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ScienceDirect
European Journal of Medicinal Chemistry 41 (2006) 1017–1024
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Preliminary communication

Synthesis of a new series of heterocyclic scaffolds for medicinal purposes

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Received 6 June 2005; received in revised form 11 March 2006; accepted 16 March 2006
Available online 22 June 2006

Abstract

A new series of substituted 8-fluro-4*H*-pyrimido[2,1-b] [1,3]benzothiazole-4-ones () substituted 7-methyl-4*H*-isoxazolo[2,3-*a*]pyrimidin-4-ones, and substituted 2-methyl-5,6,7,8-tetrahydro-9*H*-isoxazolo[2,3-*a*]pyridopyrimidin-9-ones, compounds **I–VII**, have been prepared via condensation of β-keto esters with 2-aminopyridine derivatives, in the presence of polyphosphoric acid. The same technique has also been used to prepare diazepine compounds, **VIII**–**X**, by condensation of a γ-keto ester with 2-aminopyridine derivatives. Details of synthetic procedures are shown. The new compounds have been characterized by elemental analysis, GC–MS, FT-IR and NMR spectrometry. Antibacterial, antifungal and anticancer (cytotoxic) activities, for three of these compounds, have been investigated and are presented.

Keywords: β-Keto esters; 2-Aminopyridine; Diazepine; Heterocyclic; Antifungal; Antibacterial; Cytotoxic

1. Introduction

Naturally occurring hetero-polycyclic compounds are often medically valuable [1]. Cytotoxic, anti-allergic, and anti-malarial activities are documented for hetero-polycyclic compounds such as *colchicin* [2,3], *chalcones* [3], 2-aryl-1,8-naphthyridin-4-one [2] and others [4–7]. Anti-inflammatory [8,9], cardiotonic [10], antiallergic [11], antimalarial [12] and other activities [13–16] are known for heterocyclic compounds. Therefore, it is important to find new efficient methods to synthesize new hetero-polycyclic frameworks. Different synthetic methods are known for such purpose. Examples are: cyclization via reaction of amino-heterocycles with acetylenic compounds, [17,18], base catalyzed isoxazolinyl heterocycle rearrangement [19–21] and intramolecular nucleophilic acyl substitution [22–30]. The main objective of this work is to synthesize and characterize a number of fused heterocyclic compounds that may potentially have medical value, based on structural similarities with earlier compounds.

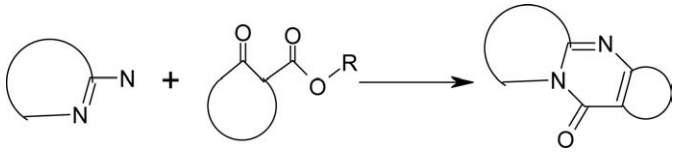
2. Chemistry

Two different groups of compounds have been prepared and characterized [27,31]. Compounds **I–VII** have been prepared by condensation of different β-keto esters with different 2-aminopyridine derivatives, in the presence of polyphosphoric acid, as exemplified in Scheme 1.

On the other hand, compounds **VIII–X** have been prepared by condensing a γ-keto ester with different 2-aminopyridine derivatives, in polyphosphoric acid, as exemplified in Scheme 2. Elemental analysis, GC–MS, FT-IR and NMR indicated the preparation of compounds **I–X** in appreciably pure forms. The structure, expected for each compound, has been confirmed. Details of preparation and characterizations of these compounds are presented in the experimental section.

3. Biological activity results

Three compounds, **I**, **IV** and **V,** have been studied for possible future biological functions.



Scheme 1. Structural formulas for I–VII here.

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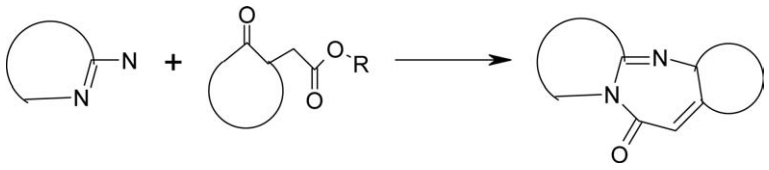
doi:10.1016/j.ejmech.2006.03.025



Scheme 2. Structural formulas for compounds VIII–X here.

3.1. Antibacterial and antifungal activities

Three compounds **I**, **IV** and **V** were all investigated for antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris* and *Candida albicans.* None of these compounds showed any significant antibacterial activity. The same compounds showed significant antifungal activity against *Microsporum canis*, *Fusarium tricincutum*, *Pythium ultimum*, *P. aphanidermatum* and *P. middletonii.* Results are shown in Tables 1 and 2.

The inhibition effects of compounds **I**, **IV** and **V**, together with the reference values, on *M. canis* and *F. tricincutumare* are shown in Table 1. The data showed significant differences between inhibition effects on *M. canis* and *F. tricincutum*, with a *P*-value < 0.001. On the other hand, all compounds, including the reference, showed same inhibition effects, with no significant differences. Thus, all compounds used including the references, had similar activity, while *M. canis* is less sensitive than the other fungus counterpart.

Inhibition effects of compounds **I**, **IV** and **V**, and references, on *P. ultimum*, *P. aphanider-matum* and *P. middletonii*, are shown in Table 2. The three fungi did not have the same sensitivity to the group of compounds, with a *P*-value < 0.001. Therefore, multiple comparisons, using Tukey's test, had been conducted and indicated that *P. ultimum* and *P. aphanidermatum* had same sensitivity, which is different from that of *P. middletonii* (*P*-value < 0.001).

Table 1
Antifungal activity results (mm day^{-1}) for compounds **I**, **IV** and **V** against *M. canis* and *F. tricincutum,* compared to reference values

Compound	% Inhibition (mean of three replicate plates ± S.E.)*	
	M. canis [a]	*F. tricincutum* [b]
I	100.00 ± 0.0	68.5 ± 1.7
IV	84.5 ± 0.0	42.6 ± 0.6
V	100.00 ± 0.0	48.9 ± 5.8
Ref.	75.5 ± 3.9	67.8 ± 1.9

Compound concentrations used are shown in section 5.4.
[a] Ref. griseofulvin;
[b] Ref. nystatin.

Table 2
Antifungal activity results (mm day^{-1}) for compounds **I**, **IV** and **V** against *P. ultimum*, *P. aphanidermatum* and *P. middletonii* compared to reference values

Compound	% Inhibition		
	P. ultimum	*P. aphanidermatum*	*P. middletonii*
I	80.4 ± 2.3	95.9 ± 0.6	44.4 ± 3.5
IV	100.00 ± 0.0	100.00 ± 0.0	80.0 ± 1.7
V	91.9 ± 0.6	100.00 ± 0.0	78.9 ± 1.0
Ref. (hymexazol)	81.1 ± 5.1	70.0 ± 3.3	24.3 ± 10.1

* Mean of three replicate plates ± S.E. Compound concentrations used are shown in Section 5.4.

Contrary to statistical results obtained from Table 1, the data in Table 2 show statistically significant differences among the group of compounds, including reference, with a *P*-value = 0.001.

Each compound activity was compared to the reference, using the Dunnett's intervals. The activity of **I** showed same activity as reference activity, whereas compound **IV** and **V** showed different activities from reference (with *P*-values < 0.01). Thus, compounds **IV** and **V** are statistically more active than compound **I** and the reference. Furthermore, *P. middletonii* is more resistant than other fungus counterparts.

This shows the future potential of using the prepared compounds in antifungal medical formulations. The hymexazole [32] (3-hydroxy-5-methylisoxazole) reference, resembles the structure of the starting material for compounds **IV** and **V**. No correlation between structures, for **I**, **II** and **V**, and their activities were constructed.

3.2. Cytotoxic activity

Three compounds **IV**, **IX** and **X** have been evaluated for cytotoxic activity. The results are summarized in Table 3. Values of percent mortality of cells against compound concentration for each compound are shown in Fig. 1. Among the three compounds used, **IV** and **X** showed significant cytotoxic activities, whereas **IX** failed to do so. In **IV** and **X**, percent cell mortality increased with compound concentration. The data indicate that **X** is potentially more favorable than **IV**. At 1.5 × 10^{-3} M concentration, **X** showed about 95% mortality, before changing into a plateau. Activity of **IV** was lower than that of **X**, within the concentration range used. To achieve mortality percent of 41%, high concentrations (~3.0 × 10^{-3} M) were needed. This limits the potential value of **IV** in medical applications. Contrary to **IV** and **X**, compound **IX** showed inconsistent correlation between percent mortality and concentration, with only a small peak (40%) for 6.25 × 10^{-4} M concentration.

4. Conclusions

Compounds **I–X** have been prepared. Two compounds, (**III** and **VIII**), occurred in relatively low yields (36% and 35%, respectively), whereas the remaining compounds occurred in high yields. Spectral analyses confirmed the proposed structures for the synthesized compounds. Compounds **I**, **IV** and **X** showed neither antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris* nor anticandidal activity against *Candida albicans.* When tested against *Microsporum canis*, *Fusarium tricincutum*, *Pythium ultimum*, *Pythium aphanidermatum* and *Pythium middletonii,* compounds **I**, **IV** and **X** showed sig-