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ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 517–520
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis, *in vitro* antibacterial and antifungal evaluations of 2-amino-4-(1-naphthyl)-6-arylpyrimidines

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Received 19 March 2006; received in revised form 22 September 2006; accepted 22 September 2006
Available online 3 November 2006

Abstract

A series of 2-amino-4-(1-naphthyl)-6-arylpyrimidines have been synthesized and characterized by IR, NMR, MS, elemental analyses and evaluated for *in vitro* antibacterial and antifungal activities. Some of the compounds were found to be active against a limited panel of bacteria and fungi. In particular, compounds **4b** and **4e** were found to be the most effective analogs against the tested bacterial and fungal strains.

Keywords: 2-Aminopyrimidines; Synthesis; Antibacterial activity; Antifungal activity

1. Introduction

Pyrimidine is the basic nucleus in nucleic acids and has been associated with a number of biological activities [1]. Pyrimidines are well known to have a number of biological and antimicrobial activities [2]. Its derivatives display a wide range of pharmacological activities [3–7]. The various methods of synthesis and reactions of aminopyrimidines are reported [8–10]. Recently, we have reported the synthesis and antibacterial activity of biphenyl, phenyl and thienyl substituted 2-aminopyrimidines [11–12]. Based on the above reports and in continuation of our research on the syntheses of biologically active heterocyclic molecules, we report here the synthesis of some naphthalene substituted 2-aminopyrimidines and their antibacterial activity.

2. Results and discussion

1-(1-Naphthyl)ethanone **2** is prepared by acetylation of naphthalene **1** in the presence of anhydrous aluminium chloride in CCl_4. The Claisen–Schmidt condensation of equimolar quantities of 1-(1-naphthyl)ethanone with different substituted benzaldehydes in the presence of alkali gives 1-(1-naphthyl)-3-arylprop-2-en-1-one **3**. When 1-(1-naphthyl)-3-arylprop-2-en-1-ones are refluxed with guanidine nitrate in presence of alkali, 2-aminopyrimidines **4a–i** are formed (Scheme 1).

The IR spectra of the compound **4** displayed characteristic absorption bands (cm^{-1}) in the regions 3150–3500 (NH stretching), 1500–1575 (C=C stretching) and 1600–1650 (C=N stretching); this gives positive evidence for the formation of the compound. Elemental analysis data of the compounds (calculated and found) are presented in Table 1.

The ^1H NMR shows characteristic peaks at δ (ppm) 5.2–5.72 (2H, s, NH_2), around 7.3 (H-5) and 7.0–8.5 (Ar–H). ^{13}C NMR displays characteristic peaks at δ (ppm) 168.5 (C-6), 108.8 (C-5), 165.9 (C-4), 163.6 (C-2), 137.1, 134.0 and 130.7 (*ipso* carbons).

The compounds **4a–i** were evaluated for antibacterial and antifungal activities against representative bacteria—*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and fungi—*Trichophyton tonsurans* and *Microsporum gypseum*. Activity was expressed as Minimum Inhibitory Concentration (MIC), the lowest concentration of the tested compound that resulted in no visible growth on

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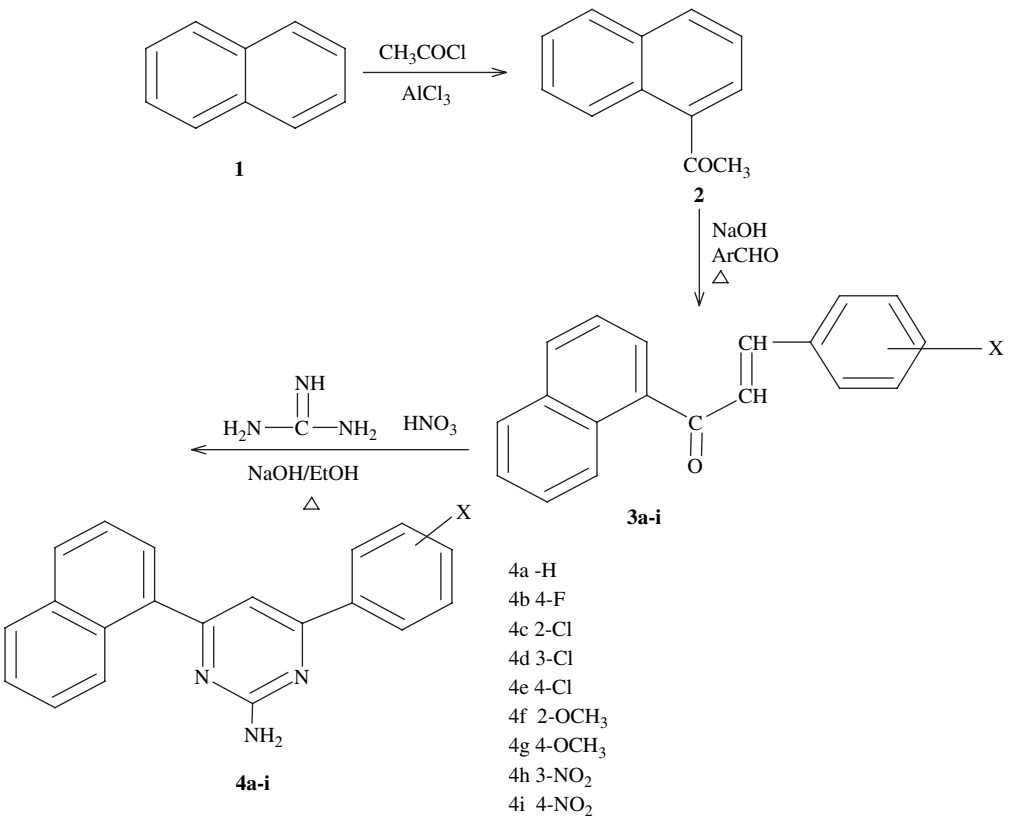
0223-5234/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.09.012



Scheme 1.

the plate. As can be seen in Table 2, although not as active as the standard ampicillin, **4a–i** were generally found to be more active against *K. pneumoniae* and *P. aeruginosa* than the other organisms in the testing panel. In particular, the 4-fluoro (**4b**) and 4-chloro (**4e**) analogs had the best overall antibacterial profile. Table 2 also contains results of antifungal activity tests of **4a–i** where, miconazole was used as the standard. Again, compounds **4b** and **4e** were more active than the other analogs in the series. Although the rest of the compounds showed varying degrees of inhibition none were as effective as miconazole. Among the three chloro isomers 4-chloro (**4e**) was more effective than the 2- or 3-chloro substituted compounds. Thus the nature and position of the substituent have strong influence on the spectrum and extent of antibacterial and antifungal activities.

3. Experimental

Melting points are determined in an open capillary and are uncorrected. ^{1}H NMR, ^{13}C NMR and SEFT spectra were recorded on Brucker (AMX-400) using $CDCl_3$ as solvent. TMS was used as internal reference for ^{1}H NMR, ^{13}C NMR and SEFT spectra. Mass spectra were recorded on a CLASS-5000 mass spectrometer with an ion source temperature of 200 °C. Elemental analyses were done on Vario EL, CHNOS elemental analyzer.

3.1. Procedure for preparation of 1-(1-naphthyl)ethanone

A mixture of 41.9 g (38 ml, 0.53 mol) of acetyl chloride and 100 ml of carbon tetrachloride was taken in a round

Table 1
Physical and analytical data of **4a–i**

Compounds	Melting point (°C)	Yield (%)	Molecular formula	Elemental analysis		
				Carbon, found (calcd) %	Hydrogen, found (calcd) %	Nitrogen, found (calcd) %
4a	80–82	60	$C_{20}H_{15}N_3$	80.60 (80.78)	5.12 (5.08)	14.23 (14.13)
4b	121–122	54	$C_{20}H_{14}N_3F$	77.61 (76.17)	4.84 (4.47)	13.72 (13.32)
4c	76–78	55	$C_{20}H_{14}N_3Cl$	72.99 (72.39)	4.83 (4.45)	12.88 (12.66)
4d	109–111	45	$C_{20}H_{14}N_3Cl$	72.87 (72.39)	4.75 (4.25)	12.96 (12.66)
4e	116–118	60	$C_{20}H_{14}N_3Cl$	72.80 (72.39)	4.43 (4.25)	12.81 (12.66)
4f	79–80	60	$C_{21}H_{17}N_3O$	77.18 (77.04)	5.63 (5.23)	13.00 (12.83)
4g	136–138	65	$C_{21}H_{17}N_3O$	77.87 (77.04)	5.73 (5.23)	13.02 (12.83)
4h	163–165	75	$C_{20}H_{14}N_4O_2$	70.35 (70.17)	4.77 (4.62)	16.98 (16.87)
4i	195–197	75	$C_{20}H_{14}N_4O_2$	70.66 (70.17)	4.61 (4.52)	16.50 (16.37)

Table 2
In vitro antibacterial and antifungal activities of **4a–i** and their standard (MIC μg ml^{-1})

Compound	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*T. tonsurans*	*M. gypseum*
4a	>60	42	5.50	6.59	25	25
4b	7.30	8.10	2.55	5.45	2.20	5.90
4c	40	>60	5.38	6.40	25	25
4d	39	41	6.38	7.42	25	25
4e	12.30	13.50	19.8	21.90	2.80	5.55
4f	>60	36	8.22	9.18	25	25
4g	>60	>60	9.31	10.21	25	25
4h	29.10	19.50	14.28	18.21	25	25
4i	25.65	16.65	15.35	11.92	25	25
Ampicillin	1	1.65	1.80	1.80	NT	NT
Miconazole	NT	NT	NT	NT	0.20	0.20

NT: not tested.

bottom flask. About 70 g (0.52 mol) of powdered dry $AlCl_3$ was added slowly to the vigorously stirred above mixture. The mixture becomes warm and then cooled to 20 °C. A solution of naphthalene (32 g, 0.25 mol) in 100 ml of CCl_4 was added and hydrogen chloride was evolved. The mixture was warmed to 30 °C for 30 min. The resulting mixture was decomposed with ice and concentrated hydrochloric acid. The product was separated and the crude was distilled under reduced pressure.

*3.2. General procedure for preparation of 1-(1-naphthyl)-3-arylprop-2-en-1-ones (**3a–i**)*

A solution of substituted benzaldehyde (0.01 mol) and 1-(1-naphthyl)ethanone (0.01 mol) in 65% aqueous ethanol (60 ml) containing sodium hydroxide (0.5 g) was heated over a water bath for 2 h. The solution was then cooled. The product thus obtained was filtered and recrystallised from ethanol.

*3.3. General procedure for preparation of 2-amino-4-(1-naphthyl)-6-arylpyrimidine (**4a–i**)*

A mixture of 1-(1-naphthyl)-3-aryl-prop-2-en-1-one (0.01 mol) and guanidine nitrate (0.01 mol) in ethanol (75 ml) was refluxed, while a solution of sodium hydroxide (0.5 mol) in water (10 ml) was added portion wise for 2 h. Refluxing was continued for further 10 h and the mixture was poured into ice cold water. The formed solid was separated by filtration. The 2-aminopyrimidines are purified from the solid mixture by column chromatography using benzene and ethyl acetate mixture as eluting solvent.

*3.3.1. 2-Amino-4-(1-naphthyl)-6-(phenyl)pyrimidine (**4a**)*

^{1}H NMR, δ (ppm) 5.72 (2H, s, NH_2), 7.32 (1H, s, H-5), 7.30–8.25 (Ar–H). ^{13}C NMR (100.6 MHz, $CDCl_3$), δ (ppm) 168.50 (C-6), 108.83 (C-5), 165.91 (C-4), 163.65 (C-2), 137.65, 137.10, 134.01 and 130.78 (quaternary carbons), 124.35–130.62 (Ar–C).

*3.3.2. 2-Amino-4-(1-naphthyl)-6-(4-fluorophenyl) pyrimidine (**4b**)*

^{1}H NMR, δ (ppm) 5.34 (2H, s, NH_2), 7.26 (1H, s, H-5), 6.97–8.21 (Ar–H). ^{13}C NMR, δ (ppm) 168.13 (C-6), 108.07 (C-5), 165.36 (C-4), 163.47 (C-2), 137.23, 133.98, 129.65, 130.79 (quaternary carbons), 125.28–128.77 (Ar–C).

*3.3.3. 2-Amino-4-(1-naphthyl)-6-(2-chlorophenyl) pyrimidine (**4c**)*

^{1}H NMR, δ (ppm) 5.66 (2H, s, NH_2), 6.96–8.29 (Ar–H), singlet for H-5 proton is merged with other aromatic protons. ^{13}C NMR, δ (ppm) 167.70 (C-6), 109.11 (C-5), 165.52 (C-4), 163.36 (C-2), 137.60, 136.33, 138.86, 133.98 and 133.83 (quaternary carbons), 124.36–132.29 (Ar–C).

*3.3.4. 2-Amino-4-(1-naphthyl)-6-(3-chlorophenyl) pyrimidine (**4d**)*

^{1}H NMR, δ (ppm) 5.32 (2H, s, NH_2), 7.30 (1H, s, H-5), 6.83–8.19 (Ar–H). ^{13}C NMR, δ (ppm) 168.73 (C-6), 108.65 (C-5), 164.28 (C-4), 163.41 (C-2), 139.27, 137.46, 136.66, 134.94, 134.09 (quaternary carbons), 123.47–134.02 (Ar–C).

*3.3.5. 2-Amino-4-(1-naphthyl)-6-(4-chlorophenyl) pyrimidine (**4e**)*

^{1}H NMR, δ (ppm) 5.2 (2H, s, NH_2), 7.2–8.1 (Ar–H), the singlet for H-5 proton is merged with aromatic protons. ^{13}C NMR, δ (ppm) 162.27 (C-6), 109.17 (C-5), 161.20 (C-4), 157.86 (C-2), 137.30, 152.3, 140.3, 134.5, 133.7, 130.6 (quaternary carbons), 124.4–129.8 (Ar–C).

*3.3.6. 2-Amino-4-(1-naphthyl)-6-(2-methoxyphenyl) pyrimidine (**4f**)*

^{1}H NMR, δ (ppm) 5.63 (2H, s, NH_2), 3.84 (3H, s, OCH_3), 6.97–8.34 (Ar–H), singlet of H-5 proton is merged in aromatic region. ^{13}C NMR, δ (ppm) 166.97 (C-6), 111.67 (C-5), 164.50 (C-4), 157.86 (C-2), 137.30, 137.02, 132.35, 134.04 (quaternary carbons), 122.61–131.32 (Ar–C), 55.72(–OCH_3).

*3.3.7. 2-Amino-4-(1-naphthyl)-6-(4-methoxyphenyl) pyrimidine (**4g**)*

^{1}H NMR, δ (ppm) 5.41 (2H, s, NH_2), 7.26 (1H, s, H-5), 6.98–8.22 (Ar–H), 3.86 (3H, s, OCH_3). ^{13}C NMR, δ (ppm) 168.21 (C-6), 108.10 (C-5), 163.47 (C-4), 161.87 (C-2), 137.26, 133.99, 130.78, 129.98 (quaternary carbons), 125.28–129.64 (Ar–C), 55.42 (–OCH_3), M^+ 327, 77 (100).

*3.3.8. 2-Amino-4-(1-naphthyl)-6-(3-nitrophenyl) pyrimidine (**4h**)*

^{1}H NMR, δ (ppm) 5.2 (2H, s, NH_2), 7.2–8.5 (Ar–H), the singlet for H-5 proton may be merged with the aromatic protons. ^{13}C NMR, δ (ppm) 163.0 (C-6), 108.7 (C-5), 163.4 (C-4), 169.5 (C-2), 148.9, 136.5, 139.3, 134.0, 130.6 (quaternary carbons), 122.2–129.7 (Ar–C), M^+ 342, 77 (100).

*3.3.9. 2-Amino-4-(1-naphthyl)-6-(4-nitrophenyl)pyrimidine (**4i**)*

^{1}H NMR, δ (ppm) 5.3 (2H, s, NH_2), 7.3–8.4 (Ar–H), the singlet for H-5 proton is merged with aromatic protons. ^{13}C NMR, δ (ppm) 163.1 (C-6), 109.2 (C-5), 163.5 (C-4), 169.4 (C-2), 149.1, 136.5, 139.3, 134.0, 130.5 (quaternary carbons), 123.9–128.6 (Ar–C).

3.4. Microbiology

3.4.1. Antibacterial activity [13]

The disk diffusion method was used for the preliminary antibacterial evaluation. The MIC was determined by the microbroth dilution technique using Mueller–Hinton broth. Serial two-fold dilutions of the test compounds in DMSO were prepared. The inoculum was prepared in broth, which had been kept at 37 °C overnight, and was diluted with broth to give a final concentration of 10^5 cfu/ml in the test tray. The trays were covered to prevent drying. After incubation at 37 °C for about 24 h the trays were examined for growth. The lowest concentration of the test compounds inhibiting visible growth was taken as the MIC value.

3.4.2. Antifungal activity [14]

All the compounds to be tested were dissolved in DMSO at a concentration of 5000 μg/ml and the final concentration was reduced to 100 μg/ml with sterile distilled water. No effect of DMSO (5%) was observed. The strains were grown on slant medium of Sabouraud (Difco), transferred to 3.5 ml nutrient broth (NB, Himedia) and incubated for 3–5 days at 25 °C. At the end of the incubation period these strains were transferred into screw-capped bottles containing sterilized beads and shaken for 4–5 min in a vortex. The suspensions of the cultures were adjusted to have an absorbance degree of 0.5 at 500 nm in the spectrophotometer. Eight different dilutions of the test compounds were prepared in microplates by serial dilutions from top to bottom. Then all the wells except the positive control were filled with 10 μl of the standardized strains. These plates were incubated at 25 °C for 7 days. The minimum concentration at which no growth was observed was taken as the MIC value.

Acknowledgements

Authors are thankful to NMR Research Center, Indian Institute of Science, Bangalore for NMR spectral measurements.

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