ELSEVIER

Il Farmaco 53 (1998) 113–117

IL FARMACO

Synthesis and anti-microbial activity of some imidazo[1′,2′:5,6]pyrimido[4,5-*c*]pyridazines and related heterocycles

Sh.M. Radwan, H.S. El-Kashef *

Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt

Received 30 June 1997; accepted 1 December 1997

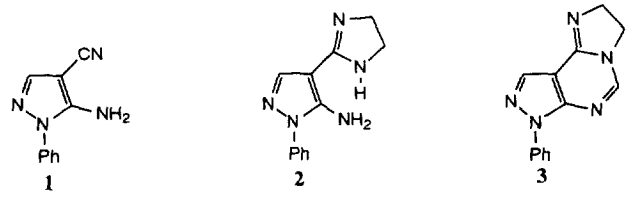
Abstract

The synthesis of the title heterocycles was achieved using 3-amino-5,6-diphenylpyridazine-4-carbonitrile (**4**) as a starting material. This compound was converted into the corresponding 4-imidazolinyl derivative **5** which was then subjected to cyclization reactions to afford the title compounds.

Keywords: Pyridazine; Imidazole; Imidazolopyrimidopyridazine

1. Introduction

Derivatives of pyrazolopyrimidine (a purine isostere) have long been known for their anti-tumor and cytostatic activity [1–5]. Several reports have indicated that pyrazolotriazolopyrimidines possess adenosine receptor antagonist activity [6–9]. In an earlier paper [10], we have described the synthesis of some imidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidines of pharmacological interest. This synthesis involved the conversion of the cyano group of 5-amino-1-phenylpyrazole-4-carbonitrile (**1**) into an imidazolinyl group followed by ring closure of the obtained product **2** into the imidazopyrazolopyrimidine **3**. The synthesis of other derivatives of **3** was also described (Scheme 1).



Scheme 1.

2. Chemistry

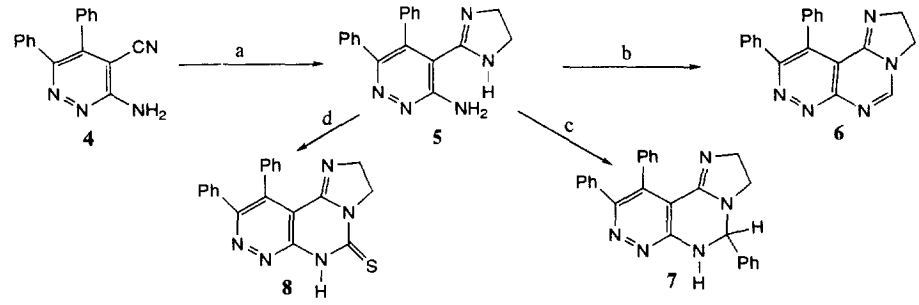
As an extension of the above work and for the sake of the biological evaluation, it was deemed of interest to substitute the pyrazole moiety of the heterocyclic system **3** by a pyridazine ring. Accordingly, this paper is devoted to the synthesis of the imidazopyrimidopyridazine isostere namely imidazo[1′,2′:1,6]pyrimido[4,5-*c*]pyridazine (**6**) and other related derivatives.

Thus, 3-amino-5,6-diphenylpyridazine-4-carbonitrile (**4**), prepared according to the method of Gewald and Oelsner [11], was allowed to interact with ethylenediamine in the presence of a catalytic amount of carbon disulfide to give the imidazolinylpyridazine **5**. The latter compound could be ring closed into the tricyclic imidazopyrazolopyrazine system in different ways. The parent tricyclic heterocycle **6** was obtained when **5** was treated with triethylorthoformate. When **5** was interacted with benzaldehyde in a similar procedure to that of Ried and Russ [12] the product was the derivative **7**. Finally the interaction of **5** with carbon disulfide gave the thione derivative **8** (see Scheme 2).

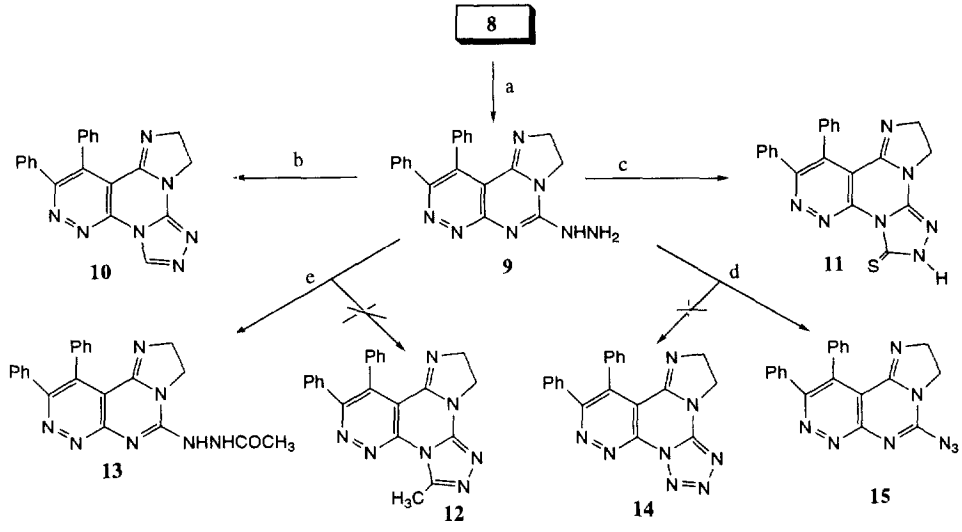
The thione **8** could easily be converted into the hydrazino derivative **9** by treatment with hydrazine hydrate. This hydrazino derivative proved to be a versatile compound that can be converted into tetracyclic heterocyclic systems (see Scheme 3). Thus the interaction of **9** with triethylorthoformate afforded the triazolo derivative **10** while its interaction with carbon disulfide in alcoholic potassium hydroxide yielded the thione derivative **11**. However, the hydrazino derivative **9** failed to produce the methyl derivative **12** when heated in refluxing glacial acetic acid, and the product was analyzed for the acetyl hydrazino derivative **13**. Also, the treatment of **9** with nitrous acid gave a product whose IR spectrum showed a band at 2150 cm^{-1} characteristic of an

* Corresponding author.

0014-827X/98/$19.00
PII S0014-827X(97)00018-9



Scheme 2. a, $H_2N(CH_2)_2NH_2/CS_2$; b, $CH(OEt)_3$; c, PhCHO; d, CS_2.



Scheme 3. a, H_2NNH_2; b, $CH(OEt)_3$; c, CS_2/KOH; d, HNO_2; e, CH_3COOH.

azide grouping which is in favor of structure **15** and eliminates the other possible tetrazolo structure **14**.

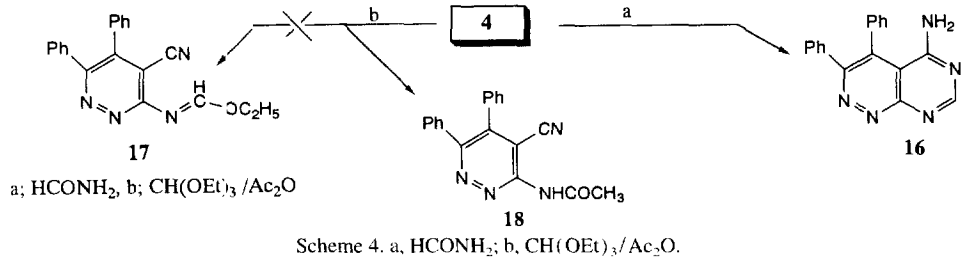
On the other hand, the pyrimidopyridazine **16** was obtained through the interaction of **4** with formamide (see Scheme 4). However, in an attempt to obtain the ethoxymethyleneimino derivative **17** via the interaction of **4** with neat triethylorthoformate the starting material was recovered unchanged. When this latter reaction was repeated in the presence of a few milliliters of acetic anhydride, the product was identified as the acetylamino derivative **18**.

3. Biological studies

3.1. Anti-bacterial and anti-fungal activities

The newly synthesized compounds were screened for their anti-bacterial activity against four different species of bacteria namely *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* and for their anti-fungal activity against five species of fungi namely *Aspergillus flavus*, *Fusarium solani*, *Penicillium citrinum* and *Trichoderma pesii* using the disc-diffusion method [13,14].

The tested compounds were dissolved in DMSO to get a solution of 1% concentration. Filter paper discs (Whatman No. 3 filter paper, 5 mm diameter) were saturated with this former solution. The saturated filter paper discs were placed on the surface of solidified Nutrient agar dishes seeded by the test bacteria and Czapek's Dox agar dishes seeded by the test fungi. The inhibition zones were measured in mm at the end of an incubation period of 48 h (at 37°C for the bacteria and at 28°C for the fungi). The results of the anti-bacterial and anti-fungal activities are collected in Table 1. All compounds under investigation were inactive against the two species of



Scheme 4. a, $HCONH_2$; b, $CH(OEt)_3/Ac_2O$.

Table 1
Anti-bacterial and anti-fungal screening of the prepared compounds [a]

Compound	*B. cereus*	*S. aureus*	*Alternaria alternata*	*Aspergillus flavus*	*Penicillium citrinium*	*Trichoderma pesii*
5	10	13	25	20	35	10
9		15				
10		15				20
16		20				

[a] The diameters of the inhibition zones are measured n mm. Only the active compounds are indicated.

Gram negative bacteria studied. However, concerning the Gram positive bacteria four compounds (**5**, **9**, **10** and **16**) were active against *S. aureus*, and only compound **5** was active against *B. cereus*. On the other hand, except *Fusarium solani*, compound **5** showed inhibition zones against all the other species of fungi under investigation. Compound **10** was active only against *Trichoderma pesii*. It seems that the presence of the dihydroimidazole ring as a substituent and not as a fused ring is responsible for the relative higher anti-microbial activity of compound **5**.

4. Experimental

All melting points are uncorrected and were determined on a Mel-Temp II melting point apparatus. IR spectra were recorded on a Pye-Unicam SP3-100 spectrophotometer using the KBr Wafer technique. ^{1}H NMR spectra were recorded on a 90 MHz Varian EM-390 NMR spectrometer in suitable deuterated solvents using TMS as an internal standard. Elemental analyses were determined on a Perkin-Elmer 240 C microanalyzer and the results were within $\pm 0.4\%$ of the calculated value.

4.1. 3-Amino-5,6-diphenylpyridazine-4-carbonitrile (4)

This compound was prepared according to a reported method [11].

4.2. 3-Amino-4-(4,5-dihydro-1H-imidazol-2-yl)-5,6-diphenylpyridazine (5)

To a mixture of 3-amino-5,6-diphenylpyridazine-4-carbonitrile (**4**) (2.7 g, 0.01 mol) and ethylenediamine (5 ml), carbon disulfide (0.9 ml) was added dropwise. The resulting reaction mixture was heated on a water bath for 8 h. After cooling, the reaction mixture was diluted with water and the solid precipitate was filtered off, washed with water and recrystallized from ethanol as white crystals, m.p. 214–216°C, yield 1.8 g (57%). IR (cm^{-1}): ν 3400, 3250, 3100 (NH_2), 1600 (C=N). ^{1}H NMR ($CDCl_3$): δ 3.35 (s, 4H, imidazole), 6.5 (s, 2H, NH_2), 6.7 (s, 1H, NH imidazole), 7–7.6 (m, 10H, ArH).

Analysis (C, H, N) for $C_{19}H_{17}N_5$.

4.3. 3,4-Diphenyl-6,7-dihydroimidazo[1',2':1,6]pyrimido-[4,5-c]pyridazine (6)

A mixture of **5** (0.3 g, 0.001 mol), triethylorthoformate (5 ml) and glacial acetic acid (0.2 ml) was heated under reflux for 4 h. The solid precipitate was collected and recrystallized from ethanol as brownish-yellow crystals, m.p. 292–294°C, yield 0.25 g (78%). IR (cm^{-1}): ν 1610 (C=N). ^{1}H NMR ($CDCl_3$): δ 3.8 (s, 4H, imidazole), 7.1–7.5 (m, 10H, ArH), 7.9 (s, 1H, pyrimidine).

Analysis (C, H, N) for $C_{20}H_{15}N_5$.

4.4. 3,4,9-Triphenyl-6,7,8,9-tetrahydro-imidazo-[1',2':1,6]pyrimido[4,5-c]pyridazine (7)

To a mixture of **5** (0,3 g, 0.001 mol) and benzaldehyde (0.3 ml, 0.003 mol) in absolute ethanol (10 ml), concentrated HCl (0.2 ml) was added and the mixture was stirred at 50–60°C for 5 h. After cooling, the mixture was neutralized with an aqueous sodium carbonate solution. The solid product obtained was filtered off and recrystallized from methanol–benzene mixture as cream crystals, m.p. 294–296°C, yield 0.2 g (50%). IR (cm^{-1}): ν 3150 NH, 1610 (C=N). ^{1}H NMR (CF_3COOH): δ 3.8–4.0 (m, 4H, imidazole), 6.5 (s, 1H, H_5), 7.1–7.7 (m, 15H, ArH).

Analysis (C, H, N) for $C_{26}H_{21}N_5$.

4.5. 3,4-Diphenyl-6,7,9,10-tetrahydro-imidazo-[1',2'-1,6]pyrimido[4,5-c]pyridazine-9-thione (8)

A mixture of **5** (1.57 g, 0.005 mol) and carbon disulfide (8 ml) in dry pyridine (20 ml) was heated on a water bath for 16 h, then the reaction mixture was left to cool. The solid precipitate was collected and recrystallized from acetic acid as yellow crystals, m.p. > 300°C, yield 0.9 g (50%). IR (cm^{-1}): ν 3300–3100 (NH), 1630 (C=N), 1140 (C=S).

Analysis (C, H, N) for $C_{20}H_{15}N_5S$.

4.6. 3,4-Diphenyl-9-hydrazino-6,7-dihydro-imidazo-[1',2'-1,6]pyrimido[4,5-c]pyridazine (9)

A mixture of **8** (1.8 g, 0.005 mol) and excess of hydrazine hydrate (8 ml, 85%) was heated under reflux for 15 min, then ethanol (15 ml) was added and the reaction mixture was further heated under reflux for 5 h. After cooling, the solid

precipitate was collected and recrystallized from ethanol as yellow crystals, m.p. 250–252°C, yield 1.2 g (66%). IR (cm^{-1}): ν 3300–3100 (NH), 1620 (C=N). ^1H NMR (CF_3COOH): δ 3.6 (s, 4H, imidazole), 7.1–7.3 (m, 10H, ArH).

Analysis (C, H, N) for $C_{20}H_{17}N_7$.

*4.7. 3,4-Diphenyl-6,7-dihydro-1,2,4-triazolo[3'',4'':2,3]-imidazo[1',2':1,6]pyrimido[4,5-c]pyridazine (**10**)*

A mixture of **9** (0.35 g, 0.001 mol) and excess triethylorthoformate (10 ml) was heated under reflux for 4 h, then allowed to cool. The solid product was collected and recrystallized from ethanol as canary-yellow crystals, m.p. 280–282°C, yield 0.3 g (75%). IR (cm^{-1}): ν 1610 (C=N). ^1H NMR (DMSO-d_6): δ 3.8 (s, 4H, imidazole), 7.1–7.4 (m, 10H, ArH), 9.4 (s, 1H, CH triazole).

Analysis (C, H, N) for $C_{21}H_{15}N_7$.

*4.8. 3,4-Diphenyl-2,3-dihydro-1,2,4-triazolo[3'',4'':2,3]-imidazo[1',2'-5,6]pyrimido[4,5-c]pyridazine-7-(6H)thione (**11**)*

To a mixture of **9** (0.71 g, 0.001 mol) and carbon disulfide (5 ml) in ethanol (15 ml), two pellets of potassium hydroxide were added. The reaction mixture was then heated on a water bath for 6 h and then allowed to cool. The solid precipitate was collected and dissolved in water (20 ml) and then acidified with acetic acid where a yellow solid was obtained. This solid was recrystallized from acetic acid as yellow crystals, m.p. > 300°C, yield 0.5 g (63%). IR (cm^{-1}): ν 3400 (NH), 1630 (C=N), 1160 (C=S). ^1H NMR (CF_3COOH): δ 3.75 (s, 4H, imidazole), 7.1–7.5 (m, 10H, ArH).

Analysis (C, H, N) for $C_{21}H_{15}N_7S$.

*4.9. 3,4-Diphenyl-9-acetylhydrazino-6,7-dihydro-imidazo[1',2'-1,6]pyrimido[4,5-c]pyridazine (**13**)*

A solution of **9** (0.35 g, 0.001 mol) in glacial acetic acid (10 ml) was heated under reflux for 10 h. After cooling, the reaction mixture was poured onto ice cold water with stirring. The solid precipitate obtained was collected and recrystallized from ethanol–benzene mixture as pale yellow crystals, m.p. > 300°C, yield 0.3 g (85%). IR (cm^{-1}): ν 3150 (NH), 1680 (C=O), 1640 (C=N). ^1H NMR (CF_3COOH): δ 2.8 (s, 3H, CH_3), 4.2–4.5 (m, 4H, imidazole), 7.2–7.6 (m, 10H, ArH).

Analysis (C, H, N) for $C_{22}H_{19}N_7O$.

*4.10. 3,4-Diphenyl-9-azido-6,7-dihydro-imidazo[1',2'-1,6]-pyrimido[4,5-c]pyridazine (**15**)*

To a well stirred solution of **9** (0.35 g, 0.001 mol) in acetic acid (10 ml), a solution of sodium nitrite (0.2 g/5 ml of water) was added dropwise with stirring at room temperature. Stirring was continued for a further 4 h. The solid product obtained after neutralization with sodium carbonate solution was filtered and recrystallized from benzene as pale-buff crystals, m.p. 232–234°C, yield 0.2 g (51%). IR (cm^{-1}): ν 2150 (N_3), 1630 (C=N).

Analysis (C, H, N) for $C_{20}H_{14}N_8$.

*4.11. 5-Amino-3,4-diphenylpyrimido[4,5-c]pyridazine (**16**)*

A mixture of **4** (0.55 g, 0.002 mol) and formamide (10 ml) was heated under reflux for 1 h. After cooling the solid product was filtered off, washed with water, and recrystallized from ethanol–benzene mixture as buff crystals, m.p. 278–280°C, yield 0.3 g (50%). IR (cm^{-1}): ν 3400, 3050 (NH_2), 1630 (C=N). ^1H NMR (CF_3COOH): δ 7.3–7.7 (m, 10H, ArH), 8.5 (s, 1H, pyrimidine).

Analysis (C, H, N) for $C_{18}H_{13}N_5$.

*4.12. Acetylamino-5,6-diphenylpyridazine-4-carbonitrile (**18**)*

A mixture of **4** (1.9 g, 0.007 mol) triethylorthoformate (15 ml) and acetic anhydride (5 ml) was heated under reflux for 4 h. After cooling, the solid product obtained was filtered off and recrystallized from ethanol as white fluffy crystals, m.p. 260–262°C, yield 4.13 g (61%). IR (cm^{-1}): ν 3200, (NH), 2200 (C=N), 1700 (C=O). ^1H NMR (CF_3COOH): δ 2.50 (s, 3H, CH_3) 7.3–7.7 (m, 10H, ArH).

Analysis (C, H, N) for $C_{19}H_{14}N_4O$.

References

[1a] Y. Makisumi, Pyrazolo[2,3-*a*]pyrimidines, Jpn. Patent No. 298 362 (1963).

[1b] Y. Makisumi, Chem. Abstr. 59 (1963) 8764.

[2a] I. Ito, 1,9-Dimethyl-2,8-diphenyl-1,2,4,6,9,10,10a,11-octahydro-3*H*-diphenylpyrazolo[4,3-*b*:4',3'-g]quinolizine-3,7(8*H*)-dione, Jpn. Patent No. 7030101 (1971).

[2b] I. Ito, Chem. Abstr. 74 (1971) 22827.

[3] J.M. Kaufman, W.O. Foye, in: W.O. Foye (Ed.), Principles of Medicinal Chemistry, 2nd ed., Lea and Febiger, Philadelphia, PA, 1981, pp. 837–861.

[4] E. Badawey, S.M. Rida, A.A. Hamza, H.T. Fahmy, Y.M. Gohar, Potential antimicrobials I. Synthesis and structure-activity studies of some new thiazolo[4,5-*d*]pyrimidine derivatives, Eur. J. Med. Chem. 28 (1993) 91–96.

[5a] M.Z. Rizk, A.Z. Abdelhamid, L.H. Faddah, Effect of some newly synthesized purine analogs on nucleic acids in mice, Egypt. J. Pharm. Sci. 34 (1993) 57–66.

[5b] M.Z. Rizk, A.Z. Abdelhamid, L.H. Faddah, Chem. Abstr. 121 (1994) 292143.

[6] P.G. Baraldi, B. Cacciari, A. Dalpiaz, S. Dionisotti, C. Zocchi, M.J. Penida de Las Infantas, G. Spalluto, K. Varani, 1*H*-Pyrazolo[4,3-*d*]-pyrimidine-7-(6*H*)-dione and 5*H*-pyrazolo[4,3-*d*]1,2,3-triazin-

4(3*H*)-one derivatives. Synthesis and in vitro biological activity at adenosine A_1 and A_{2a} receptors, Arzneim. Forsch./Drug Res. 46 (1996) 365–368.

[7] F. Gatta, M.R. Del Giudice, A. Borione, P.A. Borea, A. Dionisotti, E. Ongini, Synthesis of imidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidines, pyrazolo[4,3-*e*]1,2,3-trizolo[5,1-*i*]purines: new potent adenosine A_2 receptor antagonists, Eur. J. Med. Chem. 28 (1993) 569–576.

[8] P.G. Baraldi, B. Cacciari, G. Spalluto, M.J. Penida de Las Infantas, C. Zocchi, S. Ferrara, S. Dionisotti, 1,2,3-Triazolo[4,5-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine derivatives: a new class of A_{2A} adenosine receptor antagonists, Farmaco 51 (1996) 297–300.

[9] P.G. Baraldi, B. Cacciari, G. Spalluto, M.J. Penida de LasInfantas, C. Zocchi, S. Dionisotti, E. Ongini, Pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidine derivatives: potent and selective A_{2A} adenosine antagonists, J. Med. Chem. 39 (1996) 1164–1171.

[10] A.M. Kamal El-Dean, H.S. El-Kashef, Synthesis of some imidazopyrazolopyrimidine derivatives, Die Pharmazie 51 (1996) 155–158.

[11] K. Gewald, J. Oelsner, 3-Amino-4-cyanopyridazine durch cyclizierung von nitrilen, J. Prakt. Chem. 321 (1979) 71–74.

[12] W. Ried, T. Russ, Fused imidazo-, pyrimido- and 1,3-diazepino-[1,2-*c*]pteridines from *o*-aminopyrazinecarbonitriles, Coll. Czech., Chem. Commun. 56 (1991) 2288–2297.

[13] L.P. Carrod, F.D. Grady, Antibiotic and Chemotherapy, 3rd ed., Churchill Livingston, Edinburgh, 1972, p. 477.

[14] A. Cremer, Antibiotic Sensitivity and Assay Tests, 4th ed., Butterworth, London, 1980, p. 521.