Pyrazolopyranopyrimidines as a Class of Anti-Inflammatory Agents

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Pyrazolopyranopyrimidines **6a–c** and **8a–c** were prepared from the reaction of compounds **4a–c** or **7a–c** with methylamine or ammonium hydroxide solutions. Treatment of compounds **6a–c** or **8a–c** with 2-chloroethyl methyl ether afforded their corresponding acyclonucleosides **9a–c** or **10a–c**, respectively, as a new class of acyclonucleosides. All prepared compounds were tested as anti-inflammatory agents and some of them revealed moderate to potent anti-inflammatory activity.

Key words: Pyrazolopyranopyrimidines, Acyclonucleosides, Anti-Inflammatory Activity

Introduction

Pyranopyrazoles and pyrazolopyranopyrimidines have attracted the attention of many authors due to their chemical and biological interest (Chantegrel *et al.*, 1985; El-Assiery *et al.*, 2004), especially their anti-inflammatory activity (Kuo *et al.*, 1984). Recently, we have been involved in a program (Rashad *et al.*, 2005a, b; Swelam *et al.*, 1999; Zaki, 1998; Zaki *et al.*, 2004) aimed at developing new, simple and efficient syntheses of biologically active heteroaromatic compounds utilizing inexpensive starting materials. In conjunction to these efforts we describe here a synthetic route for preparation of some pyrazolopyranopyrimidines and some of their corresponding acyclonucleosides.

Results and Discussion

Chemistry

Treatment of 3-methyl-2-pyrazolin-5-one (**1**) with α,β-unsaturated nitriles **2a–c** afforded their corresponding 6-amino-4-aryl-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile derivatives **3a–c**, respectively, as the starting materials for the synthesis (Scheme 1). The structure of the latter compounds was confirmed on the basis of their elemental and spectral data, since the IR spectrum of compound **3a**, as a representative example, revealed the presence of cyano and amino groups. Also, its ^{1}H NMR spectrum revealed signals at (δ, ppm): 1.60 (s, 3H, C_3-CH_3), 4.10 (s, 1H, C_4-H), 6.40–6.60 (brs, 2H, NH_2, D_2O exchangeable), 6.70–7.20 (m, 3H, Ar-H), 12.10 (s, 1H, NH-pyrazole, D_2O exchangeable), and its MS gave fragments showing the isotopic pattern due to the presence of chlorine atoms.

Treatment of compounds **3a–c** with an equimolar amount of triethyl orthoformate in the presence of acetic anhydride gave a major product, which could be assigned the structure of **4a–c** or **5a–c**, respectively, due to possible acetylation on either one of the pyrazole nitrogens (Scheme 1). Inspection of ^{1}H NMR spectra of isomer **4a** or **5a**, as a representative example, revealed that the signal of C3-methyl protons appeared at δ 2.20 ppm. This is in favor of structure **4a** since the C3-methyl protons of structure **5a** are adjacent to the N2-acetyl group and so they are more deshielded and their signal would have been observed at lower field. Moreover, the formation of *N*-acylpyrazoles is governed by the steric interaction between the ring substituents groups and the acyl moiety, and it isomerizes slowly into the more thermodynamically stable form N1-acetyl products (Arakawa *et al.*, 1974a; Kashima, 2003). This assignment is in accordance with ^{1}H NMR spectral data given by many authors for alkylation and acylation reactions (Arakawa *et al.*, 1974b; Kuo *et al.*, 1984; Reddy *et al.*, 1963; Zaki *et al.*, 2004). Also, the ^{13}C NMR spectrum of compound **4a** gives further confirmation for the assigned structure since it

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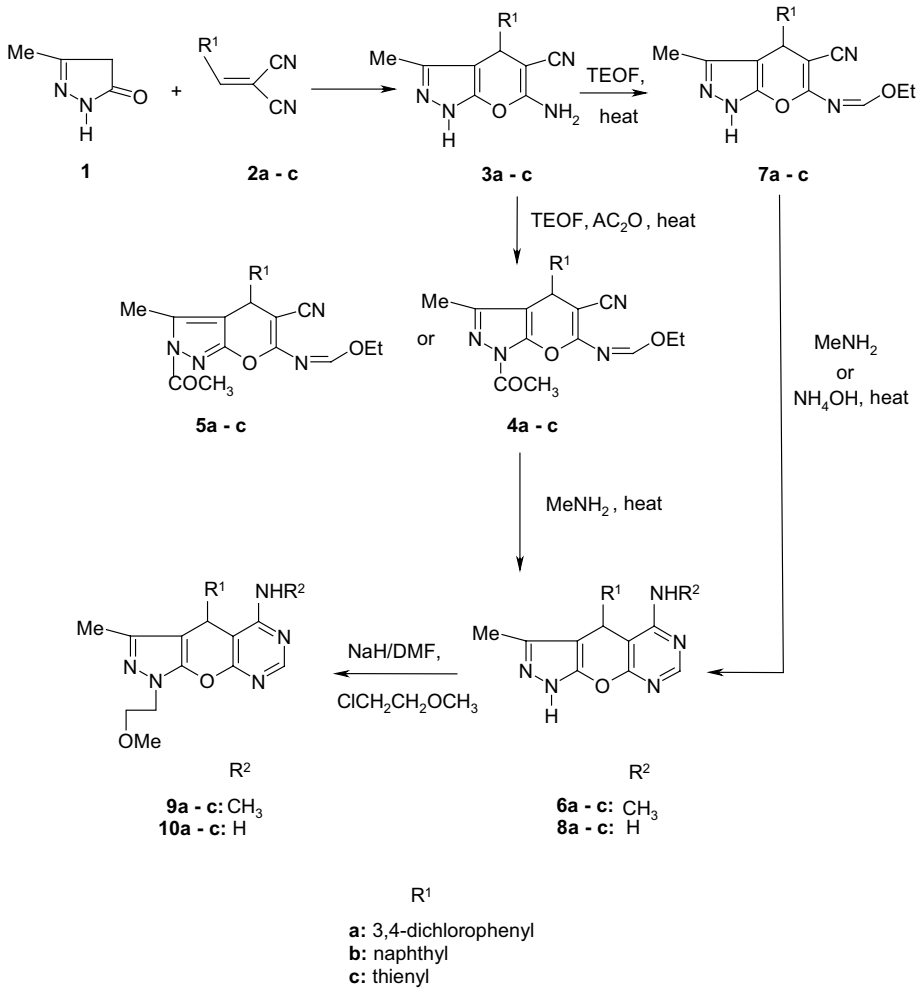

Scheme 1.

showed the following signals at (δ, ppm): 156 (C3), 153.86 (C7a), 13.89 (C10). These spectral data are in agreement with the observed data for the C3, C7a, and C10 of related N1-alkyl compounds (Kuo *et al.*, 1984; Zaki *et al.*, 2004).

As a typical chemical behavior of *N*-acylpyrazoles towards simple nucleophiles (Zaki *et al.*, 2004), treatment of compounds **4a**–**c** with methylamine solution afforded *N*-{4-(substituted)-pyrano[2,3-*d*]pyrimidin-5-yl}-methylamine derivatives **6a**–**c**, respectively, as target compounds for nucleosidation. The IR spectrum of **6a**, as a representative example, revealed the absence of a cyano group and the presence of two NH groups; also the ^{1}H NMR spectrum revealed signals at (δ, ppm): 3.15 (s, 3H, NCH_3), 6.30 (s, 1H, NH, D_2O exchangeable).

Actually, a problem was encountered in this stage of research that the yield of compounds **6a**–**c** was low, so it is hard to consider this route as a convenient pathway and that was solved by reacting compounds **3a**–**c** with neat triethyl orthoformate to give **7a**–**c**, then followed by nucleophilic substitution using methylamine to afford compounds **6a**–**c** in quantitative yield (Zaki *et al.*, 2004). These results prompted us to continue our trials to prepare other pyrazolopyranopyrimidines as targets for nucleosidation. Thus, treatment of compounds **7a**–**c** with ammonium hydroxide solution gave products assigning the structures of pyrazolopyranopyrimidin-5-ylamine derivatives **8a**–**c**, respectively. The structure of the aforementioned derivatives was verified with spectral data, since the IR spectrum of **8a**, as a representative exam-

ple, revealed the absence of a cyano group and the presence of a NH and NH_2 group. Also the ^1H NMR spectrum revealed signals at (δ, ppm): 5.40–5.60 (brs, 2H, NH_2, D_2O exchangeable), 8.30 (s, 1H, C_7-H).

Since the synthesis of acyclovir, as one of the potent antiviral drugs, by Schaeffer *et al.* (1978), many attempts have been directed by nucleoside chemists to prepare a lot of relative compounds with various side chain and glycons (Wamhoff *et al.*, 1994; Zeid *et al.*, 1999), however, acyclonucleosides of pyrazolopyranopyrimidine derivatives were not reported in the literature (to the best of our knowledge). Thus, and in continuation of our previous work (Abdel-Megeid *et al.*, 1998; Rashad and Ali, 2006; Shamroukh *et al.*, 2004) in preparing various cyclic and acyclic nucleosides of different heterocyclic compounds, we describe here the synthesis of some acyclonucleosides of pyrazolopyranopyrimidines by treating the sodium salts of compounds **6a–c** or **8a–c** (generated *in situ*) with 2-chloroethyl methyl ether to give the corresponding acyclonucleosides **9a–c** or **10a–c**, respectively (Scheme 1). The presence of methoxyethyl protons in the ^1H NMR spectra of compounds **9a** and **10a** as representative examples, and the absence of the NH signals in the IR and ^1H NMR spectra confirmed their structures.

Deprotection using ammonia/methanol did not affect the acyclonucleosides **9a–c** or **10a–c**, while attempts to deprotect them using alcoholic potassium hydroxide, unfortunately, broke the nucleosidic linkage and gave **6a–c** and **8a–c** again. We believe that compounds **9a–c** and **10a–c**, were the first acyclonucleosides of pyrazolopyranopyrimidines reported in the literature (to the best of our knowledge).

Pharmacology

Animals

Albino male rates (100–120 g) were used in the pharmacological test. They were kept under constant conditions and allowed free access to water and standard diet. Biological experiments were performed in accordance with the international guidelines for experimental animal use (WHO, 1982).

Carrageenan foot paws oedema

The procedure of Winter *et al.* (1962) was adopted. Albino male rates (100–120 g) were dosed orally with the tested compounds dissolved in 5% DMSO in a dose of 40 mg kg^{-1} body mass one hour before carrageenan challenge. Foot paw oedema were induced by injecting 0.10 ml of carrageenan solution subcutaneously into the planter portion in the right hind paw of each rat under light anesthesia. Initial foot paw was weighed immediately following carrageenan challenge. The anti-inflammatory activity was calculated as the percent inhibition after administration of the reference and the tested compounds compared to the control group:

$$\text{inhibition} = \frac{\text{\% increase in volume of reference} - \text{\% increase in volume of tested compound}}{\text{\% increase in volume of reference}}.$$

A pharmacological study to evaluate the anti-inflammatory effects of the newly synthesized compounds was performed and the results are summarized in Tables I and II. Compound **6a**, showed no anti-inflammatory activity with a lower inhibitory effect compared to diclofenac. Compound **6c** reduced oedema more effectively than compound **6a** with percentage inhibition of 50.40%. Compound **6b** reduced oedema more effectively than **6a** and **6c** with percentage inhibition of 60.10% indicating a high anti-inflammatory effect. Thus, the order of increasing the anti-inflammatory effect is as follows: **6b** > **6c** > **6a**.

According to the mentioned results we can conclude that the anti-inflammatory activity of the tested compounds is enhanced with increasing the aromaticity of the substituted group, where the 3,4-dichlorophenyl group in compound **6a**, as an example, showed nonsignificant anti-inflammatory effects compared to the reference (diclofenac) while substitution with a thienyl group slightly increased the anti-inflammatory activity. Further substitution with a naphthyl group gave the highest anti-inflammatory activity. In the course of evaluating the anti-inflammatory activity, we could not detect any significant anti-inflammatory activity for the rest of the tested compounds compared to the reference compound (Table I) except that for compound **8b**. These results are in accordance with our findings represented in Table I and indicate the improvement of the anti-inflammatory activity with increasing the aromaticity of the substituted group. Moreover, we can conclude that, the pyranopyrazoles did not reveal any anti-inflammatory activity. Introducing of a pyrimidine ring fused to the pyranopyrazole moiety increases the anti-inflammatory activity. However, further sub-

Table I. Pharmacological and some toxicological properties of the newly synthesized compounds.

Compound	R^1	Solubility[a]	LD_{50}[b] p.o. [mg/kg]	Anti-inflammatory activity[c]
3a	3,4-Dichlorophenyl	DMSO	> 500	–
3b	Naphthyl	DMSO	> 500	–
3c	Thienyl	DMSO	> 500	–
4a	3,4-Dichlorophenyl	DMSO	300	–
4b	Naphthyl	DMSO	450	–
4c	Thienyl	DMSO	360	–
6a	3,4- Dichlorophenyl	DMSO	> 500	+
6b	Naphthyl	DMSO	> 500	++
6c	Thienyl	DMSO	> 500	+
7a	3,4- Dichlorophenyl	DMSO	> 500	–
7b	Naphthyl	DMSO	450	+
7c	Thienyl	DMSO	250	–
8a	3,4- Dichlorophenyl	DMSO	390	–
8b	Naphthyl	DMSO	> 500	+
8c	Thienyl	DMSO	> 500	–
9a	3,4- Dichlorophenyl	DMSO	500	–
9b	Naphthyl	DMSO	420	–
9c	Thienyl	DMSO	500	–
10a	3,4- Dichlorophenyl	DMSO	500	–
10b	Naphthyl	DMSO	500	–
10c	Thienyl	DMSO	500	–

[a] Other solvents as Tween 80 (10%) and propylene glycol (40%) were tried. All compounds were completely soluble in DMSO. 5% DMSO solution in dist. H_2O was used to prepare doses for oral administration.
[b] Concentration higher than the above mentioned could not be prepared due to precipitation of the compound in the solvent used, 5% DMSO.
[c] 50, 100 and 150 mg/kg were tried for all compounds. The 100 mg/kg dose was used to test and compare the anti-inflammatory activity between tested compounds, control and reference compound used (diclofenac). +, slightly active; ++, active; –, inactive.

Table II. Anti-inflammatory activity of derivatives **6a**–**c**.

Experimental group	Anti-inflammatory activity as % inhibition
Control (Carrageenan)	71.0 ± 2.40
Reference compound (Diclofenac)[a]	68.80 ± 8.50**
6a	50.40 ± 12.50
6b	60.10 ± 5.80**
6c	59.20 ± 17.0*

Number of animals $n = 5$.
* Significantly different from control value at $p < 0.01$.
** Significantly different from control value at $p < 0.001$.
[a] [(2,6-Dichlorophenyl)amino]benzeneacetic acid monosodium or monopotassium salt).

stitution with a methoxyethyl group at *N*1 of the pyrazole ring decreases the anti-inflammatory activity. Thus, the order of increasing the anti-inflammatory effect is as follows: pyrazolopyranopyrimidines > substituted pyrazolopyranopyrimidines > pyranopyrazoles.

Experimental

All melting points are uncorrected and were measured using an Electrothermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed using a Vario El-Mentar apparatus. The results were found to be in agreement with the calculated values (± 0.3). The IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer. ^1H and ^{13}C NMR spectra were determined on a Jeol 300 MHz in DMSO-d_6, and the chemical shifts were expressed in ppm relative to TMS as internal reference. Mass spectra were recorded at 70 eV using an EI Ms-QP 1000 EX instrument (Shimadzu, Japan). Column chromatography was performed on Silica gel 60 (particle size 0.06–0.2 mm; Merck, Germany).

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