New Anti-Inflammatory Agents

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The pyrrole derivatives **1a**, **b** and **2a**, **b** were used as precursors for the preparation of *N*-substituted pyrrole derivatives **3a**, **b**–**9a**, **b** and pyrrolo[2,3-*d*]pyrimidines **13**–**16**. Also, all the newly prepared products were tested for anti-inflammatory activity as analogues to fenamates, and some of them revealed moderate anti-inflammatory activity compared to the standard drug indomethacin.

Key words: Pyrrole, Pyrrolo[2,3-*d*]pyrimidine, Fenamates, Anti-Inflammatory Activity

Introduction

Pyrrole and pyrrolopyrimidine derivatives have attracted the attention of many authors due to their pharmacological effects (Traxler *et al*., 1996; Wang and Folkes, 2004), especially their anti-inflammatory activity (Jarvis *et al*., 2002). Also, in continuation to our previous work about preparation of new, simple and efficient syntheses of biologically active pyrroles and fused pyrimidine compounds utilizing inexpensive starting materials (Mohamed *et al*., 2005; Rashad *et al*., 2005a–c; Rashad and Ali, 2006; Shamroukh *et al*., 2005; Zaki *et al*., 2006), we describe here efficient methods for preparation of some pyrroles and pyrrolopyrimidines and their evaluation as anti-inflammatory agents.

PNU-142731A (Fig. 1) (Chin *et al*., 1999) is a novel anti-inflammatory pyrrolopyrimidine that inhibits the production of cytokines *in vivo*. This compound is a potent and efficacious inhibitor of eosinophilic lung inflammation and is currently in Phase II Clinical Evaluation for the potential treatment of asthma. The fenamates (Fig. 1) (Boschelli *et al*., 1993) are a class of non-steroidal anti-inflammatory drugs (NSAIDs) that share as their common structural feature *N*-arylanthranilic acids. These agents were originally found to be effective anti-inflammatory agents.

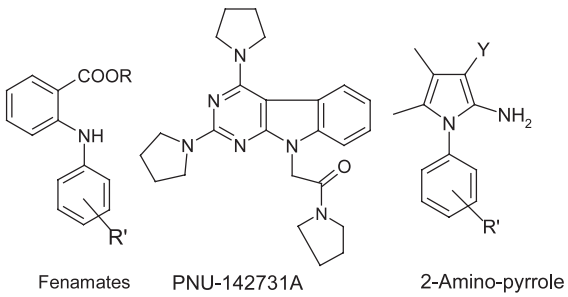

Fig. 1. Structure of some anti-inflammatory drugs.

In search of new polyheterocyclic compounds with potential pharmaceutical value, we planned to use the heterocyclic ring (pyrrole ring) as an analogue to the aryl moiety of fenamates as a way to study the anti-inflammatory activity. Also, we used a novel synthesis for some new pyrrolo[2,3-*d*]pyrimidine and fused pyrrolo[2,3-*d*]pyrimidine derivatives in the hope that they could have promising chemical and biological interest as anti-inflammatory agents.

Experimental

All melting points are uncorrected and measured using an electrothermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA) at National Research Centre, Cairo, Egypt. ^{1}H NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and chemical shifts were expressed as ppm against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on a 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan) instrument at

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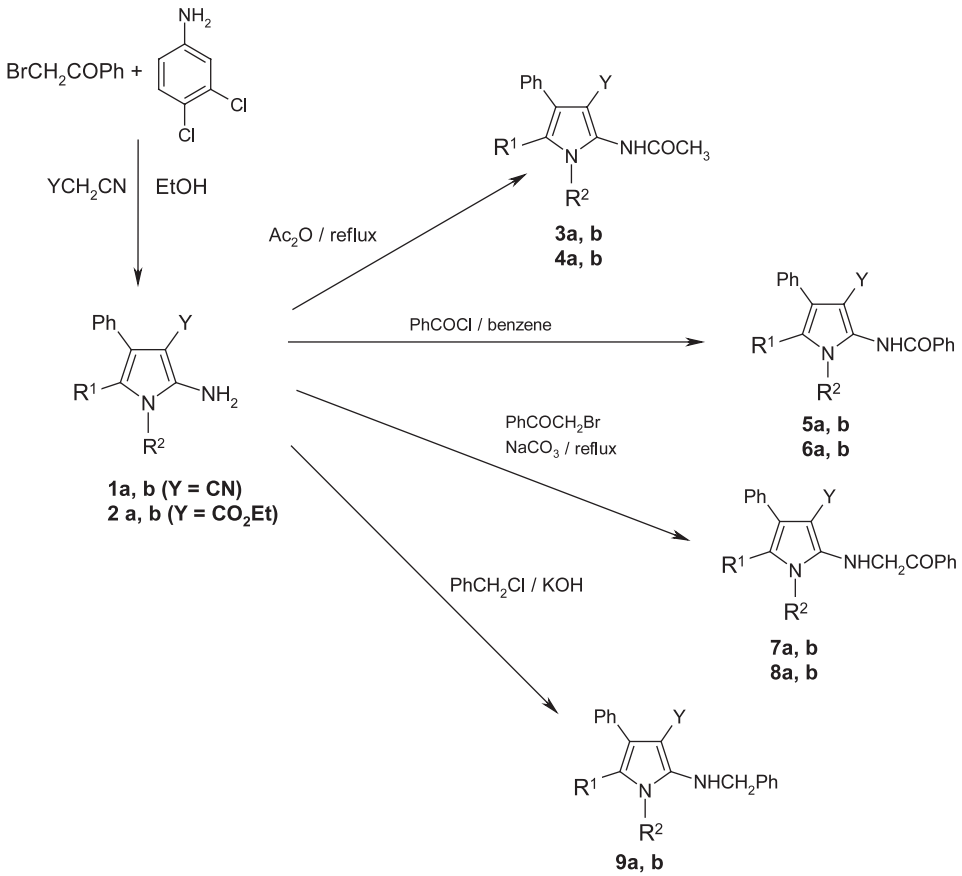
National Research Centre, Cairo, Egypt. Microanalyses were operated using a Vario, El-Mentar apparatus (Shimadzu, Japan) (Organic Microanalysis Unit, National Research Centre, Cairo, Egypt) and the results were within the accepted range (± 0.30) of the calculated values. Column chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm). For synthesis of compounds **1a**, **b**, **2a**, **b**, **10a**, **b**, **11a**, **b**, **12a**, **b** and **16a**, **b** see Mohamed *et al.* (2005).

Results and Discussion

Chemistry

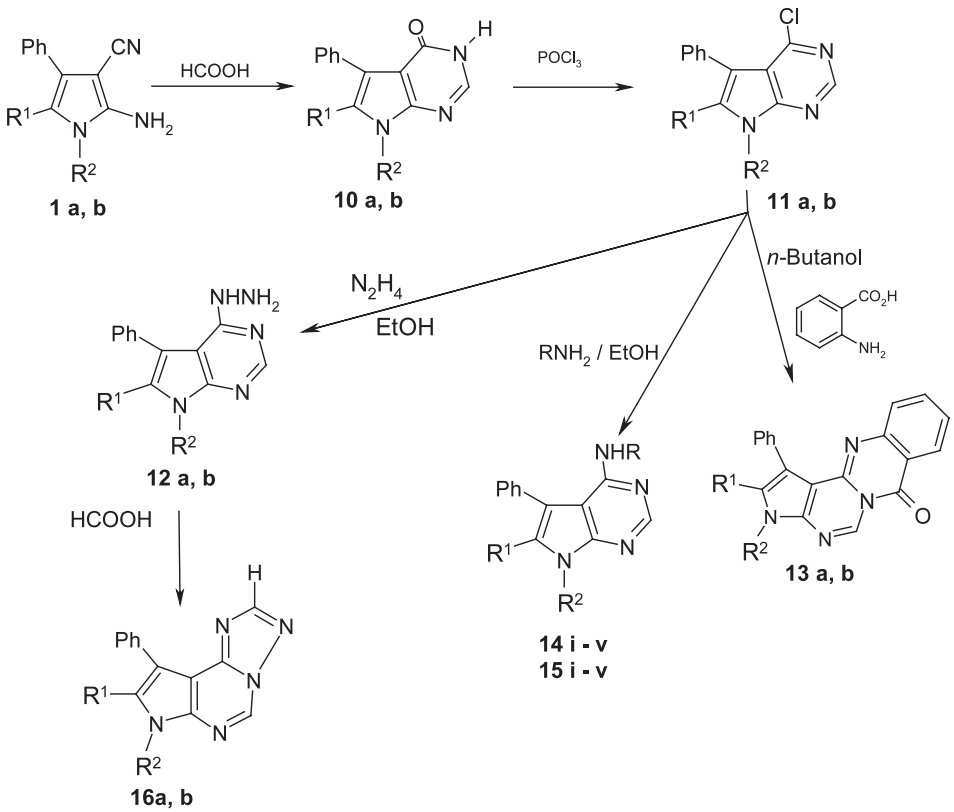
In this investigation, compound **2b** was prepared similar to compounds **1a** and **1b** (Mohamed *et al.*, 2005). The structure of compound **2b** was confirmed by spectral data, since the IR spectrum of compound **2b**, as representative example, revealed the presence of NH_2 and CO. Also, its ^1H NMR spectrum in $CDCl_3$ revealed signals at (δ, ppm): 1.3–1.8 (t, 3H, CH_3), 3.5–3.8 (q, 2H, CH_2), 4.20 (brs, 2H, NH_2, D_2O exchangeable), 6.8–7.5 (m, 8H, Ar-H), 7.9 (s, 1H, C_5-H) and its mass spectrum gave fragments showing the isotopic pattern due to the presence of chlorine atoms.

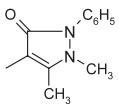
When compounds **1a**, **b** or **2a**, **b** were refluxed with acetic anhydride or benzoyl chloride in dry benzene, they afforded the corresponding 2-acylamino derivatives **3a**, **b**–**6a**, **b** (Scheme 1). The spectral data of compounds **3a**, **b**–**6a**, **b** assigned their structure. On the other hand, when com-



	R^1	R^2
a	C_6H_5	$CH_2C_6H_5$
b	H	3,4-$Cl_2C_6H_3$

Scheme 1



	R^1	R^2	R
i	C_6H_5 , H	CH_2 C_6H_5, 3,4-$Cl_2C_6H_3$	2,3$(OCH_3)_2C_6H_3$
ii	C_6H_5, H	CH_2 C_6H_5, 3,4-$Cl_2C_6H_3$	$CH_2CO_2C_2H_5$
iii	C_6H_5	CH_2 C_6H_5 , 3,4-$Cl_2C_6H_3$	2-CH_3-C_6H_4
iv	C_6H_5	CH_2 C_6H_5, 3,4-$Cl_2C_6H_3$	4- CH_3-C_6H_4
v	C_6H_5	CH_2 C_6H_5 , 3,4-$Cl_2C_6H_3$	

Scheme 2

pounds **1a**, **b** were refluxed with phenacyl bromide or benzyl chloride in ethanol, they afforded derivatives **7a**, **b**–**9a**, **b**, respectively (Scheme 1). The synthesis of compounds **13a**, **b** was achieved by refluxing the 4-chloro derivatives **11a**, **b** (Mohamed *et al.*, 2005) with *o*-aminobenzoic acid (anthranilic acid). Compounds **11a**, **b** were dissolved in ethanol and refluxed with different amines [antipyrine (2-aminophenazone), *o*-toludine, 2,3-dimethoxyaniline and glycine], respectively, with a catalytic amount of triethylamine, to afford the corresponding substituted pyrrolo[2,3-*d*]pyrimidine derivatives **14i**–**v** and **15i**–**v**, respectively (Scheme 2). The structure of the latter compounds was confirmed on the basis of their elemental analysis and spectral data, since the IR spectrum of compounds **14i**–**v**, as representative example, revealed the presence of NH.

Pharmacology

Animals: Female rats (180–200 g) were used taking into account the international principles and local regulations concerning the care and the use of laboratory animals by Saxena (1960). The animals had free access to a standard commercial diet and water, and were kept at about 25 °C.

Sponge implantation technique: The sponge implantation technique was described first by Saxena (1960). Sponges used were prepared from polyvinyl foam sheets (thickness 5 mm). Discs were punched out to a standard size and weight [(2.5 ± 0.05) mg] using a 5.0 mm cork borer. The sponges were then soaked in 70% v/v ethanol for 30 min, rinsed four times with distilled water and heated at 80 °C for 2 h. Sponges were implanted in female rats under diethyl ether anesthesia. Four 5 mm ventral incisions were made, 2 in both groins and axellae on both sides, and the dermis separated from the underlying muscle layer by insertion of blunt forceps to form separate cavities into which the sponges were inserted. Four sponges were implanted per rat and each incision was closed by two stitches.

The sponges were lifted for 8 days during which the tested compounds and indomethacin (reference standard) were injected intraperitoneally. Indomethacin was given at a dose level of 20 mg/kg and test compounds were given at equimolar dose levels. Control (10% aqueous solution of Tween 80) was given at a dose volume comparable to the tested doses. The animals were sacrificed, the sponges prepared and dried until the weight remained constant. The net dry weight after subtracting the weight of the sponge was determined.

One-way analysis of variance (ANOVA): $F \pm 2.985$. The p-value was 0.0086, considered very significant.

Tukey-Kramer multiple comparison test: If the value of q is greater than 4.889, the p-value is less than 0.05 (Table I).

Table I. The effect of the tested compounds and reference drug in the sponge implantation test of rats.

Group	Mean difference	q-Value	p-Value	Conclusion
Control (c) *vs* indomethacin	20.51	5.26	$p < 0.05$	significant
c *vs* **1a**	13.50	4.41	$p > 0.05$	not significant
c *vs* **1b**	12.50	3.21	$p > 0.05$	not significant
c *vs* **2a**	16.75	4.30	$p > 0.05$	not significant
c *vs* **2b**	20.95	5.38	$p < 0.05$	significant
c *vs* **3a**	11.53	2.96	$p > 0.05$	not significant
c *vs* **3b**	11.53	2.96	$p > 0.05$	not significant
c *vs* **4a**	13.53	3.96	$p > 0.05$	not significant
c *vs* **4b**	14.53	2.90	$p > 0.05$	not significant
c *vs* **5a**	16.53	3.96	$p > 0.05$	not significant
c *vs* **5b**	12.53	3.01	$p > 0.05$	not significant
c *vs* **6a**	12.59	3.24	$p > 0.05$	not significant
c *vs* **6b**	13.58	4.24	$p > 0.05$	not significant
c *vs* **7a**	11.53	2.97	$p > 0.05$	not significant
c *vs* **7b**	11.53	2.96	$p > 0.05$	not significant
c *vs* **8a**	13.59	4.24	$p > 0.05$	not significant
c *vs* **8b**	11.56	2.97	$p > 0.05$	not significant
c *vs* **9a**	13.59	4.24	$p > 0.05$	not significant
c *vs* **9b**	11.53	2.96	$p > 0.05$	not significant
c *vs* **10a**	13.59	4.24	$p > 0.05$	not significant
c *vs* **10b**	12.59	3.25	$p > 0.05$	not significant
c *vs* **11a**	16.23	4.96	$p > 0.05$	not significant
c *vs* **11b**	16.53	3.96	$p > 0.05$	not significant
c *vs* **12a**	13.56	3.48	$p > 0.05$	not significant
c *vs* **12b**	12.59	3.26	$p > 0.05$	not significant
c *vs* **14iii**	20.81	5.34	$p < 0.05$	significant
c *vs* **14iv**	16.81	4.34	$p > 0.05$	not significant
c *vs* **15i**	5.00	1.28	$p > 0.05$	not significant
c *vs* **15ii**	11.53	2.96	$p > 0.05$	not significant
c *vs* **16a**	15.06	3.87	$p > 0.05$	not significant
c *vs* **16b**	8.63	2.21	$p > 0.05$	not significant

Conclusion

The results in Table I reveal that compounds **2b** (ethyl ester of the dichloro series) and **14iii** (*o*-tolyl amine of the benzyl series) were active compared to indomethacin®, while all other compounds showed no significant difference to the reference drug. We can say that the ester group increased the activity more than the carbonitrile group for the dichloro series which are considered as heteroanalogues to anthranilate ester which open a new pathway for fenamates heteroanalogues. On the other hand, the amino group increased the activity over the ester in the benzyl series. Addition of another fused ring (triazole ring) or replacement of the aryl moiety in the amino derivatives with another NH_2 (hydrazino) group masked the activity for both series against the standard drug used.

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