Heterocycles of Biological Importance. Part 1. Novel Synthesis and Biological Activity Study of 5-Alkyl and 5-Aryl-7-aminopyrazolo[1,5-*a*]-pyrimidines from Allenic or Acetylenic Nitriles and 3-Aminopyrazole

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Allenic nitriles react with 3-aminopyrazole in ethanol or dimethylformamide to give 5-alkyl-7-aminopyrazolo[1,5-*a*]pyrimidines **5** of pharmaceutical interest in good yields. 3-Phenylpropynenitrile reacts with 3-aminopyrazole in a similar manner to give the aryl compound **8**.

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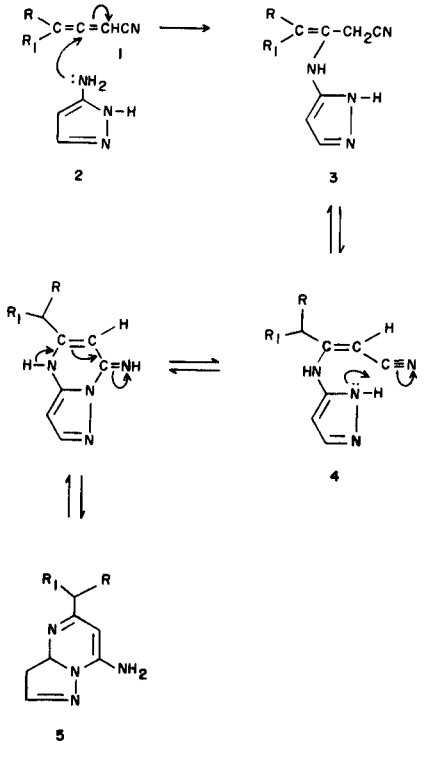
Pyrazolopyrimidines and their derivatives have been shown to exhibit important drug properties [1-5]. As a continuation of an on-going programme [6-8] on the syntheses of heterocycles from allenic and acetylenic nitriles, we treated the allenic nitriles **1** with 3-aminopyrazole **2** in ethanol and found that they reacted to give first the isolatable unconjugated enenitrile enamine **3** [9] which slowly isomerised to the conjugated adduct **4**. The adduct **4** then underwent ring closure by nucleophilic attack at the nitrile to give the pyrazolopyrimidine **5** in very good yields (Scheme 1).

Scheme I



The reaction in ethanol was slow, complete cyclization being acheived only after 21 days of refluxing. When the reaction was stopped after, say, 14 days, the cyclized product was obtained along side with some of the uncyclized intermediates **3** and **4**. When the reaction was allowed to go to completion, crude products were quantitative and were obtained pure in > 80% yield (Table 1). When the reaction was carried out in dimethylformamide it went much faster and was complete in 4-5 days. However, lower yields were obtained because the nucleophilic attack at the Michael carbon of the allenic nitrile to give **3** competed with dimerization of the allenic nitrile to the cyclobutane dimer [10]. Thus the yield of the pyrazolopyrimidine, **5**, was dependent on the rate of dimerization of the allenic nitriles.

When the reaction was carried out in ethanol for 14 days, the reaction product was a mixture of **3**, **4** and **5**. Compounds **3** and **4** thus obtained were easily converted to compound **5** by further reflux in ethanol.

The formation of the unconjugated adducts **3** which then rearrange to the conjugated adducts **4** follow the mechanism that has already been established for other enenitrile enamines [9]. The fact that more of compound **3c** than compound **4c** was found in the reaction mixture when the ethanol reaction was stopped after 14 days shows that, as expected [9], some unconjugated enaminic nitriles are very stable and isomerise rather slowly to the conjugated adducts. In the work reported in this paper, the rate determining step is the transformation of compounds **3** to compounds **4**. When once compounds **4** have been formed, they cyclise rather readily to the pyrazolopyrimidines **5**. Compounds **3** are relatively quite stable and are therefore found in the reaction mixture after 14 days.

The pyrazolopyrimidines **5** showed ν max 3400-3140 (NH_2 stretch), 1650-1635 (C=N), 1600 (C=C) and 1560-1550 cm^{-1} (N-H def) (Table 2) and λ max 225-226 (ϵ 28,900-31,100), 285-286 (ϵ 6,100-7,800) and λ max 304 nm (ϵ

Table 1

The Synthesis of Compounds **5**

Compound No.	R	R	Mp (°C)	Yield (%) In Ethanol	Yield (%) In DMF	M⁺	Molecular Formula	Analysis % Calcd./Found C	H	N
5a	Me	Me	175	80	30	176	$C_9H_{12}N_4$	61.36	6.82	31.82
								60.95	6.69	31.92
5b	Me	Et	158	86	50	190	$C_{10}H_{14}N_4$	63.16	7.37	29.19
								63.18	7.51	29.10
5c	Et	Et	185	89	70	204	$C_{11}H_{16}N_4$	64.71	7.84	27.45
								64.59	8.22	27.67
5d	Me	Pr	120	84	60	204	$C_{11}H_{16}N_4$	64.71	7.84	27.45
								64.51	7.69	27.60

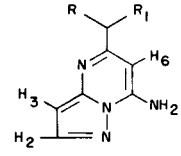
Table 2

IR and UV Data of Compounds **5**

Compound No.	R	R_1	IR (cm^{-1}) NH_2	C=N	C=C	N-H def	UV λ max	ε × 10^3	λ max	ε × 10^3	λ max	ε × 10^3
5a	Me	Me	3300, 3140	1635	1600)	1550	226	31.1	286	7.4	304	6.9
5b	Me	Et	3400, 3200	1640	1600	1560	225	28.9	285	6.1	304	5.7
5c	Et	Et	3300, 3175	1650	1600	1560	226	35.1	286	7.8	304	6.9
5d	Me	Pr	3300, 3200	1650	1600	1560	226	30.7	286	7.0	304	7.0

Table 3

NMR Data of Compounds **5**



Compound No.	R	R_1	R_2	R_3	R_6	NH_2	Others
5a	Me	Me	2.24	3.84	4.11	3.05	8.76 (6H, d), 7.40-7.04 (1H, m)
5b	Me	Et	2.14	3.82	4.10	2.85	9.18 (3H, t), 8.80 (3H, t), 8.66-8.05 (2H, m), 7.80-7.20 (1H, m)
5c	Et	Et	2.17	3.80	4.11	2.85	9.19 (6H, t), 8.70-8.15 (4H, m), 8.00-7.40 (1H, m)
5d	Me	Pr	2.22	3.80	4.16	3.06	9.18 (3H, t), 8.84 (3H, t), 9.02-8.30 (4H, m), 7.62-7.12 (1H, m)

The spectra were determined in deuteriochloroform-dimethylsulphoxide solution and the values are given as ppm in the τ scale.

5,700-6,900) (Table 2). The nmr spectra (Table 3) are consistent with the assigned structures.

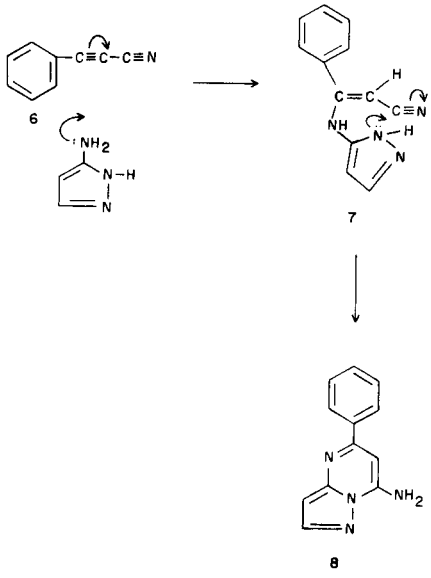
Phenylpropynenitrile **6** was similarly treated with **2** in ethanol to give **8** in 82% yield, the reaction being complete after 14 days owing to the fact that no unconjugated equivalent of **3** is formed with **6** that needs rearrangement to **7** before cyclizing to **8** (Scheme 2).

One of the pyrazolopyrimidines **5** (R = R, = Me) showed significant biological activity during primary screening (Table 4) and further tests were carried out and are summarized in Table 5. The other pyrazolopyrimidines whose synthesis is also reported in this paper were not screened for possible biological activity.

EXPERIMENTAL

The ir spectra (potassium bromide pellets) were determined with a Perkin-Elmer 337 spectrophotometer. The uv spectra were determined for ethanolic solutions on a Beckmann 25 spectrophotometer. The nmr spectra were determined with a Perkin-Elmer R 12A spectrophotometer for solutions in deuteriochloroform, or carbon tetrachloride or carbon tetrachloride-deuteriodimethylsulphoxide with tetramethylsilane as in-

Scheme 2



ternal standard and are recorded as τ values. Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. Allenic nitriles and 3-phenylpropynenitrile were prepared as previously reported [11,12].

General Procedure for the Synthesis of **5a-d**.

a) A mixture of the allenic nitrile **1** (0.02 mole) and 3-aminopyrazole **2** (0.02 mole) were dissolved in ethanol (50 ml) and the mixture refluxed for 21 days. Evaporation of the solvent gave the crude product in quantitative yield as a brown oil. This was chromatographed on neutral alumina (200 g, activity 4) and eluted with hexane-ethyl acetate to give the crude product as a solid. Recrystallization from methylene chloride-hexane gave the pure compound **5**.

When the reaction was carried out for 14 days using **1c** and **2**, compound **5c** was obtained in 65% yield as well as **3c** (26%) and a mixture of **3c** and **4c** (6%). Compound **3c** was quantitatively converted to **5c** by further refluxing in ethanol for 8 days. The mixture of **3c** and **4c** was also converted to **5c** by refluxing in ethanol for 8 days.

b) A mixture of the allenic nitrile (0.02 mole) and 3-aminopyrazole (0.02 mole) were heated in dimethyl formamide (50 ml) at 110-120° for 4-5 days. Removal of solvent under reduced pressure followed by chromatography as in a) gave compound **5**, the cyclobutane dimer and unreacted 3-aminopyrazole.

7-Amino-5-phenylpyrazole[1,5-*a*]pyrimidine (**8**).

3-Aminopyrazole **2** (1.66 g, 0.02 mole) and 3-phenylpropynenitrile **6** (2.54 g, 0.02 mole) was refluxed in 50 ml of ethanol for 14 days. Removal of the solvent gave the crude product in quantitative yield. Purification of crude product (2.0 g) by column chromatography (neutral alumina, activity 4, 150 g) and elution with ethyl acetate-hexane (7:2) gave a solid which was recrystallized from methylene chloride-hexane to give **8** (1.7 g, 85%), mp 210°; ir: ν max 3350, 3320 (NH_2), 1630 (C=N), 1590 (C=C) and 1560 cm^{-1} (N-H def); uv: λ max 209 (ϵ 18.6 × 10^3), 258 (ϵ 32.4 × 10^3), 284 (ϵ 5.2 × 10^3) and 320 nm (ϵ 3.0 × 10^3); nmr: 3.50 (1H, d, C*H*-CH=N), 3.33 (1H, s, C*H*=CNH_2), 2.60-2.35 (5H, m, aromatic), 2.28 (2H, s, =CNH_2, disappears on deuteration), 1.88 (1H, d, CH-C*H*=N); ms: m/e 210 (M^+).

Anal. Calcd. for $C_{12}H_{10}N_4$: C, 68.57; H, 4.76; N, 26.67. Found: C, 68.40; H, 4.60; N, 26.60.

BIOLOGICAL ACTIVITY TESTS

Below are brief descriptions of the tests, explanations of the criterion and a listing of suitable reference compounds and their ED_{100} (end result) value in mg/kg or mcg/ml representing the dose which is always active in the test system rather than the minimal effective dose (MED) determined blind for test compound in a given dose response experiment [13].

Test 1, Accute Toxicity.

Mice were dosed at 300 mg/kg, p.o. for observation of any toxicity symptoms or autonomic effects during the subsequent 72 hours.

Test 2, + Ionotropic.

Increase in the contractile forces of electrical stimulated guinea pig left atria by more than 40% (>40) indicates + inotropic activity. Epinephrine (0.05); isoproterenol (0.001); digitoxin (1); strophanthin (0.75); amrinone (100).

Test 3, Hypocholesterolemic.

Mice made hypercholesterolemic by being fed a high cholesterol-cholic acid diet for 7 days were dosed on the 6th and 7th days, p.o. After fasting overnight, reduction in serum cholesterol concentration by more than 15% (>15) from hypercholesterolemic control mice indicates activity. Clofibrate (400); benzafibrate (200); U-41792 (200); diethylstibestrol (200); D-thyroxine (10).

Table 4

Primary Screening of **5**, R = R_1 = Me

Test	Route	Dose (in mg)	Criterion	Response	Remarks
1. Toxicity	P.O. [a]	300		No abnormal developments	Compound not toxic
2. + Inotropic	*in vitro*	100	40	75	
3. Hypocholesterolemic	P.O.	200	15	25	HPL/CHOL = 0.95 [b]
4. HP-Betalipoprotein	P.O.	200	10	29	
5. Bronchodilator	*in vitro*	100	+	+	
6. Histamine H_2 receptor inhibition	*in vitro*	100	+	+	
7. Gastrointestinal (G.I.) motility	*in vitro*	2	+	+	
8. Antihistamine	*in vitro*	100	+	+	
9. Anticholinergic	*in vitro*	100	+	+	

[a] P.O. = Par Os *i.e.* orally administered. [b] HPL/CHOL is the heparin precipitation lipoprotein (HPL)/cholesterol (CHOL) ratio. A reduction in the HLP/CHO ratio below 0.92 suggests possible increase in serum high density lipoprotein.

Table 5

Further Tests on **5**, R = R_1 = Me

Test	Reference Compound	Route	Dose	Response
+ Inotropic		*in vitro*	50 mcg/ml	+55 MIC [b]
Inotropic		*in vitro*	25 mcg/ml	+37
Inotropic	Amrinone	*in vitro*	100 mcg/ml	+48 MIC
Bronchodilator		*in vitro*	25 mcg/ml	+ MIC
Bronchodilator		*in vitro*	10 mcg/ml	–
Bronchodilator	Aminophylline	*in vitro*	100 mcg/ml	+ MIC
Histamine H_2 receptor inhibition		*in vitro*	100 mcg/ml	+ MIC
Histamine H_2 receptor inhibition		*in vitro*	50 mcg/ml	–
Histamine H_2 receptor inhibition	Burimamide	*in vitro*	100 mcg/ml	+ MIC
Anticholinergic		*in vitro*	10 mcg/ml	+ MIC
Anticholinergic			2 mcg/ml	–
Anticholinergic	Atropine		0.1 mcg/ml	+ MIC
Hypocholesterolemic		P.O. [a]	200 mg/kg	21 MED [c]
HP-Betalipoprotein		P.O.	200 mg/kg	27
Hypocholesterolemic		P.O.	100 mg/kg	7
HP-Betalipoprotein		P.O.	100 mg/kg	0
Hypocholesterolemic	Bezafibrate	P.O.	200 mg/kg	33 ED 100 [d]
HP-Betalipoprotein	Bezafibrate	P.O.	200 mg/kg	38 ED 100

[a] P.O. = Par Os *i.e.* orally administered. [b] MIC = Minimal Inhibitory Concentration. [c] MED = minimal Effective Dose. [d] ED 100 = Dose which is always active in test system.

Test 4, HP-betalipoprotein.

Reduction of serum heparin precipitating lipoprotein (HLP) concentration in the same hypercholesterolemic mice from test 3 by more than 20% (>20) from control animals indicates activity. Reduction in the HLP/cholesterol ratio below 0.92 suggests possible increase in serum high density lipoprotein (HDL). Benzafibrate (200); D-thyroxine (10); U-41792 (200).

Test 5, Bronchodilator.

Increase by more than 50% (>50) in flow rate of Tyrodes solution through isolated perfused guinea pig lung constricted by methacholine (0.05 mcg/ml) added to the perfused fluid indicates activity (+); the test compound being injected as a bolus just proximal to entry of the cannula into the trachea. Isoproterenol (0.01); aminophylline (100); isoxuprine (50); FPL-55712 (100).

Test 6, Histamine H_2 Receptor Inhibition.

Inhibition by more than 50% of the chronotropic effect of histamine (5 mcg/ml) on spontaneously beating guinea pig right atria in the absence of intrinsic negative chronotropic action suggests activity (+). Burimamide (100); metiamide (10); cimetadine (5).

Test 7, Gastrointestinal (GI) Motility.

The effect of test compound on guinea pig ileum contractions induced by electrical transmural stimulation in oxygenated Kreb's solution at 32° is determined. The following symbols denote various effects: + = augmentation of contractions without change in basal tone (a metoclopramide effect). I = inhibition of contractions 50 percent usually accompanied by reduction in basal tone (antispasmodic effort). – = no effect.

Test 8, Antihistamine.

Inhibition of the contractile response of isolated guinea pig ileal segments to histamine (0.5 mcg/ml) indicates activity (+). Promethazine (0.1); mepyramine (0.4); cyproheptadine (0.4).

Test 9, Anticholinergic.

Inhibition by more than 80% (>80) of the contractile effect of acetylcholine (0.01 mcg/ml) on silated ileal segments indicates activity (+). Atropine (0.1); Ipratropium bromide (0.05).

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[13] All biological activity tests were performed by Panlabs, Taiwan.