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Synthesis and biological evaluation of chalcone derivatives containing aminoguanidine or acylhydrazone moieties

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Abstract

Three novel series of chalcone derivatives containing an aminoguanidine or acylhydrazone moiety were designed, synthesized and evaluated in terms of their antibacterial, antifungal and anti-inflammatory activities. Most of the synthesized compounds showed potent inhibitory activity towards various bacteria and one fungus with minimum inhibitory concentrations (MICs) ranging from 1 to 8 μg/mL. Compared with our previously reported chalcone derivatives (MICs > 64 μg/mL), these compounds exhibited improved antibacterial activities (MICs = 2 μg/mL) against Gram-negative bacterial strains (*Escherichia coli* 1924 and 1356). Compounds **4f** and **4h** were found to be the most potent with an MIC value of 1 μg/mL against the Gram-negative bacterial strains *Salmonella typhimurium* 1926 and the fungus *Candida albicans* 7535. In addition, compound **4f** displayed the most potent anti-inflammatory activity of all of the compounds prepared in the current study with 92.45% inhibition after intraperitoneal administration, making it more potent than the reference drugs indomethacin and ibuprofen. The cytotoxic activity of the compound **4f** was assessed in HeLa, Hep3B and L02 cells.

Keywords: Chalcone; Acylhydrazone; Aminoguanidine; Antibacterial activity; Antifungal activity; Anti-inflammatory activity; Cytotoxicity

Numerous research groups throughout the world are currently involved in an urgent search for novel antibacterial and antifungal agents to overcome the emergence of new infectious diseases and the increasing number of multidrug-resistant microbial pathogens.[1,2] Although several classes of antimicrobial agents are currently available, pathogenic bacteria and fungi have developed resistance to these drugs in the majority of cases.[3,4] Additionally, there is a strong relationship between bacterial infection and inflammation.[5] Furthermore, inflammation remains a common and poorly controlled clinical problem that can be life threatening in extreme cases, including allergic reaction, autoimmune disease and organ rejection following transplantation surgery.[6] There is, therefore, an urgent need to develop novel antibacterial, antifungal and anti-inflammatory agents to address these issues.

Chalcones belong to the flavonoid class of natural products and have attracted considerable interest because of their relatively simple structures and wide variety of pharmacological activities.[7-11] For example, chalcone-based compounds have been reported to exhibit anticancer,[12] anti-inflammatory,[13] anti-ulcerative,[14] analgesic,[15] antiviral,[16] antifungal,[17] antimalarial[18] and antibacterial activities.[19] Aminoguanidine derivatives have recently captured the attention of numerous researchers because of their diverse range of biological properties, including their antibacterial,[20] antifungal[21] and anti-inflammatory activities.[22] Acylhydrazones have also received considerable interest from researchers working in a variety of different fields because they possess a broad range of pharmacological properties, including antibacterial[23,24] and anticancer activities.[25] In our previous work, we reported the development of a series of chalcone derivatives that showed potent activity against Gram-positive bacterial strains, including multidrug-resistant clinical isolates.[26] Unfortunately, none of these compounds are active against Gram-negative bacteria. Aminoguanidine derivatives were reported as antifungal agents with a potency of 100 μg/mL.[21] In a continuation of our research towards the discovery and development of increasingly potent antibacterial agents, we report herein the structure-based design of chalcone derivatives containing an aminoguanidine or acylhydrazone moiety. In this way, we have developed three novel series of chalcone derivatives (Figure 1), totaling 26

compounds, which were designed, synthesized and screened for their antibacterial, antifungal and anti-inflammatory activities *in vitro*. The substituents on the phenyl ring were simultaneously changed to investigate their contribution to the biological activity.

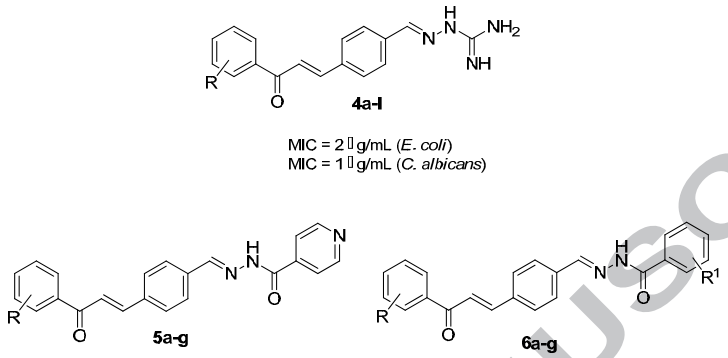
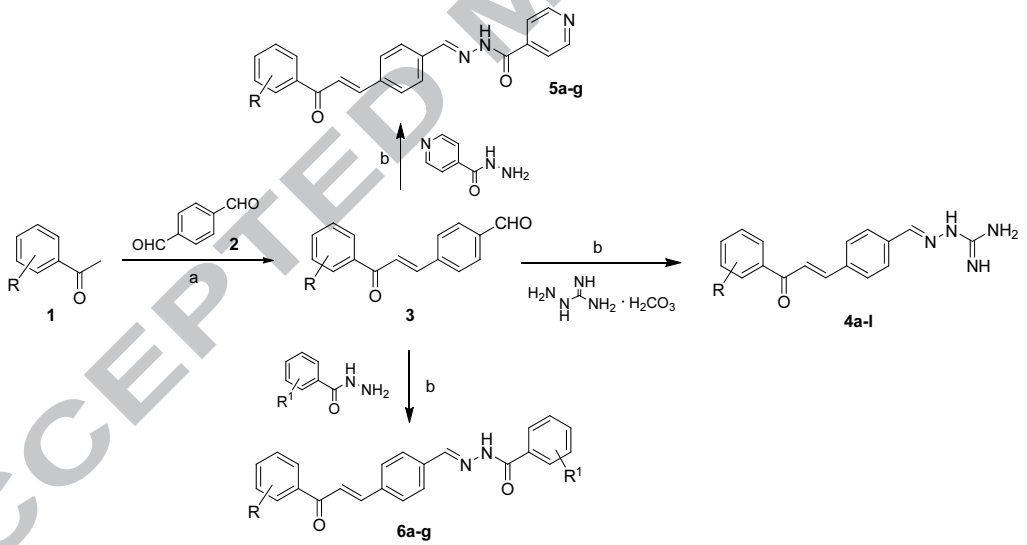


Figure 1. The structures of the target compounds.



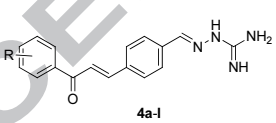
Scheme 1. Synthetic scheme for the synthesis of the target compounds. Reagents and conditions: (a) NaOH, EtOH, 23°C, 3-4 h; (b) Aminoguanidine bicarbonate or isoniazide or benzoylhydrazine, EtOH, HCl, 50-60 ^{o}C, reflux, 8-12 h.

The route used for the synthesis of compounds **4a–l**, **5a–g** and **6a–g** is shown in Scheme 1. The key intermediate **3** was prepared by the Claisen–Schmidt condensation of terephthalaldehyde (**2**) with a substituted acetophenone (**1**) using a previously

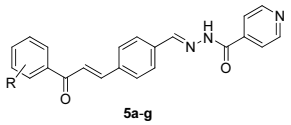
described method.[26,27] Compounds **4a–l** were prepared by the reaction of **3** with aminoguanidine bicarbonate in the presence of concentrated hydrochloric acid in refluxing ethanol. Compounds **5a–g** and **6a–g** were prepared by the reactions of compound **3** with isonicotinic acid hydrazide and substituted benzoyl hydrazine, respectively, in the presence of catalytic amounts of hydrochloric acid in ethanol.[22] The structures of the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, HRMS and MS analyses.[28]

The *in vitro* antimicrobial and antifungal activities of the synthesized compounds were evaluated against a variety of strains of bacteria and one fungus (including multidrug-resistant clinical isolates), using a 96-well microtiter plate screening format to obtain their minimum inhibitory concentration (MIC) values.[29] Gatifloxacin, moxifloxacin, fluconazole, norfloxacin and oxacillin were used as positive controls. The compounds were screened against four Gram-positive strains (*S. aureus* 4220, *S. aureus* 209, *S. aureus* 503 and *Streptococcus mutans* 3065), four clinical isolates of multidrug-resistant Gram-positive bacterial strains (MRSA 3167 and 3506, and QRSA 3505 and 3519), four Gram-negative strains (*Escherichia coli* 1924, *E. coli* 1356, *Salmonella typhimurium* 1926 and *Pseudomonas aeruginosa* 2742) and one fungus (*Candida albicans* 7535).

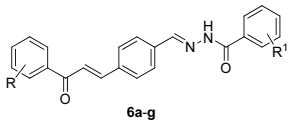
Table 1. Inhibitory activities (MIC[a], μg/mL) of compounds **4a**–**l**, **5a**–**g** and **6a**–**g** against various bacteria.



4a-l



5a-g



6a-g

Compd	R	R^1	Gram-positive strains				Gram-negative strains				Fungus
			4220[b]	503[c]	209[d]	3065[e]	1924[f]	1356[g]	1926[h]	2742[i]	7535[j]
4a	2-F	—	4	8	8	8	8	16	4	4	8
4b	4-F	—	4	8	8	8	4	8	4	4	4
4c	2-Cl	—	8	8	8	8	8	16	8	8	8
4d	3-Cl	—	4	4	4	4	4	8	2	4	4
4e	4-Cl	—	2	4	4	4	2	8	4	2	4
4f	2,4-Cl_2	—	2	2	2	2	2	8	1	2	1
4g	2-Br	—	4	8	8	8	8	16	4	8	8
4h	3-Br	—	2	2	2	2	2	8	1	2	2

4i	4-Br	—	2	4	4	4	2	32	2	2	2
4j	4-CH_3	—	4	4	4	8	4	8	4	2	2
4k	2,4-$(CH_3)_2$	—	4	8	8	8	8	8	8	4	8
4l	4-OCH_3	—	16	16	16	8	16	32	2	8	8
5a	2-F	—	64	64	64	16	64	64	>64	>64	>64
5b	4-F	—	32	64	32	64	64	64	>64	>64	>64
5c	2-Br	—	64	>64	64	32	>64	>64	>64	>64	64
5d	3-Br	—	64	64	64	>64	>64	>64	>64	>64	>64
5e	4-Br	—	>64	>64	>64	>64	>64	>64	>64	>64	>64
5f	2,4-Cl_2	—	8	16	16	32	32	32	>64	>64	4
5g	2,4-$(CH_3)_2$	—	>64	>64	>64	>64	>64	>64	>64	>64	>64
6a	2-F	H	>64	>64	>64	>64	>64	>64	>64	>64	>64
6b	2-Cl	H	>64	64	64	>64	>64	>64	>64	>64	>64
6c	2-Br	H	>64	>64	>64	>64	>64	>64	>64	>64	>64
6d	2,4-$(CH_3)_2$	H	>64	>64	>64	>64	>64	>64	>64	>64	>64
6e	2-Cl	3-Cl	32	32	32	>64	>64	>64	>64	>64	>64
6f	3-Cl	3-Cl	32	32	32	>64	32	32	>64	>64	>64
6g	4-Cl	3-Cl	32	32	32	>64	32	32	>64	>64	>64
	Gatifloxacin		0.25	4	2	0.5	2	16	2	1	n.d.[k]
	Moxifloxcin		0.25	2	2	0.25	2	128	1	1	n.d.
	Fluconazole		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1

[a] The antibacterial tests was carried out three times, and the average values were taken as the MICs.
[b] *Staphylococcus aureus* 4220. [c] *Staphylococcus aureus* 503. [d] *Staphylococcus aureus* 209.
[e] *Streptococcus mutans* 3065. [f] *Escherichia coli* 1924. [g] *Escherichia coli* 1356. [h] *Salmonella typhimurium* 1926.
[i] *Pseudomonas aeruginosa* 2742. [j] *Candida albicans* 7535. [k] n.d.: Not determined.

Table 2. Inhibitory activities (MIC[a], μg/mL) of compounds **4a**–**l**, **5a**–**g** and **6a**–**g** against the clinical isolates of multidrug-resistant Gram-positive bacterial strains.

Compd	R	R^1	Multidrug-resistant Gram-positive strains			
			MRSA		QRSA	
			3167[b]	3506[c]	3505[d]	3519[e]
4a	2-F	—	8	4	8	8
4b	4-F	—	8	4	8	8
4c	2-Cl	—	8	8	8	16
4d	3-Cl	—	4	2	4	8
4e	4-Cl	—	4	2	4	8
4f	2,4-Cl_2	—	8	8	8	16
4g	2-Br	—	8	8	8	16
4h	3-Br	—	2	2	2	4
4i	4-Br	—	8	2	4	16
4j	4-CH_3	—	8	2	4	4
4k	2,4-$(CH_3)_2$	—	8	4	8	8

4l	4-OCH_3	—	16	8	16	16
5a	2-F	—	64	64	64	64
5b	4-F	—	32	32	32	32
5c	2-Br	—	>64	>64	>64	>64
5d	3-Br	—	32	32	64	64
5e	4-Br	—	>64	64	>64	64
5f	2,4-Cl_2	—	16	16	16	16
5g	2,4-$(CH_3)_2$	—	64	64	>64	>64
6a	2-F	H	64	64	>64	>64
6b	2-Cl	H	64	64	64	64
6c	2-Br	H	>64	>64	>64	>64
6d	2,4-$(CH_3)_2$	H	>64	>64	>64	>64
6e	2-Cl	3-Cl	32	32	32	32
6f	3-Cl	3-Cl	32	32	32	32
6g	4-Cl	3-Cl	32	32	32	32
	Gatifloxacin		2	2	8	4
	Norfloxacin		8	4	>64	>64
	Oxacillin		>64	>64	1	1

[a] The antibacterial tests were carried out three times, and the average values were taken as the MICs.
[b] Methicillin-resistant *Staphylococcus aureus* 3167. [c] Methicillin-resistant *Staphylococcus aureus* 3506.
[d] Quinolone-resistant *Staphylococcus aureus* 3505. [e] Quinolone-resistant *Staphylococcus aureus* 3519.

The *in vitro* antibacterial and antifungal activities of the synthesized compounds are shown in Tables 1 and 2. Most of the synthesized compounds showed potent inhibitory activities against the different bacteria and the one fungus tested in the current study with MICs ranging from 1 to 64 µg/mL. Almost all of the compounds in series **4** exhibited potent antibacterial activity with MICs ranging from 1 to 16 µg/mL, except for **4i** and **4l** against the *E. coli* 1356 strain (MIC = 32 µg/mL). Compounds **4f** and **4h** exhibited the highest activity of all the compounds synthesized in series **4** against *S. typhimurium* 1926 with an MIC value of 1 µg/mL, making them 2-fold more potent than gatifloxacin and equipotent to moxifloxacin. Against the Gram-positive strains (*S. aureus* 503 and *S. aureus* 209) and Gram-negative *E. coli* 1924, these two compounds showed equipotent or more potent to the standard drugs gatifloxacin and moxifloxacin with an MIC value of 2 µg/mL. Against the fungus *C. albicans* 7535, compound **4f** displayed the strongest potency of all of the compounds synthesized in series **4** with an MIC value of 1 µg/mL, which was equal to that of fluconazole. Notably, we have shown for the first time the development of chalcone

derivatives exhibiting good antibacterial activity against four Gram-negative bacteria, especially *S. typhimurium* 1926 and *P. aeruginosa* 2742, with MICs ranging from 1 to 16 μg/mL (except **4i** and **4l**). These compounds also exhibited antifungal activity against *C. albicans* 7535 with MICs ranging from 1 to 8 μg/mL, making them more potent than our previously reported chalcone derivatives.[26] The position of the substituents on the phenyl ring of compounds **4a–l** had a pronounced effect on their activities, which varied according to the following orders: 3-Br > 4-Br > 2-Br for the bromo-substituted compounds; 2,4-Cl_2 > 4-Cl > 3-Cl > 2-Cl for the chloro-substituted compounds; and 4-CH_3 > 2,4-$(CH_3)_2$ > 4-OCH_3 for compounds bearing an electron-donating group. Compounds in series **5** and **6** generally showed weak activity against all of the strains tested in the current study with MICs ranging from 16 to 64 μg/mL. Notably, compound **5f** bearing a 2,4-Cl_2 substituted phenyl ring showed moderate activity against all of the strains tested in this study with MICs ranging from 4 to 32 μg/mL, except for the Gram-negative strains *S. typhimurium* 1926 and *P. aeruginosa* 2742. For the compounds in series **6**, only those bearing a Cl substituent on the phenyl ring of their benzoyl hydrazone (**6e**, **6f**, **6g**) showed moderate antimicrobial activity.

As shown in Table 2, most of the compounds exhibited good inhibitory activity towards the multidrug-resistant Gram-positive bacterial strains (MRSA 3167 and 3506, QRSA 3505 and 3519) with MIC values in the range of 2–64 μg/mL. The bromo-substituted compound **4h** exhibited the most potent activity against the MRSA and QRSA strains with MIC values of 2–4 μg/mL, making more potent or equipotent to gatifloxacin and norfloxacin (MICs of 8 and 4 μg/mL against MRSA 3167 and 3056).

In general, the antimicrobial activity results revealed that the compounds in series **4** were much more potent than the compounds in the other two series, indicating that the aminoguanidine moiety was critical to the activity of these chalcone derivatives. Furthermore, our previously reported chalcone derivatives did not show any activity against the Gram-negative bacteria tested in this study, and the introduction of an aminoguanidine moiety to these chalcone resulted in moderate to

good levels of activity against several Gram-negative strains of bacteria. It is also noteworthy that compounds **4f** and **5f**, bearing a 2,4-Cl_2 substituted phenyl ring, showed excellent antimicrobial activities with MICs ranging from 1 to 8 μg/mL and 4 to 32 μg/mL, respectively, against most of the bacteria strains and the fungus tested in this study (except *Salmonella typhimurium* 1926 and *Pseudomonas aeruginosa* 2742). These results therefore provide further evidence to suggest that the 2,4-Cl_2 substituted phenyl ring plays a critical role in the activity of these compounds, which is consistent with the results obtained for a previously reported series of rhodanine derivatives. [30]

Table 3. Anti-inflammatory activities of compounds **4a–l**, **5a–g** and **6a–g** following i.p. administration.

	R	R^1	Dose (mg/kg)	Number of mice	Edema mean ± S.D. (mg)	Inhibition rate (%)
DMSO	—	—	100	10	10.60 ± 1.35	—
Indometacin	—	—	100	10	1.12 ± 0.07***	89.38
Ibuprofen	—	—	100	10	1.33 ± 0.10***	87.36
4a	2-F	—	100	10	0.88 ± 0.28***	91.66
4b	4-F	—	100	10	0.98 ± 0.30***	90.70
4c	2-Cl	—	100	10	1.68 ± 0.53***	84.12
4d	3-Cl	—	100	10	2.05 ± 0.65***	80.66
4e	4-Cl	—	100	10	4.61 ± 0.65***	56.44
4f	2,4-Cl_2	—	100	10	0.80 ± 0.21***	92.45
4g	2-Br	—	100	10	2.30 ± 1.19***	78.30
4h	3-Br	—	100	10	5.33 ± 1.57***	49.68
4i	4-Br	—	100	10	3.30 ± 1.40***	68.86
4j	4-CH_3	—	100	10	2.36 ± 0.66***	77.66
4k	2,4-$(CH_3)_2$	—	100	10	3.80 ± 0.68***	64.15
4l	4-OCH_3	—	100	10	4.53 ± 1.00***	57.23
5a	2-F	—	100	10	5.16 ± 1.20***	51.25
5b	4-F	—	100	10	5.18 ± 1.36**	51.13
5c	2-Br	—	100	10	5.43 ± 1.50**	48.74
5d	3-Br	—	100	10	9.12 ± 1.50	13.96
5e	4-Br	—	100	10	4.70 ± 0.72***	55.66
5f	2,4-Cl_2	—	100	10	1.72 ± 0.13***	83.77
5g	2,4-$(CH_3)_2$	—	100	10	4.40 ± 1.06***	58.49
6a	2-F	H	100	10	12.79 ± 0.22	—
6b	2-Cl	H	100	10	11.42 ± 0.94	—
6c	2-Br	H	100	10	11.73 ± 0.68	—
6d	2,4-$(CH_3)_2$	H	100	10	10.58 ± 0.40	0.18
6e	2-Cl	3-Cl	100	10	6.04 ± 1.00*	43.01

6f	3-Cl	3-Cl	100	10	11.43 ± 1.08	—
6g	4-Cl	3-Cl	100	10	10.69 ± 0.62	—

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ compared with a vehicle group.
—: no anti-inflammatory activity

The anti-inflammatory effects of the synthesized compounds were also evaluated in this study, and the results are shown in Table 3. Dimethyl sulfoxide was used as the vehicle for the primary screening of the synthesized compounds, using indomethacin and ibuprofen as suitable reference drugs. Compounds were screened in a xylene-induced ear-edema model in mice, where the anti-inflammatory activity was assessed based on the ability of a test compounds to prevent edema.[31] The results revealed that most of the synthesized compounds showed pronounced anti-inflammatory effects at 100 mg/kg. It is noteworthy that compound **4f** exhibited the most potent anti-inflammatory activity of all the compounds with an activity of 92.45%, which was higher than those of ibuprofen (87.36%) and indomethacin (89.38%). Compounds **4a**, **4b** and **4f** displayed slightly improved activity compared with the reference drugs. Disappointingly, compounds **6a**, **6b**, **6c**, **6f** and **6g** did not exhibit any anti-inflammatory activity at the same dose. The anti-inflammatory activities of these three series of compounds were therefore determined to be in the order of series **4** > series **5** > series **6**, which indicated that the aminoguanidine moiety was making a bigger contribution to the biological activity than the acylhydrazone moiety.

Table 4. Anti-inflammatory activity of compound **4f** administered orally at different times before xylene application.

Time (h)	Dose (mg/kg)	Number of mice	Inhibition (%)	
			4f	Indometacin
1	100	10	20.69	11.96
2	100	10	34.19**	30.94**
3	100	10	26.42	18.65
4	100	10	16.91	15.63
5	100	10	11.28	10.28
24	100	10	5.52	5.01

**: $p < 0.01$ compared with a vehicle group.

Table 5. Anti-inflammatory activity of compound **4f** administered orally at different doses.

Time (h)	Dose (mg/kg)	Number of mice	Inhibition (%)	
			4f	Indometacin
2	100	10	43.45	33.32
2	50	10	33.70	22.76
2	25	10	17.64*	4.85*

*: $0.01 < p < 0.05$ compared with a vehicle group.

Based on their promising anti-inflammatory activity, compound **4f** was chosen for further evaluation. A dose of 100 mg/kg was orally administered at different time intervals (1, 2, 3, 4, 5 and 24 h) for xylene application. As shown in Table 4, the activity profile of compound **4f** was consistent with that of indomethacin. Notably, the activity of compound **4f** (34.19%) reached its peak at 2 h and was found to be more potent than indomethacin at this time point (30.94%). The effect of the dosage on the activity of compound **4f** was also evaluated at concentrations of 25, 50 and 100 mg/kg at 2 h after oral administration (Table 5). The results showed a maximal effect with an ear inflammation inhibition rate of 43.45% at 100 mg/kg.

Table 6. Cytotoxic activity (IC_{50}[a], μg/mL) of compound **4f** against human cell lines.

Compound	Substituents	*In vitro* cytotoxicity IC_{50}[a] (μg/mL)		
	R	HeLa[b]	Hep3B[c]	L02[d]
4f	2,4-Cl_2	8.7	15.3	18.1

[a] IC_{50} is the concentration required to inhibit the cell growth by 50%. Data represent the average of three independent experiments running in triplicate. Variation was generally between 5–10%.
[b] Human cervical cancer cells.
[c] Human liver cancer cells.
[d] Human normal hepatic cells.

To determine whether the antibacterial and antifungal activities of the synthesized compounds were selectively toxic towards bacteria and the one fungus, we evaluated the cytotoxicity of the compound **4f** using a standard technique.[32] As shown in Table 6, compound **4f** gave IC_{50} values of 8.7, 15.3 and 18.1 μg/mL against HeLa, Hep3B and L02 cells, respectively, which were slightly higher than its MIC values. The result showed the compound **4f** does not have much selectivity. Hence it is difficult to deduce that the promising antibacterial activity of these compounds is

not due to their toxicity, but some unknown mechanism of action.[33]

The mechanisms of action responsible for the antimicrobial and anti-inflammatory activities of these compounds remain unknown. Lee *et al* reported the chalcone derivative cardamomin (2′,4′-dihydroxy-6′-methoxychalcone) as an inhibitor of NF-κB activation and demonstrated the anti-inflammatory activity of this compound by blocking the nuclear factor-κB signaling pathway.[34] This result therefore suggests that a similar mechanism could be involved for our synthesized compounds. Further study towards determining the mechanisms of action of these compounds is currently underway in our laboratory.

In summary, we have synthesized three series of chalcone derivatives containing an aminoguanidine or acylhydrazone moiety and evaluated their antibacterial, antifungal and anti-inflammatory activities. Compounds **4f** and **4h** showed the highest levels of activity against the Gram-negative strain *S. typhimurium* 1926 and the fungus *Candida albicans* 7535 with an MIC value of 1 μg/mL, making them 1- and 2-fold more potent than the standard drugs. Furthermore, compound **4f** showed the most potent inhibitory activity of all of the synthesized compounds towards ear inflammation (92.45%), which was higher than those of ibuprofen (87.36%) and indomethacin (89.38%) at 100 mg/kg (i.p.). These results therefore suggest that chalcone derivative **4f** could potentially be used to develop potent antibacterial, antifungal and anti-inflammatory agents for the clinical treatment of numerous diseases. The mechanisms of action of these compounds are currently being elucidated in our laboratories and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found in the online version.

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28. Preparation of **4f**: Terephthalaldehyde **2** (6 mmol) with 2,4-Dichloroacetophenone (5 mmol) in refluxing ethanol (50 mL) at 23 ℃ in the presence of NaOH (0.4g, 10 mmol) for 3–4 h afforded (*E*)-4-(3-(2,4-dichlorophenyl)-3-oxoprop-1-en-1-yl)benzaldehyde in 60%–70% yields,[26,27] which reacted with aminoguanidine hicarbonate (3 mmol) in refluxing ethanol (20 mL) in the presence of 5 drops of concentrated hydrochloric acid at 60–70 ℃ for 8–12 h. The solution was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography with dichloromethane: methanol (50:1) to afforded yellow solid **4f**. Yield 71%; m.p. 203–205 ^{o}C; IR (KBr cm^{-1}): 3373 (NH_2), 3271 (C=NH), 3174(NH), 1680 (C=O), 1597 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (br.s, 1H, NH), 8.19 (s, 1H, CH=N), 7.95-7.80 (m, 7H, CH, NH, NH_2 and Ar-H), 7.66-7.58 (m, 2H, CH and Ar-H), 7.49-7.32 (m, 3H, Ar-H). ^{13}C NMR (DMSO–d_6, 75 MHz): δ 192.88,

156.01, 146.43, 146.15, 137.73, 136.32, 136.20, 136.12, 131.69, 131.21, 130.15, 129.75, 129.75, 128.48, 128.48, 128.14, 127.29. HRMS (MALDI) calcd for $C_{17}H_{15}Cl_2N_4O$ $(M + H)^+$: 361.0621, found: 361.0624.

29. Evaluation of anti-bacterial activity *in vitro*: The micro-organisms used in the present study were *S. aureus* 4220, *S. aureus* 209, *S. aureus* 503, *Streptococcus mutans* 3065, *Escherichia coli* 1924, *Escherichia coli* 1356, *Pseudomonas aeruginosa* 2742, *Salmonella typhimurium* 1926 and one fungus (*Candida albicans* 7535). The strains of multidrug-resistant clinical isolates were methicillin-resistant *Staphylococcus aureus* (MRSA 3167 and 3506) and quinolone-resistant *Staphylococcus aureus* (QRSA 3505 and 3519). Clinical isolates were collected from various patients hospitalized in several clinics. The *in vitro* antimicrobial activity was evaluated using the minimum inhibitory concentration (MIC) with different strains. Gatifloxacin, moxifloxacin, fluconazole, norfloxacin, and oxacillin were used as positive controls. The bacteria were grown to mid-log phase in Mueller-Hinton broth and diluted 1000-fold in the same medium. Stock solutions of the test compounds in dimethyl sulfoxide were prepared and then poured into 96-well plates. The final concentration of 0.5-64 μg/mL underwent a twofold serial dilution. The microbacteria were suspended and contained approximately 10^5 CFU/mL. These were applied to 96-well plates with a serial dilution and incubated at 37 °C for 24 h. The microbacterial growth was measured from the absorption at 630 nm. This was done using a microtiter, enzyme-linked immunosorbent assay (ELISA) reader. All experiments were carried out in triplicate.
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31. Anti-inflammatory assay: Assay in the xylene-induced ear edema method via the intraperitoneal route: The anti-inflammatory activity was evaluated by an *in vivo* inhibition assay by monitoring xylene-induced ear edema in mice. In the primary screening, all tested compounds, ibuprofen and indomethacin were freshly prepared (dissolved with DMSO) prior to administered i.p. at a dose of 100 mg/kg

to mice and at a concentration of 0.1 mL/20 g body weight. Control mice received the vehicle only (DMSO, 0.1 mL/20 g of body weight). Thirty minutes after administration i.p., animals were used in the xylene-induced ear edema test, 20 μL xylene was applied to the surface of the right ear of each mouse by a micropipette. After keeping them from struggling for 30 min, a cylindrical plug (diameter, 7 mm) was excised from each of the treated and untreated ears. Edema was quantified by the difference in weight between the two plugs. Anti-inflammatory activity was expressed as percent reduction in edema compared with the DMSO-administered control group. The NSAID ibuprofen and indomethacin were tested in parallel as reference.

Assay in the xylene-induced ear edema method via the oral route: In the latter evaluation, tested compounds and indometacin were homogenized with 0.5% sodium carboxymethylcellulose (CMC-Na) and administered *via* the oral route to mice at a concentration of 0.2 mL/20 g mice weight. Control mice received the vehicle only (0.5% CMC-Na, 0.2 mL/20 g). To explore the peak activity of the compound, edema was quantified at different intervals (1, 2, 3, 4, 5, and 24h). Compounds **4f** and indomethacin homogenized with 0.5% CMC-Na were administered orally to mice (lower doses of 50 mg/kg and 25 mg/kg and 0.2 mL/20 g mice body weight). Control mice received 0.5% CMC-Na (0.2 mL/20 g body weight) and edema quantified at the peak interval of 3 h. Edema was quantified by the difference in weight between the two plugs. Edema values, expressed as mean standard deviation, were compared statistically using one-way-ANOVA followed by Dunnet's test. A level of $p < 0.05$ was adopted as the test of significance.

32. Cytotoxicity on human cancer cells: The cytotoxicity test of selected compounds was measured through the colorimetric MTT assay. Human cervical cancer cells (HeLa) and liver cancer cells (Hep3B) suspension in DMEM medium supplemented with 10% FBS and antimycotic was added in 96-well microplates at 1.8×10^4 cells/well. A variety of concentrations of the test compounds (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.625 μM/L) dissolved by distilled 10% DMSO was added to each well. Incubation for 24h at 37℃ under 5% CO_2, 2.5mg/mL of MTT

solution was added to each well. Further the plate was incubated for 4h. Then, the medium was removed and the resulting formazan crystals were dissolved with 100 μL DMSO. After shaking 10 min, the optical density was measured at 570 nm using a microtiter ELISA reader. The selected compounds were used as positive control, whereas untreated cells were used as negative controls. The IC_{50} values were defined as the concentrations inhibiting 50% of cell growth. All experiments were performed in triplicate.

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Synthesis and biological evaluation of chalcone derivatives containing aminoguanidine or acylhydrazone moieties

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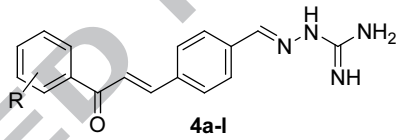
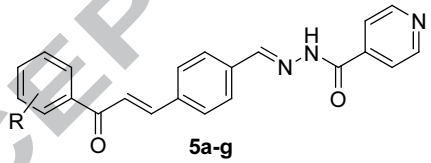
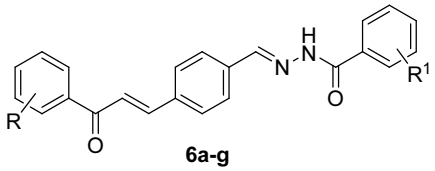


4a-l

MIC = 2 g/mL (*E. coli*)
MIC = 1 g/mL (*C. albicans*)



5a-g



6a-g

Three novel series of chalcone derivatives containing aminoguanidine or acylhydrazone moieties were designed, synthesized, characterized and evaluated for their antibacterial activity, antifungal activity and anti-inflammatory activity.