Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 703–706

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Suitably Functionalised Pyrimidines as Potential Antimycotic Agents†

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Received 22 December 1999; accepted 22 January 2000

Abstract—Various suitably functionalised pyrimidine derivatives have been synthesized to explore their potential as antimycotic agents. Some of the synthesized compounds **4c**, **4d**, **8a–e** have shown highly significant in vitro antifungal activity against five human pathogenic fungi.

Introduction

The incidence of invasive fungal infections has largely been attributed to the rising number of immunocompromised patients. The commonest cause of invasive fungal infections in human are the species of *Aspergillus* followed by *Candida* and *Cryptococcus neoformans*. The superficial fungal infections, very common in major populations is caused by species of *Trichophyton mentagrophytes*, *T. rubrum* and *Sporothrix schenckii*. Because of a wide prevelence of mycoses due to soil borne or airborne transmission of the systemic fungal pathogens and lack of broad spectrum antifungals led to the search for newer prototype molecules to exhibit highly significant antimycotic activity.

Among various nitrogen heterocycles, derivatives of azole such as imidazole and triazole are proved to be clinically potent and useful antifungal agents.[1–7] Pyrimidine, an enlarged ring size of imidazole by one carbon atom is not extensively exploited as antimycotic agents. Except for a few pyrimidine derivatives such as trimethoprim and 5-fluorocytosine[8] none of the other compounds are found clinically useful. Only 5-fluorocytosine alone is in clinical use against systemic fungal infections.The antifungal activity of 5-fluorocytosine is due to its inhibitory property of a significant enzyme thymidylate synthase, responsible for DNA synthesis.[8]

This paper reports on the synthesis of various pyrimidine derivatives with different substituents across the ring skeleton to explore their therapeutic potential as antifungal agents. Among all the synthesized 2,4,6-trisubstituted pyrimidine-5-carbonitriles, 4-chloro-2,6-disubstituted pyrimidine-5-carbonitriles are found significantly active against five human pathgoenic fungi, *Aspergillus fumigatus* (Af), *Candida albicans* (Ca), *Cryptococcus neoformans* (Cn), *Trichophyton mentagrophytes* (Tm) and *Sporothrix schenckii* (Ss) in in vitro screen. The in vitro screening of all the synthesized compounds was determined by 2-fold serial dilution technique. The activity of these compounds was expressed in terms of minimum inhibitory concentration (MIC), considering ketoconazole as a standard drug. The most potent compound amongst all the screened compounds was **4d** which displayed significant activity against Af, Tm and Cn at 0.002, 0.005 and 1.5 μg/ml concentration respectively. In general 4-chloro-2,6-disubstituted pyrimidines **4a–e** and **8a–d** were found highly active against Af and Tm. Replacement of 4-chloro group by nucleophilic substituents such as NHR, and SH group, a complete loss of activity profile of all the synthesized compounds is presented in the Table 1.

Synthesis

Various pyrimidine derivatives of the prototypes **4**, **8**, **9** and **10** have been synthesized[9] to explore their antifungal activity. 4-Chloro-6-methylthio-2*H*/substituted pyrimidine-5-carbonitrile (**4a–e**) have been synthesized by two different routes. In both the routes appropriate

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0960-894X/00/$ - see front matter
PII: S0960-894X(00)00091-3

Table 1. In vitro antifungal activity of pyrimidine derivatives **4a–e** and **8a–f**, **9** and **10** against human pathogenic fungi

Compound no.	Minimum inhibiting concentration (MIC) in μg/ml				
	Af	Tm	Ca	Cn	Ss
4a	0.39	0.39	25	1.56	6.25
4b	6.25	1.56	25	1.56	12.5
4b	0.02	12.5	>100	>100	>100
4d	0.002	0.005	>100	1.56	50
4e	0.78	0.19	50	6.25	50
8a	0.09	0.09	>100	50	>100
8b	1.56	0.39	>100	3.12	50
8c	0.04	0.09	>100	50	>100
8d	0.09	0.39	>100	1.56	>100
8e	0.04	0.19	>100	>100	>100
8f	50	50	>100	>100	>100
9a	>100	>100	>100	>100	>100
9b	>100	>100	>100	>100	>100
9c	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100
Ketoconazole	0.7	0.1	0.3	0.1	25

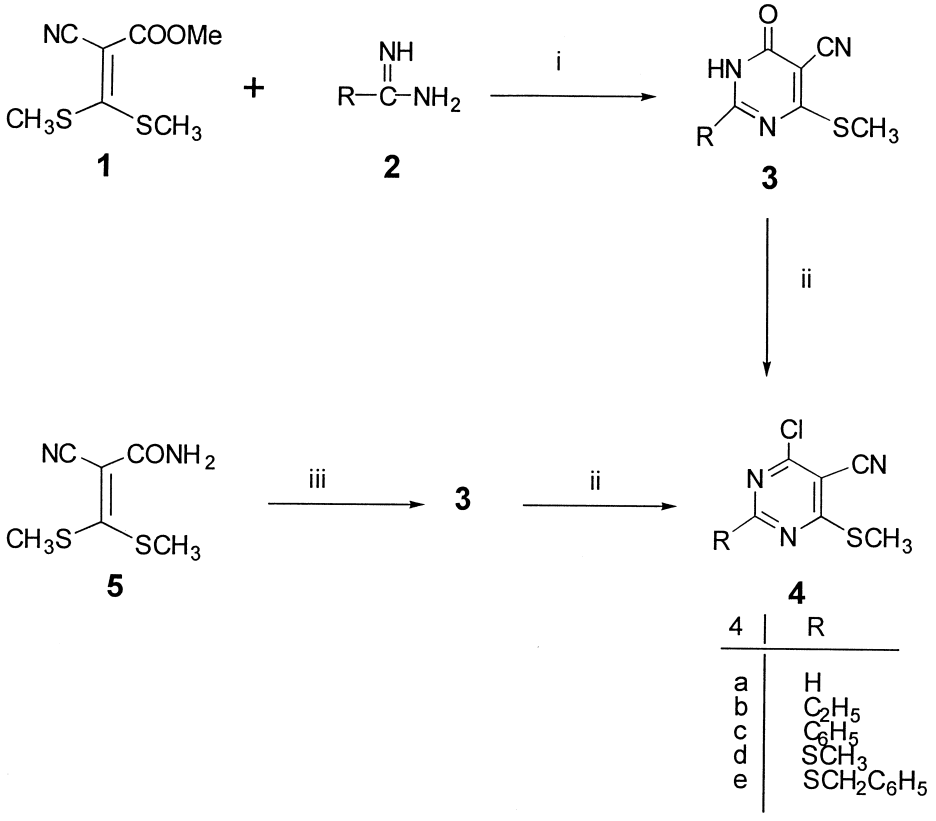
ketene dithioacetals are used as precursor. A reaction of ethyl 2-cyano-3,3-dimethylthioacrylate[10] (**1a**) with amidine in alcohol at reflux temperature yielded 6-methylthio-4-oxo-2*H*/substituted-3,4-dihydropyrimidines (**3a–e**) which were also obtained[11] from the reaction of 2-cyano-3,3-dimethylthioacrylamide and acid anhydride. The oxopyrimidines (**3a–e**) on halogenation with phosphoryl chloride provided chloropyrimidines (**4a–e**), Scheme 1.

The other prototype of chloropyrimidines (**8a–f**) were prepared starting from 6-aryl-5-cyano-2-thiouracil[9] (**6**). Regioselective alkylation of 6 with methyl iodide, led to the formation of 6-aryl-2-methylthio-4-oxo-3,4-dihydro-5-carbonitrile[12] and were halogenated by phosporyl chloride to yield 6-aryl-4-chloro-2-methylthiopyrimidine-5-carbonitrile. The halo pyrimidines are highly activated due to vicinal presence of electron withdrawing CN substituent and are prone to yield 6-aryl-4-arylamino-2-methylthio-5-carbonitriles (**9a–c**).[12] A reaction with thiourea provided corresponding 6-(3-fluorophenyl-4-mercapto-3-methylthio-5-carbonitrile (**10**) Scheme 2.

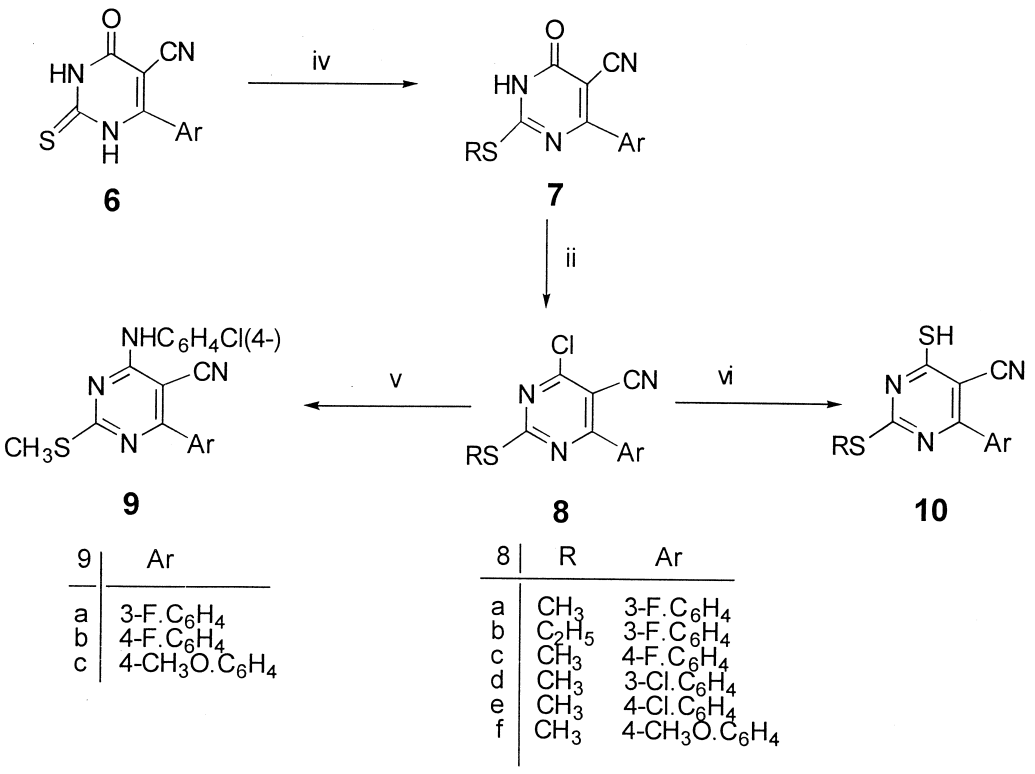
Biological Activity

The in vitro antifungal activity was evaluated by 2-fold serial dilution technique[13] against five human pathogenic fungi viz. *A. fumigatus*, *C. albicans*, *C. neoformans*, *S. schenckii* and *T. mentagrophytes*. Sabouraud's dextrose agar (SDA) slant growth of the pathogens for 24–48 h (yeasts) or 7 days (mycelial fungi) were suspended in Sabouraud's dextrose broth (SDB). The colony forming units (cfu) of the seeded broth were estimated by dilution and plating technique and adjusted in the range of 10^4–10^5 cfu/ml.

The test compounds were dissolved in DMSO to get a stock solution of 1 mg/ml. To 1.8 ml seeded broth was added 0.1 ml of the stock test solution. One milliliter of



Scheme 1.



Scheme 2. Reagents/conditions: (i) EtOH/100 °C; (ii) $POCl_3$/reflux; (iii) $(RCO)_2O$/reflux; (iv) RX/Na_2CO_3/DMF/0 °C; (v) arylamine/EtOH/100 °C; (vi) thiourea/EtOH/reflux.

this solution was further diluted with 1 ml of seeded broth to make second dilution and so on until 16 such dilutions were obtained. A set of assay tubes with seeded broth and solvent were kept as control. The tubes were incubated at 28 ± 1 °C and observed for MIC after 24–96 h depending upon the time required for optimum growth of the test organism. Ketoconazole was used as the standard drug.

The antifungal activity profile of various pyrimidine derivatives **4a–e**, **8a–f**, **9** and **10** revealed that 4-chloro substituent in the pyrimidine skeleton is a must to express significant activity. Substituents of positions 2 and 6 either ameliorate or attenuate the antimycotic activity depending upon the nature and size of groups. As evident from the activity of **4a–e**, a mere change in the size of substituent at position 2, greatly influences the activity. The most active compound in 4-chloropyrimidines (**4a–e**, **8a–f**), **4d** is highly active in in vitro screen against Af and Tm. Except amphotericine B none of the clinically used antifungals known so far which have MIC of 0.002 and 0.005 μg/ml concentration in in vitro screening against Af and Tm. An increase in the size of the substituent at position 2 from S-CH_3 to S-$CH_2C_6H_5$ in **4d**, attenuated the activity. Similarly in other 4-chloropyrimidines **8a–b**, an increase in the size of alkyl substituent at position 2 decrease in the activity was observed. Pyrimidines with or without alkyl or aryl substituent at position 2 in **4a–c**, displayed significant activity. However, 4-chloro-6-methylthio pyrimidine-5-carbonitrile (**4a**) was found highly active against all the five test fungi. Substituents at position 6 in **8a–f** also influence MIC of the test compounds. Halophenyl substituent at position 6 in **8a–f** ameliorates the activity while the phenyl group with electron donating substituent (**8f**), attenuates it. Pyrimidines (**9a–c**, **10**) with nucleophilic substituents (NHAr,SH) at position 4 without any change in other substituents, completely lost the antifungal activity.

Acknowledgements

Nidhi Agarwal and Sandeep K. Raghuwanshi are thankful to ICMR for financial support of the project.

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