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Il Farmaco 60 (2005) 279–282
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Microwave-assisted synthesis and anti-bacterial activity of some 2-Amino-6-aryl-4-(2-thienyl)pyrimidines

S. Chandrasekaran, S. Nagarajan *

Department of Chemistry, Annamalai University, Annamalai Nagar – 608 002, India

Received 2 December 2004; received in revised form 21 January 2005; accepted 31 January 2005

Abstract

Some novel 2-amino-6-aryl-4-(2-thienyl)pyrimidines were synthesized from 3-aryl-1-thien-2ylprop-2-en-1-ones and guanidine hydrochloride in presence of alkali by conventional heating in alcoholic medium and microwave heating in solvent-free conditions. The compounds were evaluated for in vitro anti-bacterial activity. The anti-bacterial data revealed that compounds **5a–e** had better activity against tested Gram-positive organisms than the reference ciprofloxacin and norfloxacin. However, the compounds were nearly inactive against Gram-negative bacteria. Compounds **5c** and **e** were the most active compounds against Gram-positive bacteria.

Keywords: Microwave; 2-thienylpropen-2-ones; 2-aminopyrimidines; anti-bacterial activity

1. Introduction

Microwave heating has emerged as a powerful technique to promote a variety of chemical reactions [1]. Microwave reactions under solvent-free conditions are attractive in offering reduced pollution, low cost and offer high yields together with simplicity in processing and handling [2]. The recent introduction of single-mode technology [3] assures safe and reproducible experimental procedures and microwave synthesis has gained acceptance and popularity among the synthetic chemist community.

Pyrimidine is the parent heterocycle of a very important group of compounds that have been extensively studied due to their occurrence in living systems. Pyrimidine moieties were reported to have anti-bacterial, anti-fungal and anti-HIV activities [4–7]. Substituted aminopyrimidine structures are common in marketed drugs, such as anti-atherosclerotic aronixil, anti-histaminic thonzylamine, anti-anxielytic buspirone, anti-psoriatic enazadrem, and other medicinally relevant compounds [8,9]. Thienyl compounds are also reported for their anti-microbial and pharmaceutical activities [10–13]. The increase in drug-resistant bacterial strain isolates during recent years presents a therapeutic challenge to physicians selecting anti-microbial agents.

Thus, the development of new agents with potent anti-bacterial activities and fewer adverse effects is urgently desired. In the present investigation, we report here the microwave-mediated synthesis of some thienyl aminopyrimidines and their anti-bacterial activity.

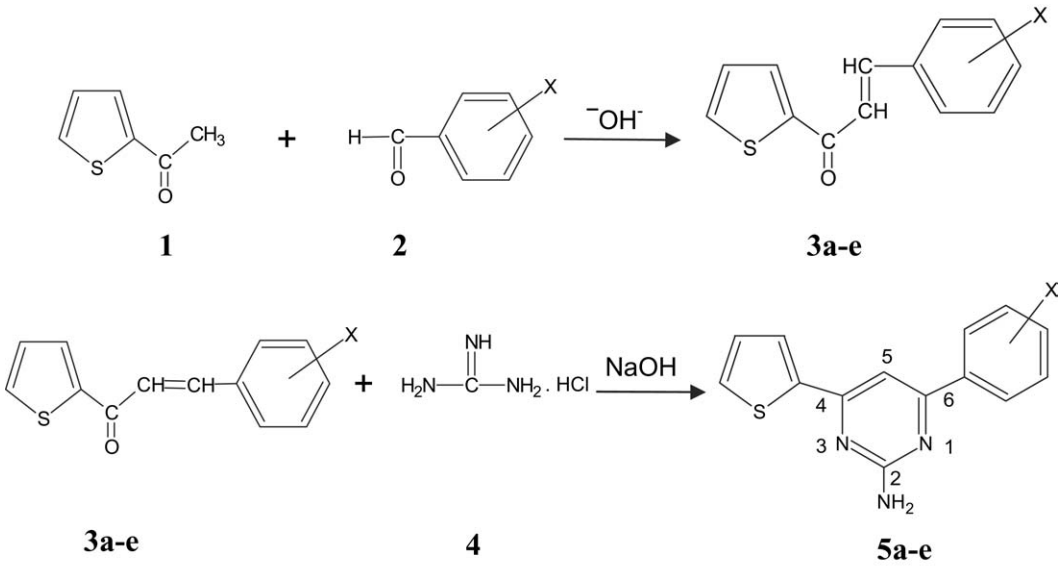
2. Results and Discussion

The equimolar quantities of 1-(2-thienyl)ethanone **1** with a suitably substituted benzaldehyde **2** in the presence of alcoholic alkali undergoes Claisen–Schmidt condensation and yield 3-aryl-1-thien-2-ylprop–2-en-1-ones **3a–e**. When 3-aryl-1-thien-2ylprop-2-en-1-ones **3a–e** are irradiated in the domestic microwave oven or on refluxing [10] in alcohol conventionally with guanidine hydrochloride **4** in presence of sodium hydroxide gave the corresponding 2-amino-6-aryl-4-(2-thienyl)pyrimidines **5a–e.** The formed compounds are characterized through analytical and ^{1}H-NMR,^{13}C/SEFT-NMR, FT-IR, UV and Mass spectral techniques. The formation of these compounds is given in Scheme 1.

In comparison to conventional (thermal) heating method, microwave heating offers more advantages such as reduced

* Corresponding author. Tel.:+91 4144 238734. Fax: + 91 4144 238080.
E-mail address: drsn@sify.com (S. Nagarajan).

0014-827X/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.farmac.2005.01.012



Where,

X = a: H; b: 4-N(CH_3)$_2$; c: 4-F; d: 4-Cl; e:4-Br

Scheme 1.

reaction time (1–1.1 min), low cost, simplicity in processing, reduced pollution and high yield (Table 1).

The ^{1}H-NMR spectrum of the compounds show a singlet for two protons in the range of 5.16–5.53 ppm is assigned to the amino protons. The singlet for H-5 proton is observed at 7.13–7.36 ppm. The aromatic protons resonate in the region of 7.0–8.1 ppm. The ^{13}C spectrum shows the signal at 101.0–102.6 ppm for C-5. The signal observed at 159.9–161.9 ppm is assigned to C-2. The signals of C-6 and C-4 are observed at 163.4–165.9 ppm. The aromatic carbons are observed in the region of 126.9–136.8 ppm.

The FT-IR spectrum shows the absorptions at 3480–3280 cm^{-1} due to N-H asymmetric and symmetric stretching vibrations of the primary amino group. The absorption frequency at 2930–2900 cm^{-1} is assigned to aromatic C-H stretching vibration. The band at 1644–1607 cm^{-1} indicates N-H in plane bending vibrations of the primary amino group. The absorption band at 1240–1225 cm^{-1} is consistent with C-N stretching vibration. The absorptions at 820–810 cm^{-1} are due to the C-H out-of-plane bending in 2-substituted thiophene. The molecular weight of the compounds are determined by the atmospheric pressure chemical ionization mass spectrometry (APcI) at 10 eV. The UV absorption spectrum shows λ_{max}(Benzene): 343–371.5 nm; λ_{max}(1,4-Dioxane): 348–371.0 nm and λ_{max}(Methanol): 351–374 nm. These absorptions are due to π–π^* transition. The λ_{max} is shifted to a higher wavelength with the increase in polarity of solvent.

The anti-bacterial activity of **5a–e** was assessed in side-by-side comparison with ciprofloxacin and norfloxacin against some Gram-positive (*S. aureus, and B. subtilis*) and Gram-negative (*E. coli, K. peneumoniae* and *P. aeruginosa*) bacteria using conventional agar dilution procedure and the results are summarized in Table 2. The anti-bacterial data indicated that compounds **5a–e** had a better activity against tested Gram-positive organisms. However, all the compounds were nearly inactive against tested Gram-negative bacteria. The anti-

Table 1
The molecular formula, microwave irradiation/thermal heating time and yield of 5a–e.

Compound	Molecular formula	Microwave irradiation		Thermal heating	
		Time (sec) 320 W	Yield %	Refluxing time (h)	Yield %
5a	$C_{14}H_{11}SN_3$	60	85	18	30
5b	$C_{16}H_{16}SN_4$	70	90	18	25
5c	$C_{14}H_{10}SN_3F$	65	90	17	35
5d	$C_{14}H_{10}SN_3Cl$	60	85	12	40
5e	$C_{14}H_{10}SN_3Br$	60	85	14	30

Table 2
In vitro anti-bacterial activity of **5a–e** and standards (MIC μg ml^{-1})

Compounds 5	*S. aureus*	*B. subtilis*	*E. coli*	*K. peneumoniae*	*P. aeruginosa*
a	0.59	0.55	32	>60	>60
b	0.40	0.47	18	24	42
c	0.15	0.024	40	>60	>60
d	0.22	0.044	39	>60	>60
e	0.18	0.028	8	>60	>60
Ciprofloxacin	0.55	0.03	0.13	0.07	1
Norfloxacin	1	0.05	0.48	0.32	4.5

bacterial data revealed that the compounds **5a–e** possesses similar anti-bacterial profiles. The selective anti-bacterial activity against Gram-positive bacteria is in contrast to the good anti-bacterial activity of ciprofloxacin against both Gram-positive and Gram-negative bacteria. The compounds **5c** and **e** are more active than the rest of the compounds tested. Among the halogenated compounds 4-fluoro **5c** is more effective than 4-bromo and 4-chloro substituted compounds. Thus the nature of the group has strong influence on the spectrum and extent of anti-bacterial activity.

3. Experimental

All melting points were taken in open capillary tubes and are uncorrected. Purity of the compounds was checked by TLC. IR spectra were recorded in an AVATAR 330 FT-IR instrument. NMR spectra were recorded on BRUCKER AMX 400 MHz spectrometer using $CDCl_3$ solvent for ^1H NMR and DMSO-d_6 solvent for ^{13}C-SEFT NMR. Mass spectra were recorded on a Q-Tof microhybrid quadrupole time of flight mass spectrometer by atmospheric pressure chemical ionization [APcI]. The UV-Visible absorption spectra were recorded on HITACHI U-2001 double beam spectrometer using analar grade Benzene, 1,4-Dioxane and Methanol.

3.1. General procedure for preparation of 3-aryl-1-thien-2ylprop-2-en-1-ones 3a–e

The compounds were prepared as per standard procedure [14,15]. It involves a solution of suitably substituted benzaldehyde **2** (0.01 mol) and 1-(2-thienyl)ethanone **1** (0.01 mol) in distilled ethanol (50 ml) containing aqueous sodium hydroxide (0.5 mol in 5 ml of water) was heated over a water bath for half-an-hour. The solution gradually turned red and yellow crystals were formed, then heating was stopped and cooled to room temperature. The product thus obtained was filtered, washed with distilled water, dried and crystallized from ethanol. Yields were in the range of 90–95%. The formed products were confirmed through FT-IR spectra.

3.2. General methods for preparation of 2-amino-6-aryl-4-(2-thienyl)pyrimidines 5a–e

3.2.1. A. Microwave irradiation

A mixture of 3-aryl-1-thien-2ylprop-2-en-1-one 3a**–e** (0.01 mol), guanidine hydrochloride **4** (0.01 mol) and sodium hydroxide pellets (0.5 mol) was mixed and finely powdered in a pestle and mortar. The mixture was transferred into a beaker and irradiated in the domestic microwave oven (LG Grill, MG395 WA). The mixture was irradiated at 320 W for 60–70 sec. Then, distilled water was added to remove the excess of alkali and then filtered and dried.

3.2.2. B. Thermal heating

A mixture of 3-aryl-1-thien-2ylprop-2-en-1-one **3a–e** (0.01 mol), guanidine hydrochloride **4** (0.01 mol) in ethanol (50 ml) while a solution of NaOH (0.5 mol in 5 ml of water) was added portion-wise for two hours. Refluxing was continued for further 12–18 h, and the mixture was concentrated. Then, the product was filtered and dried.

The products obtained by the above methods A and B were separated and purified by column chromatography using benzene and ethyl acetate mixture as eluting solvent.

3.3. 2-Amino-6-phenyl-4-thien-2ylpyrimidine 5a

^1H NMR ($CDCl_3$), δ, ppm: 5.53 (S, 2H: NH_2); 7.26 (S, 1H: H-5); 7.1–8.0 (aromatic protons); ^{13}C NMR (DMSO-d_6), δ, ppm: 101.9 (C-5); 160-6 (C-2); 164.8 and 163.4 (C-6 and C-4); 126.9–129.1 (aromatic carbons); Mass, APcI, 10 eV: 266 (M + H); IR (KBr), cm^{-1}: 3480 and 3340 γ(N-H); 2923 γ(C-H); 1225 γ(C-N); 1615 γ(N-H in plane bending); 820 γ(C-H out-of-plane bending in 2-substituted thiophene); UV, nm: λ_{max} (Benzene): 343.0; λ_{max} (1,4-Dioxane): 348.0 and 293.5; λ_{max} (Methanol): 345.5 and 257.0; m.p. 130–132 °C.

3.4. 2-Amino-6-[4-(dimethylamino)phenyl]-4-thien-2ylpyrimidine 5b

^1H NMR ($CDCl_3$), δ, ppm: 5.13 (S, 2H: NH_2); 7.30 (S, 1H: H-5); 7.1–8.0 (aromatic protons); 3.04 (S, 6H; N $(CH_3)_2$); SEFT (DMSO-d_6), δ, ppm: 101.08 (C-5); 159-9 (C-2); 163.4 and 165.9 (C-6 and C-4); 124.8–128.5 (aromatic carbons); 40.1 (CH_3; $N(CH_3)_2$); Mass, APcI, 10 eV: 297.1 (M + H); IR (KBr), cm^{-1}: 3398 and 3319 γ(N-H); 2907 γ(C-H); 1241 γ(C-N); 1607 γ(N-H inplane bending); 810 γ(C-H out-of-plane bending in 2-substituted thiophene); UV, nm: λ_{max} (Benzene): 371.5; λ_{max} (1, 4-Dioxane): 371.0 and 293.5; λ_{max} (Methanol): 374.0, 290.0 and 240.5; m.p. 174–176 °C.

3.5. 2-Amino-6-(4-fluorophenyl)-4-thien-2ylpyrimidine 5c

^1H NMR ($CDCl_3$), δ, ppm: 5.24 (S, 2H: NH_2); 7.36 (S, 1H: H-5); 7.0–8.1(aromatic protons); SEFT (DMSO-d_6), δ,

ppm: 102.6 (C-5); 161-9 (C-2); 165.4 and 164.2(C-6 and C-4); 127.9–136.8 (aromatic carbons); Mass, APcI, 10 eV: 272 (M + H); IR (KBr), cm^{-1}: 3394 and 3368 γ(N-H); 2930 γ(C-H); 1240 γ(C-N); 1635 γ(N-H inplane bending); 818 γ(C-H out-of-plane bending in 2-substituted thiophene); 1038 γ(C-F); UV, nm: λ_{max} (Benzene): 349.0;λ_{max}(1, 4-Dioxane): 356.0 and 293.0; λ_{max} (Methanol): 356.5 and 263.0; m.p. 172–174 °C.

3.6. 2-Amino-6-(4-chlorophenyl)-4-thien-2ylpyrimidine 5d

^{1}H NMR ($CDCl_3$), δ, ppm: 5.16 (S, 2H: NH_2); 7.13 (S, 1H: H-5); 7.1–7.9 (aromatic protons); SEFT (DMSO-d_6), δ, ppm: 102.2 (C-5); 160-9 (C-2); 164.8 and 163.4 (C-6 and C-4); 127.0–136.0 (aromatic carbons); Mass, APcI, 10 eV: 287.1 (M + H); IR (KBr), cm^{-1}: 3382 and 3333 γ(N-H); 3103 and 2925 γ(C-H); 1235 γ(C-N); 1644 γ(N-H inplane bending); 813 γ(C-H out-of-plane bending in 2-substituted thiophene); 724 γ(C-Cl); UV, nm: λ_{max} (Benzene): 346.0; λ_{max} (1, 4-Dioxane): 351.0 and 293.0; λ_{max} (Methanol): 351.0 and 261.0; m.p. 164–166 °C.

3.7. 2-Amino-6-(4-bromophenyl)-4-thien-2ylpyrimidine 5e

^{1}H NMR ($CDCl_3$), δ, ppm: 5.20 (S, 2H: NH_2); 7.32 (S, 1H: H-5); 7.0–8.0 (aromatic protons); SEFT (DMSO-d_6), δ, ppm: 102.3 (C-5); 161.4 (C-2); 164.9 and 163.8 (C-6 and C-4); 127.2–136.7 (aromatic carbons); Mass, APcI, 10 eV: 333 (M + H); IR (KBr), cm^{-1}: 3382 and 3346 γ(N-H); 2928 γ(C-H); 1238 γ(C-N); 1630 γ(N-H inplane bending); 815 γ(C-H out-of-plane bending in 2-substituted thiophene); 668 γ(C-Br); UV, nm: λ_{max} (Benzene): 347.0; λ_{max} (1, 4-Dioxane): 353.0 and 293.5; λ_{max} (Methanol): 353.0 and 262.5; m.p. 168–170 °C.

4. Anti-bacterial activity

The in-vitro anti-bacterial activity of the synthesized compounds against Gram-positive organisms (Staphylococcus *aureus*, and *Bacillus subtilis)* and Gram-negative (Escherichia *coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) organisms by the conventional agar dilution procedures [16] and compared with that of ciprofloxacin and norfloxacin. Two-fold serial dilutions of the compounds and reference drugs were prepared in Muller–Hinton agar. Drugs were dissolved in dimethylsulfoxide (DMSO; 1 ml) and the solution was diluted with water (9 ml). Further progressive double dilution with melted Muller–Hinton agar were performed to obtain the required concentrations.

The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

Acknowledgements

The authors are extremely grateful to the reviewers for their valuable suggestions and comments.

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