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Simple, Multicomponent, Ecofriendly, Microwave-Mediated Route for the Synthesis of Antimicrobial 2-Amino-6-aryl-4-(3H)-pyrimidinones

Ivangela E. A. da Silva [a], Vanildo M. L. Braga [a], Mychely S. Melo [b], Maria Tereza dos S. Correia [b] & Janaína V. dos Anjos [a]

[a] Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Brazil

[b] Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife, Brazil

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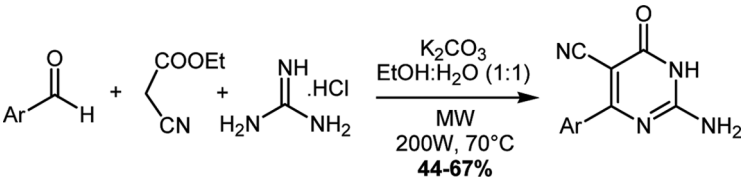
SIMPLE, MULTICOMPONENT, ECOFRIENDLY, MICROWAVE-MEDIATED ROUTE FOR THE SYNTHESIS OF ANTIMICROBIAL 2-AMINO-6-ARYL-4-(3*H*)-PYRIMIDINONES

Ivangela E. A. da Silva,[1] Vanildo M. L. Braga,[1] Mychely S. Melo,[2] Maria Tereza dos S. Correia,[2] and Janaína V. dos Anjos[1]

[1]*Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Brazil*

[2]*Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife, Brazil*

GRAPHICAL ABSTRACT



Abstract *In this work, we describe a multicomponent microwave-mediated synthesis of 10 2-amino-6-aryl-4-oxo-1,6-dihydro-pyrimidine-5-carbonitriles in good chemical yields (44–67%), four of them not related in earlier literature. All pyrimidinones synthesized herein had their antimicrobial activity evaluated against the following microorganisms:* Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*, and* Staphylococcus aureus*. Two of the synthesized substances displayed good antimicrobial activity against* P. aeruginosa *and* S. aureus*, two bacteria responsible for nosocomial infections.*

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Keywords Antimicrobial activity; microwaves; multicomponent synthesis; 4-(3*H*)-pyrimidinones

INTRODUCTION

Pyrimidinones are described in the literature as presenting a wide array of biological activities such as antihypertensive,[1] potassium channel antagonism,[2] anti-HIV,[3] antitumoral,[4] antiepileptic,[5] antimalarial,[6] antimicrobial,[7]

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Address correspondence to Janaína V. dos Anjos, Departamento de Química Fundamental, Universidade Federal de Pernambuco, 50670 910, Recife, PE, Brazil. E-mail: janaina.anjos@ufpe.br

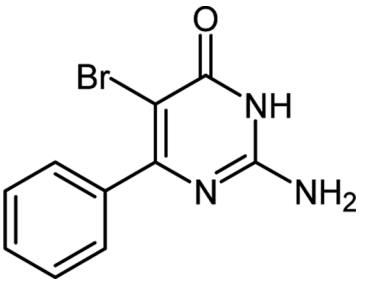


Figure 1. Bropirimine, a bioactive pyrimidinone.

anti-inflammatory,[8] anti-tubercular,[9] and antibacterial[10] activites, among others.[11] Such diversity of activities, as well as relative amiable synthetic procedures, have made them valuable targets worth pursuing and testing for more than a century.

Bropirimine (Fig. 1) is a successful example of a drug that contains the 2-amino-6-aryl-4-(3*H*)-pyrimidinone scaffold.[12] It was first synthesized by Stringfellow and coworkers and became known because of its anticancer and antiviral properties.[13] The most employed route of synthesis of this pyrimidinone derivative is a Biginelli-like reaction.[13] The original Biginelli reaction is a multicomponent reaction where an aldehyde, a β-keto ester, and an urea derivative can be used to provide a vast array of synthetic derivatives.[14] A variation of this methodology for the synthesis of the title compounds employs a Michael adduct and an uronium donor system, such as urea,[15] thiourea,[16] and guanidine[17] or their derivatives.[18] This reaction results in an alicyclic compound that can be converted to the pyrimidinone of interest by employing thermal cyclization.

Usually, for the synthesis of the pyrimidone scaffold, the first step is a Knoevenagel reaction between an aldehyde and a species with an acid hydrogen (such as ethyl cyanoacetate). The resulting Michael adduct can be reacted with uronium donors species to produce the aimed heterocycle.[19] In most cases, this reaction requires long refluxing periods. One of the alternatives for the improvement of this process lies in the use of microwave in organic chemistry. This type of radiation can be focused to the reaction medium and transfer energy directly to the species, superheating them and accelerating the process.[20] This transference of energy can be particularly important in multicomponent reactions.

Multicomponent reactions (MCRs) are procedures in which three or more reagents are mixed together to yield a product, in a cascade process.[21] The use of microwave energy may accelerate this type of reaction, because the conductive heating could improve the rate-determining step for the overall process.[22] Microwave-mediated MCRs have shown many advantages when compared to usual synthetic routes, such as simplification of the experimental procedure, shortening of the reaction time, better global yields, and duly noted water economy (because the long reflux periods are drastically reduced).[23] As an example, Stadler and Kappe made use of microwave radiation to synthesize 48 derivatives of dihydropyrimidine through the use of Biginelli's multicomponent reaction.[24] This way, microwave-mediated MCRs are an outstanding strategy, not only because of their convergent

nature, atom economy, and simplification of experimental procedure but also because of their value to the pharmaceutical industry regarding the construction of low-molecular-weight libraries.[23]

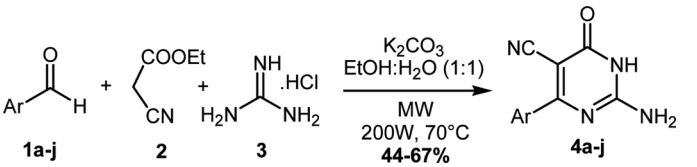
Such positive impacts on MCRs by the use of microwave methodology have prompted us to investigate and compare the methodologies (reflux and microwaves) in the synthesis of pyrimidinones, as well as to test the synthesized compounds against some bacteria including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two of the main pathogens involved in nosocomial infections, affecting mainly immunossupressed patients.[25]

RESULTS AND DISCUSSION

To optimize the conditions for this reaction some preliminary tests were conducted to evaluate some effects, such as solvent, initial power, temperature, and time. First, water was used as a solvent and temperature was set at 50 °C, for a period of 30 min. These conditions were unsatisfactory, once the consumption of the aldehyde was far from complete. Even when performed for longer periods, for became clear that a second factor in the reaction methodology should be altered. The next factor to be studied was the initial power, which was increased from 150 W to 300 W, changing the temperature of the vessel to 70 °C, for 30 min. This led to the decomposition of guanidine to ammonia. Initial power was then readjusted to 200 W, keeping temperature and time at the same values as the previous reaction. These conditions have shown some improvement when examined by thin-layer chroamtography (TLC), even though the aldehyde was not completely consumed. In fact, aldehyde consumption was only complete when the reaction was left to continue for two more 30-min cycles, totaling 90 min of reaction time. Although the completion of the starting material was perceived, the yields were poor after workup and crystallization (under 30%).

Aiming for better yields, changes on the solvent systems were proposed. Besides water, acetonitrile and ethanol were also tested, revealing that ethanol was a better fit. However, potassium carbonate and guanidine hydrochloride are not soluble in ethanol. This problem was easily fixed using equal quantities of water and ethanol in the reaction media. After the conditions were optimized, the condensation was carried out in a sealed vessel at a monomode microwave reactor in a water–ethanol mixture (1:1, v/v) at 70 °C (initial power 200 W) for 90 min, with yields varying from 44 to 67%, after purification (Scheme 1, Table 1).

To evaluate the efficiency of microwave irradiation in the preparation of the title compounds, we also performed the same reaction without microwave



Scheme 1. Multicomponent synthesis of pyrimidinones **4a–j**.

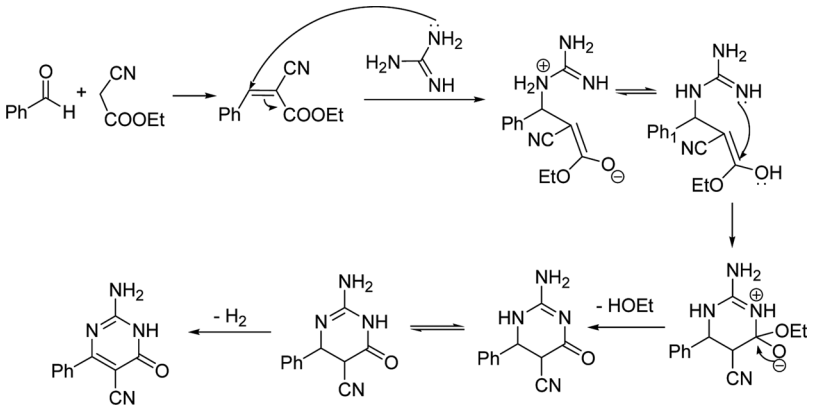
Table 1. Melting points and yields of the synthesized pyrimidinones

Compound	Ar	Yield (%)	Mp (°C)
4a	C_6H_5	62	172–174
4b	4-Cl-C_6H_4	46	233–235
4c	4-F-C_6H_4	61	249–251
4d	4-Br-C_6H_4	47	210–212
4e	4-CH_3-C_6H_4	47	197–199
4f	3-CH_3-C_6H_4	55	131–133
4g	2-CH_3-C_6H_4	67	194–197
4h	4-OCH_3-C_6H_4	46	207–210
4i	3-NO_2-C_6H_4	44	317–319
4j	3,4-diCl-C_6H_3	54	153–155

irradiation, using the same solvent (water–ethanol) and the same quantities for the reagents **2** and **3** and benzaldehyde (**1a**), in reflux. Twenty-four hours after the beginning of the reaction, there still was a huge quantity of benzaldehyde, evidenced by TLC. This experiment shows that conventional heating has not the same effect that microwave-induced heating for the synthesis of the pyrimidinone scaffold.

The formation of products **4a–j** can be explained by the occurrence of two subsequent reactions in reaction media: the first one is the Knoevenagel reaction,[19] where an aromatic aldehyde reacts with ethyl cyanoacetate, producing Michael's intermediate. Once formed, the Knoevenagel adduct can react with guanidine: Michael's adduct is attacked by the lone electron pair of a nitrogen atom in the uronium portion of guanidine. There is a sequence of additions with a subsequent ring closure to form the pyrimidinone nucleus. The last step in this mechanistic explanation is quite intriguing. According to Mendonça and coworkers,[26] atmospheric oxygen is responsible for this oxidation and loss of a H_2 molecule, leading to the heterocycle (Scheme 2).

The synthesized pyrimidinones had their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determined using previously related protocols.[27] The results are depicted in Table 2.



Scheme 2. Proposed mechanism of formation of **4a** via multicomponent reaction of **1a, 2**, and **3**.

Table 2. Antimicrobial activity for **4a–j**

	Bacillus subtilis		*Escherichia coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
Pyrimidinone	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]
4a	10	>10	1.25	2.5	2.5	5	0.625	2.5
4b	2.5	5	2.5	2.5	5	5	5	5
4c	2.5	>10	2.5	2.5	2.5	5	2.5	>10
4d	2.5	5	1.25	1.25	0.625	5	2.5	5
4e	1.25	1.25	1.25	2.5	2.5	5	2.5	5
4f	5	5	2.5	2.5	10	>10	5	>10
4g	10	>10	1.25	2.5	2.5	5	5	5
4h	2.5	5	2.5	2.5	1.25	1.25	1.25	1.25
4i	5	5	2.5	2.5	2.5	5	5	5
4j	5	10	2.5	>10	5	>10	2.5	5
Clindamycin[b]	0.039	0.039	0.019	0.039	0.019	0.039	0.019	0.078

[a]MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.
[b]Positive control.

It can be observed that the values for MIC and MBC are lower for *P. aeruginosa* and *S. aureus*. More specifically, the best results are related to pyrimidinones **4a** (for *S. aureus*) and **4d** (for *P. aeruginosa*), the latter presenting a MIC value of 0.625 mg/mL. These results indicate that these substances have a bacteriostatic profile, decreasing the multiplication of microbial cells. These results are encouraging, because these microorganisms are often cited as responsible for nosocomial infections and antibiotic resistance.[25]

Another interesting feature is that the synthesized substances show results for both Gram-positive (*S. aureus*) and Gram-negative microorganisms (*P. aeruginosa*). Gram-positive and Gram-negative bacteria differ in cell wall composition, with Gram-negative organisms more difficult to eliminate once they have an extra lipopolysaccharide outer membrane. These preliminary results may indicate that the synthesized pyrimidine derivatives are potential broad-spectrum antimicrobial agents.[28]

In summary, we have developed a multicomponent microwave-mediated strategy for the synthesis of 10 2-amino-6-aryl-4-oxo-1,6-dihydro-pyrimidine-5-carbonitriles in good chemical yields (44–67%). These substances were tested against four microorganisms and two of the synthesized pyrimidinones were active against *S. aureus* and *P. aeruginosa*.

EXPERIMENTAL

General Experimental Procedures

All reagents were obtained from commercial sources and used without further purification. Infrared (IR) spectra of the compounds were recorded on a Perkin-Elmer model 283 spectrometer employing KBr pellets. ^{1}H NMR spectra were obtained with a Varian 400-MHz instrument using tetramethylsilane (TMS) as the internal standard. ^{13}C NMR spectra were recorded on a Varian 100-MHz spectrometer. Exact mass measurements of the molecular ions were obtained on a Shimadzu Eletrospray LC/MS-IT-TOF instrument. Thin-layer chromatography

(TLC) was performed on plates coated with silica gel containing GF_{254} as fluorescent indicator. Microwave reactions were carried out in a Discover SP (CEM) microwave instrument, equipped with a 35-mL vessel. Compounds **4a** and **h**[29] and **4b, d, e, and i**[30] had their physical-chemical data compared to earlier literature for these substances.

Typical Experimental Procedure

To a sealed vessel suitable for reactions in microwave reactors (capacity: 35 mL) were added 0.94 mmol of the aromatic aldehyde (1.0 eq) and 1.88 mmol (0.2 mL, 2.0 eq) of ethyl cyanoacetate, dissolved in 4 mL of ethanol. To this solution were added 1.13 mmol (108 mg, 1.2 eq) of guanidine hydrochloride and 1.88 mmol (250 mg, 2.0 eq) of potassium carbonate dissolved in 4 mL of water. The mixture was reacted under the following conditions: temperature of 70 °C and 200 W of initial power for 90 min. The completion of the reaction was verified by TLC utilizing as eluting system hexanes / ethyl acetate 7:3 (v/v). To the reaction medium was added HCl 37% until its complete acidification. The mixture was then cooled to room temperature, and ice was poured into it, causing the precipitation of the product. The precipitate was then filtrated under vacuum, washed with water (50 mL), and subsequently recrystallized from chloroform to yield the pure pyrimidinone derivatives.

2-Amino-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (4a)

White solid; yield: 62%; R_f (ethyl acetate–methanol, 7:3 v/v): 0.37; mp: 169–170 °C. ^{1}H NMR ($DMSO_{d6}$, 400 MHz) δ: 7.56–7.62 (m, 3H), 8.03 (d, 2H, $J = 8.4$ Hz), 8.34 (s, 2H, NH_2), 13.95 (s, 1H, NH). ^{13}C NMR ($DMSO_{d6}$, 100 MHz) δ: 117.7, 128.6, 128.7, 131.4, 136.9, 155.6, 156.7, 162.1, 172.1. I. R. (KBr) $\nu_{max/cm^{-1}}$: 1684 (C=O); 2224 (C≡N); 1600 (C=N); 3138 (N-H).

Supporting Information

Full experimental details and spectroscopic data (^{1}H and ^{13}C NMR spectra) for compounds **4a–j** can be found in the Supplementary Content section of this article's Web page.

ACKNOWLEDGMENTS

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