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Solid-phase synthesis and evaluation of libraries of substituted 4,5-dihydropyridazinones as vasodilator agents

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Abstract

The solid-phase parallel preparation of a library of 4,5-dihydropyridazin-3(*2H*)-one derivatives substituted at position 6 with piperazinylmethyl or tetrahydroquinolinylmethyl groups and analogues (**3**) is reported. Polymer-supported γ-keto-δ-aminoesters prepared from Wang resin reacted with hydrazine or methylhydrazine to afford pyridazinones in good yields after a cyclization cleavage approach. We have evaluated these novel analogues and several compounds of other series (**1, 2**) for their vasorelaxant effect. Among the products tested, **3l** and **3d** proved to be efficacious and potent relaxant agents of the isolated rat aorta. Inhibitors of phosphodiesterase (PDE3), responsible for the breakdown of cyclic AMP in the vascular smooth muscle, are currently developed for cardiac heart failure because of their inotropic effect and coronary vasodilatation. We had expected that the vasodilatation induced by **3l**, as efficient as reference PDE3 inhibitors, milrinone or CI-930, to be due to PDE3 inhibition. However **3l** and **3d** exhibited a low inhibitory effect against PDE3 isoenzyme activity. These compounds induced a significant vasorelaxation, which could be of therapeutic interest even if their mechanism of action remains to be determined.

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Introduction

Pyridazinone derivatives belong to a new class of cardiac inotropic drugs. Besides their cardiac effects, they are also vasodilators both in-vitro (Gruhn et al 1998) and in-vivo (Pagel et al 1996). It was previously demonstrated that the vasodilation induced by pyridazinone derivatives may partially be due to potential inhibition of phosphodiesterase (PDE) 3 (Vegh et al 1995). Furthermore it has been reported that most of the pyridazinone derivatives, bearing a 6-aryl or heteroaryl moiety, exhibit excellent PDE3 inhibitory activity (Sircar et al 1986; Combs et al 1990; Kato 1997). The secondary messengers cyclic AMP and cyclic GMP, responsible for the regulation of numerous intracellular processes, are regulated by PDEs that hydrolyse them to the corresponding inactive 5′-monophosphate nucleotides. Eleven PDE gene families have been identified to date, varying in substrate specificity, inhibitor sensitivity and regulatory characteristics (Polson & Strada 1996). Due to their crucial role in regulation of cell function, PDEs have become good clinical targets for the treatment of inflammation (Barnes 2000), asthma (Giembycz 2000), erectile dysfunction (Corbin et al 2002) and heart failure (Young 2001). Among the large family of PDE3 inhibitors, compounds containing the 3(*2H*)-pyridazinone ring, such as CI-930, MCI-154 and pimobendan (Figure 1), have been extensively studied and have vasodilatory effects on peripheral vasculature in addition to their positive inotropic properties (Silver 1989). However, to the best of our knowledge, no report on the synthesis of 6-aminomethylsubstituted pyridazinone analogues and evaluation of their vasorelaxant action has been mentioned so far.

Solid phase organic synthesis (SPOS) has become an important tool in both drug discovery and chemical biology studies (Balkenhohl et al 1996; Hall et al 2001; Ley & Baxendale 2002) and its continued success is dependent, in part, on the possibility of

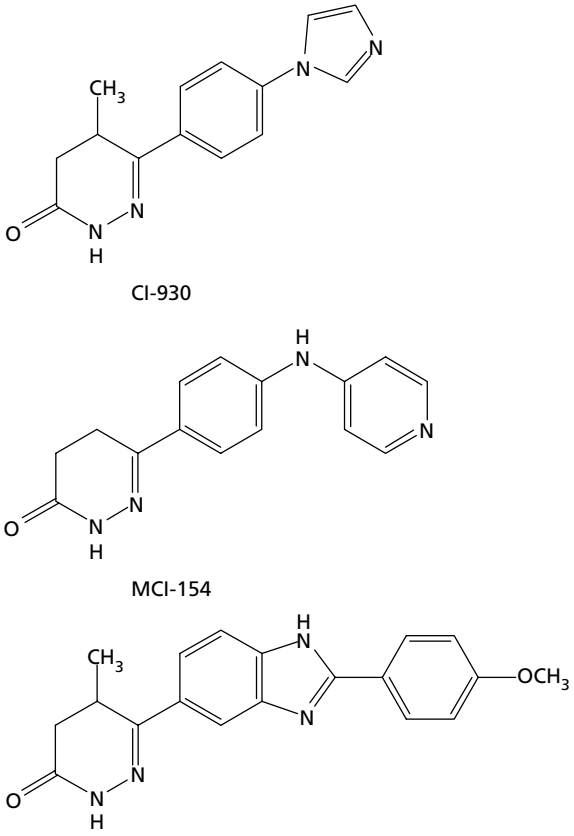


Figure 1 PDE3 inhibitors containing the 3(*2H*)-pyridazinone ring, CI-930, MCI-154 and pimobendan.

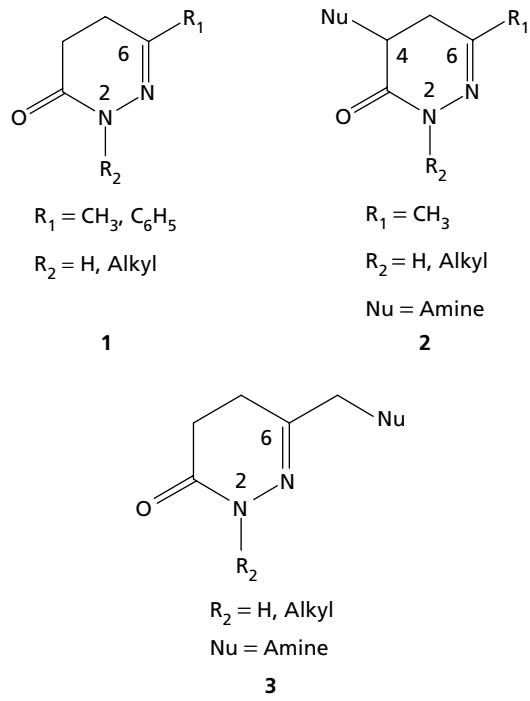


Figure 2 General structures of compounds **1, 2** and **3**.

rapid generation of many structurally related compounds from which, in conjunction with a suitable screening technique, potential drug candidates can be rapidly identified. As part of our efforts towards the solid-phase synthesis and biological evaluation of diverse heterocycles, we have previously described a convenient procedure for the preparation of 4,5-dihydropyridazinones on Wang resin (Gouault et al 2001) represented by compound **1** (Figure 2). We have also developed an efficient method starting from a polymer-supported Michael adduct to introduce diversity at the C-4 position of the final ring (Gouault et al 2002) (compound **2**). This study describes the solid-phase synthesis of new 6-substituted derivatives (**3**) and the evaluation of the vaso-relaxant effect of the whole series (**1, 2** and **3**) to initiate a more comprehensive investigation of substituents on the dihydropyridazinone core.

Materials and Methods

All reagents were purchased from Aldrich and used directly unless otherwise stated. Dimethyl formamide (DMF), tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were distilled from CaH_2, Na/benzophenone and P_2O_5, respectively, and stored under N_2. Wang resin was commercially available. Infrared spectra were recorded on a 16PC FTIR Perkin Elmer spectrometer. Solids were examined with a diffuse reflectance accessory. For liquids, a horizontal attenuated total reflectance (HATR) with a ZnSe crystal was used. ^{1}H NMR and ^{13}C NMR were recorded using a Bruker DMX (at 500 MHz and 125 MHz, respectively). Tetramethylsilane was used as the internal standard; chemical shifts (δ) were in ppm. The high-resolution mass spectra (HRMS) were recorded at the CRMPO (Centre Regional de Mesures Physiques de l'Ouest) on a Varian MAT 311 double-focusing instrument with a source temperature of 140°C, an ion accelerating potential of 3 kV and ionizing electrons of 70 eV and 300 μA or on a ZabSpec Micromass spectrometer using an electrospray ionisation mode (ESI). Melting points were determined using a Kofler hot-stage apparatus and are uncorrected.

Procedures for the formation and characterisation data for compounds **1a–f** were previously described (Gouault et al 2001).

Preparation of starting material R1

4-Oxo-pent-2-enoic acid was prepared according to the literature (Porter et al 1991) starting from levulinic acid by treatment with bromine in conc. HCl then elimination with sodium acetate. A solution of 4-oxo-pent-2-enoic acid (1.25 mmol) in anhydrous CH_2Cl_2 (3 mL) was added to a suspension of Wang resin (1 g) (Sigma-Aldrich, loading of 0.62 mmol g^{-1}) in anhydrous CH_2Cl_2 (8 mL) in the presence of a catalytic amount of dimethylaminopyridine (DMAP, 1%) and diisopropylcarbodiimide (DIC, 1.25 mmol). After agitating for 18 h at room temperature,

resin R1 was washed successively with CH_2Cl_2 (2 × 5 mL), THF (2 × 5 mL), THF–H_2O (1:1, 10 mL), H_2O (10 mL), THF (2 × 5 mL), CH_2Cl_2 (2 × 5 mL) and then dried under reduced pressure at 40°C for 2 h. IR-FT (ν cm^{-1}): 1730 (CO ester), 1700 (CO ketone).

Preparation of starting material R2

5-Bromo-4-oxo-pentanoic acid was prepared starting from levulinic acid by treatment with bromine in methanol affording methyl 5-bromolevulinate and methyl 3-bromo-levulinate (50:50) (MacDonald 1974), which were separated by distillation under reduced pressure. Methyl 5-bromolevulinate: bp 46°C/1 mmHg; methyl 3-bromo-levulinate: bp 28°C/1 mmHg.

Methyl 5-bromolevulinate was then hydrolysed with conc. HCl at 50°C for 18 h to give 5-bromo-levulinic acid. This acid was anchored to Wang resin in the same conditions as for R1, to afford R2. IR-FT (ν cm^{-1}): 1734 (CO ester), 1702 (CO ketone).

General procedure for synthesis of pyridazinones 2

Resin R1 (500 mg, 0.31 mmol) was shaken with amine (3.1 mmol) in dimethyl sulfoxide (DMSO) (6 mL) for 1 h at room temperature. This resin was washed successively with DMSO (2 × 5 mL), THF (2 × 5 ml), CH_2Cl_2 (2 × 5 ml) and then dried under reduced pressure at 40°C for 2 h. To the resin obtained (500 mg, 0.31 mmol) in THF (4 mL) was added a solution of a hydrazine (3.1 mmol) in ethanol (4 mL). The resulting mixture was stirred for 1 h at 70°C. The polymer was removed by filtration and washed with CH_2Cl_2 (3 × 3 mL). The combined filtrates were concentrated to dryness to give the crude product.

*4-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-6-methyl-4,5-dihydropyridazin-3(2*H*)-one,* ***2a***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (73%). Mp 160°C; ^{1}H NMR ($CDCl_3$): δ 2.08 (s, 3H, CH_3), 2.68 (d, J = 7.6 Hz, 2H, CH_2), 2.83 (m, 4H, CH_2), 3.12 (m, 4H, CH_2), 3.39 (t, J = 7.6 Hz, 1H, CH), 6.86 (m, 2H, H_{arom}), 6.95 (t, 2H, H_{arom}), 8.75 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.0, 29.9, 49.2, 50.5, 58.1, 116.5 (d, $^2J_{CF}$ = 21.8 Hz), 118.0 (d, $^3J_{CF}$ = 7.3 Hz), 147.9, 153.0, 157.2 (d, $^1J_{CF}$ = 238.6 Hz), 165.1; HRMS: for $C_{15}H_{19}N_4OF$ m/z calculated 290.1543, m/z found 290.1534.

*4-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-2-methyl-6-methyl-4,5-dihydropyridazin-3(2*H*)-one,* ***2b***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (66%). Mp 122–123°C; ^{1}H NMR ($CDCl_3$): δ 2.09 (s, 3H, CH_3), 2.65 (d, J = 7.1 Hz, 2H, CH_2), 2.79 (m, 4H, CH_2), 3.10 (m, 4H, CH_2), 3.31 (t, J = 7.1 Hz, 1H, CH), 3.34 (s, 3H, CH_3), 6.86 (m, 2H, H_{arom}), 6.94 (t, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 23.2, 30.4, 36.2, 49.4, 50.5, 58.6, 115.5 (d, $^2J_{CF}$ = 21.8 Hz), 117.9 (d, $^3J_{CF}$ = 7.3 Hz), 147.9, 152.8, 157.1 (d, $^1J_{CF}$ = 238.6 Hz), 163.4; HRMS: for $C_{16}H_{21}N_4OF$ m/z calculated 304.1699, m/z found 304.1703.

*4-(2,2-Diethoxy-ethylamino)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one,* ***2c***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (80%). Mp 72°C; ^{1}H NMR ($CDCl_3$): δ 1.22 (t, 3H, CH_3), 1.23 (t, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.25 ("s", 1H, NH), 2.41–2.46 (ABX, J_{AB} = 16.8 Hz, J_{AX} = 12.4 Hz, J_{BX} = 7.0 Hz, 1H, CH_2), 2.67–2.73 (ABX, 1H, CH_2), 2.78–2.84 (m, 2H, CH_2), 3.33–3.38 (ABX, 1H, CH), 3.52–3.58 (m, 2H, CH_2), 3.68–3.74 (m, 2H, CH_2), 4.58 (t, 1H, CH), 8.90 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 15.4, 23.3, 33.0, 49.7, 51.9, 62.3, 62.5, 102.1, 153.2, 167.9; HRMS: for $C_9H_{16}N_3O_2$ $[M - C_2H_6O]^{+\circ}$ m/z calculated 198.1242, m/z found 198.1245.

*4-(2,2-Diethoxy-ethylamino)-2-methyl-6-methyl-4,5-dihydropyridazin-3(2*H*)-one,* ***2d***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (70%). ^{1}H NMR ($CDCl_3$): δ 1.22 (t, 3H, CH_3), 1.23 (t, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.15 ("s", 1H, NH), 2.37–2.43 (ABX, J_{AB} = 16.8 Hz, J_{AX} = 12.7 Hz, J_{BX} = 6.7 Hz, 1H, CH_2), 2.64–2.70 (ABX, 1H, CH_2), 2.74–2.80 (m, 2H, CH_2), 3.27–3.31 (ABX, 1H, CH), 3.32 (s, 3H, CH_3), 3.53–3.58 (m, 2H, CH_2), 3.69–3.74 (m, 2H, CH_2), 4.58 (t, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 15.4, 23.5, 33.2, 36.2, 49.8, 52.3, 62.3, 62.5, 102.2, 153.0, 166.1; HRMS: for $C_{10}H_{17}N_3O_2$ $[M - C_2H_6O]^{+\circ}$ m/z calculated 211.1321, m/z found 211.1336.

General procedure for synthesis of pyridazinones 3

The resin R2 (500 mg, 0.31 mmol) was next shaken with amine (3.1 mmol) in DMSO (6 mL) for 1 h at room temperature. Resin was washed successively with DMSO (2 × 5 mL), THF–H_2O (1:1, 10 mL), H_2O (2 × 10 mL), THF (2 × 5 mL), CH_2Cl_2 (2 × 5 mL) and then dried under reduced pressure at 40°C for 2 h. To the resin **7** (500 mg, 0.31 mmol) in THF (4 mL) was added a solution of a hydrazine (3.1 mmol) in ethanol (4 mL). The resulting mixture was stirred for 1 h at 70°C. The polymer was removed by filtration and washed with CH_2Cl_2 (3 × 3 mL). The combined filtrates were concentrated to dryness to give the crude product.

*6-[4-(4-Fluoro-phenyl)-piperazin-1-ylmethyl]-4,5-dihydropyridazin-3(2*H*)-one,* ***3a***
Purified by crystallisation from Et_2O, a white solid was obtained (82%). Mp 180–182°C; ^{1}H NMR ($CDCl_3$): δ 2.47 (t, J = 8.2 Hz, 2H, CH_2), 2.63–2.66 (m, 4H, CH_2), 2.65 (t, J = 8.2 Hz, 2H, CH_2), 3.10–3.14 (m, 4H, CH_2), 3.19 (s, 2H, CH_2), 6.85–6.88 (m, 2H, H_{arom}), 6.95 (m, 2H, H_{arom}), 8.96 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.2, 26.2, 50.1, 53.3, 62.7, 115.5 (d, $^2J_{CF}$ = 21.8 Hz), 117.8 (d, $^3J_{CF}$ = 8.5 Hz), 147.8, 153.3, 157.2 (d, $^1J_{CF}$ = 239.8 Hz), 167.7; HRMS: for $C_{15}H_{19}N_4OF$ m/z calculated 290.1543, m/z found 290.1534.

6-[4-(4-Fluoro-phenyl)-piperazin-1-ylmethyl]-2-methyl-4,5-dihydropyridazin-3(2H)-one, ***3b***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a yellow solid was obtained (78%). Mp 86°C; ^{1}H NMR ($CDCl_3$): δ 2.45 (t, J = 8.6 Hz 2H, CH_2), 2.59 (t, J = 8.6 Hz 2H, CH_2), 2.60–2.64 (m, 4H, CH_2), 3.10–3.14 (m, 4H, CH_2), 3.20 (s, 2H, CH_2), 3.34 (s, 3H, CH_3), 6.86–6.89 (m, 2H, H_{arom}), 6.96 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 23.4, 26.8, 36.2, 50.0, 53.3, 62.7, 115.5 (d, $^2J_{CF}$ = 21.8 Hz), 117.8 (d, $^3J_{CF}$ = 7.5 Hz), 147.8, 154.4, 157.2 (d, $^1J_{CF}$ = 239.1 Hz), 166.1; HRMS: for $C_{16}H_{21}N_4OF$ m/z calculated 304.1699, m/z found 304.1703.

6-(4-Phenyl-piperazin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-one, ***3c***
Purified by crystallisation from Et_2O, a white solid was obtained (80%). Mp 195°C; ^{1}H NMR ($CDCl_3$): δ 2.46 (t, 2H, CH_2), 2.58 (t, 2H, CH_2), 2.59–2.68 (m, 4H, CH_2), 3.20 (s, 2H, CH_2), 3.20–3.25 (m, 4H, CH_2), 6.86 (t, 1H, H_{arom}), 6.94 (d, 2H, H_{arom}), 7.25 (t, 2H, H_{arom}), 8.75 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.3, 26.2, 49.1, 53.4, 62.8, 116.1, 119.9, 129.1, 151.2, 153.4, 167.6; HRMS: for $C_{15}H_{20}N_4O$ m/z calculated 272.1637, m/z found 272.1643.

6-(4-Phenyl-piperazin-1-ylmethyl)-2-methyl-4,5-dihydropyridazin-3(2H)-one, ***3d***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (80%). Mp 90°C; ^{1}H NMR ($CDCl_3$): δ 2.46 (t, 2H, CH_2), 2.48 (t, 2H, CH_2), 2.59–2.65 (m, 4H, CH_2), 3.20 (s, 2H, CH_2), 3.20–3.25 (m, 4H, CH_2), 6.86 (t, 1H, H_{arom}), 6.94 (d, 2H, H_{arom}), 7.25 (t, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 23.6, 26.8, 36.2, 49.1, 53.4, 62.8, 116.1, 119.9, 129.1, 151.2, 153.8, 165.9; HRMS: for $C_{16}H_{22}N_4O$ m/z calculated 286.1794, m/z found 286.1799.

6-(4-Cyclohexyl-piperazin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-one, ***3e***
Purified by crystallisation from Et_2O, a white solid was obtained (50%). Mp 130–132°C; ^{1}H NMR ($CDCl_3$): δ 1.10–1.18 (m, 1H, CH_2), 1.20–1.32 (m, 4H, CH_2), 1.62–1.70 (m, 1H, CH_2), 1.82–1.88 (m, 2H, CH_2), 1.95–2.05 (m, 2H, CH_2), 2.38–2.42 (m, 1H, CH), 2.45 (t, 2H, CH_2), 2.60 (t, 2H, CH_2), 2.60–2.66 (m, 4H, CH_2), 2.73–2.78 (m, 4H, CH_2), 3.18 (s, 2H, CH_2), 8.75 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.4, 25.9, 26.1, 28.5, 48.7, 49.1, 52.9, 62.5, 64.3, 153.8, 168.7; HRMS: for $C_{15}H_{26}N_4O$ m/z calculated 278.2107, m/z found 278.2114.

6-(4-Cyclohexyl-piperazin-1-ylmethyl)-2-methyl-4,5-dihydropyridazin-3(2H)-one, ***3f***
Purified by crystallisation from Et_2O, a white solid was obtained (65%). Mp 98–100°C; ^{1}H NMR ($CDCl_3$): δ 1.07–1.12 (m, 1H, CH_2), 1.18–1.28 (m, 4H, CH_2), 1.59–1.65 (m, 1H, CH_2), 1.77–1.81 (m, 2H, CH_2), 1.88–1.92 (m, 2H, CH_2), 2.30–2.36 (m, 1H, CH), 2.43 (t, 2H, CH_2), 2.49–2.52 (m, 4H, CH_2), 2.58 (t, 2H, CH_2), 2.60–2.64 (m, 4H, CH_2), 3.13 (s, 2H, CH_2), 3.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 23.6, 25.8, 26.8, 28.9, 36.2, 48.8, 53.7, 53.9, 62.8, 63.6, 154.1, 165.8; HRMS: for $C_{16}H_{28}N_4O$ m/z calculated 292.2263, m/z found 292.2285.

6-(4-Benzyl-piperazin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-one, ***3g***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (79%). Mp 155–157°C; ^{1}H NMR ($CDCl_3$): δ 2.43 (t, 2H, CH_2), 2.45–2.52 (m, 4H, CH_2), 2.56 (t, 2H, CH_2), 2.60–2.65 (m, 4H, CH_2), 3.12 (s, 2H, CH_2), 3.51 (s, 2H, CH_2), 7.26–7.32 (m, 5H, H_{arom}), 8.76 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.2, 26.2, 52.3, 53.3, 62.7, 62.9, 127.0, 128.2, 129.2, 137.9, 153.7, 167.7; HRMS: for $C_{16}H_{22}N_4O$ m/z calculated 286.1794, m/z found 286.1799.

6-(4-Benzyl-piperazin-1-ylmethyl)-2-methyl-4,5-dihydropyridazin-3(2H)-one, ***3h***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (45%). Mp 107–109°C; ^{1}H NMR ($CDCl_3$): δ 2.41 (t, 2H, CH_2), 2.45–2.52 (m, 4H, CH_2), 2.56 (t, 2H, CH_2), 2.60–2.65 (m, 4H, CH_2), 3.13 (s, 2H, CH_2), 3.31 (s, 3H, CH_3), 3.51 (s, 2H, CH_2), 7.26–7.33 (m, 5H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 23.6, 26.8, 36.2, 52.9, 53.3, 62.8, 62.9, 127.1, 128.2, 129.2, 137.9, 154.1, 165.9; HRMS: for $C_{17}H_{24}N_4O$ m/z calculated 300.1950, m/z found 300.1960.

6-(3,4-Dihydro-2H-quinolin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-one, ***3i***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a yellow oil was obtained (75%). ^{1}H NMR ($CDCl_3$): δ 1.97 (m, 2H, CH_2), 2.38–2.42 (m, 2H, CH_2), 2.47–2.51 (m, 2H, CH_2), 2.77 (t, J = 5.6 Hz, 2H, CH_2), 3.31 (t, J = 6.3 Hz, 2H, CH_2), 4.02 (s, 2H, CH_2), 6.57–6.62 (m, 2H, H_{arom}), 6.95–7.05 (m, 2H, H_{arom}), 9.15 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 22.2, 22.4, 26.0, 27.9, 50.4, 56.1, 111.0, 116.8, 122.8, 127.2, 129.3, 145.1, 154.0, 167.7; HRMS: for $C_{14}H_{17}N_3O$ m/z calculated 243.1372, m/z found 243.1363.

6-(3,4-Dihydro-2H-quinolin-1-ylmethyl)-2-methyl-4,5-dihydropyridazin-3(2H)-one, ***3j***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), an orange oil was obtained (80%). ^{1}H NMR ($CDCl_3$): δ 1.97 (m, 2H, CH_2), 2.36–2.41 (m, 2H, CH_2), 2.44–2.49 (m, 2H, CH_2), 2.78 (t, 2H, CH_2), 3.31 (t, 2H, CH_2), 3.35 (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 6.59–6.64 (m, 2H, H_{arom}), 6.92–7.04 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 22.2, 22.7, 26.6, 27.9, 36.3, 50.4, 56.1, 111.0, 116.8, 122.8, 127.2, 129.3, 145.1, 154.4, 165.6; HRMS: for $C_{15}H_{19}N_3O$ m/z calculated 257.1528, m/z found 257.1509.

6-(3,4-Dihydro-2H-isoquinolin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-one, ***3k***
Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a white solid was obtained (80%). Mp 128°C; ^{1}H NMR ($CDCl_3$): δ 2.46 (t, 2H, CH_2), 2.65 (t, 2H, CH_2), 2.75 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 7.00–7.13 (m, 4H, H_{arom}), 9.05 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.0, 26.2, 29.0, 50.9, 56.2, 62.6, 125.7,

126.3, 126.5, 128.7, 134.0, 134.2, 153.8, 168.0; HRMS: for $C_{14}H_{17}N_3O$ $[M\text{-}H^{\cdot}]^+$ calculated 242.1293, found 242.1285.

*6-(3,4-Dihydro-2H-isoquinolin-1-ylmethyl)-2-methyl-4,5-dihydropyridazin-3(2*H*)-one,* ***3l***

Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a yellow oil was obtained (75%). ^{1}H NMR ($CDCl_3$): δ 2.44 (t, 2H, CH_2), 2.62 (t, 2H, CH_2), 2.76 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.37 (s, 3H, CH_3), 3.65 (s, 2H, CH_2), 7.00–7.15 (m, 4H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 23.4, 26.8, 29.1, 50.9, 56.2, 62.6, 125.7, 126.3, 126.5, 128.7, 134.0, 134.3, 154.1, 165.9; HRMS (ESI): for $C_{15}H_{19}N_3O$ $[M+H^+]$ calculated 258.1606, m/z found 258.1609.

*6-(2,3-Dihydro-indol-1-ylmethyl)-4,5-dihydropyridazin-3(2*H*)-one,* ***3m***

Purified by crystallisation from Et_2O, a white solid was obtained (70%). Mp 116–118°C; ^{1}H NMR ($CDCl_3$): δ 2.48 (t, 2H, CH_2), 2.62 (t, 2H, CH_2), 3.00 (t, 2H, CH_2), 3.37 (t, 2H, CH_2), 3.84 (s, 2H, CH_2), 6.50–7.15 (m, 4H, H_{arom}), 9.40 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 22.8, 26.1, 28.6, 54.2, 54.5, 107.0, 118.5, 124.7, 127.4, 129.9, 151.9, 153.5, 167.4.

*6-(2,3-Dihydro-indol-1-ylmethyl)-2-methyl-4,5-dihydropyridazin-3(2*H*)-one,* ***3n***

Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a yellow oil was obtained (55%). ^{1}H NMR ($CDCl_3$): δ 2.46 (t, 2H, CH_2), 2.59 (t, 2H, CH_2), 3.00 (t, 2H, CH_2), 3.36 (s, 3H, CH_3), 3.37 (t, 2H, CH_2), 3.84 (s, 2H, CH_2), 6.50–7.15 (m, 4H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 23.1, 26.7, 28.6, 36.3, 54.1, 54.5, 107.0, 118.4, 124.7, 127.4, 129.8, 151.9, 153.7, 165.7.

*6-[(Benzyl-ethylamino)-methyl]-4,5-dihydropyridazin-3(2*H*)-one,* ***3o***

Purified on silica gel eluting with CH_2Cl_2–MeOH (90:10), a yellow solid was obtained (78%). ^{1}H NMR ($CDCl_3$): δ 1.08 (t, 3H, CH_3), 2.34 (t, 2H, CH_2), 2.54 (m, 2H, CH_2), 2.56 (t, 2H, CH_2), 3.18 (s, 2H, CH_2), 3.58 (s, 2H, CH_2), 7.20–7.32 (m, 5H, H_{arom}), 8.90 ("s", 1H, NH); ^{13}C NMR ($CDCl_3$): δ 11.9, 23.0, 26.1, 48.3, 58.3, 127.1, 128.3, 128.8, 139.3,155.1, 167.9; HRMS: for $C_{14}H_{19}N_3O$ m/z calculated 245.1528, m/z found 245.1531.

Biological assays

Preparation of rat aortic rings and vasorelaxation measurement

Thoracic aortae from male Wistar rats, 400–450 g, were removed, cleaned of fat and adventitial connective tissues and cut into rings of 4–5 mm length. Rings of aorta were suspended into organ bath chambers containing Krebs solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.25, KH_2PO_4 1.14, $MgSO_4$ 2.4, glucose 10, $NaHCO_3$ 25) maintained at 37°C and continually oxygenated with 95% O_2 and 5% CO_2. Changes in tension were measured isometrically by transducer and amplified and enregistered by computer (IOSLAB, EMKA Technologies, France). Experiments were done on sets of 4 rings from the same aorta. Tissues were pre-contracted with 0.3 μM phenylephrine. When the contractile response reached a steady state, compounds were added cumulatively. The relaxation produced by each concentration of compounds was measured after 5 min of incubation and the value was expressed as a percentage of the initial phenylephrine-induced tone. The compounds were dissolved in DMSO; the final concentration of DMSO (0.1%) in the assay did not affect vasorelaxant effect (Vehicle, Table 1). When possible, the concentration of drug that produced 50% of the vasorelaxation expressed vs contraction induced by phenylephrine (ED50) was calculated by non-linear regression analysis from concentration–response curves.

Phosphodiesterase assay

PDE3 activity was determined according to Lugnier et al (1986). Cytosolic PDE3 was isolated by DEAE-sephacel chromatography from media layer of bovine aorta. PDE3 activity was measured by radioenzymatic assay (Lugnier et al 1986), at a substrate concentration of 1 μM cAMP in the presence of 10 000 counts min^{-1} [^{3}H]-cAMP as a tracer. The buffer solution was of the following composition (in mM): Tris-HCl (pH 7.5) 50, magnesium acetate 2, EGTA 1. To prevent the cross influence of reciprocal cross-contamination, PDE3 studies were always carried out in the presence of 50 μM rolipram (specific PDE4 inhibitor). The compounds were dissolved in DMSO; the final concentration of DMSO (1%) in the assay did not affect PDE activity. The concentration of drug that produced 50% inhibition of substrate hydrolysis (IC50) was calculated by non-linear regression analysis from concentration–response curves (Prism software).

Statistical analysis

Statistical analysis was performed with the StatView software on an Apple computer. Statistical comparisons in multiple groups were performed by a two-way analysis of variance. Estimation of the probability value was made by Student's significant difference *t*-test, with $P < 0.05$ being considered as statistically significant. Data are presented as the mean values ± s.e.m. of n experiments as indicated.

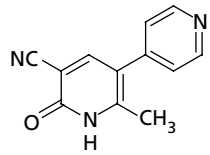
Results and Discussion

Chemistry

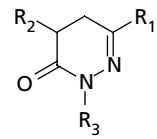
The synthetic strategy used for the preparation of compounds **1a**–**f** is summarized in Figure 3. Polymer-supported γ-ketoesters prepared from Wang resin reacted with several hydrazines to afford the desired pyridazinones in good yields after a cyclization cleavage approach (Gouault et al 2001).

Drawing from this successfully realized cyclorelease strategy, libraries of 4- and 6-substituted analogues (**2** and **3**) were developed by variation of the structure of the building blocks R1, and R2 (Figure 4). Bromination could not be accomplished starting from polymer-supported levulinic

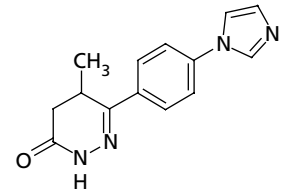
Table 1 Vasodilator efficacy of pyridazinones **1**, **2** and **3** on isolated rat aorta pre-contracted with phenylephrine (0.3 μM)



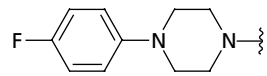
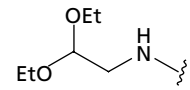
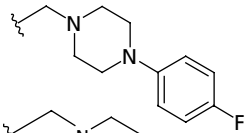
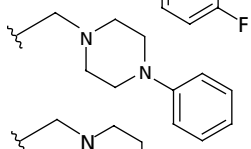
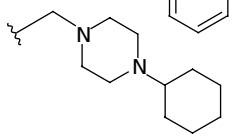
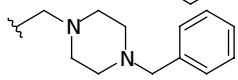
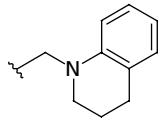
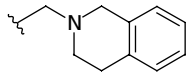
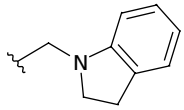
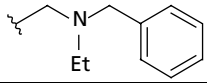
Milrinone



General structure of **1, 2, 3**



CI-930

Compound	Substituent			E_{max} (%)	n
	R_1	R_2	R_3		
Milrinone	—	—	—	67.6 ± 1.7	26
CI-930	—	—	—	73.9 ± 1.9	6
Vehicle	—	—	—	14.7 ± 2.8	26
1a	CH_3	H	H	24.4	1
1b	CH_3	H	CH_3	26.7	1
1c	CH_3	H	CH_2CH_2OH	22.7	1
1d	C_6H_5	H	H	35.55	2
1e	C_6H_5	H	CH_3	26.2	1
1f	C_6H_5	H	CH_2CH_2OH	27.0	1
2a	CH_3		H	21.1	1
2b	CH_3		CH_3	22.25	1
2c	CH_3		H	29.5	1
2d	CH_3		CH_3	9.5	1
3a		H	H	20.0	1
3b		H	CH_3	44.1	2
3c		H	H	28.15	1
3d		H	CH_3	47.1	1
3e		H	H	7.4	2
3f		H	CH_3	17.3	2
3g		H	H	19.3	2
3h		H	CH_3	18.0	2
3i		H	H	33.1	2
3j		H	CH_3	21.9	1
3k		H	H	39.6	1
3l		H	CH_3	70.5	1
3m		H	H	23.6	2
3n		H	CH_3	8.3	2
3o		H	H	23.2	2

E_{max} value, the percent of the maximal vasorelaxation; n, the number of experiments; vehicle, 0.1% DMSO.

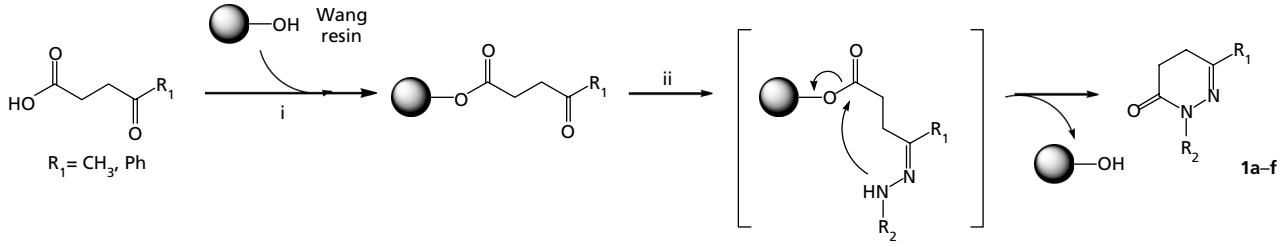


Figure 3 Synthetic routes to substituted 4,5-dihydropyridazin-3(2*H*)-ones (**1**). Reagents and conditions: i, Wang resin (0.65 mmol g^{-1}), γ-ketoacid (2 equiv.), DCI (2 equiv.), DMAP (1%), CH_2Cl_2, r.t., 18 h; ii, R_2NHNH_2 (20 equiv.), THF, 60°C, 1 h.

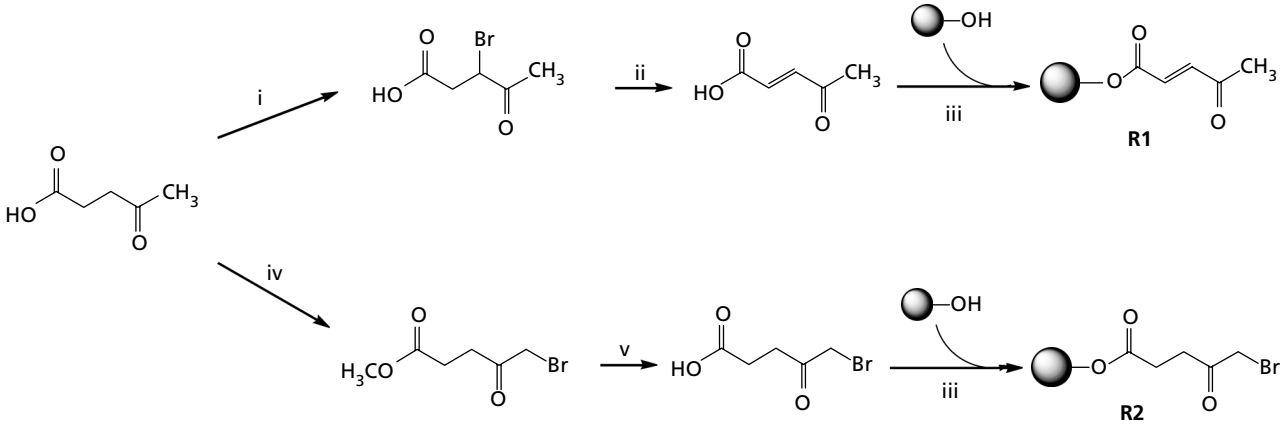


Figure 4 Synthetic routes to polymer-supported starting materials (R1, R2). Reagents and conditions: i, Bromine, conc. HCl, −15°C → r.t., 5 h; ii, Sodium acetate, glacial acetic acid, r.t. then 100°C, 45 min; iii, Wang resin (0.65 mmol g^{-1}), γ-ketoacid (2 equiv.), DCI (2 equiv.), DMAP (1%), CH_2Cl_2, r.t., 18 h; iv, Bromine (1 equiv.), MeOH, 60°C, 2 h; NEt_3 (10 equiv.), CH_2Cl_2, r.t., 1 h; v, conc. HCl, 50°C, 18 h.

acid since a mixture of bromide derivatives was obtained. Consequently, 4-oxo-pent-2-enoic acid was prepared in solution phase from levulinic acid (Porter et al 1991) (bromination and elimination of HBr) followed by anchoring to Wang resin in useful conditions providing R1. For the synthesis of resin R2, bromination in methanol (MacDonald 1974) gave a mixture of methyl 3-bromo- and 5-bromolevulinate. After separation by distillation under reduced pressure, methyl 5-bromolevulinate was converted to the corresponding acid then coupled to the solid support.

Once the starting materials were prepared, it was planned to introduce diversity on R1 via Michael addition of diverse amines. As expected, 4-amino-pyridazinones were obtained after condensation with hydrazines then intracyclative cleavage (Gouault et al 2002) (**2a–d**, Figure 5).

Following the idea of generating diversity on the pyridazinone ring, a library of 6-substituted compounds (**3a–o**) was prepared from polymer-supported 5-bromolevulinic acid R2. Displacement of bromide with excess of diverse amines (i.e., 4-substituted piperazines, tetraisoquinoline . . .) in DMSO at room temperature gave the corresponding amine intermediates. In the final step (condensation with hydrazine or methylhydrazine), the cyclorelease approach previously developed (Gouault et al 2001) was used to afford the desired pyridazinones **3a–o** (Figure 6) in good yields and purities.

Biology

The novel 4,5-dihydropyridazinones **3** and aforementioned compounds **1** and **2** were tested for their vasorelaxant effect at 10 μM on phenylephrine (0.3 μM)-precontracted thoracic aorta removed from a male Wistar rat (Lugnier et al 1986). Milrinone (Sigma, St Louis, MO) and CI-930 (gift from Pfizer, Fresnes, France), well known PDE3 inhibitors and vasorelaxant agents, were used as standard agents. Concentration–response curves (0.01–100 μM) were realized for more effective compounds (Figure 7). The ED50 value denoted the dose of drug producing 50% of the vasorelaxation expressed vs contraction induced by phenylephrine.

At the test concentration of 10 μM, some compounds showed vasorelaxant effects (Table 1). Compound **3l** exhibited the highest efficacy as a vasorelaxant, its activity being similar to that of the references milrinone and CI-930 at 10^{-4} M (Figure 7, Student's *t*-test). The other pyridazinones (**3b, 3d, 3k**) induced a moderate vasorelaxation of isolated aorta lower than that produced by the reference milrinone

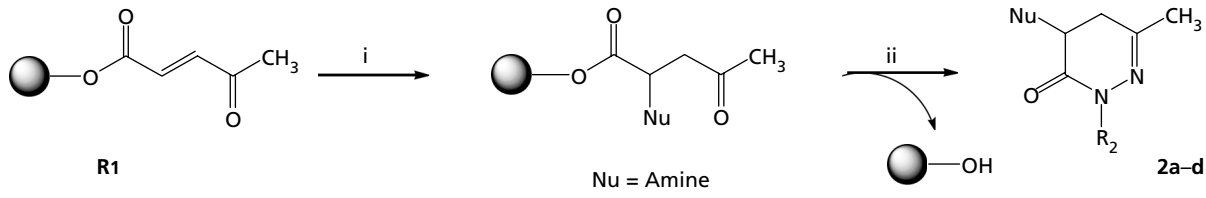


Figure 5 Synthetic routes to substituted 4,5-dihydropyridazin-3(2*H*)-ones (**2a**–**d**). Reagents and conditions: i, Amine (10 equiv.), DMSO, r.t., 1 h; ii, R_2NHNH_2 (10 equiv.), THF:EtOH (1:1), reflux, 1 h.

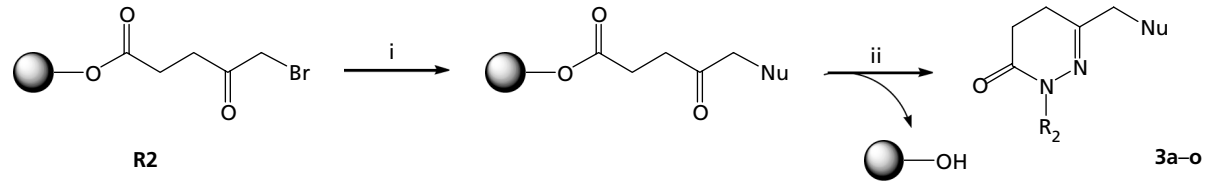


Figure 6 Synthetic routes to substituted 4,5-dihydropyridazin-3(2*H*)-ones (**3a**–**o**). Reagents and conditions: i, Amine (10 equiv.), DMSO, r.t., 1 h; ii, R_2NHNH_2 (10 equiv.), THF:EtOH (1:1), reflux, 1 h.

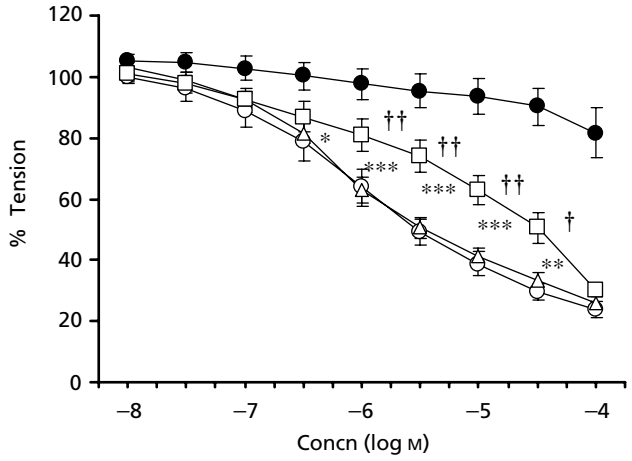


Figure 7 Relaxation of phenylephrine-pre-contracted (0.3 μM) rat aortic rings by compound **3l** (□), milrinone (○), CI-930 (△) and vehicle (●). Values are means ± s.e.m., n = 6. The effect of concentration of each compound and compound type was significant (two-way analysis of variance, $P < 0.001$). Milrinone and CI-930 induced a higher significant vasorelaxation than **3l** except at 10^{-4} M ($^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs CI-930; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs milrinone; Student's *t*-test).

(Table 1). The potency of the highest efficacious compounds was evaluated by the calculation of ED50. ED50 values (n = 6) of the compounds **3l** and **3d** ($30.5 \pm 8.4\,\mu$M ($P < 0.05$) and $42.1 \pm 1.1\,\mu$M ($P < 0.01$), respectively) were significantly higher than those of milrinone and CI-930 ($4.3 \pm 1.6\,\mu$M and $4.4 \pm 1.2\,\mu$M, respectively), showing that these pyridazinones were less potent than the reference agents (Figure 1).

These biological results indicate (see Table 1), in terms of structure–activity relationship, that the most active compounds (**3b, 3d, 3l**) belong to the third series. Structures **1** and **2** revealed, in general, a weak vasorelaxant effect. Additionally, compounds **3b, 3d** and **3l**, having a methyl group on nitrogen 1, exhibited a more potent vasorelaxant activity than their corresponding N-unsubstituted analogues, **3a, 3c** and **3k**. An examination of the data presented in Table 1 for the derivatives **3i**–**n**, bearing bicyclic moieties, indicates that the position of nitrogen on the heterocycle (**3j** and **3l**) and the size of the ring (**3l** and **3n**) had a profound influence on activity. Additionally, ring opening of the bicyclic moiety resulted in loss of activity (**3l** and **3o**). A comparison of the vasodilator efficacy of the pyridazinone derivatives **3a**–**h** demonstrated that the N-phenyl substituent was important for activity (**3d**). Substitution of the phenyl group with fluorine had no effect (**3b**). Replacement of the phenyl moiety by cyclohexyl or benzyl substituents resulted in a loss of vasodilator effect (**3f** and **3h**).

It was previously demonstrated that pyridazinone derivatives induced vasorelaxation (Bowman et al 1999), such as PDE3 inhibitors (Lugnier et al 1986; Nakamura et al 2001). Moreover, it was reported that the vasorelaxant effect of pyridazinone derivatives is partially due to potential PDE3 inhibition with excellent PDE3 inhibitory activity (Sircar et al 1986; Combs et al 1990; Vegh et al 1995; Kato 1997). Therefore we expected that the vasorelaxant effect of compounds **3l** and **3d** was the result of PDE3 inhibition. However, our results showed that compounds **3l** and **3d** induced an inhibition of PDE3 activity inferior to 50% even at high concentration (300 μM: $22.0 \pm 2.0\%$ and $6.3 \pm 3.0\%$, respectively). The concentration that produced 50% of inhibition of substrate hydrolysis (IC50) for these compounds was superior to 300 μM, whereas milrinone and CI-930 had an IC50 of $1.88 \pm 0.14\,\mu$M and $0.84 \pm 0.06\,\mu$M, respectively (Table 2). These results demonstrate that **3l** and **3d** do not selectively inhibit the PDE3 isoenzyme. The mechanism of these pyridazinone derivatives on vasodilatation could be also mediated by Ca^{2+} entry blockade, release of cyclooxygenase products,

Table 2 IC50 of compounds **3d** and **3l**, milrinone and CI-930 on PDE3 activity

	PDE3 IC50 (μM)
3d	>300 (n = 4)
3l	>300 (n = 3)
Milrinone	1.88 ± 0.14 (n = 3)
CI-930	0.84 ± 0.06 (n = 3)

Data are shown as means ± s.e.m. (n = 3–5). IC50, concentration of drug that produced 50% inhibition of substrate hydrolysis.

opening of K^+ channels, nitric oxide release or β-adrenoceptor stimulation. Additional experimentation is needed to resolve their mechanism of vasorelaxation.

Conclusion

We have developed an efficient method for the rapid preparation of 4,5-dihydropyridazinones using a solid support and prepared a small library of compounds with diverse substituents. Our original pyridazinones **3d** and **3l** induced significant vasorelaxation of isolated rat aorta. Furthermore, **3l** showed a similar efficacy compared with reference PDE3 inhibitors, milrinone or CI-930. However, they did not appear to be potent selective inhibitors of PDE3 isoenzyme. Therefore, they should have a therapeutic interest as vasodilator agents but their mechanism of action remains to be demonstrated.

References

Balkenhohl, F., Bussche-Hünnefeld, C., Lansky, A. Zechel, C. (1996) Combinatorial synthesis of small organic molecules. *Angew. Chem. Int. Ed. Engl.* **35**: 2289–2237

Barnes, P. J. (2000) New directions in allergic diseases: mechanism-based anti-inflammatory therapies. *J. Allergy Clin. Immunol.* **106**: 5–16

Bowman, P., Haikala, H., Paul, R. J. (1999) Levosimendan, a calcium sensitizer in cardiac muscle, induces relaxation in coronary smooth muscle through calcium desensitization. *J. Pharmacol. Exp. Ther.* **288**: 316–325

Combs, D. W., Rampulla, M. S., Bell, S. C., Klaubert, D. H., Tobia, A. J., Falotico, R., Haertlein, B., Lakas-Weiss, C., Moore, J. B. (1990) 6-Benzoxaninylpyridazin-3-ones: potent, long-acting positive inotrope and peripheral vasodilator agents. *J. Med. Chem.* **33**: 380–386

Corbin, J. D., Francis, S. H., Webb, D. J. (2002) Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. *Urology* **60**: 4–11

Giembycz, M. A. (2000) Phosphodiesterase 4 inhibitors and the treatment of asthma: where are we now and where do we go from here? *Drugs* **59**: 193–212

Gouault, N., Cupif, J. F., Picard, S., Lecat, A., David, M. (2001) Synthesis of diverse 4,5-dihydro-3(*2H*)-pyridazinones on Wang resin. *J. Pharm. Pharmacol.* **53**: 981–985

Gouault, N., Cupif, J. F., Amoros, M., David, M. (2002) Expedient method for the solid-phase synthesis of some 4-substituted-4,5-dihydropyridazin-3(2H)-ones. *J. Chem. Soc., Perkin Trans. 1* **20**: 2234–2236

Gruhn, N., Nielsen-Kudsk, J. E., Theilgaard, S., Bang, L., Olesen S.-R., Aldershvile, J. (1998) Coronary vasorelaxant effect of levosimendan, a new inodilator with calcium sensitizing properties. *J. Cardiovasc. Pharmacol.* **31**: 741–749

Hall, D. G., Manku, S., Wang, F. (2001) Solution- and solid-phase strategies for the design, synthesis, and screening of libraries based on natural product templates: a comprehensive survey. *J. Comb. Chem.* **3**: 125–150

Kato, K. (1997) Clinical efficacy and safety of pimobendan in treatment of hearth failure – experience in Japan. *Cardiology* **88**: 28–36

Ley, S. V., Baxendale, I. R. (2002) New tools and concepts for modern organic synthesis. *Nat. Rev. Drug Discov.* **1**: 573–586

Lugnier, C., Schoeffer, P., Le Bec, A., Strouthou, E., Stoclet, J. C. (1986) Selective inhibition of cyclic nucleotide phosphodiesterases of human, bovine and rat aorta. *Biochem. Pharmacol.* **35**: 1743–1751

MacDonald, S. F. (1974) Methyl 5-bromolevulinate. *Can. J. Chem.* **52**: 3257–3258

Nakamura, T., Houchi, H., Minami, A., Sakamoto, S., Tsuchiya, K., Niwa, Y., Minakuchi, K, Nakaya, Y. (2001) Endothelium-dependent relaxation by cilostazol, a phosphodiesterase III inhibitor, on rat thoracic aorta. *Life Sci.* **69**: 1709–1715

Pagel, P. S., Hettrick, D. A., Warltier, D. C. (1996) Influence of levosimendan, pimobendan, and milrinone on the regional distribution of cardiac output in anaesthetized dogs. *Br. J. Pharmacol.* **119**: 609–615

Polson, J. B., Strada, S. J. (1996) Cyclic nucleotide phosphodiesterases and vascular smooth muscle. *Annu. Rev. Pharmacol. Toxicol.* **36**: 403–427

Porter, N. A., Scott, D. M., Rosenstein, I. J., Giese, B., Veit, A., Zeitz, H. G. (1991) Stereoselective intramolecular radical additions to amide-substituted alkenes. *J. Am. Chem. Soc.* **113**: 1791–1799

Silver, P. J. (1989) Biochemical aspects of inhibition of cardiovascular low (Km) cyclic adenosine monophosphate diesterase. *Am. J. Cardiol.* **63**: 2A–8A

Sircar, I., Bobowski, G., Bristol, J. A., Weishaar, R. E., Evans, D. B. (1986) Cardiotonic agents. 3. Synthesis and biological activity of novel 6-(substituted 1*H*-imidazol-4(5)-yl)-3(*2H*)-pyridazinones. *J. Med. Chem.* **29**: 261–267

Vegh, A., Papp, J. G., Udvary, E., Kaszala, K. (1995) Hemodynamic effects of calcium-sensitized agents. *J. Cardiovasc. Pharmacol.* **26**: S20–S31

Young, J. B. (2001) New therapeutic choices in the management of acute congestive heart failure. *Rev. Cardiovasc. Med.* **2** (Suppl. 2): S19–S24