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Synthesis and antimicrobial activity of thiophenic chalcone and pyrazole derivative

Síntese e atividade antimicrobiana de chalcona tiofênica e derivado pirazólico

Síntesis y actividad antimicrobiana de la chalcona tiofénica y el derivado de pirazol

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ABSTRACT

Objective: To analyze the antimicrobial activity of a thiophenic chalcone and a pyrazole derivative synthesized through specific chemical routes. **Methods:** This is an in vitro experimental study. Thiophenic chalcone (GA-01) was synthesized by aldol condensation, and its pyrazole derivative (GA-04) by cyclocondensation with thiosemicarbazide. The compounds were characterized by ^{1}H and ^{13}C NMR. The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans, using the plate microdilution method. To investigate the mechanism of action, an assay with 0.8 M sorbitol was performed, evaluating the osmoprotective impact on the efficacy of the compounds. **Results:** Chalcone GA-01 had an MIC of 128 µg/mL for all strains. GA-04 showed greater activity, with an MIC of 64 µg/mL for bacteria and 4 µg/mL for C. albicans. In the sorbitol assay, an increase in MICs was observed for both compounds, suggesting action on the microbial cell wall. **Conclusion:** The synthesized compounds showed promising antimicrobial potential, with emphasis on the novel pyrazole derivative (GA-04), which demonstrated greater efficacy. The results indicate these compounds as prototypes for the development of new antimicrobial agents.

Keywords: Chalcone, Pyrazole derivative, Antimicrobial activity, MIC.

RESUMO

Objetivo: Analisar a atividade antimicrobiana de uma chalcona tiofênica e de um derivado pirazólico sintetizados por meio de rotas químicas específicas. **Métodos:** Trata-se de um estudo experimental in vitro. A chalcona tiofênica (GA-01) foi sintetizada por condensação aldólica e seu derivado pirazólico (GA-04) por ciclocondensação com tiossemicarbazida. A caracterização dos compostos foi realizada por RMN de ^{1}H e ^{13}C. Avaliou-se a atividade antimicrobiana por meio da determinação da concentração inibitória mínima (CIM) frente a Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus e Candida albicans, utilizando o método de microdiluição em placa. Para investigar o mecanismo de ação, foi realizado um ensaio com sorbitol a 0,8 M, avaliando-se o impacto osmoprotetor na eficácia dos compostos. **Resultados:** A chalcona GA-01 apresentou CIM de 128 µg/mL para todas as linhagens. Já GA-04 mostrou maior atividade, com CIM de 64 µg/mL para as bactérias e 4 µg/mL para C. albicans. No ensaio com sorbitol, observou-se aumento das CIMs para ambas as substâncias, sugerindo atuação sobre a parede celular

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microbiana. **Conclusão:** As substâncias sintetizadas apresentaram potencial antimicrobiano promissor, com destaque para o derivado pirazólico (GA-04) inédito, que demonstrou maior eficácia. Os resultados apontam esses compostos como protótipos para o desenvolvimento de novos agentes antimicrobianos.

Palavras-chave: Chalcona, Derivado pirazólico, Atividade antimicrobiana, CIM.

RESUMEN

Objetivo: Analizar la actividad antimicrobiana de una chalcona tiofénica y un derivado de pirazol sintetizados a través de rutas químicas específicas. **Métodos:** Este es un estudio experimental in vitro. La chalcona tiofénica (GA-01) se sintetizó por condensación aldólica y su derivado de pirazol (GA-04) por ciclocondensación con tiosemicarbazida. Los compuestos se caracterizaron por RMN de ^{1}H y ^{13}C. La actividad antimicrobiana se evaluó determinando la concentración mínima inhibitoria (CMI) contra Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus y Candida albicans, utilizando el método de microdilución en placa. Para investigar el mecanismo de acción, se realizó un ensayo con sorbitol 0,8 M, evaluando el impacto osmoprotector en la eficacia de los compuestos. **Resultados:** La chalcona GA-01 tuvo una CMI de 128 µg/mL para todas las cepas. El GA-04 mostró mayor actividad, con una CMI de 64 µg/mL para bacterias y 4 µg/mL para C. albicans. En el ensayo con sorbitol, se observó un aumento de la CMI para ambos compuestos, lo que sugiere una acción sobre la pared celular microbiana. **Conclusión:** Los compuestos sintetizados mostraron un potencial antimicrobiano prometedor, con énfasis en el nuevo derivado de pirazol (GA-04), que demostró una mayor eficacia. Los resultados indican que estos compuestos son prototipos para el desarrollo de nuevos agentes antimicrobianos.

Palabras clave: Chalcona, Derivado de pirazol, Actividad antimicrobiana, CMI.

INTRODUCTION

Chalcones constitute a class of organic compounds widely distributed in the plant kingdom, acting as intermediates in the biosynthesis of flavonoids. Structurally, they are characterized by the presence of two aromatic rings connected by an α,β-unsaturated side chain of three carbon atoms, a structure that gives these molecules a high degree of reactivity and chemical versatility (ZYSZKA B, et al., 2017). This flexible structural conformation allows modifications to their aromatic rings through the introduction of different functional groups, which can significantly influence both their pharmacokinetic properties and biological activity (BOULEBD H, 2020; XUE Y, et al., 2018).

Among the activities attributed to chalcones, anti-inflammatory, antioxidant, antiviral (TANG X, et al., 2019), antimicrobial (LAL K, et al., 2018), antifungal, and anticancer actions stand out, widely documented in the scientific literature (VANANGAMUDI G, et al., 2017). The ease of synthesis, combined with the structural diversity achieved through functionalization reactions, makes chalcones promising targets in the development of new drugs (MACHADO C, et al., 2017). Modification of their molecular skeleton, such as the insertion of lipophilic or electronegative groups, can enhance their biological actions or mitigate possible toxic effects. Strategies such as the removal of the α,β-unsaturated system have been studied as alternatives to reduce toxicity without compromising the desired therapeutic activity (GOMES M, et al., 2017).

In recent years, scientific interest in chalcones has increased significantly, especially in the context of the discovery of new bioactive compounds for infectious and inflammatory diseases. One of the fields benefiting most from this interest is oncology, considering that chalcones such as MIPP and MOMIPP exhibit promising antitumor activity, inducing types of non-apoptotic programmed cell death, such as methuosis, in addition to interfering with cellular processes essential to tumor metabolism (HASSAN MM e FAROUK O, 2017; SHANKARAIAH N, et al., 2017).

Concurrently, in the current public health landscape, antimicrobial resistance represents one of the main challenges facing modern medicine. According to the World Health Organization (WHO), antimicrobial resistance compromises the effectiveness of conventional treatments, resulting in prolonged infections,

increased mortality rates, high hospital costs, and the risk of uncontrollable outbreaks (HELMY YA, et al., 2023). This problem is exacerbated by the indiscriminate use of antibiotics, self-medication, and the scarcity of new antimicrobial agents in development (CANO A, et al., 2020).

In this context, chalcones emerge as relevant candidates in the search for therapeutic alternatives, especially in combating bacterial and fungal resistance. These molecules have the ability to interfere with various microbial targets, including cell wall degradation, which leads to osmotic collapse and cell lysis, as well as inhibiting biofilm formation, mechanisms often associated with resistance in chronic infections. Studies also suggest that chalcones have a highly adaptable structure, allowing the production of derivatives with increased specificity and reduced toxicity (LAGU SB, et al., 2020).

Thus, the chemical synthesis of chalcones has been widely explored as a route to obtain bioactive derivatives with higher yields and purity, enabling production on a scale suitable for preclinical and clinical investigations. A deeper understanding of the mechanisms of action and the structure-activity relationship of these molecules can accelerate the development of new antimicrobial agents, meeting an urgent demand in contemporary society (OLIVEIRA JWA, et al., 2021).

This study aimed to synthesize and evaluate a new thiophenic chalcone and a pyrazole derivative using specific chemical routes. The antimicrobial activity of the synthesized substances was determined against pathogenic strains of bacteria and a fungus. The possible mechanism of action of the most active molecules was investigated, with an emphasis on the integrity of the microbial cell wall. This research aims to contribute to addressing growing antimicrobial resistance by offering promising alternatives for treating infections.

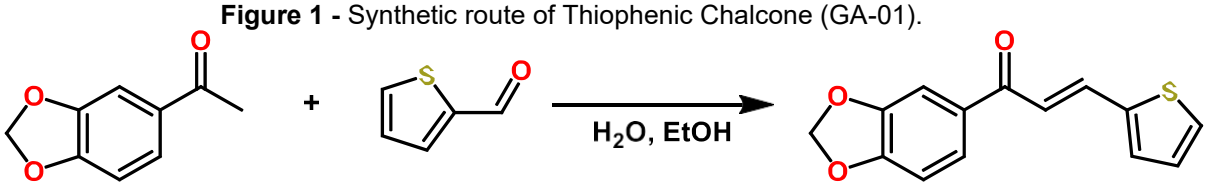
METHODS

The syntheses were performed in the Organic Chemistry Laboratory of the Biotechnology Center of the Federal University of Paraíba (CBiotec/UFPB), under controlled temperature and ventilation conditions. The reagents used were purchased from Sigma-Aldrich® (analytical grade), and the solvents from Synth® (P.A. grade).

The reactions occurred under constant magnetic stirring on a FISATOM model 752A heating mantle, with reflux using a vertical Graham condenser. The progress of the reactions was monitored by thin layer chromatography (TLC) using 60 G F254 silica gel plates (Merck®). Developments were achieved by exposure to ultraviolet light (254 nm and 366 nm) and specific chemical agents, such as vanillin-sulfuric acid solution or sublimed iodine. Pre-purification of the products was performed by vacuum filtration in a Büchner funnel with qualitative filter paper, and the solids were dried in a desiccator containing silica gel. The reaction yield was calculated based on the mass obtained in relation to the expected theoretical mass, and the products were subjected to physical and chemical characterization.

In a beaker, 150 mL of a hydroalcoholic solution (2:1) containing 10 mMol of 3,4-methylenedioxyacetophenone and 10 mmol of 2-thiophenecarboxaldehyde were added. Subsequently, 20 mMol of sodium hydroxide were added under constant stirring (**Figure 1**). The reaction mixture was kept under stirring for 6 hours at room temperature, until the formation of a yellow precipitate. The solid was filtered, washed with distilled water and dried at room temperature, resulting in 2.42 g of crude product.

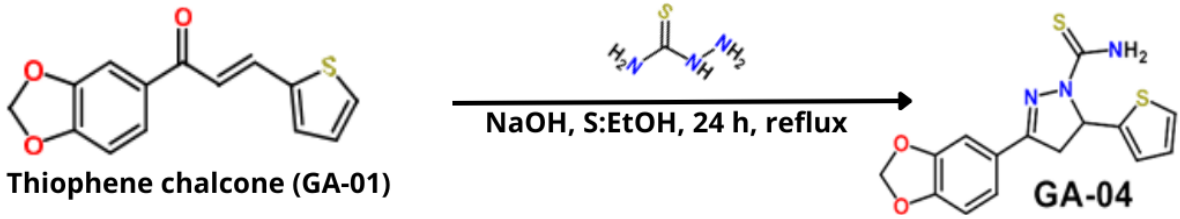
Figure 1 - Synthetic route of Thiophenic Chalcone (GA-01).



Source: Lacerda AF, et al., 2025.

Compound GA-04 was synthesized from the reaction of GA-01 (2 mmol) with thiosemicarbazide (1.75 mmol) in absolute ethanol under reflux for 24 hours (**Figure 2**). The product, a white solid, was isolated by filtration and dried at room temperature.

Figure 2 - Synthetic route for obtaining a pyrazole derivative of GA-01.



Source: Lacerda AF, et al., 2025.

Of the substances synthesized in this study, Thiophenic Chalcone (GA-01) and one of its derivatives (GA-04) were used for the antimicrobial tests performed in vitro.

For the determination of the Minimum Inhibitory Concentration (MIC), the substances GA-01 and GA-04 were dissolved in pure dimethyl sulfoxide (DMSO) to prepare 102.4 mg/mL stock solutions, which were stored at -20°C, and subsequently diluted to reduce the solution by 100 times, so that the amount of DMSO in the well did not exceed 1%. The test microorganisms were: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 13150) and *Candida albicans* (ATCC 76645), cultivated on inclined nutrient agar under refrigeration at 4°C and subjected to monthly subcultures.

The bacterial suspension was standardized to a turbidity equivalent to a McFarland tube No. 1 (≈3 × 108 CFU/mL). In 96-well microplates, serial dilutions were performed in Mueller-Hinton broth, starting at a concentration of 1024 µg/mL and ending at 1 µg/mL, from the first to the 11th column, respectively. The 12th column served as a solvent control. Growth (row G) and sterility (row H) controls were prepared accordingly. The plates were incubated at 30°C for 24 hours.

Subsequently, 10 µL of 0.1% resazurin (7-hydroxy-3H-phenoxazine-3-one 10-oxide) (redox indicator) was added to assess microbial viability. A color change from blue (resazurin) to pink (resorufin) indicated microbial growth and was analyzed by visual inspection. The lowest concentration that inhibited the color change was considered the MIC. The assays were performed in triplicate (ROA BM, et al., 2019).

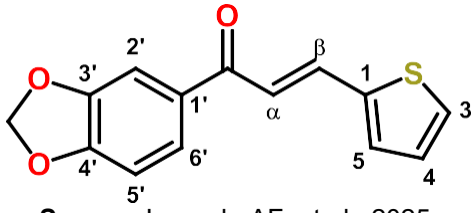
Regarding the investigation of the mechanism of action with sorbitol, the evaluation of the possible mechanism of action of substances GA-01 and GA-04 was performed using the cell wall assay using the osmoprotective agent sorbitol, as described by Krishnamurthi VR, et al. (2021). Strains of P. aeruginosa, S. aureus, and C. albicans were tested. E. coli was excluded due to nonviability during subculture.

The experiment was conducted in 96-well microplates containing nutrient broth supplemented with 0.8 M sorbitol. The substances were tested at three concentrations: lower, equal, and higher than the previously determined MIC. Each well received 10 µL of the microbial suspension and 100 µL of the substance solution in sorbitol medium. The controls were the same as those used in the MIC assay. After 24 hours of incubation at 30 °C, absorbance was measured at 630 nm in a microplate reader (Mindray MR-96A), allowing the evaluation of changes in cell viability in an osmotically protected environment.

RESULTS

The synthesis of chalcone GA-01 (**Figure 3**) was carried out via aldol condensation between 3,4-methylenedioxyacetophenone and 2-thiophenecarboxaldehyde, using an ethanolic potassium hydroxide solution as the reaction medium. The product obtained showed a yield of 93.8% and a melting point between 95–100 °C. The structure was confirmed by ^{1}H and ^{13}C NMR, demonstrating signals from the α,β-unsaturated system (δ 7.61–7.95 ppm), the carbonyl group (δ 187.73 ppm), and the methylenedioxy group (δ 6.05 ppm in ^{1}H and δ 101.98 ppm in ^{13}C) (BORCHHARDT DM, et al., 2010).

Figure 3 - (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(thiophen-2-yl)prop-2-en-1-one (GA-01).

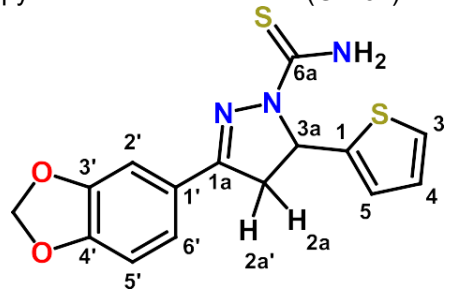


Source: Lacerda AF, et al., 2025.

The Yellow solid; MP 95–100 °C; yield 93.8%;1H NMR (400 MHz, CDCl3) δ 6.05 (s, 2H, -OCH2O-), 6.89 (dd, J 8.1; 0.3 Hz, 1H, H5'), 7.08 (dd, J 5.1; 3.6 Hz, 1H, H4), 7.28 (d,J 15.2 Hz, 1H, Hα), 7.34 (d, J 3.6 Hz, 1H, H5), 7.40 (dt, J 5.0; 1.0 Hz, 1H, H3), 7.51 (dd, J 1.7; 0.4 Hz, 1H, H2'), 7.62 (dd, J 8.2; 1.7 Hz, 1H, H6'), 7.92 (dt, J 15.3; 0.7 Hz, 1H, Hβ); 13CRMN (100 MHz, CDCl3) δ 101.98 (-OCH2O-), 108.03 (C2'), 108.46 (C5'), 120.57 (C6'), 124.68 (Cα), 128.43 (C4), 128.68 (C5), 131.97 (Cβ), 133.00 (C1'), 136.78 (C3), 140.59 (C1), 148.40 (C3'), 151.80 (C4'), 187.73 (C=O).

The GA-04 molecule (**Figure 4**) was obtained by the cyclocondensation reaction of GA-01 with thiosemicarbazide under reflux in ethanol, with a yield of 59.6% and a melting point between 160–163 °C. Spectroscopic characterization revealed the formation of the pyrazole nucleus, confirmed by the presence of geminal couplings (δ 3.32 and 3.75 ppm, J = 17.4 Hz) and the disappearance of the conjugated carbonyl signal. The signals from the carbon atoms of the thiophene group, methylenedioxy, and pyrazole ring reinforced the structural elucidation (**Figure 4**). 3-(benzo[d][1,3]dioxol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (GA-04).

Figure 4 - 3-(Benzo[d][1,3] dioxol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-carbothioamide (GA-04).



Source: Lacerda AF, et al., 2025.

The synthesized substance was characterized as a white solid, MP 160-163 °C; yield 59.60%; 1H NMR (400 MHz, CDCl3) δ 3.32 (dd, J 17.4; 2.9 Hz, 1H, H2a'), 3.75(dd, J 17.4; 10.9 Hz, 1H, H2a), 6.04 (s, 2H, -OCH2O-), 6.34 (ddd, J 10.9; 3,0; 8.1; 1.7 Hz, 1H, H6'), 7.19 (dd, J 5.1; 1.2 Hz, 1H, H3), 7.34 (d, J 1.7 Hz, 1H, H2'). 13C NMR (100 MHz, CDCl3) δ 43.10 (C2a), 59.29 (C3a), 101.90 (-OCH2O-), 106.52(C2'), 108.56 (C5'), 122.64 (C6'), 124.38 (C4), 124.86 (C1), 125.12 (C3), 126.89 (C5), 144.15 (C1'), 148.52 (C3'), 150.47 (C4'), 156.03 (C1a), 176.46 (C6a).

Regarding Minimum Inhibitory Concentration (MIC) tests, as shown in **Table 1**, the evaluation of antimicrobial activity revealed that GA-01 had an MIC of 128 µg/mL against all tested strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*). In contrast, GA-04 showed more significant activity, with an MIC of 64 µg/mL against bacteria and 4 µg/mL against C. albicans. The remarkable efficacy against yeast suggests a selective action of the compound on eukaryotic organisms, which may be related to the presence of the pyrazole ring in its structure. DMSO, used as a solvent, did not interfere with the results. The data obtained indicate that GA-04 has superior potential as an antimicrobial agent, especially as an antifungal agent.

Table 1 - Minimum inhibitory concentration of GA-01 and GA-04.

	GA-01	GA-04	SC	VC	SC
P. aeuruginosa ATCC 27853	128 µg/mL	64 µg/mL	-	+	+
E. coli ATCC 25922	128 µg/mL	64 µg/mL	-	+	+
S. aureus ATCC 13150	128 µg/mL	64 µg/mL	-	+	+
C. albicans ATCC 76645	128 µg/mL	4 µg/mL	-	+	+

Note: SC = sterility control; VC = viability control; SC = solvent control; + = presence of microbial growth; - = absence of microbial growth.
Source: Lacerda AF, et al., 2025.

The initial characterization of the antimicrobial mechanism of action of GA-01 and GA-04 was investigated using the sorbitol osmotic protection assay, which helps identify compounds that act on the cell wall. The addition of sorbitol to the culture medium resulted in an increase in the MIC for S. aureus, P. aeruginosa, and C. albicans, indicating that the compound's action involves interference with cell wall integrity (**Table 2**). Growth inhibition in the absence of sorbitol, followed by partial recovery in the presence of the osmoprotectant, suggests that the primary target of GA-01 and GA-04 is associated with cell wall biosynthesis or stability. This mode of action is consistent with the profile observed in antibiotics that affect extracytoplasmic structures, such as β-lactams and echinocandins. Although further studies are needed to further understand the mechanisms of action of these substances.

Table 2 - Evaluation of the mechanism of action of GA-01 and GA-04 on the microbial wall.

	GA–01 256 µg/mL	GA-01 128 µg/mL	GA-01 64 µg/mL	GA-04 128 µg/mL	GA-04 64 µg/mL	GA-04 32 µg/mL	SC	VC
P. aeuruginosa ATCC 27853 com sorbitol	+	+	+	+	+	+	-	+
S. aureus ATCC 13150 com sorbitol	+	+	+	+	+	+	-	+
C. albicans ATCC 76645 com sorbitol	+	+	+	+	+	+	-	+
P. aeuruginosa ATCC 27853 sem sorbitol	-	-	+	-	-	+	-	+
S. aureus ATCC 13150 sem sorbitol	-	-	+	-	-	+	-	+
C. albicans ATCC 76645 sem sorbitol	-	-	+	-	-	-	-	+

Note:SC = sterility control; VC = viability control; + = presence of microbial growth; - = absence of microbial growth.
Source: Lacerda AF, et al., 2025.

DISCUSSION

The synthesis and characterization of a thiophenic chalcone (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(thiophen-2-yl)prop-2-en-1-one (GA-01) and a new derivative were carried out, this being: 3-(benzo[d][1,3]dioxol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-carbothioamide (GA-04).

The synthesis of the pyrazole derivative containing chalcone fragments was successfully carried out using conventional aldol condensation methodologies and subsequent cycloaddition with hydrazines. The compound was obtained in satisfactory yield and presented structural characteristics compatible with the functionalized pyrazole nucleus. Analysis of the spectroscopic data confirmed the structural integrity of the synthesized molecules, serving as a basis for subsequent studies.

Pyrazoles are heterocyclic ring compounds with five atoms, two of which are adjacent nitrogen atoms, also known as azoles. They are present in several medications already on the market, such as sildenafil (phosphodiesterase inhibitor), celecoxib (anti-inflammatory and COX-2 inhibitor), and fomepizole (alcohol

dehydrogenase inhibitor) (ANSARI A, et al., 2016); they may present antimicrobial, anticorrosive (SAYED GH, et al., 2018), neuroprotective (BHAT MA, et al., 2017), anti-inflammatory (HASSAN GS, et al., 2019), antituberculosis (TAKATE SJ, et al., 2019) activity, among others.

Chalcones, as skeletons of several new synthetic compounds, can have their biological activity improved with the addition of heterocycles, demonstrating antibacterial activity (KHAN SA, et al., 2019), carcinogenesis inhibitor (HORLEY NJ, et al., 2017), antitumor (MAO Z, et al., 2016), among others (ARDIANSAH B, 2019). The addition of pharmacophores to the chalcone nucleus allows this already biologically active molecule to have greater direction of the desired activity in drug development, in addition to improving characteristics such as toxicity and exacerbated side effects that can be predicted in silico in drug design.

Thiophene chalcone GA-01 (**Figure 3**) was first synthesized by Borchhardt and collaborators in 2010 through the aldol condensation of 3,4-methylenedioxyacetophenone and 2-thiophenecarboxaldehyde. For this reaction, potassium hydroxide (KOH) was used as catalyst and methanol as solvent to obtain a yield of 91% (BORCHHARDT DM, et al., 2010).

Previous studies have shown this molecule to be a potent inhibitor of the *Trypanosoma cruzi* protein cruzain, with an IC50 value of 22 μM. The molecule has also been tested against *Mycobacterium tuberculosis* proteins (CHIARADIA LD, et al., 2012), anti-prion activity (FERREIRA NC, et al., 2014), and as an inhibitor of the *Plasmodium falciparum* falcipain 2 protein (BERTOLDO JB, et al., 2015), showing little or no activity in these studies. Furthermore, antimicrobial tests with chalcone demonstrated growth inhibition of Escherichia coli (AV12) with a minimum inhibitory concentration of 10-3 M (DUARTE GD, 2020).

Substance GA-04 (**Figure 4**) is unpublished; therefore, there are no published articles on its synthesis, biological activity, and/or activity predictions. A substructure similarity search in the Scifinder database revealed two articles on two distinct molecules with methylation of the thiophene ring at positions 3 and 5, respectively.

The methodology used to synthesize similar molecules consists of acid catalysis (acetic acid or amberlyst-15) of chalcone with thiosemicarbazide or thiosemicarbazide hydrochloride, with average yields of 72%. One of these molecules showed promising antimicrobial activity against *E. coli*, *B. subtilis*, *A. niger*, and *A. flavus*, possibly due to its ability to sequester iron from the medium, reducing its availability to microorganisms (PRABHUDEVA MG, et al., 2018). Antitumor tests were also performed with 60 cell lines, for which the compound was found to be inactive (INSUASTY B, et al., 2012).

The evaluation of antimicrobial activity by determining the minimum inhibitory concentration (MIC) revealed that the tested derivatives exhibited significant inhibitory effects against strains of *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. The compounds exhibited varied MIC values, with emphasis on the derivative containing electroattractant substituents (GA-04), which demonstrated greater potency against bacterial strains compared to GA-01, and even greater activity against C. albicans (ZHAO C, et al., 2019). These results suggest that the electronic nature of the functional groups present in the aromatic ring significantly influences biological activity, favoring more effective interactions with intracellular bacterial targets (PAN T, et al., 2017).

Investigation of the mechanism of action indicated a possible propensity of the substances to interfere with the microbial cell wall, although the exact mechanism of action remains unknown. Further studies focused solely on investigating this possible mechanism are needed (JORDA A e ZEITLINGER M, 2020). Thus, the data obtained indicate that pyrazole derivatives containing chalcones constitute a promising class of bioactive compounds, with an adequate pharmacokinetic profile, high affinity for microbial molecular targets, and multiple mechanisms of action that enhance their antimicrobial efficacy.

CONCLUSION

This study demonstrated that the pyrazole derivative containing a chalcone skeleton has great potential as an antimicrobial agent, due to its significant activity against resistant strains. The synthesis was efficient

and reproducible, with clear and consistent structural characterization. The relationship between chemical structure and activity revealed that electroattractant substituents favor antimicrobial potency. These findings demonstrate that the studied compounds can serve as prototypes for the development of new antimicrobial drugs, especially in a critical scenario of increasing bacterial resistance. Future studies should focus on structural optimization, *in vivo* toxicological evaluation, and pharmacodynamic testing to consolidate the therapeutic applicability of these derivatives.

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