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Bis-1,2,4-triazolo[4,3-*a*:3′,4′-*c*]quinoxalines of pharmaceutical interest from 1,3-dipolar cycloaddition

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Abstract

Various derivatives of the heterocyclic system 1,12,12a,12b-tetrahydrobis-1,2,4-triazolo[4,3-*a*:3′,4′-*c*]quinoxaline of pharmaceutical interest have been obtained by double site- and regio-selective 1,3-dipolar cycloaddition of arylnitrilimines to quinoxalines. No evidence for the formation of mono-adducts was obtained, at variance with literature reports. Specific studies to establish the exact stereochemistry of the bis-cycloadducts were undertaken.

In common with other nitrogen heterocycles, quinoxalines, as well as their fused-ring bioisosteric analogs, show marked activity in many biological systems. A large number of compounds incorporating these ring systems were found to possess antitumour and antibacterial activities. Thus, indolo[2,3-*b*]quinoxalines of type **1** represent an important series of DNA intercalating agents endowed with antiviral and cytotoxic activities (Fig. 1). For example, compound **2** (B-220) was found active against herpes virus[1,2] and also as chemopreventive agent in experimental tumour models.[3] The tetracyclic 1,6-diamino-bis-1,2,4-triazolo[4,3-*a*:3,4-*c*]quinoxaline (**3**) and 7-chloro-2-oxo-2*H*-pyrimido[2′,1′:5,1]-1,2,4-triazolo[4,3-*a*]quinoxalines of type **4** have shown moderate and high activity, respectively, against Gram-positive and Gram-negative microorganisms.[4]

Furthermore some 9*H*-bis-[1,2,4]triazolo[4,3-*a*:3′,4′-*d*][1,5]benzodiazepine derivatives of type **5**, evaluated for antiproliferative activity against a panel of cell lines derived from either hematological or solid human tumours, showed antiproliferative activity against either or both leukemia- and lymphoma-derived cell lines in the low micromolar range.[5] On the basis of these data, we focused our studies on bis-triazoloquinoxaline derivatives of type **6**, bioisosters of **5**, to explore the effect of the ring contraction on biological activities.

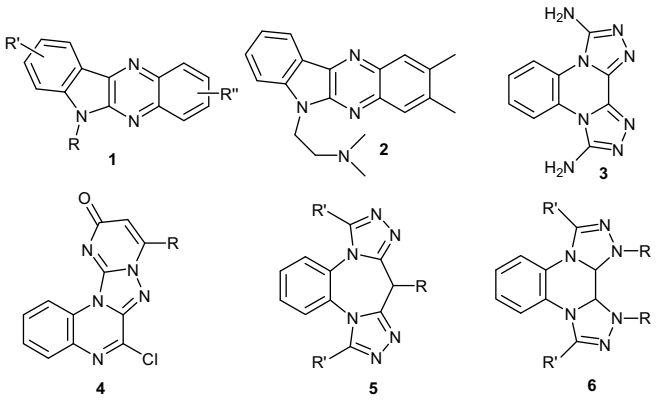

Fig. 1. Common heterocyclic quinoxalines and quinoxaline-like compounds with biological activity.

A useful synthetic strategy adopted to obtain various polyheterocyclic systems involves the use of 1,3-dipolar cycloaddition reactions.[6] To date in the literature no examples of this reaction applied to synthesize the bis-triazoloquinoxaline core structure have been reported. Quinoxalines, as other azine systems, act as dipolarophiles (2π component) in the dipolar 1,3-cycloadditions, thus

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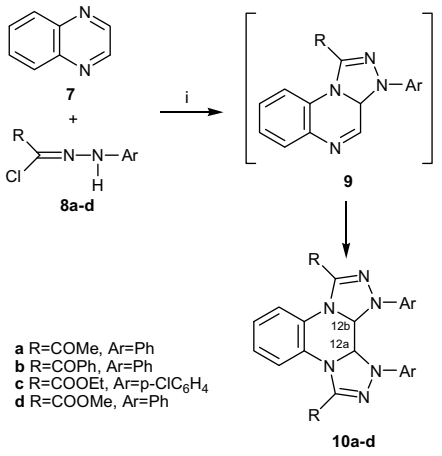
representing adequate synthons for the construction of polycyclic systems by a simple one-pot synthesis. In particular azine or diazine systems (pyridine, quinoline, isoquinoline, pyrazine, and benzodiazepine) were shown to undergo 1,3-dipolar cycloaddition in a highly site- and regio-selective fashion.[7]

All the examples of 1,3-dipolar cycloaddition to quinoxaline ring system, reported in the literature so far, demonstrate that the dipoles react with the benzodiazine ring exclusively at C=N bonds (site-selectivity) and always maintain the same orientation (regio-selectivity).[8,9] Likewise in our hands the treatment of quinoxaline **7** with two equivalents of chloroarylhydrazones **8a–d** gave rise exclusively to the bis-cycloadduct 1,12,12a,12b-tetrahydro-bis-1,2,4-triazolo[4,3-*a*:3′,4′-*c*]quinoxalines **10a–d** in satisfactory yields (Scheme 1). No increase in yields was registered by varying the reaction time or temperature. These tetracyclic derivatives formally arise from a double regio-specific 1,3-dipolar cycloaddition of the nitrilimines onto the two dipolarophile sites C=N. The structures of **10a–d**[10] were assigned by NMR spectral analysis (^{1}H and ^{13}C). In particular, the ^{1}H chemical shifts observed for the protons in 12a and 12b positions were found in the range 5.76–6.34 ppm, and those of the corresponding carbon atoms (C-12a, C-12b) in the range 72.3–84.5 ppm in the ^{13}C spectra, in agreement with the values reported for analogous azine-adducts systems,[7] unequivocally indicating double cycloaddition to the hetero-double bonds.



Scheme 1. Reaction conditions: (i) NEt_3, THF, rt, 48 h.

In contrast with the literature data, previously reported by Dalla Croce[9] for the reaction of the quinoxaline with chloroarylhydrazones **10d**, the formation of the mono-adduct of type **9** was never observed even though equimolar amount of the reactants or a large excess of dipolarophile was used. However we could verify that the concise analytical and spectroscopic data of the compound described by Dalla Croce[9] are really in agreement with the experimental data of the bis-1,2,4-triazolo[4,3-*a*:3′,4′-*c*]quinoxaline **10d** isolated by us. This should confirm that the mono-cycloadduct **9** is probably formed as an intermediate but it cannot be isolated: evidently it is much more reactive as heterodipolarophile than the starting quinoxaline.

In the case of the bis-triazoloquinoxalines **10a**,**c**,**d** from the reaction mixtures it was possible to isolate, in good yields, two different solids **A** and **B** possessing analogous properties as shown in Table 1 (solubility in common solvent, similar IR spectrum) but different melting point and R_f. In the NMR spectra of these symmetric compounds the only considerable difference is due to the resonance of two magnetic and chemical equivalent protons (H-12a, H-12b).

These experimental evidences suggested that in the reaction of **8a**,**c**,**d** with quinoxaline **7** both possible diastereoisomer 2:1 cycloadducts were obtained, arising from the double cycloaddition of the 1,3-dipole on the opposite or on the same sides of the quinoxaline ring. These findings should confirm furthermore the lack of diastereoselectivity of the 1,3-dipolar cycloadditions of nitrilimine to quinoxaline, in contrast with the literature reports for other symmetrical diazine rings: pyrazine,[7b] benzodiazine.[11] In our hand only a moderate excess of diastereoisomer **A** with respect to **B** was observed.

On the basis of the spectroscopic values reported for analogous pyrazine-bisadducts,[7b] to confirm that each fraction **A** consists of the racemic mixture of the diastereoisomers *anti*, it was chosen to use the chiral lanthanide shift reagent (CLSR) Europium tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate] [**Eu**(**hfc**)$_3$] in the NMR analysis.

Infact, in the presence of these optically active chelates, enantiomers (that respond to lanthanide NMR shift reagents by chelate formation between Eu^{3+} and probably the lone pair of nitrogen and carbonyl oxygen) realize

Table 1
Physicochemical properties

	R_f (DCM)	^{1}H NMR **12a**, **12b** δ	^{13}C NMR **12a**, **12b** δ	Mp (°C)	IR (cm^{-1})	Yield (%)
10aA	0.55	5.86	72.6	212–214	1685	44
10aB	0.29	6.31	84.5	148–152	1682	15
10cA	0.70	5.76	72.3	123–125	1729	48
10cB	0.34	6.34	83.6	191–192	1724	33
10dA	0.46	5.76	72.7	232–235	1735	30
10dB	0.19	6.34	84.1	191–194	1728	18

diastereoisomeric complexes spectroscopically separated even if in a racemic mixture.

For both fractions **A** and **B** of derivative **10d**, ^{1}H NMR spectra were registered in $CDCl_3$, adding increasing amounts (10% p/p of **Eu(hfc)**$_3$ with respect to the amount of analysed sample) before each measurements.[12] Hence it was possible to observe the splitting of the singlet due to 12a, 12b protons, only in the spectrum of **10dA**. These results confirm that fraction **A** of derivative **10d**, and of the other strictly related derivatives **10aA** and **10cA**, consists of the racemic mixture (RR and SS) of *anti* isomers.

In conclusion, 1,3-dipolar cycloadditions constitute a versatile and useful synthetic strategy to obtain polycondensed nitrogen heterocycles in one-pot reaction. The 1,3-dipolar cycloadditions of nitrilimines to quinoxalines are highly site- and regio-selective but not diastereoselective. Specific studies to establish the exact stereochemical configuration of the derivatives synthesized were carried out, by using CLSRs in NMR analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.01.047.

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10. *Experimental*: Melting points (uncorrected) were taken on a Buchi-Tottoli capillary apparatus; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz, respectively, in $(CD_3)_2SO$ solution, using a Bruker AC-E series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230–400 mesh ASTM. Chlorophenylhydrazones **8a–d** were prepared according to the procedure described in the literature.[13] *General procedure for bis-1,2,4-triazolo[4,3-a:3′,4′-c]quinoxalines* **10a–d**. Triethylamine (8.30 mmol) was added to a solution of quinoxaline (3.84 mmol) and chlorophenylhydrazones (7.68 mmol) in anhydrous tetrahydrofuran (20 mL) and the mixture was stirred at room temperature for 48 h. The solution was concentrated under reduced pressure and treated/crystallized with ethanol and collected by filtration as a colored solid. *1,12,12a,12b-tetrahydrobis-3,10-diacetyl-1,12-diphenyl-1,2,4-triazolo[4,3-a:3′,4-c]quinoxaline* (**10a**): The reaction mixture obtained from the reaction of quinoxaline **7** with (1*E*)-2-oxo-*N*-phenylpropane-hydrazonoyl chloride (**8a**) was evaporated under reduced pressure and the residue was treated with cold ethanol (5 mL). The first fraction crystallized gave **10aA** as orange solid: IR: 1685 cm^{-1} (C=O); ^{1}H NMR: δ 2.61 (s, 6H, 2 × CH_3), 5.86 (s, 2H, H-12a, H-12b), 6.72–6.82 (m, 2H, *p*-NC_6H_5) 7.03–7.11 (m, 10H, H-6, H-7, *m*-NC_6H_5, *o*-NC_6H_5), 7.55–7.61 (m, 2H, H-5, H-8). ^{13}C NMR: δ 28.5 (q), 72.6 (d), 113.1 (d), 120.9 (d), 122.1 (d), 122.5 (d), 124.8 (s), 128.8 (d), 142.6 (s), 146.1 (s), 188.4 (s). Anal. Calcd for $C_{26}H_{22}N_6O_2$: C, 69.32; H, 4.92; N, 18.65. Found: C, 69.25; H, 4.90; N, 18.39. HRMS: (M^+) calcd for $C_{26}H_{22}N_6O_2$ 450.1804; found, 450.1819. The solution was concentrated to afford **10aB** as a dark orange solid: IR: 1682 cm^{-1} (C=O); ^{1}H NMR: δ 2.35 (s, 6H, 2 × CH_3), 6.31 (s, 2H, H-12a, H-12b), 6.79–6.85 (m, 2H, *p*-C_6H_5) 7.00–7.13 (m, 8H, *m*-C_6H_5, *o*-C_6H_5), 7.21–7.26 (m, 2H, H-6, H-7), 7.40–7.47 (m, 2H, H-5, H-8). ^{13}C NMR: δ 26.7 (q), 84.5 (d), 115.4 (d), 121.8 (d), 125.8 (d), 126.0 (d), 128.4 (d), 135.0 (s), 142.7 (s), 146.4 (s), 187.6 (s). Anal. Calcd for $C_{26}H_{22}N_6O_2$: C, 69.32; H, 4.92; N, 18.65. Found: C, 69.25; H, 4.95; N, 18.39. HRMS: (M^+) calcd for $C_{26}H_{22}N_6O_2$ 450.1804; found, 450.1815. *1,12,12a,12b-tetrahydrobis-3,10-dibenzoyl-1,12-diphenyl-1,2,4-triazolo[4,3-a:3′,4-c]quinoxaline* (**10b**): The reaction mixture obtained from the reaction of quinoxaline **7** with 1(*E*)-2-oxo-*N*, 2-diphenylethane-hydrazonoyl chloride (**8b**) crystallized from ethanol giving **10b** as an orange solid: yield 23%, mp 200–201 °C; IR 1657 cm^{-1} (CO); ^{1}H NMR: δ 6.01 (s, 2H, H-12a, H-12b), 6.81–7.28 (m, 14H, H-6, H-7, 2 × NC_6H_5, *p*-COC_6H_5) 7.67, (d, 4H, *m*-COC_6H_5, $J = 7.0$ Hz), 7.78 (d, 2H, H-5, H-8) 8.20 (d, 4H, *o*-COC_6H_5, $J = 7.0$ Hz). ^{13}C NMR: δ 73.7 (d), 113.5 (d), 120.5 (d), 121.1 (d), 122.7 (d), 124.8 (s), 128.7 (d), 128.9 (d), 130.4 (d), 134.5 (d), 135.8 (s), 143.6 (s), 145.6 (s), 183.5 (s). Anal. Calcd for $C_{36}H_{26}N_6O_2$: C, 75.25; H, 4.56; N, 14.62. Found: C, 75.31; H, 4.45; N, 14.51. HRMS: (M^+) calcd for $C_{36}H_{26}N_6O_2$ 574.2117; found, 574.2112. *1,12,12a,12b-tetrahydrobis-3,10-diethoxycarbonyl-1,12-4-chlorophenyl-1,2,4-triazolo[4,3-a:3′,4-c]quinoxaline* (**10c**): The reaction mixture obtained from the reaction of quinoxaline **7** with ethyl (2*Z*)-chloro[(4-chlorophenyl)hydrazono]acetate (**8c**) was evaporated under reduced pressure, the residue was washed with ethanol (5 mL) and chromatographed on a silica gel column using DCM as eluent. The first fraction eluted gave **10cA** as a clear yellow solid: IR: 1729 cm^{-1} (C=O); ^{1}H NMR: δ 1.32 (t, 6H, $J = 6.6$ Hz, 2 × CH_3), 4.39 (q, 4H, $J = 6.6$ Hz, 2 × CH_2), 5.76 (s, 2H, H-12a, H-12b), 7.00–7.20 (m, 10H, *m*-C_6H_5, *o*-C_6H_5, H-6, H-7), 7.45–7.49 (m, 2H, H-5, H-8); ^{13}C NMR: δ 13.8 (q), 62.6 (t), 72.3 (d), 114.7 (d), 121.1 (d), 123.2 (d), 124.4 (s), 124.5 (s), 128.7 (d), 141.7 (s), 142.4 (s), 158.1 (s). Anal. Calcd for $C_{28}H_{24}Cl_2N_6O_4$: C, 58.04; H, 4.17; N, 14.50. Found: C, 58.09; H, 4.13; N, 14.41. HRMS: (M^+) calcd for $C_{28}H_{24}Cl_2N_6O_4$ 578.1236; found, 578.1244. The second fraction eluted afforded **10cB** as a clear yellow solid: IR: 1724 cm^{-1} (C=O); ^{1}H NMR: δ 1.15 (t, 6H, $J = 7.0$ Hz, 2 × CH_3), 4.16 (q, 4H, $J = 7.0$ Hz, 2 × CH_2), 6.34 (s, 2H, H-12a, H-12b), 6.93 (d, 4H, 4*o*-C_6H_5, $J = 8.4$ Hz), 7.10 (d, 4H, 4*m*-C_6H_5, $J = 8.4$ Hz), 7.32–7.39 (m, 2H, H-6, H-7), 7.45–7.51 (m, 2H, H-5, H-8); ^{13}C NMR: δ 13.7 (q), 61.7 (t), 83.6 (d), 116.2 (d), 124.7 (s), 126.7 (d), 126.9 (d), 128.3 (d), 134.9 (s), 141.81 (s), 141.83 (s), 156.9 (s). Anal. Calcd for $C_{28}H_{24}Cl_2N_6O_4$: C, 58.04; H, 4.17; N, 14.50. Found: C, 57.99; H, 4.19; N, 14.35. HRMS: (M^+) calcd for $C_{28}H_{24}Cl_2N_6O_4$ 578.1236; found, 578.1229. *1,12,12a,12b-tetrahydrobis-3,10-dimethoxycarbonyl-1,12-diphenyl-1,2,4-triazolo[4,3-a:3′,4-c]quinoxaline* (**10d**): The reaction mixture obtained from the reaction of quinoxaline **7** with methyl (2*E*)-chloro (phenylhydrazono)-acetate

(**8d**) was evaporated under reduced pressure. The residue was washed with ethanol (5 mL) and chromatographed on a silica gel column using DCM as eluent. The first fraction eluted gave **10dA** as a clear yellow solid: IR: 1735 cm^{-1} (C=O); ^1H NMR: δ 3.91 (s, 6H, 2 × CH_3), 5.76 (s, 2H, H-12a, H-12b), 6.74–6.81 (m, 2H, *p*-C_6H_5) 7.03–7.15 (m, 10H, *m*-C_6H_5, *o*-C_6H_5, H-6, H-7), 7.46–7.51 (m, 2H, H-5, H-8); ^{13}C NMR: δ 53.2 (q), 72.7 (d), 113.2 (d), 120.9 (d), 121.1 (d), 123.1 (d), 124.5 (s), 128.9 (d), 141.3 (s), 143.8 (s), 158.6 (s). Anal. Calcd for $C_{26}H_{22}N_6O_4$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.66; H, 4.63; N, 17.32. HRMS: (M^+) calcd for $C_{26}H_{22}N_6O_4$ 482.1702; found, 482.1695. The second fraction eluted afforded **10dB** as a clear yellow solid: IR: 1728 cm^{-1} (C=O); ^1H NMR: δ 3.74 (s, 6H, 2 × CH_3), 6.34 (s, 2H, H-12a, H12b), 6.76–6.83 (m, 2H, *p*-C_6H_5) 6.95–7.10 (m, 8H, *m*-C_6H_5, *o*-C_6H_5), 7.27–7.32 (m, 2H, H-6, H-7), 7.41–7.51 (m, 2H, H-5, H-8); ^{13}C NMR: δ 52.6 (q), 84.1 (d), 114.9 (d), 121.3 (d), 125.77 (d), 126.4 (d), 128.4 (d), 134.7 (s) 141.0 (s), 143.3 (s), 157,7 (s). Anal. Calcd for $C_{26}H_{22}N_6O_4$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.80; H, 4.67; N, 17.34. HRMS: (M^+) calcd for $C_{26}H_{22}N_6O_4$ 482.1702; found, 482.1714.

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