.83–3.72 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.72–3.63 (m, 4H, 2×CH_2–Cl), and 3.19 (dd, J = 17.1, 6.6 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 160.6 (C), 146.7 (C), 144.4 (C), 135.8 (C), 131.9 (CH), 131.3 (C), 129.3 (CH), 127.4 (CH), 124.8 (C), 120.5 (CH), 119.2 (s), 114.0 (CH), 58.0 ($C_{5'}$), 54.3 (CH_2), 41.6 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 556/558/560/562/564 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+] (9/15/9/3/1), 521/523/525/527 (10/13/6/1), 97 (60), and 57 (100). Anal. calcd. for $C_{22}H_{20}BrCl_3N_4S$: C, 47.29; H, 3.61; N, 10.03; S, 5.74. Found: C, 47.32; H, 3.59; N, 10.01; S, 5.78.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl]thiazol-2-amine (**8c**)*

Pale yellow solid; 61% yield; m.p. 134–136°C. FTIR (ATR) ν (cm^{-1}): 3,021 (=C–H), 2,961 and 2,935 (C–H), and 1,597 and 1,530 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.72 (dd, J = 8.9, $^4J_{H-F}$ = 5.4 Hz, 2H, H_{Bo}), 7.28 (t, J = 7.3 Hz, 2H, H_{Cm}), 7.17 (d, J = 7.7 Hz, 2H, H_{Co}), 7.10 (t, J = 8.7 Hz, 2H, H_{Bm}), 6.89 (t, J = 7.3 Hz, 1H, H_{Cp}), 5.50 (dd, J = 11.7, 6.7 Hz, 1H, $H_{5'}$), 3.78–3.73 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.72–3.67 (m, 4H, 2×CH_2–Cl), and 3.19 (dd, J = 17.1, 6.7 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.2 (C), 163.3 (d, $^1J_{C-F}$ = 232.5 Hz, C), 147.0 (C), 144.8 (C), 132.2 (C), 129.3 (CH), 128.7 (d, $^4J_{C-F}$ = 3.0 Hz, C), 127.9 (d, $^3J_{C-F}$ = 8.3 Hz, CH), 120.4 (CH), 119.5 (C), 115.9 (d, $^2J_{C-F}$ = 21.9 Hz, CH), 114.0 (CH), 58.1 ($C_{5'}$), 54.3 (CH_2), 41.9 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 496/498/500/502 [M^+/M + 2^+/M + 4^+/M + 6^+] (6/6/1/1), 461/463/465 (5/3/1), 97 (44), 57 (93), and 43 (100). Anal. calcd. For $C_{22}H_{20}Cl_3FN_4S$: C, 53.08; H, 4.05; N, 11.25; S, 6.44. Found: C, 53.04; H, 4.02; N, 11.22; S, 6.49.

*5-[3-(Benzo[d][1,3]dioxol-5-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl]-4-chloro*-N,N-*bis(2-chloroethyl)thiazol-2-amine (**8d**)*

Pale yellow solid; 57% yield; m.p. 177–180°C. FTIR (ATR) ν (cm^{-1}): 3,018 (=C–H), 2,961 and 2,910 (C–H), and 1,597 and 1,526 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.40 (d, J = 1.4 Hz, 1H, $H_{Bo'}$), 7.26 (t, J = 7.9 Hz, 2H, H_{Cm}), 7.16 (d, J = 7.9 Hz, 2H, H_{Co}), 7.05 (dd, J = 8.1, 1.4 Hz, 1H, H_{Bo}), 6.90–6.80 (m, 2H, H_{Bm}, H_{Cp}), 6.00 (s, 2H, –OCH_2O–), 5.46 (dd, J = 11.6, 6.6 Hz, 1H, $H_{5'}$), 3.81–3.73 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.72–3.65 (m, 4H, 2×CH_2–Cl), and 3.15 (dd, J = 17.0, 6.6 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.2 (C), 148.8 (C), 148.3 (C), 147.8 (C), 144.9 (C), 136.5 (C), 129.2 (CH), 126.8 (C), 120.6 (CH), 120.2 (CH), 119.7 (C), 114.0 (CH), 108.3 (CH), 106.0 (CH), 101.5 (–OCH_2O–), 58.00 ($C_{5'}$), 54.26 (CH_2–N), 42.02 ($C_{4'}$), and 40.75 (CH_2–Cl). MS (EI): *m/z* 522/524/526/528 [M^+/M + 2^+/M + 4^+/M + 6^+] (21/24/9/2), 487/489/491 (11/9/2), 91 (52), 57 (94), and 43 (100). Anal. calcd. for $C_{23}H_{21}Cl_3N_4O_2S$: C, 52.73; H, 4.04; N, 10.69; S, 6.12. Found: C, 52.71; H, 4.07; N, 10.72; S, 6.09.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl]thiazol-2-amine (**8e**)*

White solid; 50% yield; m.p. 150–152°C. FTIR (ATR) ν (cm^{-1}): 3,031 (=C–H), 2,956 and 2,901 (C–H), and 1,595 and 1,533 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.68 (d, J = 8.8 Hz, 2H, H_{Bo}), 7.27 (t, J = 7.6 Hz, 2H, H_{Cm}), 7.17 (d, J = 7.9 Hz, 2H, H_{Co}), 6.94 (d, J = 8.8 Hz, 2H, H_{Bm}), 6.87 (t, J = 7.2 Hz, 1H, H_{Cp}), 5.46 (dd, J = 11.6, 6.7 Hz, 1H, $H_{5'}$), 3.85 (s, 3H, OCH_3), 3.80–3.72 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.71–3.66 (m, 4H, 2×CH_2–Cl), and 3.19 (dd, J = 17.0, 6.7 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.2 (C), 160.6 (C), 148.0 (C), 145.1 (C), 132.0 (C), 129.2 (CH), 127.6 (CH), 125.1 (C), 120.1 (CH), 119.9 (C), 114.2 (CH), 114.0 (CH), 57.9 (OCH_3), 55.5 ($C_{5'}$), 54.3 (CH_2), 42.0 ($C_{4'}$), and 40.8 (CH_2). MS (EI): *m/z* 508/510/512/514 [M^+/M + 2^+/M + 4^+/M + 6^+] (27/35/12/3), 473/475/477 (14/8/1), 91 (72), 63 (100), and 43 (81). Anal. calcd. for $C_{23}H_{23}Cl_3N_4OS$: C, 54.18; H, 4.55; N, 10.99; S, 6.29. Found: C, 54.14; H, 4.57; N, 10.96; S, 6.31.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[1-phenyl-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]thiazol-2-amine (**8f**)*

White solid; 58% yield; m.p. 144–145°C. FTIR (ATR) ν (cm^{-1}): 3,032 (=C–H), 2,955 and 2,920 (C–H), and 1,591 and 1,530 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.29 (t, J = 7.3 Hz, 2H, H_{Cm}), 7.18 (d, J = 7.7 Hz, 2H, H_{Co}), 6.97 (s, 2H, H_{Bo}), 6.89 (t, J = 7.3 Hz, 1H, H_{Cp}), 5.51 (dd, J = 11.7, 6.6 Hz, 1H, $H_{5'}$), 3.93 (s, 6H, 2×OCH_3), 3.89 (s, 3H, OCH_3), 3.85–3.71 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.73–3.64 (m, 4H, CH_2–Cl), and 3.21 (dd, J = 17.0, 6.6 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.2 (C), 153.5 (C), 147.7 (C), 144.7 (C), 139.4 (C), 132.1 (C), 129.3 (CH), 128.0 (C), 120.4 (CH), 119.6 (C), 114.0 (CH), 103.4 (CH), 61.1 (OCH_3), 58.0 ($C_{5'}$), 56.4 (OCH_3), 54.3 (CH_2), 42.0 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 568/570/572/574 [M^+/M + 2^+/M + 4^+/M + 6^+] (57/61/24/4), 533/535/537 (17/10/3), 91 (40), and 63

(100). Anal. calcd. for $C_{25}H_{27}Cl_3N_4O_3S$: C, 52.69; H, 4.78; N, 9.83; S, 5.63. Found: C, 52.71; H, 4.75; N, 9.87; S, 5.67.

4-Chloro-N,N-*bis(2-chloroethyl)-5-(1,3-diphenyl-4,5-dihydro-1*H-*pyrazol-5-yl)thiazol-2-amine (**8g**)*

White solid; 56% yield; m.p. 144–146°C. FTIR (ATR) ν (cm^{-1}): 3,038 (=C–H), 2,938 and 2,924 (C–H), and 1,595 and 1,528 (C=N and C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.74 (d, J = 8.5 Hz, 2H, H_{Bo}), 7.44–7.34 (m, 3H, H_{Bm}, H_{Bp}), 7.29 (t, J = 7.4 Hz, 1H, H_{Cm}), 7.19 (d, J = 7.7, 1.2 Hz, 2H, H_{Co}), 6.89 (t, J = 7.2 Hz, 1H, H_{Cp}), 5.51 (dd, J = 11.7, 6.6 Hz, 1H, $H_{5'}$), 3.83–3.72 (m, 5H, $H_{4b'}$, 2×CH_2–N), 3.72–3.66 (m, 4H, 2×CH_2–Cl), and 3.22 (dd, J = 17.1, 6.6 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 166.2 (C), 147.8 (C), 144.8 (C), 132.4 (C), 132.2 (C), 129.3 (CH), 129.2 (CH), 128.8 (CH), 126.1 (CH), 120.3 (CH), 119.6 (C), 114.0 (CH), 57.9 ($C_{5'}$), 54.2 (CH_2), 41.8 ($C_{4'}$), and 40.7 (CH_2). MS (EI): *m/z* 478/480/482/484 [M^+/M + 2^+/M + 4^+/M + 6^+] (10/13/6/1), 443/445/447 (9/14/1), 91 (46), 69 (100), and 43 (80). Anal. calcd. for $C_{22}H_{21}Cl_3N_4S$: C, 55.07; H, 4.41; N, 11.68; S, 6.68. Found: C, 55.02; H, 4.45; N, 11.62; S, 6.71.

4.1.6 | General procedure for the synthesis of *N*-3,5-dichlorophenyl pyrazolines (9a–g)

A mixture of the selected chalcone **5a–g** (0.42 mmol) and 3,5-dichlorophenylhydrazine hydrochloride (2.1 mmol) in MeOH (5.0 ml) was heated under reflux for 4–12 hr. The precipitate formed was filtered, dried, and recrystallized from ethanol to afford derivatives **9a–g**.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[3-(4-chlorophenyl)-1-(3,5-dichlorophenyl)-4,5-dihydro-1*H-*pyrazol-5-yl]thiazol-2-amine (**9a**)*

Pale yellow solid; 88% yield; m.p. 187–189°C. FTIR (ATR) ν (cm^{-1}): 3,074 (=C–H), 2,964 and 2,922 (C–H), and 1,583 and 1,524 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.82 (d, J = 8.3 Hz, 2H, H_{Bo}), 7.52 (d, J = 8.3 Hz, 2H, H_{Bm}), 7.03 (d, J = 1.3 Hz, 2H, H_{Co}), 6.95 (t, J = 1.3 Hz, 1H, H_{Cp}), 5.78 (dd, J = 11.7, 4.0 Hz, 1H, $H_{5'}$), 3.87 (dd, J = 17.8, 11.7 Hz, 1H, $H_{4b'}$), 3.80–3.74 (m, 4H, 2×CH_2–Cl), 3.73–3.63 (m, 4H, 2×CH_2–N), and 3.39 (dd, J = 17.8, 4.0 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.8 (C), 149.7 (C), 145.3 (C), 134.6 (C), 134.2 (C), 132.2 (C), 130.1 (C), 128.8 (CH), 128.0 (CH), 118.2 (CH), 116.3 (C), 111.5 (CH), 56.5 ($C_{5'}$), 52.2 (CH_2), 41.0 (CH_2), and 40.7 ($C_{4'}$). MS (EI): *m/z* 580/582/584/586/588/590/592 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+/M + 10^+/M + 12^+] (57/95/76/32/9/1/0.3), 545/547/549/551/553/555 (66/100/66/24/5/0.6), 323/325/327/329 (84/66/17/2), 274/276/278 (77/32/3), and 63 (16). Anal. calcd. for $C_{22}H_{18}Cl_6N_4S$: C, 45.31; H, 3.11; N, 9.61; S, 5.50. Found: C, 45.30; H, 3.12; N, 9.62; S, 5.53.

*5-[3-(4-Bromophenyl)-1-(3,5-dichlorophenyl)-4,5-dihydro-1*H-*pyrazol-5-yl]-4-chloro*-N,N-*bis(2-chloroethyl)thiazol-2-amine (**9b**)*

Pale yellow solid; 90% yield; m.p. 188–190°C. FTIR (ATR) ν (cm^{-1}): 3,076 (=C–H), 2,963 and 2,922 (C–H), and 1,584 and 1,522 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.77 (d, J = 7.3 Hz, 2H, H_{Bo}), 7.67 (d, J = 7.4 Hz, 2H, H_{Bm}), 7.05 (d, J = 1.5 Hz, 2H, H_{Co}), 6.97 (t, J = 1.5 Hz, 1H, H_{Cp}), 5.80 (dd, J = 11.6, 4.5 Hz, 1H, $H_{5'}$), 3.89 (dd, J = 17.8, 11.6 Hz, 1H, $H_{4b'}$), 3.82–3.76 (m, J = 8.5 Hz, 4H, 2×CH_2–Cl), 3.77–3.64 (m, 4H, 2×CH_2–N), and 3.41 (dd, J = 17.8, 4.5 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.8 (C), 149.8 (C), 145.3 (C), 134.6 (C), 132.2 (C), 131.8 (CH), 130.5 (C), 128.3 (CH), 123.0 (C), 118.3 (CH), 116.3 (C), 111.6 (CH), 56.6 ($C_{5'}$), 52.2 (CH_2), 41.0 (CH_2), and 40.7 ($C_{4'}$). MS (EI): *m/z* 624/626/628/630/632/634/636 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+/M + 10^+/M + 12^+] (40/73/66/34/11/2/0.5), 589/591/593/595/597/599 (53/100/81/35/8/1), 367/369/371/373 (60/86/32/6), and 63 (89). Anal. calcd. for $C_{22}H_{18}BrCl_5N_4S$: C, 42.10; H, 2.89; N, 8.93; S, 5.11. Found: C, 42.13; H, 2.90; N, 8.90; S, 5.16.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[1-(3,5-dichlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H-*pyrazol-5-yl]thiazol-2-amine (**9c**)*

Pale yellow solid; 76% yield; m.p. 143–145°C. FTIR (ATR) ν (cm^{-1}): 3,074 (=C–H), 2,966 and 2,924 (C–H), and 1,583 and 1,533 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.86 (dd, J = 8.5, $^4J_{H-F}$ = 5.6 Hz, 2H, H_{Bo}), 7.30 (t, J = 8.8 Hz, 2H, H_{Bm}), 7.02 (d, J = 1.3 Hz, 2H, H_{Co}), 6.94 (t, J = 1.3 Hz, 1H, H_{Cp}), 5.76 (dd, J = 11.7, 4.6 Hz, 1H, $H_{5'}$), 3.87 (dd, J = 17.8, 11.7 Hz, 1H, $H_{4b'}$), 3.80–3.74 (m, 4H, 2×CH_2–Cl), 3.75–3.64 (m, 4H, 2×CH_2–N), and 3.40 (dd, J = 17.8, 4.6 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.8 (C), 162.9 (d, $^1J_{C-F}$ = 247.8 Hz, C), 149.9 (C), 145.4 (C), 134.5 (C), 132.1 (C), 128.6 (d, $^3J_{C-F}$ = 8.6 Hz, CH), 127.8 (d, $^4J_{C-F}$ = 3.1 Hz, C), 118.0 (CH), 116.4 (C), 115.8 (d, $^2J_{C-F}$ = 21.9 Hz, CH), 111.4 (CH), 56.5 ($C_{5'}$), 52.2 (CH_2), 41.3 (CH_2), and 40.7 ($C_{4'}$). MS (EI): *m/z* 564/566/568/570/572/574 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+/M + 10^+] (1.8/3.8/2.5/0.8/0.5/0.3), 529/531/533/535/537 (2.3/3.1/1.8/0.7/0.5), 369 (6), 97 (43), 83 (50), and 69 (100). Anal. calcd. for $C_{22}H_{18}Cl_5FN_4S$: C, 46.62; H, 3.20; N, 9.89; S, 5.66. Found: C, 46.60; H, 3.17; N, 9.86; S, 5.69.

*5-[3-(Benzo[*d*][1,3]dioxol-5-yl)-1-(3,5-dichlorophenyl)-4,5-dihydro-1*H-*pyrazol-5-yl]-4-chloro*-N,N-*bis(2-chloroethyl)thiazol-2-amine (**9d**)*

Pale yellow solid; 77% yield; m.p. 171–173°C. FTIR (ATR) ν (cm^{-1}): 3,068 (=C–H), 2,967 and 2,895 (C–H), and 1,582 and 1,530 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.44 (d, J = 1.7 Hz, 1H, $H_{Bo'}$), 7.24 (dd, J = 8.1, 1.7 Hz, 1H, H_{Bo}), 7.01 (d, J = 1.8 Hz, 2H, H_{Co}), 6.99 (d, J = 8.1 Hz, 1H, H_{Bm}), 6.90 (t, J = 1.8 Hz, 1H, H_{Cp}), 6.10 (s, 2H, –OCH_2O–), 5.71 (dd, J = 11.6, 4.6Hz, 1H, $H_{5'}$), 3.81 (dd, J = 16.8, 11.6 Hz, 1H, $H_{4b'}$), 3.78–3.74 (m, 4H, 2×CH_2–Cl), 3.74–3.66 (m, 4H, 2×CH_2–N), and 3.34 (d, J = 16.8, 4.6 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.7 (C), 150.5 (C), 148.7 (C), 147.8 (C), 145.5 (C), 134.5 (C), 132.0 (C), 125.4 (C), 121.4 (CH), 117.8 (CH), 116.5 (C), 111.3 (CH), 108.4 (CH), 105.8 (CH), 101.5 (–OCH_2O–), 56.2 ($C_{5'}$), 52.2 (CH_2), 41.4 (CH_2), and 40.7 ($C_{4'}$). MS (EI): *m/z* 590/592/594/596/598/600 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+/M + 10^+] (13/19/12/3/1/0.2), 555/557/559 (7/7/3), 368 (12), 97 (36), 83 (40), 57 (85), and 43 (100). Anal. calcd. for $C_{23}H_{19}Cl_5N_4O_2S$: C, 46.60; H, 3.23; N, 9.45; S, 5.41. Found: C, 46.58; H, 3.22; N, 9.44; S, 5.39.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[1-(3,5-dichlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]thiazol-2-amine (**9e**)*

White solid; 59% yield; m.p. 146–148°C. FTIR (ATR) ν (cm^{-1}): 3,005 (=C–H), 2,957 and 2,899 (C–H), and 1,581 and 1,533 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.73 (d, J = 8.9 Hz, 2H, H_{Bo}), 7.00 (d, J = 8.9 Hz, 2H, H_{Bm}), 6.98 (d, J = 1.8 Hz, 2H, H_{Co}), 6.88 (t, J = 1.8 Hz, 1H, H_{Cp}), 5.68 (dd, J = 11.4, 4.5 Hz, 1H, $H_{5'}$), 3.82 (dd, J = 17.7, 11.4 Hz, 1H, $H_{4b'}$), 3.80 (s, 3H, OCH_3), 3.77–3.73 (m, 4H, 2×CH_2–Cl), 3.71–3.66 (m, 4H, 2×CH_2–N), and 3.33 (dd, J = 17.7, 4.5 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.9 (C), 160.7 (C), 150.8 (C), 145.8 (C), 134.6 (C), 132.2 (C), 128.1 (CH), 123.8 (C), 117.8 (CH), 116.6 (C), 114.4 (CH), 111.4 (CH), 56.3 ($C_{5'}$), 55.4 (OCH_3), 52.3 (CH_2), 41.5 (CH_2), and 40.8 ($C_{4'}$). MS (EI): *m/z* 576/578/580/582/584/586 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+/M + 10^+] (49/79/52/19/3.5/0.6), 541/543/545/547/549 (39/44/21/6/2), 381 (60), 319 (49), 124 (69), and 57 (100). Anal. calcd. for $C_{23}H_{21}Cl_5N_4OS$: C, 47.73; H, 3.66; N, 9.68; S, 5.54. Found: C, 47.70; H, 3.68; N, 9.65; S, 5.56.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[1-(3,5-dichlorophenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]thiazol-2-amine (**9f**)*

White solid; 81% yield; m.p. 166–167°C. FTIR (ATR) ν (cm^{-1}): 3,005 (=C–H), 2,959 and 2,937 (C–H), and 1,584 and 1,539 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.04 (s, 2H, H_{Bo}), 7.00 (d, J = 1.4 Hz, 2H, H_{Co}), 6.88 (t, J = 1.4 Hz, 1H, H_{Cp}), 5.71 (dd, J = 11.3, 4.2 Hz, 1H, $H_{5'}$), 3.89–3.77 (m, 7H, $H_{4b'}$, 2×OCH_3), 3.78–3.72 (m, 4H, 2×CH_2–Cl), 3.72–3.66 (m, 7H, 2×CH_2–N, OCH_3), and 3.42 (dd, J = 17.8, 4.2 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.1 (C), 153.3 (C), 151.1 (C), 145.6 (C), 139.3 (C), 134.8 (C), 132.4 (C), 127.0 (C), 118.2 (CH), 116.7 (C), 111.7 (CH), 104.0 (CH), 60.4 (OCH_3), 56.6 ($C_{5'}$), 56.3 (OCH_3), 52.5 (CH_2), 41.6 (CH_2), and 41.0 ($C_{4'}$). MS (EI): *m/z* 636/638/640/642/644/646 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+/M + 10^+] (19/36/25/9/2/1), 601/603/605/607/609 (8/10/5/1/0.2), 441 (14), 193 (15), and 63 (100). Anal. calcd. for $C_{25}H_{25}Cl_5N_4O_3S$: C, 47.00; H, 3.94; N, 8.77; S, 5.02. Found: C, 47.05; H, 3.97; N, 8.73; S, 4.98.

4-Chloro-N,N-*bis(2-chloroethyl)-5-[1-(3,5-dichlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl]thiazol-2-amine (**9g**)*

White solid; 79% yield; m.p. 152–154°C. FTIR (ATR) ν (cm^{-1}): 3,089 (=C–H), 2,968 and 2,900 (C–H), and 1,585 and 1,537 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.78 (dd, J = 7.8, 1.7 Hz, 2H, H_{Bo}), 7.49–7.39 (m, 3H, H_{Bm}, H_{Bp}), 7.00 (d, J = 1.6 Hz, 2H, H_{Co})), 6.90 (t, J = 1.6 Hz, 1H, H_{Cp}), 5.73 (dd, J = 11.6, 4.3 Hz, 1H, $H_{5'}$), 3.85 (dd, J = 17.8, 11.6 Hz, 1H, $H_{4b'}$), 3.77–3.70 (m, 4H, 2×CH_2–Cl), 3.70–3.64 (m, 4H, 2×CH_2–N), and 3.35 (dd, J = 17.8, 4.3 Hz, 1H, $H_{4a'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 167.1 (C), 151.0 (C), 145.7 (C), 134.8 (C), 132.5 (C), 131.4 (C), 130.1 (CH), 129.1 (CH), 126.5 (CH), 118.3 (CH), 116.6 (C), 111.7 (CH), 56.6 ($C_{5'}$), 52.5 (CH_2), 41.4 (CH_2), and 41.0 ($C_{4'}$). MS (EI): *m/z* 546/548/550/552/554/556 [M^+/M + 2^+/M + 4^+/M + 6^+/M + 8^+/M + 10^+] (41/70/48/17/3/0.5), 511/513/515/517/519 (70/80/40/11/1.2), 351 (30), 289 (86), 240 (66), 124 (85), and 63 (100). Anal. calcd. for $C_{22}H_{19}Cl_5N_4S$: C, 48.15; H, 3.49; N, 10.21; S, 5.84. Found: C, 48.19; H, 3.52; N, 10.20; S, 5.87.

4.1.7 | Preparation of 3-(4-chlorophenyl)-1-(3,5-dichlorophenyl)-1*H*-pyrazole (10a)

A mixture of chalcone **5a** (0.42 mmol) and 3,5-dichlorophenylhydrazine hydrochloride (2.1 mmol) in AcOH (5.0 ml) was heated under reflux for 8 hr. After cooling, the precipitate formed was filtered and washed with ethanol.

*3-(4-Chlorophenyl)-1-(3,5-dichlorophenyl)-1*H*-pyrazole (**10a**)*

Yellow solid; 72% yield; m.p. 137–138°C. FTIR (ATR) ν (cm^{-1}): 3,146, 3,093 and 3,061 (=C–H), and1,589 and 1,531 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.73 (d, J = 2.6 Hz, 1H, $H_{5'}$), 8.05 (d, J = 1.8 Hz, 2H, H_{Co}), 7.99 (d, J = 8.5 Hz, 2H, H_{Bo}), 7.56 (d, J = 1.8 Hz, 1H, H_{Cp}), 7.52 (d, J = 8.5Hz, 2H, H_{Bm}), and 7.15 (d, J = 2.6Hz, 1H, $H_{4'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 151.7 (C), 141.2 (C), 135.1 (C), 133.1 (C), 131.0 (C), 130.4 (CH), 128.9 (CH), 127.5 (CH), 125.4 (CH), 116.7 (CH), and 106.6 (CH). MS (EI): *m/z* 322/324/326/328 [M^+/M + 2^+/M + 4^+/M + 6^+] (100/94/21/4), 252/254 (9/3), 145 (10), 111 (15), and 75 (13). Anal. calcd. for $C_{22}H_{18}Cl_6N_4S$: C, 55.68; H, 2.80; N, 8.66. Found: C, 55.72; H, 2.77; N, 8.64.

4.2 | Biological assays

4.2.1 | Antifungal activity

Microorganisms and media

For the antifungal evaluation, standardized strains from the American Type Culture Collection (ATCC, Rockville, MD), *C. albicans* ATCC 10231 and *C. neoformans* ATCC 32264, were used. Strains were grown on Sabouraud chloramphenicol agar slants for 48 hr at 30°C, and they were maintained on slopes of Sabouraud dextrose agar (Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Inocula were obtained according to reported procedures[54] and adjusted to 1–5 × 10^3 cells with colony-forming units (CFU)/ml.

Fungal growth inhibition percentage determination

Broth microdilution techniques were performed in 96-well microplates according to the Clinical and Laboratory Standards Institute—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, M27* (4th ed.).[54] For the assay, compound test wells (CTWs) were prepared with stock solutions of each compound in DMSO (maximum concentration ≤ 1%), diluted with Roswell Park Memorial Institute (RPMI)-1640, to final concentrations of 250–3.9 μg/ml. An inoculum suspension (100 μl) was added to each well (final volume in the well = 200 μl). A growth control well (GCW; containing medium, inoculum, and the same amount of DMSO used in a CTW, but compound-free) and a sterility control well (SCW; sample, medium, and sterile water instead of inoculum) were included for each fungus tested. Microtiter trays were incubated in a moist, dark chamber at 30°C for 48 hr for both yeasts. Microplates were read in a VERSA Max microplate reader (Molecular Devices, Sunnyvale, CA).

Amphotericin B (Sigma-Aldrich, St. Louis, MO) was used as a positive control. Tests were performed in triplicate. Reduction of growth for each compound concentration was calculated as follows: % of inhibition = 100 − (OD_{405} CTW − OD_{405} SCW)/(OD_{405} GCW − OD_{405} SCW). The means ± SEM was used to construct the dose–response curves representing % inhibition versus concentration of each compound.

4.2.2 | Antibacterial activity

All the synthesized compounds were tested to determine the antibacterial activity against Gram-negative and -positive bacteria. Wild-type and multidrug-resistant strains were included as follows: MSSA ATCC 25923, MRSA ATCC 43300, VISA, *E. coli* ATCC 25922, carbapenemase-positive *K. pneumoniae* BAA 1705, *K. pneumoniae* ATCC 700603 (extended-spectrum β-lactamase, ESBL positive), *P. aeruginosa* ATCC 27853, and *N. gonorrhoeae* ATCC 31426 (β-lactamase positive). Stock solutions (100 mg/ml) of the compounds were prepared in DMSO and diluted to a final screening concentration of 1 mg/ml. An initial screening of bacterial inhibition was performed by the agar diffusion method. Briefly, sterile Mueller Hinton agar (MHA, BBL) was prepared in Petri dishes and inoculated with a bacterial suspension prepared in trypticase soy broth and adjusted to 1.5×10^8 CFU/ml (i.e., 0.08–0.1 OD at 600 nm).[62] Wells (6 mm in diameter) were punched in the agar and 10 µl of each compound (stock solution) was filled into each well. Dimethyl sulfoxide and trypticase soy broth were included as negative controls (i.e., no inhibition of bacterial growth). Gentamicin and polymyxin B (Sigma-Aldrich) were included as positive controls of growth inhibition. Compounds showing growth inhibition were tested at least twice before being selected for microdilution testing. For *N. gonorrhoeae*, the agar diffusion method was also used in the screening process with some modifications. For this method, 200 µl of a bacterial suspension (1.5×10^8 CFU/ml) was inoculated in gonococcal (GC) agar (BBL) supplemented with 1% isovitalex (BBL), and then the compounds were added to the wells, as mentioned above, and incubated at 35–36.5°C in 5% CO_2 atmosphere for 48 hr. Penicillin, ceftriaxone, and ciprofloxacin (BBL) were used as controls.[63]

Microdilution test

MIC was determined in those compounds with visible and reproducible bacterial inhibition by the screening test. Bacterial suspensions were adjusted with Mueller Hinton broth (MHB) to a concentration of 5×10^5–8×10^5.[62] Stock solutions of the newly synthesized compounds were diluted in MHB, containing 5% DMSO and 0.1% Tween 80,[61] and added to 90 µl of the bacterial inoculum. The microplates were incubated for 24 hr at 35–37°C. MICs were defined as the lowest concentration with visible inhibition of bacterial growth[64] and/or detection using resazurin (1 mg/ml). Gentamicin (Sigma-Aldrich) was included as control of inhibition growth; MHB and DMSO were used as bacterial growth controls. Experiments were performed in duplicate and replicated at least three times.

For *N. gonorrhoeae*, those compounds with visible growth inhibition in the screening test were further tested for MIC on agar plates, as described by the Centers for Disease Control and Prevention (CDC)[56] and the Clinical and Laboratory Standards Institute (CLSI)[63] with modifications. Briefly, GC agar supplemented with 1% isovitalex was prepared, containing increasing concentrations of the novel compounds, and it was inoculated with 5 µl of the bacterial suspension (i.e., 1×10^4 CFU).[56] The lowest concentration of the compound that inhibited bacterial growth was determined as the MIC. Bacterial growth was examined and verified using the oxidase test. Experiments were performed in duplicate and replicated at least three times.

4.2.3 | Hemolytic activity

The ability to induce hemolysis was evaluated following the method of cytotoxicity by spectrophotometry on a 96-well plate. Human red blood cells (RBC) were adjusted to 5% hematocrit in RPMI-1640 medium; 500 µl of RBCs were placed into each well of a 24-well plate and subsequently exposed against 200 µg/ml. Detection of free hemoglobin, after 48 hr of incubation at 37°C, is the evidence that the compound induces hemolysis. The concentration of free hemoglobin was performed spectrophotometrically at 542 nm (Varioskan; Thermo Fisher Scientific), and intensity of color (absorbance) was registered as optical densities (OD). Nonspecific absorbance was corrected by subtracting the absorbance of the blank. Determinations were done by triplicate in at least two independent experiments.[65]

4.2.4 | Antiprotozoal activity

Compounds storage

All compounds were stored at room temperature until use. Before the biological evaluation, each compound was solubilized in 0.5% DMSO and then diluted to the appropriate concentration in culture media.

Cytotoxic activity

Cytotoxicity of the compounds was evaluated over human monocytes (U-937 ATCC CRL-1593.2) in an exponential growth phase and adjusted at 1×10^5 cells/ml in RPMI-1640 medium enriched with 10% fetal bovine serum (FBS). Also, 100 µl of cell suspension was dispensed in each well of a 96-well microplate, and then, 100 µl of 200, 50, 12.5, and 3.125 µg/ml concentration of each compound or standard drugs, chloroquine (malaria), benznidazole (Chagas disease), and amphotericin B (leishmaniasis), was added. Cells exposed to compounds or standard drugs were incubated for 72 hr at 37°C and with 5% of CO_2. Cytotoxic activity of each compound was determined according to the effect on the cell viability by the MTT microenzymatic method in which 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is reduced to a purple product, named

formazan by mitochondrial enzyme succinate dehydrogenase. Thus, 10 μl/well was added to each well of exposed and unexposed cells, and plates were incubated at 37°C and with 5% CO_2 for 3 hr. The reaction was stopped by adding 100 μl/well of isopropanol with 50% and 10% of sodium dodecyl sulfate. The concentration of formazan was determined spectrophotometrically at 570 nm (Varioskan; Thermo Fisher Scientific), and intensity of color (absorbance) was registered as OD. Cells exposed to control drugs (chloroquine, amphotericin B, and benznidazole) were used as control for toxicity (positive control), whereas cell incubated in the absence of any compound or drug was used as control for viability (negative control). Nonspecific absorbance was corrected by subtracting absorbance (OD) of the blank. Determinations were done by triplicate in at least two independent experiments.[66]

Antiplasmodial activity

The antiplasmodial activity was evaluated in vitro on asynchronic cultures of *P. falciparum* (3D7 strain), maintained in standard culture conditions. The effect of each compound over the growth of the parasites was determined by quantification of parasite death, based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. Briefly, unsynchronized *P. falciparum* cultures were adjusted to 0.5% parasitemia and 1% hematocrit in RPMI medium enriched with 3% lipid-rich bovine serum albumin—Albumax II. Then, in each well of a 96-well plate, 100 μl of parasite suspension was dispensed and subsequently exposed against 100 μl of four serial dilutions of compounds (100, 25, 6.25, and 1.56 μg/ml). Dilutions were prepared from a stock solution of 1,000 μg/ml. CQ was used as positive antiplasmodial drug control. Parasites unexposed to any compound were used as control of both growth and viability (negative control). Plates were incubated for 48 hr at 37°C in N_2 (90%), CO_2 (5%), and O_2 (5%) atmosphere. After incubation, plates were harvested and parasites were subjected to three 20-min freeze–thaw cycles. Meanwhile, 100 μl of Malstat reagent (400 μl Triton X-100 in 80 ml deionized water, 4 g L-lactate, 1.32 g Tris buffer, and 0.022 g acetylpyridine adenine dinucleotide in 200 ml deionized water; pH 9.0), and 25 μl of NBT/PES solution (0.16 g nitroblue tetrazolium salt and 0.08 g phenazine ethosulphate in 100 ml deionizated water) were added to each well of a second flat-bottom 96-well microtiter plate. After freeze–thaw cycles, culture in each of the wells of the first plate was resuspended by pipetting and 15 μl of each well was taken and added to the corresponding well of the second plate (containing Malstat and NBT/PES reagents). After an hour of incubation in the dark, color development of the LDH reaction was monitored colorimetrically in a spectrofluorometer (Varioskan; Thermo Fisher Scientific) reading at 650 nm. The intensity of color in each experimental condition was registered as OD. Nonspecific absorbance was corrected by subtracting the OD of the blank. Determinations were done by triplicate in at least two independent experiments.[67]

Antileishmanial activity

Antileishmanial activity of compounds was determined according to the ability of the compound to reduce the infection by *L. panamensis* parasites. For this, the antileishmanial activity was tested on intracellular amastigotes of *L. panamensis* transfected with the green fluorescent protein gene (MHOM/CO/87/UA140-EpiR–GFP strain).[5] Briefly, U-937 human cells at a density of 3×10^5 cells/ml in RPMI-1640 and 0.1 μg/ml of phorbol-12-myristate-13-acetate (PMA) were dispensed on a 24-well microplate and then infected with stationary-phase *L. panamensis* promastigotes in a 15:1 parasites per cell ratio. Plates were incubated at 34°C and 5% CO_2 for 3 hr and then cells were washed twice with phosphate-buffered solution (PBS) to eliminate not internalized parasites. Fresh RPMI-1640 was added into each well (1 ml) and plates were incubated again to complete infection. After 24 hr of infection, the RPMI-1640 medium was replaced by fresh culture medium containing each compound at four serial dilutions (50, 12.5, 3.125 and 0.78 μg/ml), and plates were then incubated at 37°C and 5% CO_2 during 72 hr. Then, cells were removed from the bottom plate with a trypsin/ethylenediaminetetraacetic acid (250 mg) solution. The cells were centrifuged at 1,100 rpm during 10 min at 4°C, the supernatant was discarded, and cells were washed with 1 ml of cold PBS and centrifuged at 1,100 rpm for 10 min at 4°C. Cells were washed two times employing PBS, as previously, and after the last wash, the supernatant was discarded and cells were suspended in 500 μl of PBS.

Cells were analyzed by flow cytometry employing a flow cytometer (cytomics FC 500MPL) reading at 488 nm (exciting) and 525 nm (emitting) over an argon laser and counting 10,000 events. Infected cells were determined according to the events for green fluorescence (parasites). All determinations for each compound and standard drugs were carried out by triplicate, in two experiments. Infected cells exposed to control drugs (amphotericin B) were used as control for antileishmanial activity (positive control), whereas infected cells incubated in the absence of any compound or drug were used as a control for infection (negative control). Nonspecific fluorescence was corrected by subtracting the fluorescence of unstained cells. Determinations were done by triplicate in at least two independent experiments.[5,66]

Antitrypanosomal activity

The effectiveness of synthesized compounds was determined according to the ability of the compound to reduce the infection by *T. cruzi* parasites. For this, the antitrypanosomal activity was tested on intracellular amastigotes of *T. cruzi* (Tulahuen strain transfected with β-galactosidase gene, donated by Dr. F. S. Buckner, University of Washington).[68] For this purpose, 100 μl of U-937 human cells at a concentration of 2.5×10^5 cells/ml in RPMI-1640, 10% fetal bovine serum, and 0.1 μg/ml of PMA were placed in each well of a 96-well microplate. Cells were then infected with epimastigotes (24 hr of growing) in 5:1 parasites per cell relation. Infected cells were incubated with each compound at four concentrations (50, 12.5, 3.125, and 0.78 μg/ml) using benznidazole as trypanocidal control. After 72 hr of incubation, the effect of all compounds on intracellular amastigotes viability was determined by measuring the β-galactosidase activity by colorimetric method. For this assay, CPRG at 100 μM and Nonidet P-40 at 0.1% were added, and after 3 hr of

incubation, measurement was performed at 570 nm in a spectrophotometer (Varioskan; Thermo Fisher Scientific) and intensity of color (absorbance) was registered as OD. Infected cells exposed to control drugs (benznidazole) were used as a control for antitrypanosomal activity (positive control), whereas infected cells incubated in the absence of any compound or drug were used as control for infection (negative control). Nonspecific absorbance was corrected by subtracting the absorbance (OD) of the blank. Determinations were done by triplicate in at least two independent experiments.[69]

Statistical analysis

Cytotoxicity was determined according to viability and mortality percentages obtained for each experimental condition (synthesized compounds, amphotericin B, benznidazole, chloroquine and culture medium). Results were expressed as mean lethal concentrations (LC_{50}), concentration necessary to kill 50% of cells, calculated by Probit analysis (parametric method of linear regression that permits dose–response analysis).[70]

Initially, viability percentages were calculated by the following equation, where the OD of control well corresponds to 100% of viability. In turn, the mortality percentage corresponds to 100% – % viability.

$$\%\ \text{Viability} = \frac{\text{OD Exposed cells}}{\text{OD Control cells}} \times 100. \quad (1)$$

Then, the percentage of cell growth inhibition was calculated using the following equation:

$$\%\ \text{Cell growth inhibition} = 100\%\ \text{Viability}. \quad (2)$$

The toxicity was defined according to LC_{50} values, using the following scale: Toxic: $LC_{50} < 100\ \mu M$, moderately toxic: $LC_{50} > 100\ \mu g/ml$ and $<200\ \mu M$, and potentially nontoxic: $LC_{50} > 200\ \mu M$.

The antiplasmodium activity of each evaluated compound was evidenced by the reduction of the absorbance (OD). Indeed, the viability percentage was calculated by the following equation:

$$\text{Viability}\ (\%) = \frac{\text{OD of parasites exposed to compounds} - \text{OD of culture medium}}{\text{OD of parasites unexposed to compounds} - \text{OD of culture medium}} \times 100. \quad (3)$$

Then, the inhibition growing percentage was calculated according to the following formula:

$$\text{Inhibition}(\%) = 100 - \text{Viability}(\%). \quad (4)$$

The inhibitory concentration (IC_{50}), that is, the concentration of the compound that produces a 50% reduction of growth of the parasite, was determined by the statistical program Probit analysis.

Antileishmanial activity was determined according to the reduction of infected cells percentage obtained for each experimental condition by the cytometer. The parasitemia values for each concentration of compound tested were calculated by the following equation, where the % of infected cells in the control well corresponds to 100% of parasitemia.

$$\%\ \text{Infection} = \frac{\%\ \text{Infected cells in compound exposed well}}{\%\ \text{Infected cells in control well}} \times 100. \quad (5)$$

Then, the inhibition percentage was calculated with formula:

$$\text{Inhibition}\ (\%) = 100 - \text{Parasitemia}(\%). \quad (6)$$

For trypanosomal activity, the parasitemia percentage and parasitemia inhibition were calculated by Equation (5) and Formula (6).

Results of antileishmanial and antitrypanosomal activities were expressed as being half of the maximal effective concentrations (EC_{50}) measured by the Probit method. The activity of each compound was established according to EC_{50} values, using the following scale: Active: $EC_{50} < 50\ \mu g/ml$; moderately active: $EC_{50} > 50\ \mu g/ml$ and $<100\ \mu g/ml$, and potentially not active: $EC_{50} > 100\ \mu g/ml$.

SI was calculated by dividing the cytotoxic activity and the plasmodium, leishmanicidal, or trypanocidal activities using the following equation:

$$SI = \frac{LC_{50}}{EC_{50}}. \quad (7)$$

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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