Synthesis of 3-Amino-5*H*-pyrimido[5,4-*b*]indol-4-one Derivatives

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Starting with ethyl 3-aminoindole-2-carboxylate, the synthesis of 3-amino-5*H*-pyrimido[5,4-*b*]indole-2,4-dione **5**, 3-amino-5*H*-pyrimido[5,4-*b*]indol-4-one **10** and some related compounds is described. Preliminary results about the inhibition of platelet aggregation by these compounds is reported.

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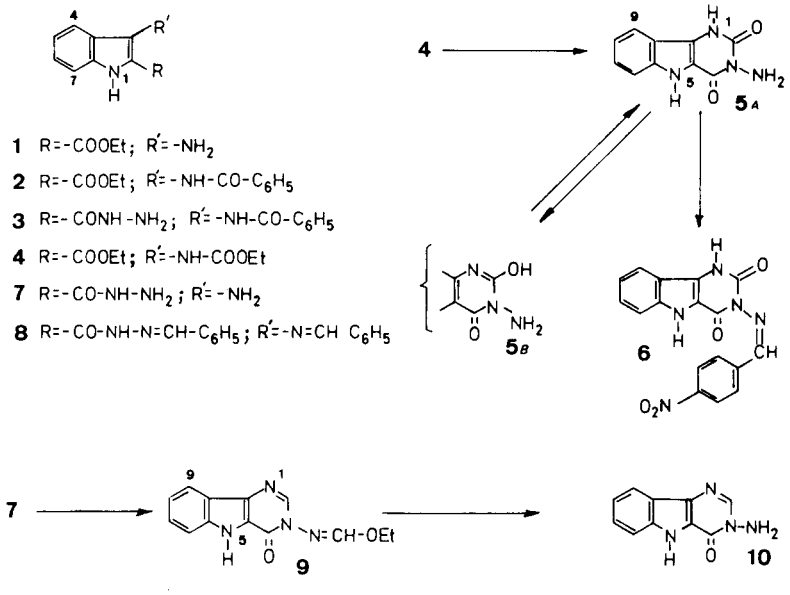
During the last years, and due to their potential pharmacological interest, special attention has been focused on the synthesis and biological properties of pyrimidin-4-ones, and particularly on their 3-amino derivatives, fused with different carbocyclic or heterocyclic systems, as benzene (quinazolinones) [1,6], benzothiophene [7], pyrazole [8,9], imidazole [9,10] and 1,2,3-triazole [9]. These compounds are generally obtained by the cyclization of *o*-aminoaroylhidrazines with orthoesters [1-10]. However, this reaction may yield different products, namely the 3-aminopyrimidin-4-ones, the respective triazepinones or also oxadiazoles, depending on the reagents, their molar proportion, the solvents and other conditions. The most suitable conditions to obtain each product, and the mechanisms, have been studied in detail with the anthranilhydrazides [1-4].

As a continuation of our previous work on the synthesis and biological properties of fused systems of indole and different nitrogenous heterocycles, we have been interested [11,12] in the last years in the synthesis and biological properties of 5*H*-pyrimido[5,4-*b*]indole derivatives. In this paper we report our results on the synthesis of 3-amino-5*H*-pyrimido[5,4-*b*]indol-4-one derivatives and some preliminary results of their study as inhibitors of the platelet aggregation induced by arachidonic acid. At present, only a small number of pyrimido[5,4-*b*]indole derivatives have been reported [11,12].

The compounds were obtained as illustrated in the Scheme. Compound **1** was benzoylated to **2** (78%) and the hydrazide **3** (55%) was obtained in the usual way. Boiling a solution of **1** and ethoxycarbonyl chloride in xylene, **4** (95%) was obtained. This last compound cyclized to **5** with hydrazine hydrate. From the elemental analysis and ir spectra, the compound **5** would be 3-aminopyridazine-2,4-

SCHEME



dione or the isomeric triazepinedione resulting in the cycle expansion. The ^{1}H-nmr spectra showed that the correct structure is the aminopyridazinedione. According to the literature [1,3,8-10] 3-aminopyridazin-4-ones show in the ^{1}H-nmr spectra a signal at about δ = 5.0-6.0 (s or bs, 2H, 3-NH_2), which disappears by the addition of deuterium oxide. In the isomeric triazepinones the signals for the protons of the system -CO-NH-NH-CX- (X = N, O) appear at values of δ >8.0 [2] as two different signals (s or bs). The ^{1}H-nmr spectra of **5** shows a signal at about δ = 5.56 (bs, 2H), which was assigned to the 3-amino group. However, this spectra does not show any expected signal for the proton of the group -N(1)H-CO-. This proton seems to interchange very easily with the protons of water in the solvent (DMSO-d_6) and the tautomeric structure **5b** seems to us the most probable. The structure **5b** (or **5a**) for this compound was confirmed by the preparation of the *p*-nitrobenzylidene derivative **6** (60%).

When **1** or **2** was treated with hydrazine hydrate, **7** was obtained and its bisbenzylidene derivative **8** prepared in the usual way. Compound **7** reacted with an excess [1] of ethyl orthoformiate to give **9** (80%). Hydrolysis of **9** with alcoholic potassium hydroxide gave **10** (90%). As expected, the ^{1}H-nmr spectra of **10** shows a signal (s, 2H) at about δ = 5.95 for the 3-amino group.

All the compounds **2** are new and they were characterized by elemental analysis and spectral data (ir, ^{1}H-nmr).

The compounds **1-9** have been assayed as inhibitors of human platelet aggregation induced by arachidonic acid. In preliminary experiments, in conditions previously described [13], we obtained the following results in percentage of inhibition at a concentration of 0.5 m*M*: **1** (100%), **2** (31%), **3** (43%), **4** (24%), 5 (13%), **7** (100%), **8** (53%), **9** (15%) and **10** (18%). These results show that the presence of a primary amino group on C-3 of the indole system is important for that biological activity.

EXPERIMENTAL

Melting points were determined in a Kofler apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3-4 mm Hg, 2-3 hours, at about 60-70°). Infrared spectra were recorded on a Perkin-Elmer 681 apparatus, using potassium bromide tablets for solid products and sodium chloride plates for liquid products; the frequencies were expressed in cm^{-1}. The ^{1}H-nmr spectra were obtained on a Perkin-Elmer R-32 (90 MHz) instrument, with TMS as the internal reference, at a concentration of about 0.1 g/ml and solvent as indicated; the chemical shifts are reported in ppm from TMS and are given in δ units.

Thin-layer chromatography (tlc) was carried out in silica gel (DSF-5, Cammaga 0.3 mm. thickness) with benzene:dioxane:acetic acid (90:25:4) as solvent and the plates were scanned under ultraviolet light, λ = 254 and 366 nm.

Materials.

Compound **1** (mp 150-152°) was prepared by reported methods from 2-aminobenzonitrile [13] or ethylindole-2-carboxylate [11].

Ethyl 3-Benzoylaminoindole-2-carboxylate (**2**).

To a boiling solution of **1** (1.02 g, 5 mmoles) in dioxane (20 ml) benzoyl chloride (5 ml) was slowly dropped. Then, the mixture was boiled for 3 hours and cooled. The precipitate was collected and recrystallized, mp 160-163° (ethanol/water), yield about 1.20 g (78%) of white crystals; ir (potassium bromide): 3320 (s, NH), 1690 and 1650 (s, CO), 740 and 695 (s, aromatic substitutions); ^{1}H-nmr (DMSO-d_6): 1.24 (t, 3H, CH_3), 4.28 (c, 2H, CH_2), 6.90-7.80 (m, 7H, H-4, H-5, H-6, H-7 of indole and H-3', H-4', H-5', of Ph), 10.35 (s, 1H, NH-CO), 12.00 (s, 1H, NH-indole). The signals assigned to the NH groups disappeared by the addition of deuterium oxide.

Anal. Calcd. for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.49; H, 5.27; N, 9.02.

3-Benzoylaminoindole-2-carbohydrazide (**3**).

To a stirred solution of **2** (3.08 g, 10 mmoles) in dioxane (25 ml), 80% hydrazine hydrate (5 ml) was slowly added. The mixture was stirred at room temperature for 3 hours and then poured into crushed ice (300 g). The precipitate was collected and recrystallized, mp 224-226° (ethanol), yield 1.52 g (55%); ir (potassium bromide): 3250 (s, NH), 1620 and 1610 (s, CO), 735 and 685 (s, aromatic substitutions); ^{1}H-nmr (DMSO-d_6): 4.60 (bs, 2H, NH_2), 7.00-7.80 (m, 7H, H-4, H-5, H-6, H-7 of indole and H-3', H-4', H-5' of Ph), 7.90-8.15 (m, 2H, H-2', H-6' of Ph), 9.35 (bs, 1H, CO-NH-N), 10.68 (s, 1H, NH-CO-Ph), 11.55 (s, 1H, NH-indole). The signals assigned to the groups NH and NH_2 disappeared by the addition of deuterium oxide.

Anal. Calcd. for $C_{16}H_{14}N_4O$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65,13; H, 4.84; N, 18.73.

Ethyl 3-Ethoxycarbonylaminoindole-2-carboxylate (**4**).

To a suspension of **1** (2.04 g, 10 mmoles) in xylene (10 ml), ethoxycarbonyl chloride (3 ml) was added, and the mixture was boiled for 10 hours. On cooling, compound **4** crystallized, mp 157-158° (xylene), yield 2.5 g (95%) as white needles; ir (potassium bromide): 3330 and 3260 (s, NH), 1690 (multiple, CO), 735 (aromatic 1,2-substituted); ^{1}H-nmr (DMSO-d_6): 1.20 (t, 3H, CH_3), 1.30 (t, 3H, CH_3), 4.10 (c, 2H, CH_2), 4.30 (c, 2H, CH_2), 6.90-7.50 (m, 3H, H-5, H-6, H-7), 7.62 (dd, 1H, H-4), 8.75 (s, 1H, CO-NH), 11.65 (s, 1H, NH-indole). The signal assigned to the group NH disappeared by the addition of deuterium oxide.

Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.87; H, 5.80; N, 10.04. Found: C, 61.11; H, 6.09; N, 10.09.

3-Amino-5*H*-pyrimido[5,4-*b*]indole-2,4-dione (**5**).

A mixture of **4** (2.76 g, 10 mmoles) and 100% hydrazine hydrate (5 ml) was boiled for 10 hours. Solvent was removed in vacuum and the solid residue recrystallized, mp >300° (DMF/ethanol), yield 1.70 g (80%) as pale brown crystals; ir (potassium bromide): 3340 (w, NH), 3190 (s, NH), 1730 (m, CO), 1630 (s, CO), 740 (aromatic 1,2-disubstituted); ^{1}H-nmr (DMSO-d_6): 5.56 (s, 2H, NH_2), 7.05-7.55 (m, 3H, H-5, H-6, H-7), 8.00 (dd, 1H, H-4), 12.05 (bs, 1H, NH-indole). Any signal was observed for -N(1)H-; this proton seems to interchange with the protons of traces of water in the solvent. The signals at about δ = 5.56 (s) and 12.05 (bs) disappeared by the addition of deuterium oxide.

Anal. Calcd. for $C_{10}H_8N_4O_2$: C, 55.56; H, 3.73; N, 26.02. Found: C, 55.34; H, 3.92; N, 26.02.

3-(4-Nitrobenzilideneamino)-5*H*-pyrimido[5,4-*b*]indole-2,4-dione (**6**).

A mixture of **5** (2.16 g, 0.1 mole), *p*-nitrobenzaldehyde (4.50 g, 0.3 moles) and DMF (15 ml) was boiled for 6 hours. Solvent was removed in vacuum and the residual material suspended in ethanol. The solid material was collected by filtration, washed with ethanol and recrystallized, mp 235° (DMF), yield 2.10 g (60%) as brown crystals; ir (potassium bromide): 3170 (bs, NH), 1720 (s, CO), 1630 (w, CO, C=N), 845 (aromatic 1,4-disubstituted), 740 (aromatic 1,2-disubstituted); ^{1}H-nmr (DMSO-d_6, 35°): 7.02-7.57 (m, 3H), 7.87-8.50 (m, 5H), 9.00 (s, 1H, -CH=N-), 11.90 (s, 1H, NH), 12.40 (bs, 1H, NH). These two last signals for NH groups dropped out by the addition of deuterium oxide.

Anal. Calcd. for $C_{17}H_{11}N_5O_4$: C, 58.45; H, 3.15; N, 20.05. Found: C, 58.14; H, 3.38; N, 19.84.

3-Aminoindole-2-carbohydrazide (**7**).

A suspension of **2** (3.08 g, 10 mmoles) or **1** (2.05 g, 10 mmoles) and 80% hydrazine hydrate (10 ml) was boiled for 2 hours. On cooling the reaction mixture, an oily solid precipitated. Solvent was poured out and the oily solid crystallized, mp 165° (ethanol/water), pale brown needles, yield 1.50 g (79%) from **1** and 0.80 g (35%) from **2**: ir (potassium bromide): 3450 (w, NH), 3320 and 3260 (s, NH), 1640 (s, CO), 730 (aromatic 1,2-disubstituted); ^{1}H-nmr (DMSO-d_6): 4.35 (bs, 2H, NH_2), 5.45 (bs, 2H, NH_2), 6.75-7.25 (m, 3H, H-5, H-6, H-7), 7.60 (dd, 1H, H-4), 8.60 (bs, 1H, NH-CO), 10.25 (s, 1H, NH-indole). The signals assigned to the groups NH and NH_2 disappeared by the addition of deuterium oxide.

Anal. Calcd. for $C_9H_{10}N_4O$: C, 56.83; H, 5.30; N, 29.46. Found: C, 56.89; H, 5.37; N, 29.18.

2-Benzylidene-1-(3-benzylidenaminoindole)-2-carbohydrazine (**8**).

A mixture of **6** (1.90 g, 10 mmoles), benzaldehyde (1.50 g, 15 mmoles) and ethanol (15 ml) was boiled for 0.5 hours. On cooling the reaction mixture, compound **8** crystallized, mp 258° (ethyl acetate) as yellow crystals, yield about 3.2 g (85%); ir (potassium bromide): 3230 (s, NH), 1650 (s, CO), 725 and 680 (aromatic substitutions): ^{1}H-nmr (DMSO-d_6): 7.00-7.80 (m, 12H), 7.85-8.15 (m, 2H), 8.28 (s, 1H, CH=N), 9.20 (s, 1H, CH=N), 12.0 (bs, 1H, NH), 12.25 (bs, 1H, NH). The signals assigned to the NH groups disappeared by the addition of deuterium oxide.

Anal. Calcd. for $C_{23}H_{18}N_4O$: C, 75.82; H, 4.94; N, 14.83. Found: C, 75.79; H, 5.09; N, 15.19.

3-Ethoxymethyleneamino-5*H*-pyrimido[5,4-*b*]indol-4-one (**9**).

A solution of **7** (1.90 g, 10 mmoles) and ethyl orthoformiate (3.0 g, 20 mmoles) in *N*,*N*,-dimethylformamide (10 ml) was boiled for 10 hours. Solvent was removed in vacuum, the residue was dissolved in ethyl acetate (10 ml) and the solid material collected and recrystallized, mp 233-235° (DMF/ethanol), yield about 2.0 g (80%) as white needles; ir (potassium bromide): 3210 (s, NH), 1640 (s, CO), 735 (aromatic 1,2-disubstituted); ^{1}H-nmr (DMSO-d_6): 1.40 (t, 3H, CH_3), 4.40 (c, 2H, CH_2), 7.05-7.60 (m, 3H, H-6, H-7, H-8), 8.00 (dd, 1H, H-9), 8.20 (s, 1H, H-2), 8.65 (s, 1H, CH=), 12.33 (s, 1H, NH-indole).

Anal. Calcd. for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.74; H, 4.90; N, 21.61.

3-Amino-5*H*-pyrimido[5,4-*b*]indol-4-one (**10**).

A mixture of **9** (2.56 g, 0.1 moles) and 0.1 *M* potassium hydroxide in ethanol (20 ml) was boiled for 20 hours. Solvent was removed in vacuum and the residue suspended in water. The solid material was collected by filtration, washed with water and recrystallized, mp 250° (DMF/ethanol) as white crystals, yield 1.8 g (90%); ir (potassium bromide): 3160-3080 (broad, NH), 1680 (s, C=O), 730 (aromatic 1,2-disubstituted); ^{1}H-nmr (DMSO-d_6, 35°): 5.95 (s, 2H, NH_2), 7.10-7.60 (m, 3H, H-6, H-7, H-8), 8.03 (dd, 1H, H-9), 9.00 (s, 1H, H-2), 12.15 (s, 1H, NH). The signals assigned to the groups NH and NH_2 disappeared by addition of deuterium oxide.

Anal. Calcd. for $C_{10}H_8N_4O_2$: C, 60.00; H, 4.08; N, 27.99. Found: C, 60.18; H, 4.03; N, 27.65.

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