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Research Article

Synthesis and Pharmacological Evaluation of 6-Arylpyridazinones as Potent Vasorelaxants

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT As part of a research program to identify compounds with potent antihypertensive properties, a new series of dihydropyridazin-3(2*H*)-one analogs was synthesized and evaluated for vasodilator activity in rat thoracic aortic rings. Most of the newly synthesized compounds displayed good vasorelaxant activity as compared with SK&F-93741 and hydralazine, with the *N*2-unsubstituted 4-isobutyramidophenylpyridazinone derivative **5** being the most potent vasorelaxant, producing vasorelaxation greater than that of hydralazine and equipotent to SK&F-93741. A significant effect of the 6-phenyl substituents and substitution at *N*-2 position of pyridazinone nucleus on the vasodilatory activity was observed. Drug Dev Res 74 : 296–305, 2013.

Key words: vasodilators; dihydropyridazin-3(2*H*)-one; hypertension

INTRODUCTION

Significant efforts have been made to obtain novel antihypertensive agents acting by diverse mechanisms to control blood pressure [Demirayak et al., 2004a], with a wide variety of heterocyclic systems having been explored [Khalil et al., 1980]. The pyridazinones have been investigated in recent decades for biological, medicinal, and agricultural reasons. Pyridazinone derivatives exhibit diverse pharmacological properties [Bansal and Thota, 2012]—for example, antidepressant, antihypertensive, antithrombotic, anticonvulsant, cardiotonic, antibacterial, and anticancer actions [Rubat et al., 1990; Matyus, 1998; Coelho et al., 2003; Siddiqui and Wani, 2004; Thota and Bansal, 2010]. Based on earlier studies and recent work, it has been observed that various pyridazinone derivatives possess antihypertensive activity due to their vasorelaxant activity [Demirayak et al., 2004b; Kumar et al., 2008; Bansal et al., 2009; Costas et al., 2010; Asif et al., 2012]. In this context, our research has focused on the discovery and development of novel pyridazinone-based molecules as potent vasorelaxants.

Because the 6-arylpyridazinone structure is essential for activity in the cardiovascular system, a number of studies with regard to substitutions on both pyridazinone and aryl residues have been performed [Curran and Ross, 1974; Lee et al., 2004] that indicate that amino, acylamino, cyano, and halogen substituents on the phenyl ring produce interesting antihypertensive activity. In an effort to identify novel compounds with

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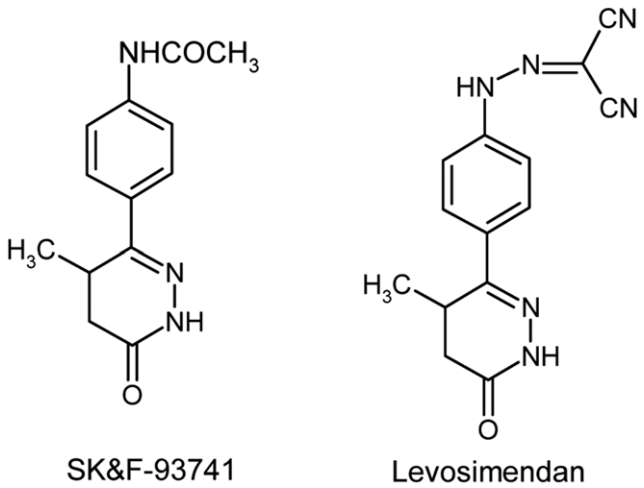


Fig. 1. Structures of pyridazinone-based vasorelaxants.

vasodilatory and antihypertensive activities, our group initially focused on the modestly selective structure of SK&F-93741 (Fig. 1), a dihydropyridazinone derivative possessing vasodilatory properties [Curran and Ross, 1974]. We have also reported the vasorelaxant effect of 6-(4-acetamidophenyl)-2-substituted-4,5-dihydropyridazin-3(2*H*)-ones [Kumar et al., 2008]. The present study extends the exploration of the vasorelaxant activity of dihydropyridazinones by synthesizing a series of derivatives with increased chain length at the acetamido group of phenyl ring.

Literature reports have also revealed that cyano group-containing pyridazinones exhibit appreciable cardiotonic activity with dual inotropic and vasodilatory properties, as exemplified by levosimendan (Fig. 1), a drug used for the treatment of congestive heart failure [Montes et al., 2006]. In view of this and in continuation of our efforts toward synthesis of pyridazinone-based vasorelaxants, several newer derivatives possessing a cyano moiety were also synthesized. As position 2 had remained relatively unexplored in the context of vasorelaxant effects, several 2-substituted pyridazinone derivatives were also prepared.

MATERIALS AND METHODS

The m.p. reported is uncorrected; ^{1}H NMR spectra were recorded on a 300 MHz Brucker AC-300F instrument (Bruker AG, Fallanden, Switzerland) using Me_4Si (TMS) as the internal standard (chemical shifts in δ, ppm). IR spectra were recorded on a Perkin–Elmer 882 and Perkin–Elmer Spectrum RX 1 FT-IR spectrophotometers (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, UK) using potassium bromide pellets (υ_{max} in per cm). Elemental analyses were carried out on a Perkin–Elmer 2400 CHN analyzer. Thin-layer chromatography (TLC) plates were prepared with silica gel G (E. Merck, India) according to Stahl's method using EtOAc as solvent (activated at 110°C for 30 min) and visualized by exposure to iodine vapors. Anhydrous sodium sulfate was used as drying agent. All solvents were dried and freshly distilled prior to use according to standard procedures.

Synthesis

Procedure for synthesis of *N*-phenyl isobutyramide (1)

Isobutyryl chloride (2.5 mL, 23.86 mmol) was added to a stirred solution of aniline (2.0 mL, 21.95 mmol) in dichloromethane (30 mL). The reaction mixture was stirred at room temperature for 1 h under anhydrous conditions. TLC was used to monitor the completion of the reaction. The solvent was removed under reduced pressure to obtain a solid residue. Ice-cold water was added to it, and the obtained precipitate was filtered off, washed with ice-cold water, dried, and recrystallized from a mixture of acetone and petroleum ether (60–80°C).

Yield: 44.66%. m.p.: 108–109°C. IR (KBr), υ, per cm: 3299, 2970, 1662, 1600, 1546, 1441, 1099, 758. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 9.70 ppm (s, 1H, -N*H*COCH$(CH_3)_2$, disappeared on deuterium exchange), 7.53 (d, 2H, aromatic protons *ortho* to isobutyramide, J_o = 7.83 Hz), 7.31 (m, 2H, aromatic protons *meta* to isobutyramide), 7.09 (t, 1H, aromatic proton *para* to isobutyramide, J_o = 7.41 Hz), 2.51 (m, 1H, -C*H*$(CH_3)_2$), 1.25 (d, 6H, -CH(C*H*$_3)_2$, J = 6.87 Hz).

General procedure for synthesis of *N*-phenyl isobutyramide-substituted γ-keto acids (2–4)

A mixture of **1** (2.0 g, 12.25 mmol) and the requisite anhydride, succinic anhydride (1.4 g, 14.00 mmol)/ methylsuccinic anhydride (1.4 g, 12.27 mmol), was added to a stirred solution of aluminum chloride (8 g) in purified carbon disulfide (20 ml). The reaction mixture was stirred manually under anhydrous conditions for 20 min and allowed to stand at room temperature for 48 h. The mixture was decomposed with crushed ice. The solid product obtained was filtered and washed thoroughly with distilled water. The residual solid was dissolved in 5% aqueous sodium bicarbonate solution and the insoluble part was filtered off. Acidification with concentrated HCl resulted in a precipitate, which was filtered, washed with ice-cold water, dried, and recrystallized from methanol to afford the corresponding γ-keto acids, **2** and **3**. Compound **2** (0.5 g, 1.9 mmol)

was added in small portions to the stirred ice-cold mixture of concentrated HNO_3–H_2SO_4 (3:1, 8 mL). The reaction mixture was further stirred in ice for 30–45 min and then at room temperature for 15 min to obtain a yellow, sticky product, 4-(4-isobutyramido-3-nitrophenyl)-4-oxobutyric acid (**4**), which was used in further reactions.

4-(4-Isobutyramidophenyl)-4-oxobutyric acid (2)

Yield: 40.29%. m.p.: 192–193°C. IR (KBr), υ, per cm: 3350, 3290, 2900, 1725, 1678, 1600, 1410, 1210, 840. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 9.49 ppm (s, 1H, -N*H*, disappeared on deuterium exchange), 7.91 (d, 2H, aromatic protons *meta* to isobutyramide, J_o = 8.75 Hz), 7.75 (d, 2H, aromatic protons *ortho* to isobutyramide, J_o = 8.73 Hz), 3.25 (t, 2H, -COC*H*$_2$CH$_2$COOH, J = 6.61 Hz), 2.70 (t, 2H, -COCH$_2$C*H*$_2$COOH, J = 6.57 Hz), 2.66 (m, 1H, -C*H*(CH$_3$)$_2$), 1.21 (d, 6H, -CH(C*H*$_3$)$_2$, J = 6.93 Hz).

4-(4-Isobutyramidophenyl)-3-methyl-4-oxobutyric acid (3)

Yield: 47.08%. m.p.: 188–190°C. IR (KBr), υ, per cm: 3308, 2976, 1728, 1670, 1535, 1409, 1172, 832. ^{1}H NMR (400 MHz, $CDCl_3$ + CF_3COOD, TMS): δ 8.01 ppm (d, 2H, aromatic protons *meta* to isobutyramide, J_o = 8.36 Hz), 7.87 (s, 1H, -N*H*, disappeared on deuterium exchange), 7.67 (d, 2H, aromatic protons *ortho* to isobutyramide, J_o = 8.58 Hz), 3.57 (m, 1H, -COC*H*(CH$_3$)-CH$_2$COOH), 3.20 (m, 2H, -COCH(CH$_3$)C*H*$_2$COOH), 2.71 (sept, 1H, -NHCOC*H*(CH$_3$)$_2$, J = 6.11 Hz), 1.39 (d, 3H, -COCH(C*H*$_3$)CH$_2$COOH, J = 6.92 Hz), 1.30 (d, 6H, -NHCOCH(C*H*$_3$)$_2$, J = 6.80 Hz).

General procedure for synthesis of 6-(4-isobutyramidophenyl)-substituted 4,5-dihydropyridazin-3(2*H*)-ones (5–11)

The requisite hydrazine derivative (3.5 mmol) was added to a stirred and refluxing solution of substituted γ-keto acids **2–4** (3.8 mmol) in aldehyde-free ethanol (25 ml). The reaction mixture was further refluxed with stirring for 7 h (12 h for compounds **6** and **7**). The completion of the reaction was monitored by TLC. The resulting solution was concentrated to half of the volume and left overnight in the refrigerator to yield crystals of the corresponding dihydropyridazinone derivatives, **5–11**. The crystals obtained were washed with cold ethanol, dried, and recrystallized from suitable solvent. The yields of compounds **10** and **11** were calculated from compound **2**, as the nitro derivative **4** was obtained as an oily residue.

6-(4-Isobutyramidophenyl)-4,5-dihydropyridazin-3(2*H*)-one (5)

Recrystallization: methanol. Yield: 57.88%. m.p.: 260–262°C. IR (KBr), υ, per cm: 3309, 2971, 1672, 1615, 1590, 1515, 1346, 843. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 9.80 ppm (s, 1H, -N*H*, pyridazinone, disappeared on deuterium exchange), 9.18 (s, 1H, -N*H*COCH(CH$_3$)$_2$, disappeared on deuterium exchange), 7.68 (m, 4H, aromatic), 2.96 (t, 2H, 5-C*H*$_2$, J = 8.57 Hz), 2.60 (m, 3H, 4-C*H*$_2$ and -NHCOC*H*(CH$_3$)$_2$), 1.22 (d, 6H, -NHCOCH(C*H*$_3$)$_2$, J = 7.06 Hz). Analysis calculated for $C_{14}H_{17}N_3O_2$: C 64.84, H 6.60, N 16.20; found: C 64.92, H 6.55, N 16.42.

6-(4-Isobutyramidophenyl)-2-phenyl-4,5-dihydropyridazin-3(2*H*)-one (6)

Recrystallization: chloroform and methanol. Yield: 59.66%. m.p.: 170–171°C. IR (KBr), υ, per cm: 3330, 1660, 1600, 1530, 1325, 680. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 7.77 ppm (d, 2H, 3-C*H* and 5-C*H*, aromatic protons *meta* to isobutyramide, J_o = 8.60 Hz), 7.59 (m, 4H, 2-C*H* and 6-C*H*, aromatic, *N*-phenyl; aromatic protons *ortho* to isobutyramide), 7.41 (t, 2H, 3-C*H* and 5-C*H*, aromatic, *N*-phenyl, J_o = 7.84 Hz), 7.27 (m, 1H, 4-C*H*, aromatic, *N*-phenyl), 3.07 (t, 2H, 5-C*H*$_2$, J = 8.01 Hz), 2.78 (t, 2H, 4-C*H*$_2$, J = 8.01 Hz), 2.52 (sept, 1H, -NHCOC*H*(CH$_3$)$_2$, J = 6.83 Hz), 1.26 (d, 6H, -NHCOCH(C*H*$_3$)$_2$, J = 6.89 Hz). Analysis calculated for $C_{20}H_{21}N_3O_2$: C, 71.62; H, 6.31; N, 12.53; found: C 71.96, H 6.22, N 12.91.

6-(4-Isobutyramidophenyl)-2-(4-fluorophenyl)-4,5-dihydropyridazin-3(2*H*)-one (7)

Recrystallization: methanol. Yield: 46.36%. m.p. 213–214°C. IR (KBr), υ, per cm: 3295, 2968, 1670, 1604, 1509, 1407, 1330, 1213, 837, 504. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 7.77 ppm (d, 2H, aromatic protons *meta* to isobutyramide, J_o = 8.71 Hz), 7.61 (d, 2H, aromatic protons *ortho* to isobutyramide, J_o = 8.76 Hz), 7.55 (m, 2H, 2-C*H* and 6-C*H*, aromatic, *N*-*p*-fluorophenyl), 7.10 (t, 2H, 3-C*H* and 5-C*H*, aromatic, *N*-*p*-fluorophenyl, J_o = 8.70 Hz), 3.07 (t, 2H, 5-C*H*$_2$, J = 7.51 Hz), 2.77 (t, 2H, 4-C*H*$_2$, J = 8.03 Hz), 2.53 (sept, 1H, -NHCOC*H*(CH$_3$)$_2$, J = 6.13 Hz),1.27 (d, 6H, -NHCOCH(C*H*$_3$)$_2$, J = 6.75 Hz). Analysis calculated for $C_{20}H_{20}N_3O_2F$: C 67.97, H 5.70, N 11.89; found: C 67.63, H 5.38, N 11.89.

2-(4,5-Dihydro-1H-imidazol-2-yl)-6-(4-isobutyramidophenyl)-4,5-dihydropyridazin-3(2*H*)-one (8)

Recrystallization: methanol. Yield: 48.25%. m.p.: 170–171°C. IR (KBr), υ, per cm: 3394, 1658, 1542,

1398, 1324, 845. ^{1}H NMR (400 MHz, $CDCl_3$+ CF_3COOD, TMS): δ 9.50 ppm (s, 1H, -N*H*COCH $(CH_3)_2$, disappeared on deuterium exchange), 7.78 (d, 2H, aromatic protons *meta* to isobutyramide, $J_o = 8.77$ Hz), 7.66 (d, 2H, aromatic protons *ortho* to isobutyramide, $J_o = 8.75$ Hz), 3.75 (s, 4H, 2× -CH_2-, imidazoline), 3.18 (t, 2H, 5-CH_2, $J = 8.04$ Hz), 2.61 (m, 1H, -C*H*$(CH_3)_2$), 2.40 (t, 2H, 4-CH_2, $J = 8.01$ Hz), 1.20 (d, 6H, -CH(CH_3)$_2$, $J = 6.74$ Hz). Analysis calculated for $C_{17}H_{21}N_5O_2 \cdot H_2O$: C 59.81, H 6.71, N 20.27; found: C 59.56, H 7.0, N 20.42.

6-(4-Isobutyramidophenyl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (9)

Recrystallization: methanol. Yield: 63.14%. m.p.: 241–242°C. IR (KBr), υ, per cm: 3314, 3207, 3055, 2970, 2930, 1671, 1527, 1345, 840, 568. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 10.07 ppm (s, 1H, -N*H*, pyridazinone, disappeared on deuterium exchange), 9.37 (s, 1H, -N*H*COCH$(CH_3)_2$, disappeared on deuterium exchange), 7.67 (m, 4H, aromatic), 3.05 (m, 1H, 5-C*H*(CH_3)), 2.59 (m, 3H, -NHCOC*H*$(CH_3)_2$ and 4-CH_2), 1.27 (d, 3H, 5-CH(CH_3), $J = 3.15$ Hz), 1.21 (d, 6H, -NHCOCH(CH_3)$_2$, $J = 6.72$ Hz). Analysis calculated for $C_{15}H_{18}N_3O_2$: C 66.15, H 6.66, N 15.42; found: C 65.93, H 7.01, N 15.63.

6-(4-Isobutyramido-3-nitrophenyl)-4,5-dihydropyridazin-3(2*H*)-one (10)

Recrystallization: methanol. Yield: 45.59%. m.p.: 240–242°C. IR (KBr), υ, per cm: 3360, 3186, 3077, 2926, 1686, 1620, 1517, 1332, 1260, 1143, 826. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 10.46 ppm (s, 1H, -N*H*COCH$(CH_3)_2$, disappeared on deuterium exchange), 10.38 (s, 1H, -N*H*, pyridazinone, disappeared on deuterium exchange), 8.76 (d, 1H, aromatic proton *meta* to nitro, $J_o = 9.06$ Hz), 8.54 (d, 1H, aromatic proton *ortho* to nitro, $J_m = 2.13$ Hz, $J = 6.87$ Hz), 8.05 (dd, 1H, aromatic proton *para* to nitro, $J_o = 9.05$ Hz, $J_m = 2.12$ Hz), 3.0 (t, 2H, 5-CH_2, $J = 8.40$ Hz), 2.69 (m, 1H, -NHCOC*H*$(CH_3)_2$), 2.60 (t, 2H, 4-CH_2, $J = 8.64$ Hz),1.31 (d, 6H, -NHCOCH(CH_3)$_2$). Analysis calculated for $C_{14}H_{16}N_4O_4$: C 55.25, H 5.30, N 18.41; found: C 55.38, H 4.96, N 18.52.

6-(4-Isobutyramido-3-nitrophenyl)-2-(4-fluorophenyl)-4,5-dihydropyridazin-3(2*H*)-one (11)

Recrystallization: ethanol. Yield: 23.22%. m.p.: 238–239°C. IR (KBr), υ, per cm: 3354, 2979, 1685, 1503, 1332, 1269, 1218, 1151, 839. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 10.56 ppm (s, 1H, -N*H*COCH$(CH_3)_2$, disappeared on deuterium exchange), 8.92 (d, 1H, aromatic proton *meta* to nitro, $J_o = 9.18$ Hz), 8.59 (d, 1H, aromatic proton *ortho* to nitro, $J_m = 2.16$ Hz), 8.11 (dd, 1H, aromatic proton *para* to nitro, $J_o = 9.09$ Hz, $J_m = 2.18$ Hz), 7.53 (m, 2H, 2-C*H* and 6-C*H*, aromatic, *N*-*p*-fluorophenyl), 7.12 (m, 2H, 3-C*H* and 5-C*H*, aromatic, *N*-*p*-fluorophenyl), 3.10 (t, 2H, 5-CH_2, $J = 7.51$ Hz), 2.81 (t, 2H, 4-CH_2, $J = 7.81$ Hz), 2.68 (m, 1H, -NHCOC*H*$(CH_3)_2$), 1.32 (d, 6H, -NHCOCH(CH_3)$_2$, $J = 6.27$ Hz). Analysis calculated for $C_{20}H_{19}N_4O_4F$: C 60.29, H 4.81, N 14.06; found: C 59.99, H 4.70, N 14.40.

Procedure for synthesis of phenoxyacetonitrile (12)

Chloroacetonitrile (1.0 mL, 15.80 mmol) was added to a stirred and refluxing suspension of phenol (1.0 g, 9.08 mmol) and anhydrous potassium carbonate (2.0 g, 14.47 mmol) in ethyl methyl ketone (60 ml). The reaction mixture was further refluxed for 12 h with continuous stirring. The completion of the reaction was monitored by TLC. The reaction mixture was cooled and filtered, and the excess of solvent was removed under reduced pressure to obtain an oily residue of **12**, which was used as such for further reactions.

^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 7.35 ppm (t, 2H, aromatic protons *meta* to alkoxy, $J = 7.11$ Hz), 7.08 (m, 1H, aromatic proton para to alkoxy), 6.98 (d, 2H, aromatic protons *ortho* to alkoxy, $J_o = 8.61$ Hz), 4.74 (s, 2H, -OCH_2-).

General procedure for synthesis of 4-(4-cyanomethoxyphenyl)-substituted γ-keto acids (13, 14)

A mixture of **12** (1.3 g, 9.76 mmol) and succinic anhydride (1.3 g, 12.99 mmol)/methyl succinic anhydride (1 g, 8.76 mmol) was added to a stirred solution of aluminum chloride (5 g) in nitrobenzene (10 mL). The reaction mixture was stirred manually under anhydrous conditions for 20 min and allowed to stand for 48 h at room temperature. Reaction contents were decomposed with crushed ice and steam distilled to remove nitrobenzene. The hot solution was filtered, and the filtrate was cooled in ice for complete precipitation. The solid product obtained was filtered and washed thoroughly with distilled water. The resulting residue was dissolved in 10% aqueous sodium bicarbonate solution and the insoluble part was filtered off. Acidification with concentrated HCl gave a precipitate, which was filtered, washed with water, dried, and recrystallized from methanol to afford **13** and **14**. The yields of both compounds **13** and **14**, obtained from two-step reactions, were calculated from the starting material phenol and not from compound **12**, which was obtained as an oily residue.

4-(4-Cyanomethoxyphenyl)-4-oxobutyric acid (13)

Yield: 37.32%. m.p.: 112–114°C. IR (KBr), υ, per cm: 3020, 1700, 1680, 1600, 1440, 1240, 850. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 8.01 ppm (dd, 2H, aromatic protons *meta* to alkoxy, J_o = 6.72 Hz, J_m = 2.09 Hz), 7.05 (dd, 2H, aromatic protons *ortho* to alkoxy, J_o = 6.75 Hz, J_m = 2.05 Hz), 4.87 (s, 2H, -OC*H*$_2$-), 3.27 (t, 2H, -COC*H*$_2$CH$_2$COOH, J = 6.64 Hz), 2.75 (t, 2H, -COCH$_2$C*H*$_2$COOH, J = 6.75 Hz).

4-(4-Cyanomethoxyphenyl)-3-methyl-4-oxobutyric acid (14)

Yield: 30.16%. m.p.: 110–111°C. IR (KBr), υ, per cm: 3020, 1700, 1600, 1440, 1240, 1180, 850. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 8.08 ppm (d, 2H, aromatic protons *meta* to alkoxy, J_o = 8.78 Hz), 7.03 (d, 2H, aromatic protons *ortho* to alkoxy, J_o = 8.69 Hz), 4.95 (s, 2H, -OC*H*$_2$-), 3.44 (m, 1H, -COC*H*(CH$_3$) CH$_2$COOH), 2.97 (m, 2H, -COCH(CH$_3$)C*H*$_2$COOH), 1.28 (d, 3H, -COCH(C*H*$_3$)CH$_2$COOH, J = 7.12 Hz).

General procedure for synthesis of 6-(4-cyanomethoxyphenyl)-substituted 4,5-dihydropyridazin-3(2*H*)-one (15–18)

The requisite hydrazine derivative (3.5 mmol) was added to a stirred and refluxing solution of substituted γ-keto acids **13** and **14** (3.8 mmol) in aldehyde-free ethanol (25 mL). The reaction mixture was further refluxed with stirring. The completion of the reaction was monitored by TLC. The resulting solution was concentrated to half of the volume and left overnight in the refrigerator to afford crystals of corresponding dihydropyridazinone derivatives **15–18**. The crystals obtained were washed with cold ethanol, dried, and recrystallized from suitable solvent.

6-(4-Cyanomethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-one (15)

Recrystallization: ethanol. Yield: 58.14%. m.p.: 162–163°C. IR (KBr), υ, per cm: 3256, 2922, 1666, 1622, 1603, 1518, 1435, 1345, 1318, 1113, 1050, 990, 771. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 7.75 (dd, 2H, aromatic protons *meta* to alkoxy, J_o = 8.84 Hz, J_m = 2.97 Hz), 7.02 (dd, 2H, aromatic protons *ortho* to alkoxy, J_o = 8.87 Hz, J_m = 2.91 Hz), 4.88 (s, 1H, -OC*H*$_2$-), 2.96 (t, 2H, 5-C*H*$_2$, J = 8.18 Hz), 2.58 (t, 2H, 4-C*H*$_2$, J = 7.7 Hz). Analysis calculated for $C_{12}H_{11}N_3O_2$: C 62.87, H 4.84, N 18.33; found: C 62.54, H 4.83, N 18.32.

6-(4-Cyanomethoxyphenyl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (16)

Recrystallization: acetone. Yield: 54.20%. m.p.: 185–186°C. IR (KBr), υ, per cm: 3240, 2928, 1670, 1510, 1346, 1246, 1044, 836. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 8.64 ppm (s, 1H, N*H*, disappeared on deuterium exchange), 7.72 (dd, 2H, aromatic protons *meta* to alkoxy, J_o = 8.91 Hz, J_m = 1.82 Hz), 7.01 (dd, 2H, aromatic protons *ortho* to alkoxy, J_o = 8.99 Hz, J_m = 2.03 Hz), 4.81 (s, 2H, -OC*H*$_2$-), 3.05 (m, 1H, 5-C*H*(CH$_3$)), 2.62 (m, 2H, 4-C*H*$_2$), 1.32 (d, 3H, 5-CH(C*H*$_3$), J = 6.76 Hz). Analysis calculated for $C_{13}H_{13}N_3O_2$: C 64.18, H 5.38, N 17.27; found: C 64.01, H 5.41, N 16.82.

6-(4-Cyanomethoxyphenyl)-2-phenyl-4,5-dihydropyridazin-3(2*H*)-one (17)

Recrystallization: methanol. yield: 55.12%. m.p.: 103–104°C. IR (KBr), υ, per cm: 3062, 1672, 1599, 1507, 1325, 1246, 1184, 1043, 836, 605. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6, TMS): δ 7.81 ppm (d, 2H, aromatic protons *meta* to alkoxy, J_o = 8.81 Hz), 7.58 (d, 2H, 2-C*H* and 6-C*H*, aromatic, *N*-phenyl, J_o = 7.74 Hz), 7.42 (t, 2H, 3-C*H* and 5-C*H*, aromatic, *N*-phenyl, J_o = 7.77 Hz), 7.28 (t, 1H, 4-C*H*, aromatic, *N*-phenyl, J_o = 7.31 Hz), 7.01 (d, 2H, aromatic protons *ortho* to alkoxy, J_o = 8.84 Hz), 4.79 (s, 2H, -OC*H*$_2$-), 3.06 (t, 2H, 5-C*H*$_2$, J = 8.11 Hz), 2.78 (t, 2H, 4-C*H*$_2$, J = 8.01 Hz). Analysis calculated for $C_{18}H_{15}N_3O_2$: C 70.80, H 4.95, N 13.76; found: C 70.51, H 4.56, N 13.65.

6-(4-Cyanomethoxyphenyl)-2-(4,5-dihydro-1*H*-imidazol-2-yl)-2-phenyl-4,5-dihydropyridazin-3(2*H*)-one (18)

Recrystallization: water. Yield: 54.28%. m.p.: 204–205°C. IR (KBr), υ, per cm: 3369, 3063, 2922, 1658, 1557, 1399, 1244, 1040, 840. ^{1}H NMR (400 MHz, $CDCl_3$+ CF_3COOD, TMS): δ 7.26 ppm (s, 2H, aromatic protons *meta* to alkoxy), 7.10 (d, 2H, aromatic protons *ortho* to alkoxy, J_o = 8.22 Hz), 4.97 (s, 2H, -OC*H*$_2$-), 3.90 (brs, 4H, 2 x -C*H*$_2$-, imidazoline), 3.07 (brs, 2H, 5-C*H*$_2$), 2.83 (brs, 2H, 4-C*H*$_2$). Analysis calculated for $C_{15}H_{15}N_5O_2$:.H_2O C 60.59, H 5.43, N 22.21; found: C 60.23, H 5.22, N 22.01.

Vasodilatory Activity

The vasodilatory activity of the newly synthesized compounds was studied using descending thoracic aortic rings of Wistar rats precontracted with

phenylephrine (10^{-6} M), as previously reported (Olmo et al., 2006; Bansal et al., 2009). The pharmacological protocol was reviewed by the University of Salamanca, and animal usage was approved by the institutional ethical committee.

Wistar rats of either sex weighing 300–400 g were killed by a blow on the head. The descending thoracic aorta was rapidly dissected and placed in a physiological saline solution (PSS) of the following composition: NaCl (118 mM), KCI (4.75 mM), $NaHCO_3$ (25 mM), $MgSO_4$ (1.2 mM), $CaCl_2$ (1.8 mM), KH_2PO_4 (1.2 mM), and glucose (11 mM). After excess fat and connective tissue were removed, the aortas were cut into rings (4–5 mm in length), mounted under a basal tension of 2 g in 5-mL organ baths containing PSS and attached to force-displacement transducers to measure isometric contractile force. The tissue bath was maintained at 37°C and bubbled with an O_2/CO_2 (95:5) gas mixture. Each preparation was allowed to equilibrate for at least 90 min prior to initiation of experimental procedures, and during this period the incubation media were changed every 20 min. After equilibration, aortic rings were contracted by a single concentration of phenylephrine (10^{-6} M). When the contractions became stable, compounds were added in progressively increasing cumulative concentrations (10^{-8}–10^{-5} M) at 30-min intervals. Only one compound was tested in each ring. All compounds were initially dissolved in DMSO to prepare a 10^{-2} M stock solution. Further solutions were made in PSS. Results are expressed as means ± SEM. The compound response of the aortic rings was expressed as a percentage of the initial contraction to 10^{-6} M phenylephrine. Dose–response curves were analyzed by a sigmoidal curve-fitting analysis to give the pD_2 value (the drug concentration exhibiting 50% of the E_{max} expressed as negative log molar) and E_{max} value (the maximal effect).

RESULTS AND DISCUSSION

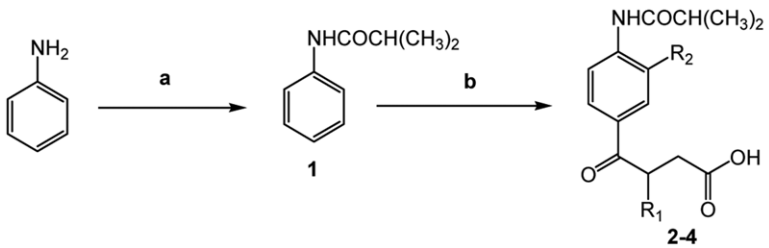
Chemistry

The synthesis of new pyridazinone derivatives has been carried out according to Figures 2 and 3. *N*-Phenylisobutyramide (**1**), which was used as starting compound for the synthesis of pyridazinone derivatives, was obtained by reacting aniline with isobutyryl chloride in dichloromethane at room temperature. The Friedel–Crafts acylation reaction of compound **1** with succinic anhydride or methylsuccinic anhydride in the presence of anhydrous aluminum chloride afforded γ-ketoacids, **2** and **3**. The 4-(4-isobutyramidophenyl)-4-oxobutyric acid (**2**) and its 3-methyl analog **3** recorded C = O absorption bands for saturated aliphatic acid near 1720 and for amide at 1680 per cm. Triplets of α and β methylene protons appeared at δ 2.70 and 3.25 ppm, respectively, in the ^{1}H NMR spectrum of compound **2**. A downfield and separated methine signal at δ 3.57 ppm in the case of **3** confirmed the formation of a β-methyl derivative. As part of the research program, the effect of introducing a nitro group *ortho* to the 4-acylamino substituent of the 6-phenyl ring on the activity profile of pyridazinones was also investigated. For this, nitration of compound **2** was carried out in an ice-cold mixture of concentrated HNO_3–H_2SO_4 (3:1), which yielded a yellow, sticky product, **4**. Infrared spectra of compound **4** showed asymmetrical and symmetrical stretching bands characteristic of a nitro group in conjugation with aromatic ring system near 1510 and 1345 per cm, respectively. Further treatment of the γ-keto acids **2–4** with the requisite hydrazine hydrate resulted in the formation of the corresponding desired pyridazinones **5–11**, which exhibited clear N-H stretching absorption frequencies, characteristic of secondary amides near 3300, and amidic C = O stretching bands of pyridazinone residue at 1660–1685 per cm.

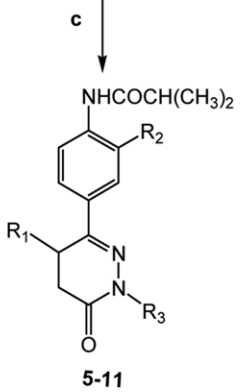
The methylene protons of the parent pyridazinone ring in compound **5** appeared as triplets at δ 2.60 (4-C*H*$_2$) and 2.96 (5-C*H*$_2$), while those of the 2-substituted pyridazinones **6** and **7** appeared downfield at 2.77 (4-C*H*$_2$) and 3.07 ppm (5-C*H*$_2$). A doublet, integrating for six protons, for two methyl of the isobutyramido moiety was recorded at δ ~1.2 ppm for all these 6-(4-isobutyramidophenyl) pyridazinones. For the *N*-imidazolinyl-substituted compound **8**, the methylene protons of C_4 and C_5 of the pyridazinone nucleus appeared as triplets at δ 2.40 and 3.18 ppm, respectively, while those of the imidazoline ring resonated downfield at δ 3.75 ppm.

Compound **9**, 6-(4-isobutyramidophenyl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one, exhibited characteristic PMR signals at δ 1.27 (d, 3H, 5-CH(C*H*$_3$)), 2.59 (m, 3H, -NHCOC*H*(CH$_3$)$_2$ and 4-C*H*$_2$), and 3.05 ppm (m, 1H, 5-C*H*(CH$_3$)). Aromatic protons of the 6-phenyl ring displayed distinct signals at δ ~8.1 (dd, 1H, aromatic proton *para* to nitro), 8.55 (d, 1H, aromatic proton *ortho* to nitro), and 8.80 ppm (d, 1H, aromatic proton *meta* to nitro) for both of the nitropyridazinones **10** and **11**.

The cyano group-containing pyridazinones were prepared by Friedel–Crafts acylation of phenoxyacetonitrile **12** with appropriate anhydride to form γ-keto acids **13** and **14**, which on cyclocondensation with various hydrazine derivatives gave the target compounds **15–18** (Fig 3). The pyridazinone C_4 protons for 6-(4-cyanomethoxy phenyl)-4,5-dihydropyridazin-3(2*H*)-one (**15**) were recorded at δ ~2.60 ppm. In case of compound **17**, protons for the *N*- substituted aromatic ring appeared in the range of δ 7.28–7.58 ppm.



Comp. No.	R_1	R_2
2	-H	-H
3	$-CH_3$	-H
4	-H	$-NO_2$



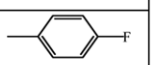
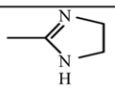
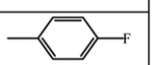
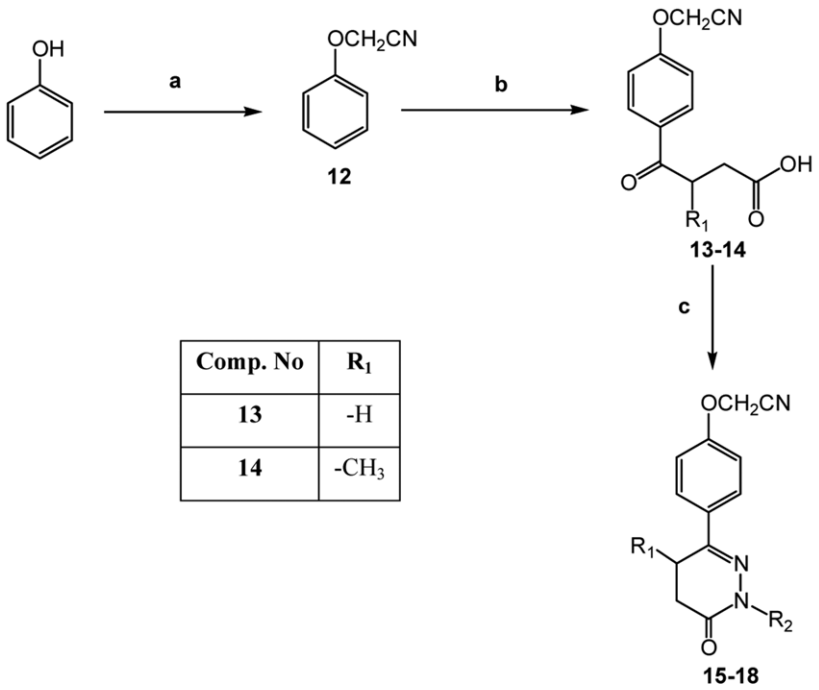
Comp. No.	R_1	R_2	R_3
5	-H	-H	-H
6	-H	-H	
7	-H	-H	
8	-H	-H	
9	$-CH_3$	-H	-H
10	-H	$-NO_2$	-H
11	-H	$-NO_2$	

Fig. 2. Reagents and conditions: (a) isobutyryl chloride, dichloromethane, stirred at room temperature; (b) aluminum chloride, succinic anhydride/methyl succinic anhydride, ice-cold mixture of concentrated HNO_3–H_2SO_4 (3:1); (c) hydrazine hydrate/phenylhydrazine hydrochloride/*p*-fluorophenylhydrazine hydrochloride/2-hydrazine-2-imidazoline hydrobromide, absolute ethanol.

Similarly, for compound **18**, the imidazolinyl protons resonated as a four-proton broad singlet at δ 3.90 ppm.

Vasodilatory Activity

The compounds **5–11** and **15–18** were evaluated for vasodilatory activity using rat thoracic aortic rings, precontracted with phenylephrine (10^{-6} M). The results were expressed as percentage of the maximal control phenylephrine-induced responses. Maximal relaxation (% of control) and pD_2 (–log IC_{50}) and IC_{50} values of various compounds to inhibit the contractions induced by phenylephrine (10^{-6} M) are shown in Table 1. Imidazolinyl derivatives **8** and **18** could not be screened for vasodilatory activity because of solubility problems.



Comp. No	R_1
13	-H
14	$-CH_3$

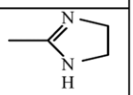
Comp. No.	R_1	R_2
15	-H	-H
16	$-CH_3$	-H
17	-H	
18	-H	

Fig. 3. Reagents and conditions: (a) Chloroacetonitrile and anhydrous potassium carbonate in ethyl methyl ketone; (b) aluminum chloride, succinic anhydride/methyl succinic anhydride; (c) hydrazine hydrate/phenylhydrazine hydrochloride/2-hydrazine-2-imidazoline hydrobromide, absolute ethanol.

Concentration–response curves for vasorelaxant activity of compounds on aortic rings, obtained from Wistar rats are depicted in Figure 4. In both series of newly synthesized 6-(*para*-substituted phenyl) pyridazinones, most of the compounds produced a concentration-dependent inhibition of the contractile response to phenylephrine and produced vasorelaxation comparable with that of hydralazine and SK&F-93741 (Fig. 4) except the *N*2-phenyl-substituted derivatives **6** and **17**. The *N*2-unsubstituted pyridazinone derivatives **5** ($IC_{50} = 0.199 \pm 0.1$ μM) and **9** ($IC_{50} = 0.158 \pm 0.2$ μM) in the isobutyramide series and **15** ($IC_{50} = 7.94 \pm 0.17$ μM) and **16** ($IC_{50} = 3.98 \pm 0.2$ μM) in the acetonitrile series exhibited potent vasorelaxant activity (Table 1). However, an unsubstituted nitrogen on the pyridazinone nucleus does not seem to be a necessary condition for a compound to be vasorelaxant, as *N*2-*p*-fluorophenyl derivatives **7** ($IC_{50} = 1.0 \pm 0.1$ μM) and **11** ($IC_{50} = 0.63 \pm 0.06$ μM) also produced good vasorelaxation.

Methyl substitution at the 5-position of the pyridazinone skeleton in compounds **9** and **16** resulted in slightly increased vasorelaxant action than 5-unsubstituted analogs **5** and **15**, respectively. This is in accord with earlier reports, indicating that methyl substitution at the 5-position of the pyridazinone results in increased antihypertensive activity [Curran and Ross, 1974].

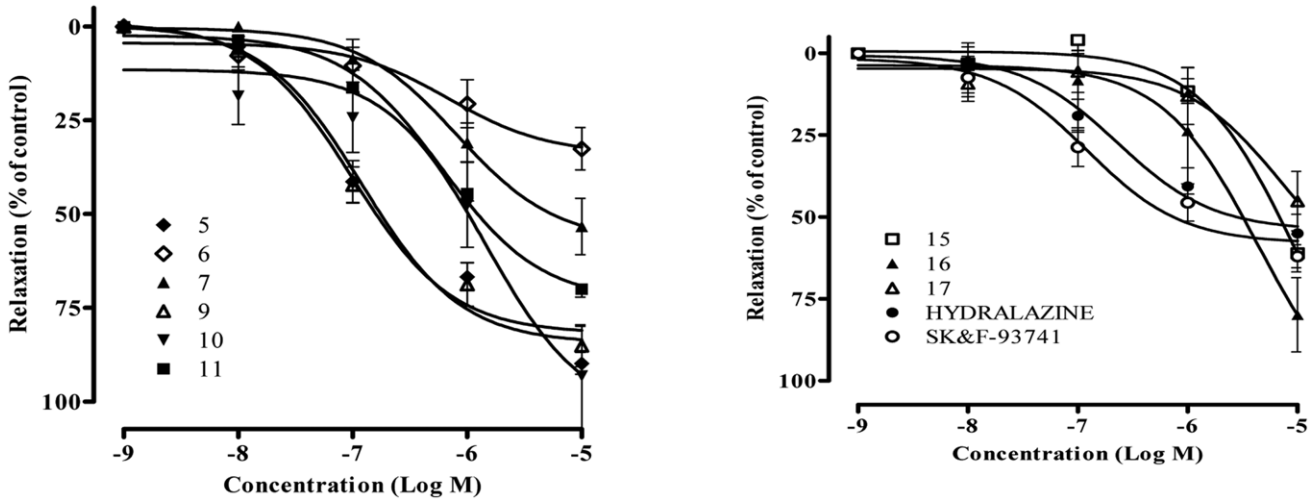


Fig. 4. Concentration–response curves for vasorelaxant activity of compounds shown on aortic rings obtained from Wistar rats. Each data point represents the mean ± SEM (n = 4–6).

TABLE 1. Maximal Response (E_{max}), pD_2, and IC_{50} (μM) Values Needed to Inhibit the Contractions Induced by Phenylephrine (10^{-6} M)

Compound (code)	E_{max} (%)	pD_2	IC_{50} (μM)
5 (DPJ-943)	89.8 ± 2.9*	6.7 ± 0.1	0.199 ± 0.1
6** (DPJ-RG-1224)	32.58 ± 5.65	—	—
7 (DPJ-RG-1225)	53.3 ± 7.5*	6.0 ± 0.1	1.0 ± 0.1
9 (DPJ-965)	85.1 ± 5.6*	6.8 ± 0.2	0.158 ± 0.2
10 (DPJ-RG-1351)	93.1 ± 13.1*	6.1 ± 0.01	0.794 ± 0.01
11 (DPJ-RG-300)	70.0 ± 2.2*	6.2 ± 0.06	0.63 ± 0.06
15 (DPJ-RG-1242)	61.06 ± 5.74*	5.10 ± 0.17	7.94 ± 0.17
16 (DPJ-RG-1352)	80.0 ± 11.31*	5.4 ± 0.2	3.98 ± 0.2
17** (DPJ-RG-1279)	45.20 ± 9.07*	—	
DMSO	1.80 ± 7.01	—	—
Hydralazine	55.0 ± 5.8*	6.5 ± 0.1	0.316 ± 0.1
SK&F-93741	62.0 ± 3.5*	6.7 ± 0.2	0.199 ± 0.2

*P < 0.05 versus DMSO.
**Compounds did not produce 50% relaxation up to 10^{-5} M.
Values are expressed as mean ± SEM, n = 4–8.

In general, compounds **5**, **7**, and **9–11**, possessing an isobutyramide substituent at the *para* position of the 6-phenyl ring, displayed better vasorelaxant effect in comparison with the acetonitrile analogs **15–17**. Introduction of a nitro functionality on the 6-phenyl ring in the case of isobutyramide-substituted pyridazinones **10** and **11** did not bring much change in vasodilatory activity. Overall, the 6-(*p*-isobutyramidophenyl) pyridazinones represent a new series of pyridazinone derivatives with potent vasorelaxant action.

In conclusion, two new series of 6-arylpyridazinone derivatives were synthesized. Their structures were confirmed using various spectral and elemental analysis data, and they were screened for vasorelaxant activity. The *N*2-unsubstituted 4-isobutyramidophenylpyridazinone derivative **5** emerged as the most potent vasorelaxant among the synthesized derivatives, producing vasorelaxation that was greater than hydralazine and equipotent to SK&F-93741, thus providing a possible lead structure for development of new vasorelaxants.

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