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Microwave-assisted synthesis of new environmentally friendly biodegradable and antibacterial heterocycles

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Abstract

The rise of bacterial resistance to common clinical antibiotics is calling for alternative approaches to synthesize new antibacterial drugs with high environmental biodegradability.

One such approach is the use of microwave-assisted synthesis, which is a powerful green synthetic technique used increasingly to accelerate organic reactions.

To discover new antibacterial compounds, we report the efficient synthesis of nineteen thiophene derivatives, five furan derivatives, and two thiazole derivatives, using Suzuki-Miyaura cross-coupling by microwave irradiation. Microwave irradiation conditions have reduced the reaction time, increased the yield and this method uses less organic solvent compared to conventional heating.

Several compounds showed broad antibacterial activity. One compound, in particular, demonstrated significant antibacterial activity against *Streptococcus pyogenes* with a minimal inhibitory concentration of 0.097 µg/mL and against *Salmonella enterica* with a minimal inhibitory concentration of 0.78 µg/mL. The biodegradability of two furan derivatives with the best antibacterial activities and their effects on environmental bacteria were studied using the closed bottle test. This test proved that the two compounds were readily biodegradable without toxic effects on environmental bacteria present in wastewater treatment plants.

Therefore our data demonstrated not only green synthesis but also environmental benignity due to the biodegradability of the novel antibacterials, which is a very desirable feature.

Key-words: Environmental biodegradability, closed bottle test, microwave-assisted synthesis, antibacterial, thiophenes, furans.

1. Introduction

Antibiotics have revolutionized medicine, turning infections that were once lethal into uncomplicated diseases. Antibiotic resistance occurs when bacteria evolve to adapt to antibiotics, resulting in drug inefficiency and persistent infections with an increase of the risk of severe diseases and transmission. Unfortunately, misuse and overuse of existing antimicrobials in humans, animals and aquaculture are accelerating the development and spread of antibiotic resistance complicated by the fact that new antibiotics are discovered at an ever-decreasing rate (Bush, 2004; Economou and Gousia, 2015; Norrby et al., 2005).

After administration, non-metabolized antibiotics and their active metabolites can be excreted by animals or humans through urine and feces (Kim and Aga, 2007; Kümmerer et al., 2000; Sarmah et al., 2006). As a result, most antibiotics are prevalent in various environments (Javid et al., 2016; Kümmerer, 2009a, 2009b; Kümmerer and Henninger, 2003; Rodríguez-Rojas et al., 2013). Even trace amounts of antibiotics induce the development of antibacterial resistance by exerting selective pressure on environmental bacteria (Allen et al., 2010; Tello et al., 2012; Wang et al., 2018). This likely contributes to the global spread of antibiotic resistance genes and ultimately poses a major threat to the health and safety of aquatic and terrestrial organisms, as well as human beings (Tan and Shuai, 2015). We need global selectivity towards bacterial strains responsible for human diseases without disrupting microorganism populations present in water treatment plants. These observations thus highlight the urgent need for new antibacterial agents with high biodegradability.

Heterocyclic compounds, containing oxygen/sulfur/nitrogen atoms, have been of significant interest, because of their common applicability (Progress in Heterocyclic Chemistry, Volume 5 - 1st Edition, 1996). Among these heterocyclic compounds thiophenes have a special place, due to their synthetic accessibility and pharmacological properties, including, e.g., antibacterial (Alsayari et al., 2020; Boibessot et al., 2016; Nasr et al., 2014), antifungal (Ibrahim et al., 2015) and antitumor (Abdel-Rahman et al., 2017; Wang et al., 2013) activities. Furan compounds, obtained from synthetic or natural sources, have been attracting much interest, due to the wide range of pharmaceutical applications (De et al., 2015), including demonstrated antimicrobial activities (Erkuş et al., 2020; Long et al., 2019). Moreover, thiazoles have attracted continuing interest because of their numerous biological properties, such as anti-inflammatory (Sharma and Sawhney, 2010) and antibacterial (Mishra et al., 2017) activities. On the other hand, microwave-assisted synthesis is a growing synthetic approach among medicinal chemists and has proven to be more efficient in terms of reaction yield, reaction time, product purity and environmental friendliness for many reactions when compared to conventional thermal methods (Abbas et al., 2016; Abedinifar et al., 2020; Saad et al., 2011).

Motivated by these findings, we reported here an improved microwave-assisted synthesis of new thiophene, furan and thiazole derivatives in moderate to good yields. These compounds were evaluated for their antibacterial properties against selected clinical isolates, and the compounds with the best minimal inhibitory concentration (MIC) have been evaluated for their environmental biodegradability.

2. Experimental

2.1. Chemistry

All reagents were purchased from commercial suppliers (Acros, Sigma Aldrich, Alfa Aesar, and TCI) and were used without further purification. Nuclear Magnetic Resonance (NMR) spectra were recorded with a Bruker Avance 300 spectrometer (300 and 75 MHz for ^1H and ^{13}C NMR, respectively) and Bruker Avance 400 spectrometer (376.5 for ^{19}F). Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively, using residual solvent signals as reference for the ^1H and ^{13}C . The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, br s = broad signal, dd = double doublet, dt = double of triplet, m = multiplet. High-resolution mass spectra (HRMS) were obtained by electrospray using a TOF analyzer Platform. Infrared (IR) spectra were obtained using a Jasco FT-IR 410 instrument as a thin film on NaCl disc as stated. Only structurally important peaks ($\bar{\nu}$) are presented in cm^{-1} . Reactions were monitored with Merck Kieselgel 60F₂₅₄ precoated aluminum silica gel plates (0.25 mm thickness). Melting points were determined on a Stuart scientific SMP10 apparatus and are uncorrected. Flash chromatography was performed on a Grace Reveleris X2 using a 40 μm packed silica cartridge. Flash chromatography experiments were carried out on Silica Gel premium Rf grade (40-63 μm) or were performed on a Grace Reveleris X2 using a 40 μm packed silica cartridge. Ultra performance liquid chromatography (UPLC) analyses were obtained on the Waters Acquity H-Class using the following conditions: Waters Acquity BEH C18 column (1.7 μm , 50 mm x 2.1 mm), 25 °C column temperature, 0.5 mL/min flow rate, photodiode array detection (TUV – 214nm) and the mobile phase consisted of a gradient of water and acetonitrile (each containing 0.1% of formic acid). High-performance liquid chromatography (HPLC) analyses were obtained on the Agilent VwD 1100 Series using the following conditions: BDS Hypersil C18 column (1.7 μm , 50 mm x 2.1 mm), 25 °C column temperature, 0.5 mL/min flow rate, photodiode array detection (TUV – 254nm), mobile phase consists of a gradient of water and acetonitrile (each containing 0.1% of trifluoroacetic acid). The purity of all synthetic compounds was determined by UPLC/HPLC analysis and was greater than 95% except for **8y**. The reaction was done in the monomode microwave Discover system from CEM (CEM MWaves SAS, Orsay, France) that has one magnetron (2450 MHz) with a maximum delivered power of 300 W in 1 W increments. The monomode microwave features an electromagnetic stirring with adjustable speeds (Teflon[®] coated stir bars are suitable), and direct control of temperature with the aid of a fiber-optic (FO).

General experimental procedure

A 2 M solution of base (1.6 mL/mmol) was added to a commercial bromo-thiophene, bromo-thiazole or bromo-furan (1 eq), respectively, with corresponding boronic acid (1 eq to 1.5 eq) and Pd(PPh₃)₄ (0.05 eq) in indicated solvent (2.5 mL/mmol). The reactor was placed into the synthesis monomode microwave oven cavity and carried out under microwave irradiation (300W) at 120 °C for 17 min and then at 130 °C for 3 min. The reaction mixture was stirred continuously during the reaction. The disappearance of starting materials was monitored by thin-layer chromatography (TLC). Once the reaction was finished, the mixture was extracted with 5x50 ml of ethyl acetate. The organic layers were washed with brine, dried with Na₂SO₄, filtered, and concentrated under pressure. The crude compound was purified by silica gel chromatography or flash chromatography, eluting petroleum ether/ ethyl acetate to generate the desired compound. Finally, the obtained powder was dissolved in a small volume of ethyl acetate and precipitated in cold pentane (-20 °C).

Selected spectra data. For all other data see the supplementary material section.

2-(5-nitrofur-2-yl)phenol (8s) Yellow powder. Yield: 39% (50 mg); mp: 218-220 °C; *R_f*: 0.28 (PE/EtOAc: 7/3); IR (cm⁻¹): 3446 (ν_{OH}); ¹H NMR (DMSO - *d*₆, 300 MHz) δ: 6.98 (td, 1H, *J*₁ = 8.1 Hz, *J*₂ = 1.2 Hz); 7.02 (dd, 1H, *J*₁ = 8.4 Hz, *J*₂ = 0.9 Hz); 7.23 (d, 1H, *J* = 3.9 Hz); 7.32 (ddd, 1H, *J*₁ = 8.7 Hz, *J*₂ = 7.2 Hz, *J*₃ = 1.5 Hz); 7.75 (dd, 1H, *J*₁ = 7.8 Hz, *J*₂ = 1.8 Hz); 7.78 (d, 1H, *J* = 3.9 Hz); 10.79 (br s, 1H, OH); ¹³C NMR (DMSO - *d*₆, 75 MHz) δ: 112.8 (CH); 114.5 (C); 115.7 (CH); 116.6 (CH); 119.7 (CH); 126.2 (CH); 131.6 (CH); 150.4 (C); 154.0 (C, C-NO₂); 155.8 (C, C-OH); UPLC: *t_R*: 2.29 min; purity: 100%; HRMS: [M + H]⁺ calculated for C₁₀H₈NO₄, 206.0453, found 206.0451 (mass error: -1.0 ppm).

3-(5-nitrofur-2-yl)phenol (8t) Yellow powder. Yield: 40% (53 mg); mp: 205-207 °C; *R_f*: 0.31 (PE/EtOAc: 7/3); IR (cm⁻¹): 3400 (ν_{OH}); ¹H NMR (DMSO - *d*₆, 300 MHz) δ: 6.89 (m, 1H); 7.26 (m, 1H); 7.34 (m, 3H); 7.81 (d, 1H, *J* = 3.9 Hz); 9.87 (br s, 1H, OH); ¹³C NMR (DMSO - *d*₆, 75 MHz) δ: 109.7 (CH); 111.4 (CH); 115.6 (CH); 116.2 (CH); 117.7 (CH); 128.7 (C); 130.5 (CH); 151.1 (C); 156.1 (C, C-NO₂); 158.0 (C, C-OH); UPLC: *t_R*: 2.15 min; purity: 100%; HRMS: [M + H]⁺ calculated for C₁₀H₈NO₄, 206.0453, found 206.0457 (mass error: 1.9 ppm).

4-(5-nitrofur-2-yl)phenol (8u) Yellow powder. Yield: 20% (63 mg); mp: 245-247 °C; *R_f*: 0.42 (PE/EtOAc: 5/5); IR (cm⁻¹): 3384 (ν_{OH}); ¹H NMR (DMSO - *d*₆, 300 MHz) δ: 6.91 (d, 2H, *J* = 8.7 Hz); 7.19 (d, 1H, *J* = 3.9 Hz); 7.74 (d, 2H, *J* = 8.7 Hz); 7.81 (d, 1H, *J* = 3.9 Hz); 10.21 (br s, 1H, OH); ¹³C NMR (DMSO - *d*₆, 75 MHz) δ: 107.5 (CH); 116.2 (2-CH); 116.3 (CH); 118.6 (C); 127.2 (2-CH); 150.7 (C); 157.2 (C, C-NO₂); 159.8 (C, C-OH); UPLC: *t_R*: 2.21 min; purity: 98%; HRMS: [M + H]⁺ calculated for C₁₀H₈NO₄, 206.0453, found 206.0453 (mass error: 0.0 ppm).

2.3 Biological evaluation

2.3.1 Disk Diffusion Assay

The Kirby-Bauer disk diffusion susceptibility assay was used to determine the sensitivity of bacteria to selected compounds, according to the Clinical Laboratory Standards Institute (CLSI) guidelines (Wayne, 2012). See supplementary material for more details.

2.3.2 Determination of the minimal inhibitory concentration and minimal bactericidal concentration

The MIC of the tested compounds was determined by the two-fold serial broth (lysogeny broth, Brain heart infusion or Todd Hewitt Broth medium) dilution method in 96 well plates (Fisher Scientific Co.), according to CLSI guidelines (Wayne, 2012). See supplementary material for more details.

2.3.3 Biodegradability and toxicity assays following the closed bottle test

The closed bottle test is known as one of the simplest tests to assess the biodegradability of low solubility or insoluble organic compounds (OECD, 1992). Following guidelines, the test was conducted in the dark in a proofer at 20 °C +/- 0.2 °C in closed bottle test flasks. The test was performed for 28 days as previously reported with compounds **8s,t** (Alexy et al., 2004; OECD, 1992). See Table S2, See supplementary material for more details.

3. Results and discussion

3.1 Synthesis of furan, thiazole, and thiophene derivatives 8a-y

Previous results reported in our laboratory by Boibessot *et al.* showed that compound **8a** had promising antimicrobial activity (Boibessot et al., 2016). Compound **8a** was first synthesized using the Boibessot *et al.* method with Suzuki-Miyaura cross-coupling starting from 2-bromo-

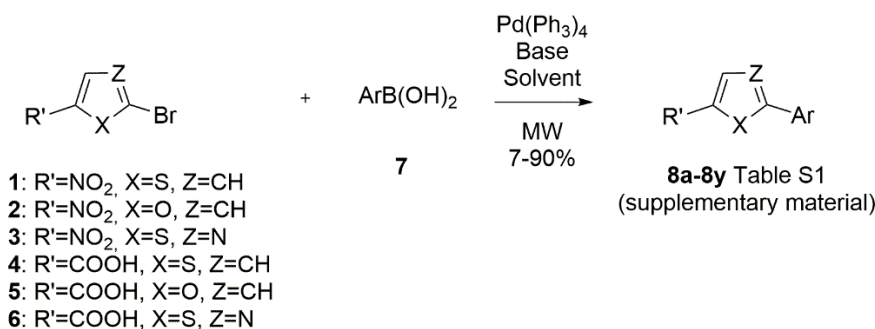
5-nitro-thiophene **1** and 2-hydroxy-phenylboronic acid in the presence of a 2 M solution of potassium carbonate in hot dioxane. Compound **8a** was obtained by conventional heating method with a 42% yield in 15 hours (Scheme 1, Table 1). Here we present improved synthetic access to this series of compounds in moderate to good yields. To increase the chemical yield and decrease the reaction time of molecule **8a**, microwave heating conditions were considered (Scheme 1). Three microwave conditions were used and are summarized in Table 1. Initially, compound **8a** was obtained with a 49% yield under stoichiometric conditions (Experiment 1) in 10 minutes. This yield was significantly improved to 72% when the reaction time was slightly increased to 20 min (Experiment 2). Finally, an excess of 2-hydroxy-phenylboronic acid (Experiment 3) was used to obtain **8a** in 76% yield in 20 min.

Table 1 Improved conditions for the synthesis of compound **8a**.

	Heating	Time	Yield	Comments
Previous work	Conventional heating	15 h	42%	Stoichiometric conditions
Experiment 1	Microwave	10 min	49%	Stoichiometric conditions
Experiment 2	Microwave	20 min	72%	Stoichiometric conditions
Experiment 3	Microwave	20 min	76%	1.5 eq of boronic acid

A total of 25 analogs of **8a** were synthesized using an improved Suzuki-Miyaura cross-coupling reaction by microwave irradiation (see supplementary material for all spectra details). Heterocycles **1-6** reacted with the corresponding boronic acids in the presence of a 2 M aqueous solution of a base in the corresponding solvent. Compounds **8a-y** were obtained with yields ranging from 7% to 90% after purification (Scheme 1). The synthesis of compound **10** was detailed in the supplementary material.

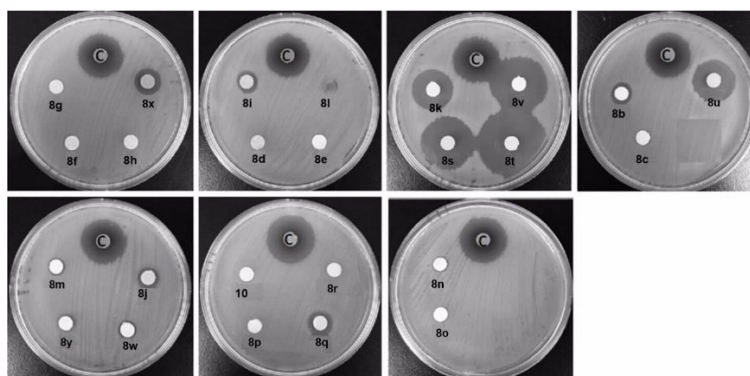
Scheme 1 Synthesis of compounds **8a-8y** from bromo-heterocycles **1-6** and boronic acids **7**. Reagents and conditions: Pd(PPh₃)₄, base and solvent using microwave heating, 7-90%. For more details see supplementary material. The use of microwave heating renders the synthetic pathway green compatible.



3.2 Antibacterial evaluation of furan, thiazole and thiophene derivatives 8a-y and 10

Antibacterial activities of the 25 compounds were evaluated by a standard Kirby-Bauer disk diffusion assay (Fig. 1). Each paper disc was impregnated with 100 µg of each of the compounds, dried and placed on plates containing *B. subtilis* laboratory strain JH642. In this assay, compounds diffuse out of the disk, building a gradient of compound surrounding the disk. A lawn of bacteria is grown, and a clearing around a disk signals that the bacteria are sensitive to growth inhibition or killing activity of the respective compound. Some clear level of growth inhibition was observed for nine compounds such as **8b**, **8i-k**, **8q**, **8s-v** and **8x**, but only compounds **8k** and **8s-v** showed inhibition rings similar to the control (ampicillin: 10 µg/disk). For some compounds, no growth inhibition was observed. It is worth noting that, eight of the nine compounds with antimicrobial activity share a NO₂ group at position 2 of thiophene, suggesting that this group is crucial for antimicrobial activity.

Fig. 1 Evaluation of the antibacterial activity of 25 compounds on *B. subtilis* strain JH642 by the Kirby-Bauer disk diffusion method. C is the positive control disk that contained 10 µg of ampicillin. All other disks contain 100 µg of the indicated compound. The inhibitory activity is represented by the dark circle of no bacterial growth around the disk.



The Kirby-Bauer assays are only semi-quantitative, since rings of inhibition are a function of both, killing activity but also solubility of the compound. Thus, by using a quantitative serial dilution assay, the MIC, as well as the minimal bactericidal concentration (MBC) of compounds **8k**, **8s-t**, and **8v** were determined for eight different bacterial strains, and the results were summarized in Table 2.

Table 2 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) result in eight bacterial strains of compounds **8a**, **8s**, and **8t**.

	<i>B. subtilis</i> (G +)	<i>S. aureus</i> (G +)	<i>B. anthracis</i> (G +)	<i>S. pyogenes</i> (G +)	<i>S. agalactiae</i> (G +)	<i>E. faecalis</i> (G +)	<i>S. enterica</i> (G -)	<i>E. coli</i> (G -)
8s MIC µg/mL	0.585	6.25	0.78	0.585	> 50	12.5	1.56	9.375
MBC	0.585	6.25	0.78	0.585	> 200	18.75	> 6.25	9.375

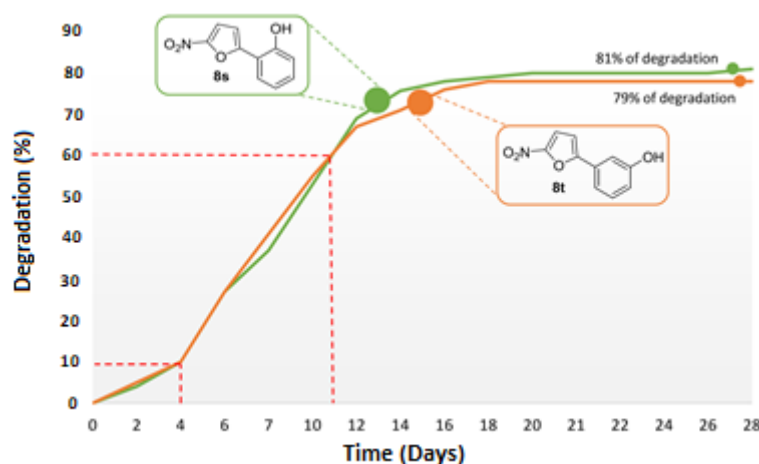
	μg/mL								
8t	MIC	0.146	1.56	0.097	0.097	25	3.125	0.78	3.125
	μg/mL								
	MBC	0.146	1.56	0.097	0.195	50	3.125	> 0.78	3.125
8a	μg/mL								
	MIC	7	16	7	10	16	32	32	32
	μg/mL								
8a	MBC	10	32	16	32	> 32	> 64	32	32
	μg/mL								
	μg/mL								

Molecules **8s,t** are analogs of molecule **8a** with a furan nucleus. Compound **8a** has antibacterial activity with MICs ranging from 7 to 32 μg/mL and MBCs ranging from 10 to > 64 μg/mL. The results obtained with the furan series **8s,t** clearly show antibacterial activity against Gram-positive and Gram-negative bacteria. Their MICs range from 0.097 to 12.5 μg/mL and MBCs range from 0.097 to 18.75 μg/mL (Table 2). These values are significantly higher than the thiophene compound **8a**. None of the new molecules show improved activity against *S. agalactiae*, when compared to compound **8a**. This suggests that either the new compounds are not able to access their cellular target as easily in this strain, perhaps due to reduced permeability, or otherwise, that the cellular target is more specific for compound **8a**, in contrast to all other tested bacterial strains.

3.3 Biodegradability and toxicity of compounds **8s,t**

One of the easiest ways to readily measure the biodegradability of a molecule is the closed bottle test described by the Organization for Economic Co-operation and Development (OECD). This experiment allows the determination of chemical oxygen demand (COD) in a mineral medium at low concentration of assay substance. To determine the biodegradability of the structures, we focused on the compounds that have shown the best antibacterial activities among the nitrofur series: **8s** and **8t**.

Fig. 2 Percentage of degradation of compounds **8s** and **8t** over time. 10% biodegradability is reached on day 4 and 60% on day 11. This result is represented by the dotted lines. The Green curve corresponds to the **8s** degradability profile while the **8t** degradability profile is represented by the orange curve. 81% biodegradability of **8s** and 79% biodegradability of **8t** was achieved over the time of the experiment.



Degradation experiments for compounds **8s** and **8t** are depicted in figure 2 and show that they are readily biodegradable. 60% of theoretical oxygen demand (ThOD) was consumed in ten days, once the ThOD reached 10% (between days 4 and 14 for **8s** and **8t**). According to OECD guidelines, the tested molecules proved to be nontoxic, since sodium acetate was degraded by more than 30% in toxicity control flasks within two weeks (Table S3, supplementary material). When sodium acetate is readily degraded in flasks in the presence of test substances, it demonstrates that the substances did not inhibit the biodegradation activity of the microorganism population. In summary, the closed bottle test assays suggest that compounds **8s** and **8t** have the desirable features of being non-toxic to the marine environment, while at the same time being readily biodegradable with a total degradation of 81% and 79% at day 28, respectively. More detailed studies on cultivatable bacteria to validate this observed absence of toxicity in an aquatic medium, however, are needed.

4. Conclusion

We prepared a new series of thiophene, thiazole and furan derivatives using Suzuki-Miyaura cross-coupling by microwave irradiation. The use of microwaves allowed to reduce the reaction time and to improve significantly the yield of the reaction. All compounds were evaluated for antimicrobial activities against Gram-positive and Gram-negative bacterial strains. The furan derivative **8t** was the most potent compound against several Gram-positive pathogens with an impressive MIC of 0.097 $\mu\text{g/mL}$ against *B. anthracis* and *S. pyogenes*. It also inhibited Gram-negative strains with a MIC of 0.78 $\mu\text{g/mL}$ against *S. enterica*. Furthermore, compound **8t** proved to be readily biodegradable, while also showing to be non-toxic to aquatic microbial environments. In conclusion, our findings present an encouraging avenue for the further development of similar antibacterial compounds that have a suggested low impact on the environment and are thus expected to not easily contribute to a quick evolution of resistant strains in microbial communities.

Supplementary Material The online version contains supplementary material available at XX
Author contributions

ZB was responsible for the overall supervision, ZB, PM, HS, wrote the manuscript, MRH synthesized the different compounds, MOO, NF and HS performed the biological experiment,

VD and DB performed the biodegradability experiments. All authors approved the final version of the manuscript.

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Availability of data and material Supporting material for characterization of each synthesized compound are provided including NMR data and HRMS.

Code availability Not applicable

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication All the authors have seen and approved the final version of the manuscript.

References

- Abbas, E.M.H., Gomha, S.M., Farghaly, T.A., 2014. Multicomponent reactions for synthesis of bioactive polyheterocyclic ring systems under controlled microwave irradiation. *Arabian Journal of Chemistry* 7, 623–629. <https://doi.org/10.1016/j.arabjc.2013.11.036>
- Abdel-Rahman, S.A., El-Gohary, N.S., El-Bendary, E.R., El-Ashry, S.M., Shaaban, M.I., 2017. Synthesis, antimicrobial, anti-quorum-sensing, antitumor and cytotoxic activities of new series of cyclopenta(hepta)[b]thiophene and fused cyclohepta[b]thiophene analogs. *Eur. J. Med. Chem.* 140, 200–211. <https://doi.org/10.1016/j.ejmech.2017.08.066>
- Abedinifar, F., Babazadeh Rezaei, E., Biglar, M., Larijani, B., Hamedifar, H., Ansari, S., Mahdavi, M., 2021. Recent strategies in the synthesis of thiophene derivatives: highlights from the 2012–2020 literature. *Mol Divers* 25, 2571–2604. <https://doi.org/10.1007/s11030-020-10128-9>
- Alexy, R., Kümpel, T., Kümmerer, K., 2004. Assessment of degradation of 18 antibiotics in the Closed Bottle Test. *Chemosphere* 57, 505–512. <https://doi.org/10.1016/j.chemosphere.2004.06.024>
- Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8, 251–259. <https://doi.org/10.1038/nrmicro2312>
- Alsayari, A., Muhsinah, A.B., Asiri, Y.I., Alfaifi, M.Y., Elbehairi, S.E.I., Alatibi, F., El-Sayed, N.N.E., Mabkhot, Y.N., 2020. Synthesis of antimicrobial agents of tetra-substituted thiophenes from ethyl 5-(2-bromoacetyl)-4-phenyl-2-(phenylamino)thiophene-3-carboxylate. *J. Heterocycl. Chem.* 57, 2911–2922. <https://doi.org/10.1002/jhet.3999>
- Boibessot, T., Zschiedrich, C.P., Lebeau, A., Bénimélis, D., Dunyach-Rémy, C., Lavigne, J.P., Szurmant, H., Benfodda, Z., Meffre, P., 2016. The Rational Design, Synthesis, and Antimicrobial Properties of Thiophene Derivatives That Inhibit Bacterial Histidine Kinases. *J. Med. Chem.* 59, 8830–8847. <https://doi.org/10.1021/acs.jmedchem.6b00580>
- Bush, K., 2004. Antibacterial drug discovery in the 21st century. *Clin. Microbiol. Infect.* 10, 10–17. <https://doi.org/10.1111/j.1465-0691.2004.1005.x>
- De, B., Sen, S., Easwari, T.S., 2015. Chemistry and Therapeutic Aspect of Furan: A Short Review. *Asian J. Res. Chem.* 8, 428. <https://doi.org/10.5958/0974-4150.2015.00069.3>
- Economou, V., Gousia, P., 2015. Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infect. Drug Resist.* 8, 49–61. <https://doi.org/10.2147/IDR.S55778>

Erkuş, B., Özcan, H., Zaim, Ö., 2020. Synthesis, antimicrobial activity, and ion transportation investigation of four new [1 + 1] condensed furan and thiophene-based cycloheterophane amides. *J. Heterocycl. Chem.* 57, 1956–1962. <https://doi.org/10.1002/jhet.3922>

Hiraishi, A., 1988. Respiratory quinone profiles as tools for identifying different bacterial populations in activated sludge. *J. Gen. Appl. Microbiol.* 34, 39–56. <https://doi.org/10.2323/jgam.34.39>

Ibrahim, S., Abdallah, H., El-Halawany, A., Mohamed, G., 2015. Naturally occurring thiophenes: isolation, purification, structural elucidation, and evaluation of bioactivities. *Phytochem. Rev.* <https://doi.org/10.1007/s11101-015-9403-7>

Javid, A., Mesdaghinia, A., Nasser, S., Mahvi, A.H., Alimohammadi, M., Gharibi, H., 2016. Assessment of tetracycline contamination in surface and groundwater resources proximal to animal farming houses in Tehran, Iran. *J. Environ. Health Sci. Eng.* 14. <https://doi.org/10.1186/s40201-016-0245-z>

Kim, S., Aga, D.S., 2007. Potential Ecological and Human Health Impacts of Antibiotics and Antibiotic-Resistant Bacteria from Wastewater Treatment Plants. *J. Toxicol. Environ. Health Part B* 10, 559–573. <https://doi.org/10.1080/15287390600975137>

Kümmerer, K., 2009a. Antibiotics in the aquatic environment--a review--part I. *Chemosphere* 75, 417–434. <https://doi.org/10.1016/j.chemosphere.2008.11.086>

Kümmerer, K., 2009b. Antibiotics in the aquatic environment--a review--part II. *Chemosphere* 75, 435–441. <https://doi.org/10.1016/j.chemosphere.2008.12.006>

Kümmerer, K., Al-Ahmad, A., Mersch-Sundermann, V., 2000. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. *Chemosphere* 40, 701–710. [https://doi.org/10.1016/S0045-6535\(99\)00439-7](https://doi.org/10.1016/S0045-6535(99)00439-7)

Kümmerer, K., Henninger, A., 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clin. Microbiol. Infect.* 9, 1203–1214. <https://doi.org/10.1111/j.1469-0691.2003.00739.x>

Long, Q.-S., Liu, L.-W., Zhao, Y.-L., Wang, P.-Y., Chen, B., Li, Z., Yang, S., 2019. Fabrication of Furan-Functionalized Quinazoline Hybrids: Their Antibacterial Evaluation, Quantitative Proteomics, and Induced Phytopathogen Morphological Variation Studies. *J. Agric. Food Chem.* 67, 11005–11017. <https://doi.org/10.1021/acs.jafc.9b03419>

Mishra, R., Sharma, P.K., Verma, P.K., Tomer, I., Mathur, G., Dhakad, P.K., 2017. Biological Potential of Thiazole Derivatives of Synthetic Origin. *J. Heterocycl. Chem.* 54, 2103–2116. <https://doi.org/10.1002/jhet.2827>

Nasr, T., Bondock, S., Eid, S., 2014. Design, synthesis, antimicrobial evaluation and molecular docking studies of some new thiophene, pyrazole and pyridone derivatives bearing sulfisoxazole moiety. *Eur. J. Med. Chem.* 84, 491–504. <https://doi.org/10.1016/j.ejmech.2014.07.052>

Norrby, S.R., Nord, C.E., Finch, R., 2005. Lack of development of new antimicrobial drugs: a potential serious threat to public health. *Lancet Infect. Dis.* 5, 115–119. [https://doi.org/10.1016/S1473-3099\(05\)01283-1](https://doi.org/10.1016/S1473-3099(05)01283-1)

OECD, 1992. OECD 301 - Ready Biodegradability. *OECD Guidel. Test. Chem.* 301, 1–62. <https://doi.org/10.1787/9789264070349-en>

Progress in Heterocyclic Chemistry, Volume 5 - 1st Edition, editors-in-chief, Alan R. Katritzky, Charles W. Rees, Eric F.V. Scriven. ed, 1996.

Rodríguez-Rojas, A., Rodríguez-Beltrán, J., Couce, A., Blázquez, J., 2013. Antibiotics and antibiotic resistance: A bitter fight against evolution. *Int. J. Med. Microbiol., Special Issue Antibiotic Resistance* 303, 293–297. <https://doi.org/10.1016/j.ijmm.2013.02.004>

Saad, H.A., Youssef, M.M., Mosselhi, M.A., 2011. Microwave Assisted Synthesis of Some New Fused 1,2,4-Triazines Bearing Thiophene Moieties With Expected Pharmacological Activity. *Molecules* 16, 4937–4957. <https://doi.org/10.3390/molecules16064937>

Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* 65, 725–759. <https://doi.org/10.1016/j.chemosphere.2006.03.026>

Sharma, P., Sawhney, S., 2010. ChemInform Abstract: Potent Antiinflammatory 3Thiazole4(5)-acetic Acids of 1,2-Benzisothiazole. *Cheminform* 29. <https://doi.org/10.1002/chin.199804121>

- Tan, D.T., Shuai, D., 2015. Research highlights: antibiotic resistance genes: from wastewater into the environment. *Environ. Sci. Water Res. Technol.* 1, 264–267.
<https://doi.org/10.1039/C5EW90011E>
- Tello, A., Austin, B., Telfer, T.C., 2012. Selective Pressure of Antibiotic Pollution on Bacteria of Importance to Public Health. *Environ. Health Perspect.* 120, 1100–1106.
<https://doi.org/10.1289/ehp.1104650>
- Wang, W., Shangguan, S., Qiu, N., Hu, C., Zhang, L., Hu, Y., 2013. Design, synthesis and biological evaluation of novel 3,4,5-trisubstituted aminothiophenes as inhibitors of p53–MDM2 interaction. Part 1. *Bioorg. Med. Chem.* 21, 2879–2885.
<https://doi.org/10.1016/j.bmc.2013.03.061>
- Wayne, P.A., 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved standards-9th ed. Clin. Lab. Stand. Inst. CLSI document M07-A9.