Condensation of Substituted 2-Aminopyridine with β-Ketocarboxylic Esters: 4*H*-Pyrido[1,2-*a*]pyrimidin-4-ones and Pyridin-2-ones

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We report the condensation of substituted 2-aminopyridines **5** with β-ketocarboxylic esters in polyphosphoric acid. In this reaction were obtained together with the target compounds, 4*H*-pyrido[1,2-*a*]pyrimidin-4-ones **6** also the pyridin-2-ones **7**. All the compounds **7** were tested for their calcium-antagonistic activity but failed to evoke any vasorelaxant response.

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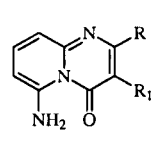
In continuing our studies directed toward the synthesis of products with potential biological activity, we are interested to prepare, *via* substituted 4*H*-pyrido[1,2-*a*]pyrimidin-4-one, some 4-hydroxy-1,8-naphthyridine derivatives and examine their affinity for adenosine receptors.

The substituted 4*H*-pyrido[1,2-*a*]pyrimidin-4-ones are generally easily accessible by condensation of a suitable 2-aminopyridine with β-ketocarboxylic esters in poliphosphoric acid [1-4].

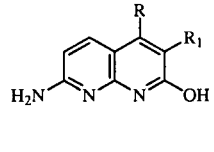
Only in the case of the condensation of 2,6-diamino- and 2-acetamido-6-aminopyridine with β-ketocarboxylic esters was generally obtained a mixture of products: the expected 4*H*-pyrido[1,2-*a*]pyrimidin-4-one **1** together with substituted 2-hydroxy-1,8-naphthyridine **2**, 4-hydroxy-1,8-naphthyridine **3** and 2-hydroxypyrimido[1,2-*a*][1,8]naphthyridin-10(10*H*)-one **4**, whose ratios depended on the substituents of the β-ketocarboxylic esters [3].

Attempts to prepare a new series of substituted 4*H*-pyrido[1,2-*a*]pyrimidin-4-ones by condensation of 6-substituted-2-aminopyridines **5** with β-ketoesters at 100° for 4 hours gave instead a mixture of the expected pyridopyrimidinones **6a-e** and pyridin-2-ones **7a-e** (Scheme 1).

In the light of these results some condensations, previously reported by us [1,2] were repeated (Table 1). In the case of condensation of 6-methyl-2-aminopyridine **5a** with ethyl benzoylacetate a mixture of pyridopyrimidinone **6f** and pyridin-2-one **7f** was obtained. Previously, compound **7f** has ot been isolated from the mixture of condensation because of the method of separation utilised [1]: in fact the products of



1



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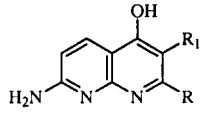

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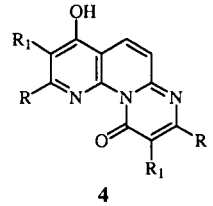

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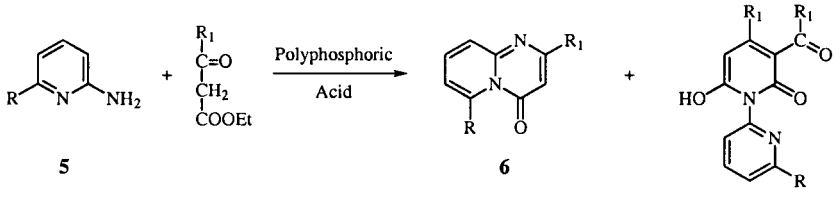
Scheme 1



5

	R
a	CH_3
b	Br
c	Cl
d	F

6

	R	R_1
a	CH_3	*p*-F-C_6H_4
b	CH_3	*o*-F-C_6H_4
c	Br	C_6H_5
d	Cl	C_6H_5
e	F	C_6H_5
f	CH_3	C_6H_5 [1]
g	CH_3	*p*-NO_2-C_6H_4 [2]
h	CH_3	CF_3 [2]
i	CH_3	CH_3 [2]

7

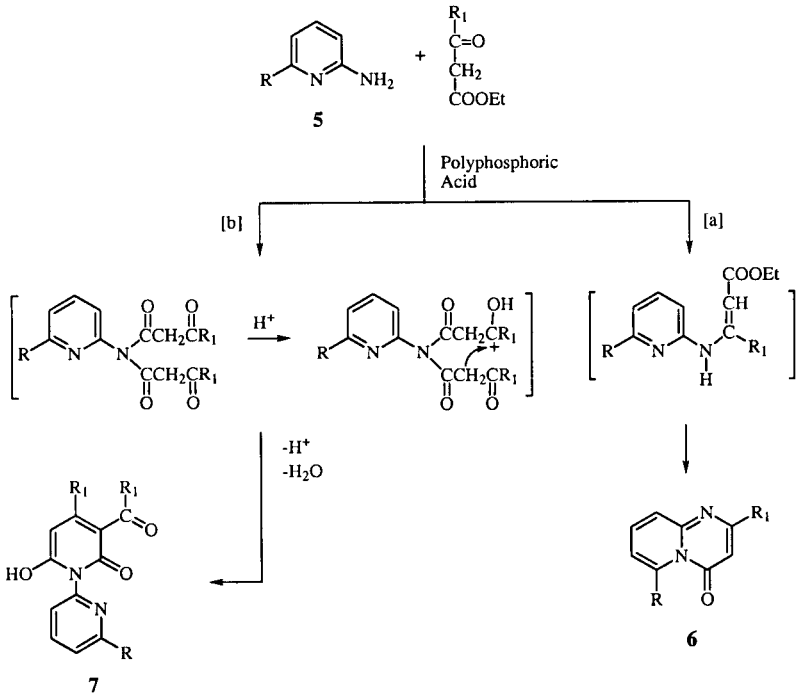
	R	R_1
a	CH_3	*p*-F-C_6H_4
b	CH_3	*o*-F-C_6H_4
c	Br	C_6H_5
d	Cl	C_6H_5
e	F	C_6H_5
f	CH_3	C_6H_5

Table 1
Physical Data of Compounds **5-7**

Compound	Yield %	mp°C [a]	MS m/z M+	Empirical Formula	Elemental Analyses Calcd./Found		
5d	70	57-59 [b,c]	112	$C_5H_5N_2F$	53.57	4.49	24.99
					53.23	4.81	25.12
5f	61	118-120 [b]	154	$C_7H_7N_2OF$	54.54	4.58	18.17
					54.86	4.58	18.19
6a	22	185-187 [b]	254	$C_{15}H_{11}N_2OF$	70.86	4.36	11.02
					71.09	4.44	11.10
6b	29	99-101 [b]	254	$C_{15}H_{11}N_2OF$	70.86	4.36	11.02
					70.94	4.40	11.84
6c	47	154-156 [b]	300	$C_{14}H_9N_2OBr$	55.84	3.01	9.30
					55.57	2.82	9.47
6d	28	133-134 [b]	256	$C_{14}H_9N_2OCl$	65.51	3.53	10.91
					65.84	3.32	10.62
6e	14	144-147 [b]	240	$C_{14}H_9N_2OF$	70.00	3.78	11.66
					69.68	3.75	11.64
6f	58	139-140 [b, d]	236	$C_{15}H_{12}N_2O$			
7a	3	255-256 [e]	417	$C_{24}H_{16}N_2O_3F_2$	68.90	3.86	6.70
					69.23	4.11	7.04
7b	3	189-191 [e]	417	$C_{24}H_{16}N_2O_3F_2$	68.90	3.86	6.70
					69.21	3.92	6.81
7c	12	255-257 [e]	445	$C_{23}H_{15}N_2O_3Br$	61.76	3.38	6.26
					61.55	3.47	6.03
7d	11	255-256 [e]	409	$C_{23}H_{15}N_2O_3Cl$	68.58	3.75	6.95
					68.44	3.51	7.09
7e	6	233-235 [e]	385	$C_{23}H_{15}N_2O_3F$	71.50	3.91	7.25
					71.34	3.90	7.27
7f	4	254-255 [e]	382	$C_{24}H_{18}N_2O_3$	75.38	4.74	7.33
					75.03	4.51	7.12

[a] Recrystallisation solvent, [b] Petroleum ether 100-140°, [c] mp 58-59° [9], [d] mp 139-140° [1]. [e] Toluene.

Scheme 2



[a] = Conrad-Limpach condensation. [b] Knorr condensation.

the reactions were separated in basic medium and under these conditions compound **7f** is soluble because of its acidity. Whereas, in the other cases, the condensation of **5a** with ethyl acetoacetate, trifluoroacetoacetate and *p*-nitrobenzoylacetate gave only the corresponding pyridopyrimidinones **6g-i**, as previously described by us [2].

Earlier, it has been reported that β-ketocarboxylic esters and 2-aminopyridines react to form crotonate derivatives (Conrad-Limpach condensation) or amido derivatives (Knorr condensation) depending on the conditions of the reaction. On cyclization, these products form the corresponding 4-hydroxy- or 2-hydroxy-1,8-naphthyridine [5].

Thus, also 4*H*-pyrido[1,2-*a*]pyrimidin-4-ones **6** and pyridin-2-ones **7** were obtained *via* crotonate and *via* amido derivative respectively.

From the present data and those reported in previous papers [2-4], it seems reasonable to suggest the following mechanism for the process of condensation of 2-aminopyridines **5** with β-ketocarboxylic esters in polyphosphoric acid (Scheme 2).

When substituents as CH_3, n-C_3H_7, CF_3 and p-NO_2-C_6H_4 (R_1) on the β-ketoesters are present, the nucleophilic attack of the amino group occurs at the more reactive keto group affording, through a Conrad-Limpach condensation, the pyridopyrimidinones **6**, as shown in the Scheme 2 (route [a]). In contrast, in the case of R_1 = C_6H_5, o-F-C_6H_4 and p-F-C_6H_4, which are able to stabilise, by a conjugative effect [6], the keto functionality and slightly to reduce the corresponding reactivity, the reaction can partially proceed by nucleophilic addition of the amino group to the ester functionality to give, together with **6**, small amounts (3-12%) of pyridinones **7**, through a Knorr-type condensation (route [b]).

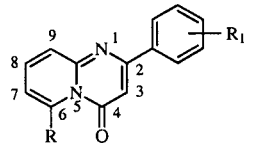
The unisolated diamido derivatives are capable of self condensation under the reaction conditions used to give pyridinones **7**, by elimination of H^+ and water. Moreover, in the reaction between 6-substituted 2-aminopyridine with β-ketocarboxylic esters, an important factor tending to obtain, *via* an amido derivative, 2-hydroxy-1,8-naphthyridine derivatives **2** or pyridin-2-ones **7**, seem to be the substituent in the 6-position of the pyridine nucleus. In fact, amino and acetamido groups seem to favour the formation of 2-hydroxy-1,8-naphthyridines **2** [3], whereas methyl, fluoro, chloro and bromo groups seem to favour the formation of pyridin-2-ones **7**.

The structures of compounds **6** and **7** were confirmed by elemental analysis, ir, ^{1}H nmr and mass spectoscopy (Table 1, 2 and 3). The ^{1}H nmr spectra of **6** show a singlet ranging at δ 6.59-6.86 due to H_3 and a multiplet at downfields due to H_7, H_8, H_9, and Ar. The ^{1}H nmr spectra of **7** show a singlet ranging at δ 10.50-11.27 due to OH and a multiplet ranging at δ 6.50-8.30 due to H_5 and Ar (Table 2 and 3).

The ir spectra of **7** show two signals ranging at 1681-1695 cm^{-1} and 1635-1643 cm^{-1} due to a carbonyl group and a group containing a carbonyl attached to a nitrogen atom (Table 3).

Table 2

^{1}H NMR and IR Data of 4*H*-Pyrido[1,2-*a*]pyrimidin-4-ones **6**

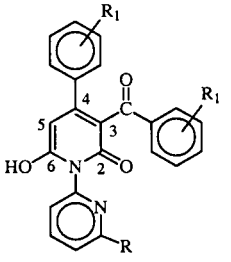


6

Compound	R	^{1}H NMR (δ) H_3(s)	$H_{7,8,9}$, A_r(m)	IR (cm^{-1}) CO
6a	3.07 (s, CH_3)	6.64	7.01-8.12	1694
6b	2.96 (s, CH_3)	6.59	6.89-8.15	1699
6c	---	6.75	7.05-8.25	1695
6d	---	6.86	7.10-8.18	1703
6e	---	6.78	6.86-8.25	1698
6f	2.96 (s, CH_3)	6.75	6.80-8.20	1688

Table 3

^{1}H NMR and IR Data of Pyridin-2-ones **7**



7

Compound	R	^{1}H NMR (δ) OH	H_5,A_r (m)	IR (cm^{-1}) CO
7a	2.45 (s, CH_3)	10.85	6.90-8.20	1695, 1635
7b	2.55 (s, CH_3)	10.80	6.66-8.06	1695, 1635
7c	---	10.90	6.80-8.15	1685, 1643
7d	---	11.27	7.00-8.15	1682, 1640
7e	---	11.14	6.85-8.20	1681, 1639
7f	2.50 (s, CH_3)	10.50	6.50-8.30	1686, 1637

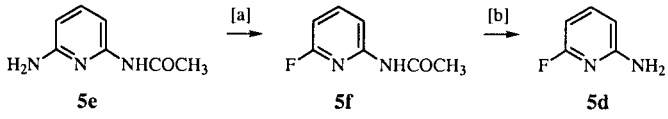
The synthesis of **5d** was carried out in the following way, in accord with that reported by us for the preparation of 2-amino-6-chloropyridine **5c** [7]. Diazotization of 2-acetamido-6-aminopyridine **5e** [8] in fluoroboric acid gave directly 2-acetamido-6-fluoropyridine **5f**, in 61% yield, that was converted to 2-amino-6-fluoropyridine **5d** [9] by hydrolysis with 20% sulfuric acid (Scheme 3).

Pharmacological Results.

It is known that 4-aryl substituted 1,4-dihydropyridines of the nifedipine-type are of great interest because of their calcium-antagonistic and -agonistic activities [10].

Compounds **7**, which are partially related to 4-aryl-1,4-dihydropyridines of the nifedipine-type, were tested for

Scheme 3



5e **5f** **5d**

[a] = HBF_4, $NaNO_2$. [b] H_2SO_4.

their calcium-antagonistic activity, but all the compounds **7** failed to evoke any vasorelaxant response, while the calcium antagonist Verapamil fully relaxed the vessels, precontracted by a potassium chloride-induced depolarization. Thus, a possible calcium entry blocker activity can be discarded.

EXPERIMENTAL

Chemistry.

All compounds were routinely checked for their structure by ir and ^1H nmr spectroscopy. Melting points were determined in a Köfler hot-stage apparatus and are uncorrected. The ir spectra were measured with ATI Mattson Genesis series FTIR™. The ^1H nmr spectra were determined in dimethyl-d_6 sulfoxide or in deuteriochloroform with tetramethylsilane as the internal standard, by Fourier transform spectrometer Varian Model CFT 20. Mass spectra were obtained by Hewlett-Packard 5988A spectrometer 70 eV. Analytical tlc was carried out on Merck 0.2 mm precoated silica-gel glass plates (60 F-254) and the location of spots was detected by illumination with a uv lamp. Elemental analyses for C, H and N of all compounds synthesized were performed by our analytical laboratory.

General Procedure for the Condensation of 6-Substituted 2-Aminopyridine **5** with β-Ketocarboxylic Esters.

A stirred mixture of 10.0 mmoles of a suitable 2-aminopyridine **5** and 11.0 mmoles of β-ketocarboxylic esters with 30 g of polyphosphoric acid was heated at 100° for 4 hours. The solution obtained, after cooling, was poured onto crushed ice.

In the case of **7a,b,f** the aqueous solution obtained was extracted with chloroform and the organic layer was dried over magnesium sulfate and evaporated to dryness *in vacuo*. In the case of **7c-e**, a precipitate was obtained which was filtered and washed with water.

All the residues obtained were extracted with petroleum ether 100-140° to obtain small amounts of 4*H*-pyrido[1,2-*a*]pyrimidin-4-ones **6a-f.** The residue was then purified by crystallisation to give derivatives **7**. Afterwards, to the aqueous solution concentrated ammonium hydroxide was added until *p*H 6. The solid compound obtained was separated by filtration, washed with water and purified by crystallisation to give pyridopyrimidinones **6a-f** (Table 1).

2-Acetamido-6-fluoropyridine **5f**.

To a cooled solution (-5°) of 10 mmoles of **5e** [8] in 75 ml of 50% aqueous fluoroboric acid was added portionwise 20 mmoles of sodium nitrite. After standing at -5° for one hour, the solution was made basic (*p*H 9) with concentrated ammonium hydroxide and extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated to dryness *in vacuo* to obtain **5f**, which was purified by crystallisation (Table 1); ^1H nmr (deuteriochloroform): δ 7.55-7.90 (m, H_4), 7.95-8.15 (m, H_3), 6.50-6.70 (m, H_5), 2.20 (s, CH_3).

2-Amino-6-fluoropyridine **5d** [9].

A solution of 10 mmoles of **5f** in 10 ml of 20% aqueous sulfuric acid was heated at 100° for 4 hours. After cooling, the solution was made basic (*p*H 9) with concentrated ammonium hydroxide and extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated to dryness *in vacuo* to provide **5d,** which was purified by crystallisation (Table 1); ^1H nmr (deuteriochloroform): δ 6.25-7.60 (m, H_3 and H_5), 6.10-6.35 (m, H_4).

Pharmacology.

All the protocols on experimental animals were performed following the guidelines of the European Community Council directive 86-609. To determine a possible calcium antagonist activity, the compounds were tested as vasodilators on isolated aortae of male Wistar rats (250-350 g). The rats were killed by cervical dislocation under light ether anaesthesia and bled. The aortae were immediately explanted, freed of extraneous tissues and endothelium and prepared as a multiple-ring preparation [11]. Then, the vessels were suspended (preload 2 g) in 10 ml organ baths containing Tyrode solution (composition of saline in m*M*: sodium chloride 136.8; potassium chloride 2.95; calcium chloride 1.80; magnesium sulfate heptahydrate 1.05; sodium dihydrogenphospate 0.41; sodium bicarbonate 11.9; Glucose 5.5), thermostated at 37° and continuously bubbled with a mixture of oxygen (95%) and carbon dioxide (5%). Changes in tension were recorded by means of an isometric transducer (Basile model 7005), connected with an unirecord microdynamometer (Basile model 7050).

After an equilibration period of 60 minutes, the absence of an acetylcholine (50 μ*M*)-induced relaxation of norepinephrine (50 n*M*)-precontracted tissues was considered as representative of a significant absence of the endothelial layer. After a further equilibration time of 30-40 minutes, the aortic preparations were contracted by treatment with a single concentration of potassium chloride (20 m*M*) and when the contraction reached a stable plateau, 3-fold increasing concentrations of the compounds under test (10 n*M*-10 m*M*) were added cumulatively. Furthermore, the known calcium channel blocker Verapamil (1 n*M*-1 μ*M*) was also used.

Preliminary experiments showed that the potassium chloride-induced contractions remained constant in a stable tonic state for at least 40 minutes.

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