Antibacterial and antifungal properties of guanylhydrazones

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Abstract: A series of novel guanylhydrazones were designed, synthesized and characterized. All the compounds were screened for their antibacterial and antifungal activity. Compounds 26 and 27 showed excellent antibacterial activities against Staphylococcus aureus ATCC 25923 and Micrococcus luteus ATCC 379 with minimal inhibitory concentrations of 4 µg mL⁻¹, and good antifungal activity against Candida parapsilosis ATCC 22019. These results suggested that the selected guanylhydrazones could serve as promising leads for improved antimicrobial development.

Keywords: guanylhydrazones; iminoguanidines; antibacterial activity; antifungal activity; Candida spp.

INTRODUCTION

Infectious diseases caused by human pathogens, both bacteria and fungi, result in significant morbidity and mortality worldwide. Treatment of these diseases is often hampered by limited therapeutic options and the development of resistance. Bacteremia is a major cause of life-threatening complications in patients in intensive care units, neonates, or cancer patients, who are at extremely high risk for infections caused by antibiotic resistant bacteria.¹ Invasive candidiasis is the fourth most common bloodstream infection with mortality rates remaining disturbingly high at 40 %.² This makes the quest for new molecules that are effective against the threat of drug resistance a significant issue in modern medicine.

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Guanylhydrazones have been a long-standing point of interest in medicinal chemistry. Recently, a two-step procedure was demonstrated for the preparation of simple guanylhydrazones 1 (Fig. 1). The synthesized compounds were evaluated for their in vitro antifungal activities against a wide range of medically important fungal strains. Among the series, compound 2 proved to be an effective, broad-spectrum antifungal compound (Fig. 1). In particular, compound 2 exhibited excellent activity against the voriconazole-resistant Candida albicans CA5 strain.

Given the good antifungal properties shown by selected guanylhydrazones and as a continuation of research on the development of new antimicrobial agents, in the present work, the synthesis, characterization and evaluation of the antibacterial and antifungal properties of new guanylhydrazone derivatives are reported.

RESULTS AND DISCUSSION

Chemistry

The Suzuki–Miyaura reaction enabled access to a range of aldehydes 4–7 from easily obtainable starting compounds (Scheme 1). The low yield of aldehyde 5 is associated with problems during purification and isolation of the desired product from the crude reaction mixture. The reported yield is also non-optimized and certainly could be further improved through variation of the reaction conditions.

Bromination of aldehydes 8 with bromine and N-bromosuccinimide afforded the corresponding monobromo derivatives 10 and 11, respectively (Scheme 2). Aldehydes 12 and 13 were prepared by a Suzuki–Miyaura reaction starting from bromide 10 (Scheme 2). Access to 4-fluoro-5-phenyl-2-furaldehyde (15) was accomplished by a halogen/metal exchange reaction. The lithiated intermediate formed in the reaction medium was trapped with the electrophilic fluor-
inacting reagent \( N \)-fluorobenzenesulfonimide (NFSI, Scheme 2). The thiophene aldehyde 8 underwent nitration with a mixture of nitric acid and acetic anhydride affording the corresponding 4-nitro derivative 16 in 71 % yield (Scheme 2). Subsequent acid hydrolysis of 16 gave the nitroaldehyde 17 (Scheme 2).

The guanylhydrazones 18–27 were synthesized using a one-step condensation reaction of aminoguanidine hydrochloride and the corresponding aldehyde in good to excellent yields (Scheme 3). All the guanylhydrazones were obtained as hydrochlorides.

Antimicrobial activity

All synthesized guanylhydrazones were assessed for their antimicrobial activity against one Gram-negative strain (\textit{Pseudomonas aeruginosa} PAO1, NCTC 10332), and three Gram-positive strains (\textit{Staphylococcus aureus} ATCC 25923, \textit{Micrococcus luteus} ATCC 379 and \textit{Listeria monocytogenes} NCTC 11994) and
three fungal strains (Candida albicans ATCC 10231, Issatchenkia orientalis ATCC 6258 and Candida parapsilosis ATCC 22019). The minimum inhibitory concentration (MIC) values obtained by the standard broth dilution method were compared to those of clinically used antibiotics (kanamycin and nystatin).

Most of the tested compounds were found to display poor to moderate activities against the tested bacterial strains, with the exception of compound 22, which displayed excellent antibacterial activity against P. aeruginosa PAO1, and compounds 26 and 27, which exhibited excellent activities against S. aureus ATCC 25923 and M. luteus ATCC 379 (Table I). It is noteworthy that compounds 22, 26 and 27 showed better antibacterial activities against these three bacterial strains in comparison to the control drug, kanamycin (Table I).

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<th>Compound</th>
<th>P. aer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S. aur&lt;sup&gt;b&lt;/sup&gt;</th>
<th>M. lut&lt;sup&gt;c&lt;/sup&gt;</th>
<th>L. mon&lt;sup&gt;d&lt;/sup&gt;</th>
<th>C. alb&lt;sup&gt;e&lt;/sup&gt;</th>
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Kanamycin<sup>h</sup> | 50 | 10 | 12.5 | 12.5 | – | – | – |

Nystatin<sup>h</sup> | – | – | – | – | 1 | 7.8 | 2 |

<sup>a</sup>Pseudomonas aeruginosa PAO1 NCTC 10332; <sup>b</sup>Staphylococcus aureus ATCC 25923; <sup>c</sup>Micrococcus luteus ATCC 379; <sup>d</sup>Listeria monocytogenes NCT 11994; <sup>e</sup>Candida albicans ATCC 10231; <sup>f</sup>Issatchenka orientalis ATCC 6258; <sup>g</sup>Candida parapsilosis ATCC 22019; <sup>h</sup>control drug

From a perusal of the data, it could be seen that all the tested compounds showed moderate antifungal activity against all the tested fungal strains, while two compounds 26 and 27 exhibited the most promising activity against C. parapsilosis ATCC 22019 (Table I).

Overall, the minimal inhibitory concentrations (MIC) values lead to the conclusion that the additional aromatic ring on thiophene was beneficial to the antibacterial and antifungal activity of compounds 26 and 27.

EXPERIMENTAL

Instrumentation

Dry-flash chromatography was performed on SiO<sub>2</sub> (0.018–0.032 mm). Melting points were determined on a Boetius PMHK apparatus and were not corrected. IR spectra were recorded on a Thermo-Scientific Nicolet 6700 FT-IR Diamond Crystal instrument. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded on a Bruker Ultrashield Advance III spectrometer (at 500 and 125 MHz, respectively) using tetramethylsilane (TMS) as the internal standard. Chemical
shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. ESI MS spectra of the synthesized compounds were recorded on an Agilent Technologies 6210 time-of-flight LC–MS instrument in the positive ion mode using MeOH/H2O = 1/1 with 0.2% HCOOH as the carrying solvent solution. The samples were dissolved in pure MeOH (HPLC grade). The selected settings were as follows: capillary voltage, 4 kV; gas temperature, 350 °C; drying gas, N2, 12 L·min⁻¹; nebulizer pressure, 45 psig*; fragmentator voltage, 70–200 V. The GC–MS spectra of the synthesized compounds were acquired on an Agilent Technologies 7890A apparatus equipped with a DB-5 MS column (30 m×0.25 mm×0.25 μm), a 5975C MSD and FID detector. Selected settings were as follows: carrier gas He (1.0 mL·min⁻¹), temperature linearly increased from 40–315 °C (10 °C min⁻¹), injection volume, 1 μL, temperature, 250 °C, temperature (FID detector), 300 °C, and EI mass spectra range: m/z 40–550. Thin-layer chromatography was performed on pre-coated Merck silica gel 60 F₂₅₄ and Merck RP-18 F₂₅₄ plates. All the reported yields refer to isolated yields. The compounds were analyzed for purity (HPLC) using a Agilent 1200 HPLC system equipped with Quat pump (G1311B), injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and detector 1260 DAD VL + (G1315C) (other details are presented in the Supplementary material to this paper). All compounds were >95% pure.

The analytical and spectral data of the synthesized compounds are given in the Supplementary material to this paper.

Chemistry

General procedure. 5-(4-Methylphenyl)furan-2-carbaldehyde (4). In a dry glass flask purged with argon, Pd(OAc)₂ (3.4 mg, 0.015 mmol) was dissolved in dry dimethoxyethane (DME) (2 mL) and PPh₃ (16.2 mg, 0.060 mmol) was added. The resultant solution was stirred at room temperature for 10 min and 3 (113.8 mg, 0.650 mmol) and Na₂CO₃ (aq.) (2M, 0.65 mL, 1.3 mmol) were added. After 5 min stirring at room temperature, a solution of (4-methylphenyl)boronic acid (111.5 mg, 0.820 mmol) was added and the reaction mixture was purged with argon and refluxed at 90 °C overnight under argon. The solution was cooled to room temperature and filtered through a Celite pad, washed with CH₂Cl₂ and dried with anh. Na₂SO₄. The organic solvent was removed under reduced pressure and the crude product was purified by dry-flash chromatography (SiO₂: hexane/EtOAc = 95/5 to 9/1) to afford the title compound 4 (113.6 mg, 94%).

5-(4-Bromophenyl)furan-2-carbaldehyde (5). To a glass flask, 3 (113.8 mg, 0.650 mmol), (4-bromophenyl)boronic acid (114.7 mg, 0.715 mmol), tetrabutylammonium bromide (209.6 mg, 0.650 mmol), Pd(OAc)₂ (2.9 mg, 0.013 mmol) and K₂CO₃ (224.6 mg, 1.63 mmol) were added and then dissolved in deionized water (3 mL). The reaction mixture was stirred vigorously for 5 h at room temperature. After the white reaction mixture had become yellow and non-homogeneous, the mixture was diluted with water (10 mL), and the product was extracted with EtOAc. The organics were separated, filtered through a Celite pad, and dried with MgSO₄. The organic solvent was removed under reduced pressure and the crude product was purified by dry-flash chromatography (SiO₂: hexane/EtOAc = 9/1 to 7/3) to afford the title compound 5 (11 mg, 7%).

5-(4-Fluorophenyl)furan-2-carbaldehyde (6). The general Suzuki coupling procedure was followed, except 4-fluorophenylboronic acid (114.7 mg) was used. The reaction mixture was refluxed for 12 h. The crude product was purified by dry-flash chromatography (SiO₂: hexane/EtOAc = 95/5 to 8/2) to afford the title compound 6 (108.3 mg, 88%).

*45 psig = 310.3 kPa
5-(4-Methoxyphenyl)furan-2-carbaldehyde (7). The general Suzuki coupling procedure was followed, except 4-methoxyphenylboronic acid (124.6 mg) was used. The reaction mixture was refluxed for 12 h. The crude product was purified by dry-flash chromatography (SiO$_2$: hexane/EtOAc = 9/1 to 6/4) to afford the title compound 7 (118 mg, 90%).

4-Bromo-5-phenylthiophene-2-carbaldehyde (10). To a solution of aldehyde 8 (76 mg, 0.40 mmol) in dry CHCl$_3$ (700 μL) was added a solution of bromine (33 μL, 0.64 mmol) in dry CHCl$_3$ (330 μL). The resulting solution was stirred at r.t. for 3 h. To the reaction mixture was added sat. Na$_2$S$_2$O$_3$ solution and the reaction mixture extracted with CH$_2$Cl$_2$ (2×10 mL). The organic layer was washed with sat. NaHCO$_3$ solution and dried over anhydrous Na$_2$SO$_4$. The organic solvent was removed under reduced pressure and the crude residue was purified by dry-flash chromatography (SiO$_2$: hexane/EtOAc = 9/1) to yield 10 (81 mg, 75%).

4-Bromo-5-phenyl-2-furaldehyde (11). To a solution of aldehyde 9 (100 mg, 0.58 mmol) in MeCN (10 mL) was added NBS (114 mg, 0.64 mmol). The resulting solution was stirred at r.t. for 24 h. To the reaction mixture was added H$_2$O (10 mL) and the reaction mixture was extracted with CH$_2$Cl$_2$ (2×10 mL). The organic layer was washed with brine and dried over anhydrous Na$_2$SO$_4$. The organic solvent was removed under reduced pressure and the crude residue was purified by dry-flash chromatography (SiO$_2$: hexane/EtOAc = 95/5) to yield 11 (72.9 mg, 50%).

4,5-Diphenylthiophene-2-carbaldehyde (12). The general Suzuki coupling procedure was followed, except phenylboronic acid (38.3 mg) was used. The reaction mixture was refluxed for 12 h. The crude product was purified by dry-flash chromatography (SiO$_2$: hexane/EtOAc = 9/1) to afford the title compound 12 (53 mg, 89%).

4-(4-Fluorophenyl)-5-phenylthiophene-2-carbaldehyde (13). The general Suzuki coupling procedure was followed, except 4-fluorophenylboronic acid (36.6 mg) was used. The reaction mixture was refluxed for 12 h. The crude product was purified by dry-flash chromatography (SiO$_2$: hexane/EtOAc = 9/1) to afford the title compound 13 (42 mg, 77%).

2-(4-Bromo-5-phenyl-2-furyl)-1,3-dioxolane (14). Aldehyde 11 (117 mg, 0.46 mmol), ethylene glycol (150 μL, 2.33 mmol), and p-toluenesulfonic acid monohydrate (1.8 mg, 9.32×10$^{-3}$ mmol) were dissolved in PhMe (4 mL). Under Dean–Stark conditions, the reaction mixture was refluxed for 3 h, and then washed sequentially three times with 3 M NaOH and water. The PhMe layer was dried, filtered, evaporated under vacuum and the crude residue was purified by dry-flash chromatography (SiO$_2$: hexane/EtOAc = 9/1) to yield 14 (109 mg, 80%).

4-Fluoro-5-phenyl-2-furaldehyde (15). To a solution of acetal 14 (49 mg, 0.167 mmol) in dry THF (2.5 mL), nBuLi (1.6 M in hexane, 125 μL, 0.2 mmol) was added dropwise at a temperature below −60 °C under Ar. After stirring the mixture for 2 h, N-fluorobenzensulfonimide (58 mg, 0.18 mmol) in THF (1 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature. H$_2$O was added to the reaction mixture, which was then extracted with CH$_2$Cl$_2$, washed with brine, dried with MgSO$_4$ and concentrated. To a stirred solution of crude residue in THF (2 mL) at 25 °C was added 2M HCl (0.6 mL), and the reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was slowly quenched with sat. aq. NaHCO$_3$ (2 mL), the biphasic mixture was extracted with CH$_2$Cl$_2$ (2×10 mL), and the combined organic layers were dried over MgSO$_4$ and concentrated. The crude residue was purified by dry-flash chromatography (SiO$_2$: hexane/EtOAc = 9/1) to yield 15 (9.4 mg, 30 % for 2 steps).

(4-Nitro-5-phenyl-2-thienyl)methylene diacetate (16). To a cold solution of aldehyde 8 (35 mg, 0.186 mmol) in Ac$_2$O (500 μL) was added a solution of HNO$_3$ (9.5 μL, 0.223 mmol)
in AcOH (190 µL). The resulting solution was stirred at r.t. for 2 h, and then ice was added and the reaction mixture was extracted with CH₂Cl₂ (2×10 mL). The organic layer was washed with sat. NaHCO₃ solution, brine and dried over anhydrous Na₂SO₄. The organic solvent was removed under reduced pressure and the crude residue was purified by dry-flash chromatography (SiO₂; hexane/EtOAc = 8/2) to yield 14 (44 mg, 71 %).

4-Nitro-5-phenylthiophene-2-carbaldehyde (17). To a solution of diacetate 16 (17 mg, 0.05 mmol) in MeOH/H₂O (1/1 volume ratio, 1 mL), was added H₂SO₄ (100 µL). The resulting solution was stirred at r.t. for 2 h, and then H₂O was added and the reaction mixture extracted with CH₂Cl₂ (2×10 mL). The organic layer was washed with brine and dried over anhydrous Na₂SO₄. The organic solvent was removed under reduced pressure and the crude product was used in the next step.

**General procedure for the preparation of guanylhydrazones.**

(2E)-2-[[5-(4-Methylphenyl)thiophene-2-yl]methylidene]hydrazinecarboximidamide hydrochloride (18). To a solution of aldehyde 4 (86.2 mg, 0.463 mmol) in absolute ethanol (10 mL), aminoguanidine hydrochloride (51.2 mg, 0.463 mmol) was added. The resultant solution was stirred at room temperature for 5 min, and a solution of concentrated HCl (5 mol %) in absolute EtOH (50 µL, 1/25, v/v) was added. The reaction mixture was heated to 90 °C, refluxed for 18 h and allowed to cool to room temperature. The solvent was removed under reduced pressure, the crude product was washed with CH₂Cl₂ (1 mL) and then crystallized from EtOH/hexane (9/1) to provide the title compound 18 (127.8 mg, 99 %).

(2E)-2-[[5-(4-Methoxyphenyl)thiophene-2-yl]methylidene]hydrazinecarboximidamide hydrochloride (19). Following the general procedure for guanylhydrazone formation, 19 (144 mg, 100 %) was obtained from 7.

(2E)-2-[[5-(4-Fluorophenyl)thiophene-2-yl]methylidene]hydrazinecarboximidamide hydrochloride (20). Following the general procedure for guanylhydrazone formation, 20 (93.2 mg, 100 %) was obtained from 6.

(2E)-2-[[5-(4-Bromophenyl)thiophene-2-yl]methylidene]hydrazinecarboximidamide hydrochloride (21). Following the general procedure for guanylhydrazone formation, 21 (17.2 mg, 100 %) was obtained from 5.

(2E)-2-[[4-(4-Fluorophenyl)-5-phenyl-2-thienyl]methylidene]hydrazinecarboximidamide hydrochloride (22). Following the general procedure for guanylhydrazone formation, 22 (7.1 mg, 79 %) was obtained from 15.

(2E)-2-[[4-(4-Bromo-5-phenyl-2-thienyl)ethylidene]hydrazinecarboximidamide hydrochloride (23). Following the general procedure for guanylhydrazone formation, 23 (41 mg, 92 %) was obtained from 10.

(2E)-2-[[4-(4-Bromo-5-phenyl-2-thienyl)methylidene]hydrazinecarboximidamide hydrochloride (24). Following the general procedure for guanylhydrazone formation, 24 (44 mg, 78 %) was obtained from 11.

(2E)-2-[[4-(4-Nitro-5-phenyl-2-thienyl)methylidene]hydrazinecarboximidamide hydrochloride (25). Following the general procedure for guanylhydrazone formation, 25 (19.5 mg, 75 %) was obtained from 17.

(2E)-2-[[4,5-Diphenyl-2-thienyl]methylidene]hydrazinecarboximidamide hydrochloride (26). Following the general procedure for guanylhydrazone formation, 26 (51.9 mg, 99 %) was obtained from 12.

(2E)-2-[[4-(4-Fluorophenyl)-5-phenyl-2-thienyl]methylidene]hydrazinecarboximidamide hydrochloride (27). Following the general procedure for guanylhydrazone formation, 27 (36.5 mg, 92%) was obtained from 13.
Antimicrobial activity

Guanylhydrazones were dissolved in DMSO in stock concentrations of 50 mg mL\(^{-1}\) and used immediately for antimicrobial activity assessments. MIC concentrations (concentration value corresponding to the lowest concentration that inhibited the growth after 24 h at 37 °C) were determined according to the standard broth microdilution assays, recommended by the National Committee for Clinical Laboratory Standards (M07-A8) for bacteria in LB (Luria-Bertani) broth and Standards of European Committee on Antimicrobial Susceptibility Testing (EDef7.1.) in SAB (Sabouraud Dextrose) broth. The highest concentration used was 500 µg mL\(^{-1}\). The test organisms included *Pseudomonas aeruginosa* PAO1 (NCTC 10332), *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 379, *Listeria monocytogenes* (NCTC 11994) *Candida albicans* (ATCC 10231), *Issatchenkia orientalis* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019). The inoculums were 10\(^5\) colony forming units, CFU mL\(^{-1}\), for the bacteria and 10\(^4\) CFU mL\(^{-1}\) for the *Candida* strains.

CONCLUSIONS

In the present work, an efficient synthesis of novel guanylhydrazones was designed. The activity of these compounds against the panel of human pathogens consisting of one Gram-negative strain, three Gram-positive strains, and three fungal strains, was assessed. Noticeably, compounds 26 and 27 showed excellent antibacterial activities against Gram-positive *S. aureus* ATCC 25923 and *M. luteus* ATCC 379, even better than the control drug, kanamycin. Furthermore, compounds 26 and 27 displayed good antifungal activity against *C. parapsilosis* ATCC 22019. Altogether, the reported results indicate that the selected guanylhydrazones could form the basis for further development of new and effective antimicrobial agents.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 172008 and 173048) and the Serbian Academy of Sciences and Arts.
Гуанилгидразони као антимикробиал

ATCC 25923 и Micrococcus luteus ATCC 379 сода присутним микробиологом. Истовремено, овај единајаша показала су и изражену антифунгалну активност према Candida parapsilosis ATCC 22019 соју.

(Примљено 13. фебруара, ревирирано 3. марта, прихваћено 7. марта 2017)

REFERENCES