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Synthesis and antimicrobial activity of new prenylated 2-pyrone derivatives

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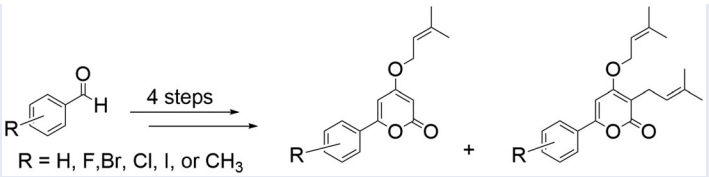
ABSTRACT

A series of new monoprenylated and diprenylated 2-pyrone derivatives with different halogen substituents were synthesized from the corresponding 6-aryl-4-hydroxy-2-pyrones by prenylation reactions. The compounds were evaluated for antibacterial activity and displayed significant *in vitro* activity with the highest activity shown by the monoprenylated 6-aryl-2-pyrones. All the compounds except the bromine-containing analogs were active against one or more tested bacteria, with *Escherichia coli* being the most susceptible of the test organisms. With the remarkable antibacterial activity of eight of the compounds against a drug-resistant β-lactamase-producing *Klebsiella pneumoniae*, a synergistic evaluation between each of these compounds and ampicillin was undertaken. Out of the eight combinations studied, synergistic effects were observed with two compounds, 4-(3-methylbut-2-enoxy)-6-phenyl-2*H*-pyran-2-one and 6-(4-fluorophenyl)-4-(3-methylbut-2-enoxy)-2*H*-pyran-2-one. Both compounds, at half the individual MIC values, were able to lower the MIC of ampicillin in combinations from 2500 to 2.4 μg/mL (1/1041 of MIC).

GRAPHICAL ABSTRACT



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Antibacterial activity; halogenated aromatics; prenylated pyrone; 2-pyrone

Introduction

Over the years, great strides have been made to combat bacterial infections, and new antibiotics were developed from different sources. Notwithstanding all the efforts and achievements, bacterial infections still constitute a threat to public health, mainly due to the continuous development and spread of multidrug-resistant bacteria. Drug resistance continues to hamper the effectiveness of available antibiotics and thereby exacerbating the burden of bacterial infections.[1,2] Furthermore, first-line drugs show no effects on

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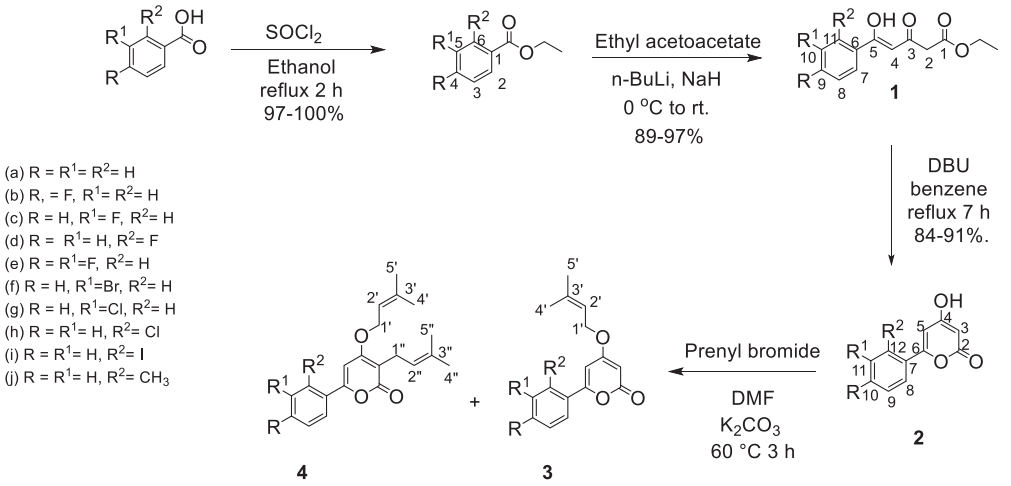


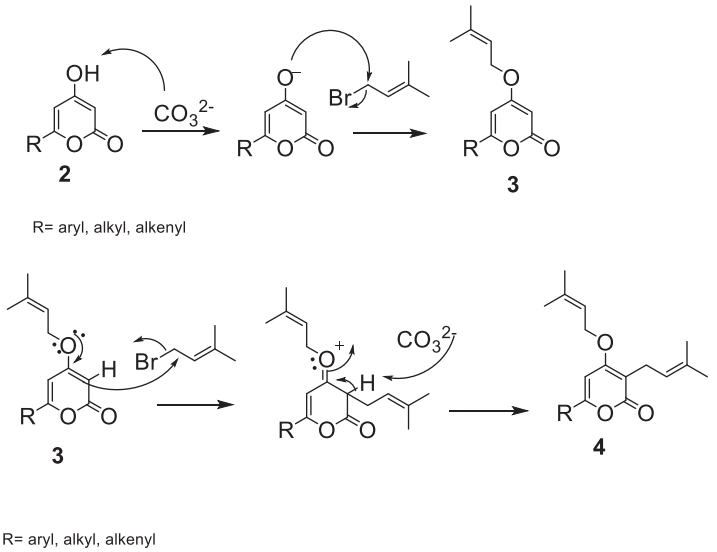
Scheme 1. Synthetic route to prenylated 2-pyrones **3a–3j** and **4a–4j**.

methicillin-resistant *Staphylococcus aureus* and in most cases, second-line drugs are administered that are more expensive and have side effects. Therefore, there is an urgent need to overcome these challenges by discovering new antibiotic drugs that are safe and inexpensive to combat antibacterial drug resistance.

The 2-pyrone moiety is present in many naturally occurring and synthetic compounds that display important medicinal and biological activities.[3–6] Some of these compounds are shown to have anti-HIV, antimycobacterial, anticancer, antimicrobial, antifungal, and antityrosinase activities.[7] The biological activity of 2-pyrone derivatives has been extensively reviewed by Bhat et al.[7] In addition, the 2-pyrone moiety has also been recognized to interact with proteins in various biological systems,[7,8] and is also an active building block for the synthesis of complex heterocycles.[9,10]

The prenyl group is a unique structural moiety present in many naturally occurring compounds with latent benefits to human health.[11–13] Prenyl may refer to several types of chains such as 3,3-dimethylallyl, geranyl (*E*-3,7-dimethyl-2,6-octadienyl), and 1,1-dimethylallyl substituents, but most commonly refers to the 3,3-dimethylallyl group.[11,14] This motif is reported to be a versatile tool for modulating different types of biological activities by enhancing the affinity to cell membranes and thus interacting with targeted proteins.[15] Prenylated aromatic natural products display a wide range of bioactivities.[14] The addition of a prenyl chain to aromatic secondary metabolites has been shown to enhance the physicochemical properties of the parent compound such as lipophilicity and increase hydrophobicity thereby resulting in increased antibacterial activity.[13,16–19] Consequently, prenylated compounds have improved membrane partitioning and interact better with the targeted sites compared to non-prenylated counterparts.[12,20] The parent structures can be *O*-prenylated where the prenyl group is incorporated on an oxygen atom or *C*-prenylated where the prenyl group is attached to a carbon atom.

The biological significance of the 2-pyrone moiety and interesting activities of prenylated compounds prompted us to combine these structural moieties to synthesize a



Scheme 2. Proposed mechanism for the synthesis of prenylated 2-pyrones.

series of prenylated (3,3-dimethylallylated) 2-pyrone derivatives and screen the compounds for *in vitro* antibacterial activity.

Results and discussion

Ten monoprenylated and ten diprenylated 2-arylpyrones, which include eight halogenated and one unsubstituted methyl derivative, were synthesized according to a four-step reaction sequence as shown in Scheme 1. The final compounds were obtained by prenylation of **2a**-**2j** with prenyl bromide and potassium carbonate in DMF. In these reactions, both monoprenylation (*O*-prenylation) and diprenylation (*O*-prenylation and *C*-prenylation on C-3) were observed. The series of 2-pyrones includes 6-aryl substituents with a fluorine atom on the *ortho*-, *meta*-, or *para*-positions (**3b–3d** and **4b–4d**), two fluorine atoms on the 3- and 4-positions (**3e** and **4e**), a bromine atom on the 3-position (**3f** and **4f**), a chlorine atom on the *ortho*- or *meta*-position (**3g–3h** and **4g–4h**), an iodine on the *ortho*-position (**3i** and **4i**) or a methyl group on the *ortho* position (**3j** and **4j**). Compounds **3a–3j**, the major products of these reactions, were obtained in yields between 64 and 74%, with the minor products **4a–4j** in 22–30% yield.

The proposed mechanism for the synthesis of monoprenylated (**3**) and diprenylated (**4**) is an S_N2 reaction.

In this reaction, there is a competition between *O*-prenylation and *C*-prenylation. According to Le Noble,[21] an aprotic polar solvent (acetone) and a large counterion (potassium) favor *O*-prenylation. Therefore, it is suggested that the *O*-prenylation reaction occurs first, followed by a *C*-prenylation. The hydroxy proton of compound **2** is removed by the strong base, resulting in a nucleophile (unstable anionic intermediate), which then attacks the carbon of the prenyl bromide to give **3**. Delocalization of lone pairs of electrons on the oxygen atom of the prenyl chain of **3** resulted in an

Table 1. Antibacterial activity of monoprenylated 2-pyrones.

	Antibacterial activity (MIC, μg/mL)			
	Gram + ve	Gram –ve		Drug-resistant Gram –ve
Compounds	Sa	Ec	Pa	Kp
3a	0.78	0.05	1.56	1.56
3b	15.63	1.56	62.50	31.25
3c	31.25	3.90	31.25	62.50
3d	31.25	1.56	31.25	31.25
3e	15.63	1.56	15.63	31.25
3f	>250.00	>250.00	>250.00	>250.00
3g	7.80	3.90	3.90	15.63
3h	3.90	0.39	15.63	7.80
3i	3.90	0.20	6.25	31.25
3j	15.63	1.56	15.63	31.25
Neomycin	0.12	0.98	0.98	0.24

MIC: minimum inhibitory concentration; Sa: *Staphylococcus aureus*; Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Kp: *Klebsiella pneumoniae*.

electrophilic aromatic substitution by the attack on the excess prenyl bromide to form the diprenylated pyrone **4**, as shown in Scheme 2. Further evidence for the proposed mechanism is the fact that mono-*O*-prenylated compounds were isolated, but no mono-*C*-prenylated analogs could be detected.

Nuclear magnetic resonance (NMR), IR and mass spectrometry were the key techniques used to confirm the structure of the products of the prenylation reactions. The chemical shifts and splitting patterns of the aryl ring of **3** and **4** are similar to those of compound **2**.[22] In the ^{1}H NMR spectrum of **3a**–**3j**, the two protons on the pyrone ring resonated as doublets at δ_H 5.39–5.54 and δ_H 6.28–6.85, with coupling constants of 1.9–2.1 Hz. Compounds **3a**–**3j** and **4a**–**4j** showed two upfield methyl singlets of the *cis*-allylic methyl protons at δ_H 1.73–1.75 and the *trans*-allylic methyl protons at δ_H 1.79–1.82. The doublet signals, assigned to two allylic protons of the *O*-prenyl chains, appeared at δ_H 4.55 ($J = 6.9$ Hz) for **3a**–**3j** and at δ_H 4.64– 4.69 ($J = 6.7$ Hz) for **4a**–**4j**. The vinylic protons signals of the *O*-prenyl chain of **3a**–**3j** and **4a**–**4j** appeared as multiplets at δ_H 5.41–5.47 and δ_H 5.39–5.45, respectively. The *C*-prenyl chains of **4a**–**4j** have two upfield methyl singlets at δ_H 1.67–1.70 and δ_H 1.75–1.78 corresponding to the *cis*-allylic methyl protons and *trans*-allylic protons, respectively, of the *C*-prenyl chains. Doublet integrating for two protons appeared at δ_H 3.17–3.19 ($J = 7.3$ Hz) for the methylene protons of the *C*-prenyl chains of **4a**–**4j**. A vinyl proton resonance for the *C*-prenyl chain was observed as multiplets at δ_H 5.18–5.28. The replacement of the hydrogen on the 2-pyrone ring of **4** with a prenyl group was confirmed by the presence of only one vinyl proton at δ_H 6.27–6.74 as a singlet. In addition, the ^{13}C NMR spectra and high-resolution mass data further confirmed the structures and all data are reported in the experimental section.

The results of the antibacterial activity of the mono- and diprenylated 2-pyrone analogs are presented in Tables 1 and 2. All the compounds, except **3f** and **4f**, were active against one or more test organisms. *Escherichia coli*, a Gram-negative bacteria, was the most susceptible of all the test organisms. It was susceptible to all the compounds, except **3f** and **4f**. Compound **3a** was the most active of all the prenylated 2-pyrones with an MIC range of 1.56–0.049 μg/mL. It exhibited a broad spectrum activity (active against all the test organisms). More important, it was also active against the drug-resistant β-lactamase-producing *Klebsiella pneumoniae* at an MIC value of 1.56 μg/mL.

Table 2. Antibacterial activity of diprenylated 2-pyrones.

	Antibacterial activity (MIC, μg/mL)			
	Gram + ve	Gram –ve		Drug-resistant Gram –ve
Compounds	Sa	Ec	Pa	Kp
4a	3.90	0.195	7.80	7.80
4b	7.80	0.39	7.80	7.80
4c	7.80	0.39	7.80	7.80
4d	7.80	0.78	7.80	7.80
4e	15.63	0.78	7.80	15.63
4f	>250.00	>250.00	>250.00	>250.00
4g	7.80	0.39	7.80	7.80
4h	7.80	0.39	3.90	7.80
4i	15.63	1.56	15.63	31.25
4j	15.63	1.56	15.63	62.50
Neomycin	0.12	0.98	0.98	0.24

MIC: minimum inhibitory concentration; Sa: *Staphylococcus aureus*; Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Kp: *Klebsiella pneumoniae*.

Table 3. Combined effects of different pyrones with ampicillin against drug-resistant *Klebsiella pneumoniae*.

Combinations	Individual MIC (μg/mL)	Combination MIC (μg/mL)	Individual FIC	FIC Index (FICI)	Interpretation
3a + ampicillin	1.56/2500	0.78/2.40	0.5/0.00096	0.50	Synergistic
3h + ampicillin	7.80/2500	3.90/2.40	0.5/0.00096	0.50	Synergistic
4a + ampicillin	7.80/2500	7.81/2.40	1.0/0.00096	1.00	No interaction
4b + ampicillin	7.80/2500	7.81/2.40	1.0/0.00096	1.00	No interaction
4c + ampicillin	7.80/2500	7.81/2.40	1.0/0.00096	1.00	No interaction
4d + ampicillin	7.80/2500	7.81/2.40	1.0/0.00096	1.00	No interaction
4g + ampicillin	7.80/2500	15.63/2.40	2.0/0.00096	2.00	No interaction
4h + ampicillin	7.80/2500	7.81/2.40	1.0/0.00096	1.00	No interaction

MIC: minimum inhibitory concentration; FIC: fractional inhibitory concentration.

Halogenation of the phenyl ring of both the mono- and diprenylated 2-pyrones seems to decrease the antibacterial activity of the compounds. The substitution of one of the hydrogen atoms on the aryl ring with bromine rendered the 2-pyrones analogs ineffective against all the test organisms (MIC >250 μg/mL).

During the past decades, combination therapy has become one of the most effective strategies in combating bacterial infections caused by drug-resistant pathogens. The rationale is to enhance the activity by the achievement of a synergistic effect. *K. pneumoniae* (ATCC 700603) is a *β*-lactamase-producing organism and will hydrolyze *β*-lactam antibiotics, such as ampicillin, thereby becoming resistant to these antibiotics.

Considering the antibacterial activity of **3a, 3h, 4a, 4b, 4c, 4d, 4g,** and **4h** against the drug-resistant *K. pneumoniae*, the synergistic evaluation of these compounds in combination with ampicillin was undertaken. The MICs obtained by the combinations of the 2-pyrones with ampicillin against drug-resistant *K. pneumoniae* are presented in Table 3. Many chemical compounds are known to possess antimicrobial activity. However, when used in combination, some of these compounds have the potential to either inhibit the modified target or exhibit a synergy by blocking one or more of the targets in the metabolic pathway, thus acting as a modifier of multidrug-resistance mechanisms.[23] A drug-resistant strain of *K. pneumoniae* was used in the present study. It is an extended-spectrum beta-lactamase strain that produces the enzyme SHV-18. Out of the eight

combinations studied, only two synergistic effects were detected (Table 3). These were the combinations **3a**/ampicillin and **3h**/ampicillin. Given the FICI values (0.5) (see Experimental section for an explanation of FICI values), the interactions could be described as borderline synergistic effects.[24] However, both compounds, at half of the individual MIC values, were able to lower the MIC of ampicillin in the combinations from 2500 to 2.4 μg/mL (1/1041 of MIC).

Conclusion

In conclusion, the syntheses of mono- and diprenylated 2-pyrone derivatives have been achieved by the prenylation of 6-aryl-4-hydroxy-2-pyrones with prenyl bromide. All compounds are reported here for the first time. The obtained compounds were evaluated for antibacterial activity, and all except the bromine-containing derivative were active against one or more of the test organisms. *E. coli*, a Gram-negative bacteria, was the most susceptible of all the test organisms. With regards to the antibacterial activity of eight of the compounds against a drug-resistant β-lactamase-producing *K. pneumoniae*, an evaluation of the synergistic effect between each of these eight pyrones with ampicillin was investigated. Out of the eight combinations studied, two synergistic effects were detected. Both compounds **3a** and **3h**, at half of the individual MIC values, were able to lower the MIC of ampicillin in the combinations from 2500 to 2.4 μg/mL (1/1041 of MIC).

Experimental

General experimental procedure

All air- and moisture-sensitive reactions were performed in flame-dried glassware under an inert gas (argon or nitrogen) atmosphere. Reactions were monitored using thin-layer chromatography (TLC, silica, aluminum-backed). Visualization of the TLC plates was achieved by using an iodine tank or fluorescence on exposure to short wavelength UV (254 nm). Solvents were dried and purified following standard procedures. Compounds were characterized using a Bruker 400 MHz Avance III (for ^{1}H and ^{13}C experiments) or a 500 MHz Avance III UltraShield NMR spectrometer (for ^{19}F experiments) at frequencies of 400 MHz (^{1}H NMR), 100.4 MHz (^{13}C NMR) and 470 MHz (^{19}F NMR) at 298 K with chemical shifts (δ) given in parts per million (ppm) downfield from tetramethylsilane as the internal standard and coupling constants (J) in Hertz (Hz). ^{1}H and ^{13}C NMR spectra were referenced to residual solvent signals, and ^{19}F NMR spectra were referenced to chlorotrifluoromethane. High-resolution mass spectrometry (HRMS) was performed on a Waters LCT Premier time-of-flight mass spectrometer with electrospray ionization in the positive mode [HRMS-ESI-(+)]. IR spectra were recorded on a Perkin Elmer Spectrometer (100 FT-IR) with universal ATR sampling accessory. Melting points were recorded using a Stuart melting point apparatus and were uncorrected.

General procedure for the preparation of compounds 3a–3j and 4a–4j

The 6-aryl-4-hydroxy-2-pyrone (1.0 mmol) was added to a suspension of dry DMF (5 mL) and potassium carbonate (0.44 g, 3.0 mmol) under nitrogen in the absence of

light. The resulting mixture was allowed to stir for 1 h at 60 °C, cooled to room temperature and prenyl bromide (0.23 g, 1.5 mol) was added dropwise. After addition, the mixture was again heated to 60 °C and stirred for another 3 h. On completion of the reaction, as indicated by TLC monitoring, the reaction mixture was cooled to room temperature and water (30 mL) was added. The aqueous phase was extracted with chloroform (4 × 30 mL) and the combined organic extracts were washed with water (5 × 30 mL), brine (40 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude residue was purified by column chromatography using hexanes–EtOAc (9:1) to afford products **3** and **4**.[25]

Determination of antibacterial activity

Preparation of microorganisms

The bacteria used in this study were one Gram-positive (*S. aureus* ATCC 12600), two Gram-negative (*Pseudomonas aeruginosa* ATCC 10145, *E. coli* ATCC 11775), and one drug-resistant Gram-negative (*K. pneumoniae* ATCC 700603) bacterial strains. The cultures of bacteria were maintained on Mueller Hinton Agar slants at 4 °C throughout the study and used as stock cultures.

Determination of minimum inhibitory concentration (MIC)

The MIC values of compounds against the bacterial strains were determined using a rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay.[26] Stock solutions of test compounds (10 mg/mL) were prepared in DMSO. Prior to the assay, stock solutions were diluted with sterilized distilled water to a concentration of 1 mg/mL. Solutions were then added to Mueller Hinton Broth (MHB) and serially diluted twofold in a 96-well microplate to a final concentration range of 250–1.95 µg/mL. Compounds with MIC values less than 1.95 µg/mL were further assayed at a final concentration range of 50–0.012 µg/mL to determine the individual MIC values. Bacterial strains were cultured overnight at 37 °C on MHB and adjusted to a final density of 10^6 cfu/mL with MHB. These were used as inocula. One hundred microliters (100 µL) of inoculum was added to each well. The plates were covered with a sterile plate sealer and then incubated at 37 °C for 20 h. Wells containing 10% DMSO, MHB and 100 µL of inoculum served as the negative controls while neomycin was used as a positive control. The total volume in each well was 200 µL. The MICs of the compounds were observed after 20 h incubation at 37 °C, and subsequent 30 min incubation after the addition of 40 µL of 0.2 mg/mL INT. Clear wells with INT after incubation indicated inhibition of bacterial growth. MIC values were recorded as the lowest concentration of the test compound that completely inhibited bacterial growth. The assay was repeated twice in duplicate per sample and values represent the averages. The standard deviations/errors were not included because they were insignificant.

Determination of *in vitro* synergistic activity

Combinations of compounds that recorded an MIC value $\leq$7.8 µg/mL and ampicillin against drug-resistant beta-lactamase-producing Gram-negative *K. pneumoniae* were

tested by the checkerboard method. For each compound and ampicillin combination, 50 µL of MHB was added in each well of a 96-well microplate. About 50 µL of each compound solution was added in row A and was twofold serially diluted down to row H. 50 µL of appropriate dilutions of ampicillin was added in columns with column one having the highest concentration and column twelve the lowest concentration of antibiotic. To the 100 µL of different combinations in each of the 96-well microplates, 100 µL of bacterial inoculum as described earlier, was added. The final concentration of the compound and ampicillin in combinations ranged from $1/64 \times$ MIC to $2 \times$ MIC. Plates were incubated for 20 h. Interpretation of the data was achieved by calculating the fractional inhibitory concentration index (FICI) as follows:

$$\text{FICI} = \text{FIC A} + \text{FIC B}.$$

where

FIC A = (MIC of compound A in combination with antibiotics/MIC of compound A alone).

FIC B = (MIC of antibiotics B in combination with compound/MIC of antibiotics B alone).

The results were interpreted as follows: FICI ≤ 0.5, synergistic; $0.5 <$ FICI < 4, no interaction; and FICI ≥ 4 antagonistic.

Funding

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