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Original research paper

Synthesis and evaluation of 2-substituted-6-phenyl-4,5-dihydropyridazin-3(2*H*)-ones as potent inodilators

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The present study describes the synthesis and pharmacological evaluation of 2-substituted-6-(4-acylaminophenyl)-4,5-dihydropyridazin-3(2*H*)-ones as potent inodilating agents. The synthesis of target compounds **2–4** and **7–11** was achieved by Friedel-Crafts acylation of appropriate anilide derivative with succinic anhydride or methylsuccinic anhydride and subsequent cyclization of intermediary keto acids with various hydrazine derivatives. The newly synthesized pyridazinone derivatives were evaluated for cardiotonic activity using isolated rat atria and for vasorelaxant activity using descending thoracic aortic rings of Wistar rats precontracted with phenylephrine (10^{-6} mol L^{-1}). 6-(4-Methanesulfonamidophenyl)-2-phenyl-4,5-dihydropyridazin-3(2*H*)-one (**7**) exhibited significant inodilatory properties and showed vasorelaxant activity in a nanomolar range (IC_{50} = 0.08 ± 0.01 μmol L^{-1}).

Keywords: pyridazinones, anilides, hydrazine derivatives, cardiotonic activity, vasodilatory activity

The inotropic and vasodilatory properties of 4,5-dihydro-6-phenylpyridazin--3(2*H*)-ones are well documented in literature (1–3). Pyridazinone derivatives like SK&F--93741, its nor-methyl derivative and levosimendan (Fig. 1) possess a substituted amino group at *para*-position of 6-phenyl ring and have emerged as potent cardiotonic agents with dual inotropic and vasodilatory properties in higher animals (4–6). These pyridazinone-based cardiotonics have shown good promise in the treatment of congestive heart failure; however, species differences in inotropic response to these agents have been observed (7). It is also evident from literature reports that the most dramatic alterations in the potency of pyridazinone based cardiotonics result from varying *para*-substituents of the phenyl ring attached to 4-position of pyridazinone nucleus. However, position 2 of the pyridazinone ring remains relatively unexplored. Pyridazin-3(2*H*)-ones further drew our attention because of their easy functionalization at various ring positions (1), which makes them attractive synthetic building blocks for designing and development of novel pyridazinone based cardiotonic agents.

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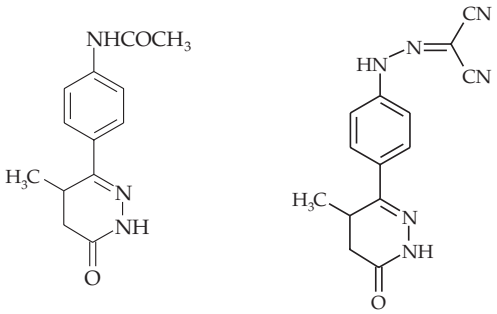


Fig. 1. Structural formulae of two pyridazinone based cardiotonic agents.

In the present study, several 2-substituted derivatives of 6-(4-acylaminophenyl)pyridazinones have been prepared and evaluated for their inotropic and vasodilatory properties to investigate the effects produced by incorporating substituents at 2-position of the pyridazinone ring. Several sulphonamido pyridazinone derivatives were also synthesized to compare their biological effects with their acetamido congeners. The synthesis of target pyridazinones was achieved by the Friedel-Crafts acylation (8, 9) of anilides with anhydrides of succinic acid and its 2-methyl derivative to afford γ-keto acids, which on subsequent cyclization using suitable hydrazine derivatives gave the target compounds **2–4** and **7–11**.

EXPERIMENTAL

Melting points were determined on a Veego melting point apparatus (India) and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 882 (UK) and a Perkin--Elmer spectrum RX 1, FT-IR spectrophotometer (Switzerland) using KBr pellets (ν_{max} in cm^{-1}). Proton (^1H) resonance spectra were recorded on a AC-300F, 300 MHz and Brucker multinuclear FT NMR spectrometer, model AV-400, 400 MHz instrument (Brucker, Switzerland) using tetramethylsilane as internal reference (chemical shifts in δ, ppm). Plates for TLC were prepared with silica gel G (Merck, India) and activated at 110 °C for 30 min. Ethyl acetate was used as solvent. Iodine was used to develop the spots. Elemental analyses were carried out on a Perkin-Elmer-2400 model CHN analyzer (USA). All solvents were distilled prior to use according to standard procedures. Anhydrous sodium sulfate was used as drying agent.

Aluminium chloride, carbon disulfide, hydrazine hydrate, phenylhydrazine hydrochloride and succinic anhydride were obtained from S.D. Fine Chemicals (India), methylsuccinic anhydride was obtained from Aldrich Chem. Co. (USA), 4-fluorophenylhydrazine from Lancaster (UK) and hydralazine hydrochloride was obtained ex gratis from Prof. Alan Harvey (UK); 2-hydrazino-2-imidazoline hydrobromide was obtained ex gratis from Organon labs (USA). Iodine, sodium bicarbonate, acetone, methanol and ether were purchased from Qualigens Fine Chemicals (India).

4-(4-Acetamidophenyl)-4-oxobutyric acid (**1**) was prepared by the reported procedure (8).

Satisfactory analysis for C, H, N, within ± 0.4% of the theoretical values, was obtained for all compounds.

Syntheses

*6-(4-Acetamidophenyl)-2-substituted-4,5-dihydropyridazin-3(2*H*)-ones* (**2–4**). – Requisite hydrazine derivative (2.14 mmol) was added to a stirred and refluxing solution of 4-(4--acetamidophenyl)-4-oxobutyric acid (**1**) (2.13 mmol) in aldehyde free ethanol (40 mL). The reaction mixture was refluxed for 8 h under continuous stirring. The reaction mixture was concentrated to half the volume and left overnight in refrigerator for crystallization. The crystals obtained were collected on a filter, washed with cold ethanol, dried and recrystallized from the appropriate solvent.

4-(4-Methanesulfonamidophenyl)-4-oxobutyric acids (**5, 6**). – A mixture of *N*-phenyl-methanesulfonamide (5.0 g, 29.20 mmol) and succinic anhydride (2.8 g, 27.98 mmol)/methyl succinic anhydride (2.8 g, 24.54 mmol) was added to a stirred solution of aluminum chloride (18 g) in purified carbon disulphide (30 mL). The reaction mixture was stirred manually under anhydrous conditions for 20 min and was allowed to stand for 48 h at room temperature. Carbon disulphide was decanted and the mixture was decomposed with crushed ice. The product obtained was filtered and washed thoroughly with distilled water. The resulting solid was dissolved in 5% aqueous sodium bicarbonate solution and filtered off the insoluble part. Acidification of the filtrate with concentrated hydrochloric acid gave a precipitate, which was collected on a filter, washed with water and dried. The solid residue so obtained was recrystallized from methanol.

*6-(4-Methanesulfonamidophenyl)-2-substituted-4,5-dihydropyridazin-3(2*H*)-ones* (**7–9**). – Requisite hydrazine derivative (1.90 mmol) was added to a stirred and refluxing solution of 4-(4-methanesulfonamidophenyl)-4-oxobutyric acid (**5**) (1.90 mmol) in aldehyde free ethanol (40 mL). The reaction mixture was further refluxed under stirring for 7 h. The resultant solution was concentrated to half the volume and left overnight in refrigerator for crystallization. The crystals obtained were collected on a filter, washed with cold ethanol, dried and recrystallized from the appropriate solvent.

*6-(4-Methanesulfonamidophenyl)-4,5-dihydropyridazin-3(2*H*)-ones* (**10**, **11**). – Hydrazine hydrate (1.90 mmol) was added to a stirred and refluxing solution of 4-(4-methanesulfonamidophenyl)-4-oxobutyric acid (**5**)/4-(4-methanesulfonamidophenyl)-5-methyl-4-oxobutyric acid (**6**) (1.90 mmol) in aldehyde free ethanol (40 mL). The reaction mixture was further refluxed under stirring for 7 h. The resultant solution was concentrated to half the volume and left overnight in refrigerator for crystallization. The crystals obtained were collected on a filter, washed with cold ethanol, dried and recrystallized from ethanol.

Pharmacology

One hundred Wistar rats of either sex, weighing 300–400 g, were bred and housed in the animal house of the University of Salamanca, Spain (P.A.E.-SA001) under standard laboratory conditions at 22 ± 3 °C, relative humidity 50–55% and 12 h light/dark cycle. Drinking water and a nutritionally balanced synthetic pelleted diet were supplied

ad libitum throughout the study period. Fifty animals were used for cardiotonic activity experiments and fifty for vasodilatory activity. In all of these experiments, the provisions regarding the protection of animals used for experimental purposes in current Spanish legislation (Real Decreto 223/1988) and European Community (EEC 86/609) specifications were applied. All animal protocols were approved by the ethical committee of the University of Salamanca.

Cardiotonic activity. – Animals were killed by cervical dislocation and their hearts were rapidly removed (10). Right and left atria were dissected and mounted vertically in 10 mL organ baths containing Tyrode solution of the composition: NaCl (137 mmol L^{-1}), KCl (5.4 mmol L^{-1}), $CaCl_2$ (1.8 mmol L^{-1}), $MgCl_2$ (1.05 mmol L^{-1}), $NaHCO_3$ (11.6 mmol L^{-1}), NaH_2PO_4 (0.42 mmol L^{-1}) and glucose (5.5 mmol L^{-1}). The solution was bubbled with O_2/CO_2 (95:5) and maintained at 34 °C. Under these conditions, the right atria beat spontaneously, while the left atria were electrically driven at a basal rate of 3 Hz through bipolar platinum electrodes with rectangular pulses (1 ms duration, twice threshold strength) delivered from a multipurpose programmable stimulator (Cibertec CS 220, Spain). The left atrium was maintained under constant electric stimulation and was used to measure the effects of compounds on the contraction force, while the spontaneously beating right atrium was used to evaluate the effect on the contraction force as well as on cardiac frequency. The assays were carried out by increasing cumulative concentrations of drugs from 10^{-9} to 10^{-3} mol L^{-1} at 20 min intervals as shown in Tables II–IV. Rate and amplitude of contractions were measured isometrically by a force-displacement transducer and recorded on a Grass Model 7B polygraph (Grass Instrument Co., USA). Resting tension was adjusted to 1 g and a 30 min equilibration period was allowed to elapse before control measurements were taken. After control values for each parameter were obtained, incremental concentrations of each product were added every 20 min to the bath to obtain a complete concentration-response curve. The values for the different parameters (rate and amplitude of contractions) obtained in the absence of each product were used as controls and compared with those obtained after each increment in product concentration.

The pyridazinone derivatives and reference compounds were dissolved in DMSO to prepare a 10^{-2} mol L^{-1} stock solution, which was further diluted with Tyrode solution to obtain final concentrations containing < 1% DMSO in the organ bath.

The results obtained from a minimum of 5 experiments were expressed as mean ± SEM. Statistical analysis of the results was performed by unpaired Student's *t*-test.

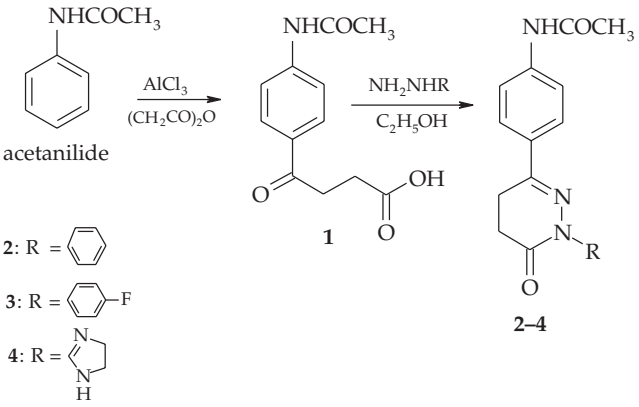
Vasodilatory activity. – Vasodilatory activity of the compounds was studied using descending thoracic aortic rings of Wistar rats precontracted with phenylephrine (10^{-6} mol L^{-1}) according to the reported method (11). Wistar rats of either sex, weighing 300–400 g, were killed by a blow on the head. The descending thoracic aorta was rapidly dissected and placed in a physiological saline solution (PSS) of the composition: NaCl (118 mmol L^{-1}), KCI (4.75 mmol L^{-1}), $NaHCO_3$ (25 mmol L^{-1}), $MgSO_4$ (1.2 mmol L^{-1}), $CaCI_2$ (1.8 mmol L^{-1}), KH_2PO_4 (1.2 mmol L^{-1}) and glucose (11 mmol L^{-1}). After excess of fat and connective tissue was removed, the aorta was cut into rings (4–5 mm in length), mounted under the basal tension of 2 g in 5 mL organ baths containing physiological saline solution and attached to force-displacement transducers to measure the 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 that period the incubation media were changed every 20 min.

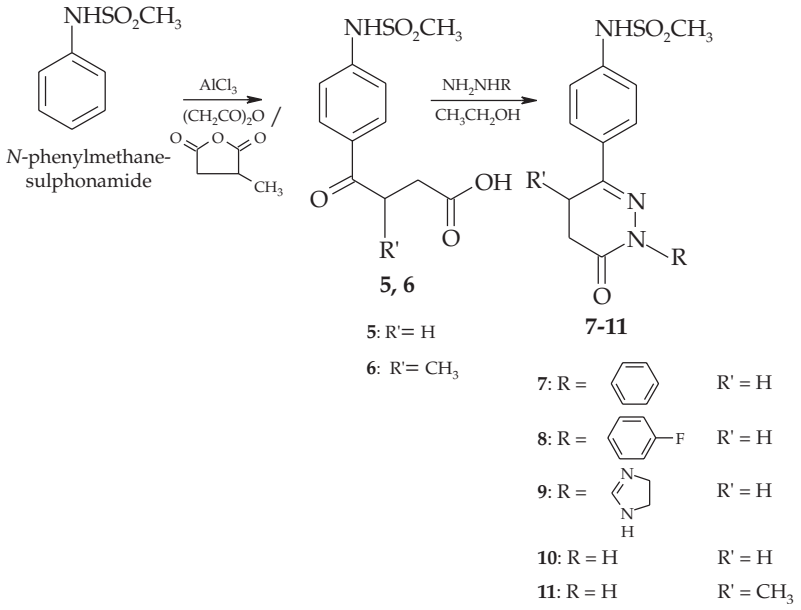
After equilibration, aortic rings were contracted with a single concentration of phenylephrine (l0^{-6} mol L^{-1}). When the contractions were stable, compounds were added in progressively increasing cumulative concentrations (10^{-8}–10^{-5} mol L^{-1}) at 30 min intervals. Only one compound was tested in each ring. All the results were expressed as a percentage of the maximal control phenylephrine-induced responses. All pyridazinone derivatives and the reference compounds were initially dissolved in dimethyl sulfoxide (DMSO) to prepare a 10^{-2} mol L^{-1} stock solution. Further solutions were made in physiological saline solution. Hydralazine (10^{-8}–10^{-5} mol L^{-1}) and SK&F-93741 (10^{-8}–10^{-5} mol L^{-1}) were used as reference compounds under the same experimental conditions.

RESULTS AND DISCUSSION

Friedel-Crafts acylation of acetanilide and *N*-phenylmethanesulfonamide using succinic anhydride or methylsuccinic anhydride in the presence of anhydrous aluminium chloride (a Lewis acid) in purified carbon disulphide at room temperature afforded the corresponding γ-keto acids (**1**, **5**) and β-methyl-γ-keto acid **6** (8, 9). Subsequent cyclization reaction of γ-keto acids **1** and **5** with various hydrazine derivatives such as phenylhydrazine or 4-fluorophenylhydrazine hydrochlorides, or 2-hydrazino-2-imidazoline hydrobromide in aldehyde free ethanol afforded the pyridazinone derivatives **2–4** and **7–9**, respectively. The synthetic pathway is given in Schemes 1 and 2 and characterization data for all new compounds are given in Table I. Methanesulfonamido substituted γ-keto acid **5** and its β-methyl analogue **6** were also treated separately with hydrazine hydrate to afford respective 2-unsubstituted pyridazinones **10** and **11**. Compound **11** was obtained as a chiral molecule with a stereogenic center in the pyridazinone ring.



Scheme 1



Scheme 2

Infrared spectrum of compounds **2–4** and **7–9** exhibited peaks in the region ~3300 cm^{-1} due to N–H stretching vibrations and characteristic amide C=O absorption bands near 1690 and 1660 cm^{-1}. In the NMR spectra of **2–4** and **7–9**, pyridazinone C_4 and C_5 protons resonated as triplets at δ 2.76 and 3.10 ppm, respectively. A downfield shift of C_4 protons was observed in these 2-substituted derivatives in comparison to unsubstituted ones (**10** and **11**) because of phenyl, 4-fluorophenyl and imidazolinyl substitution at nitrogen of the pyridazinone ring. Aromatic protons of the 6-phenyl ring were obtained as doublets with protons *ortho* to amide at an upfield position in comparison to those present at *meta* position for all the pyridazinone derivatives. 2-Unsubstituted –N*H* signal was observed at δ 10.41 ppm in the case of **10** and **11** while it was absent in the case of 2-substituted derivatives **2–4** and **7–9**. The ratio of two enantiomers in 5-methyl derivative **11** could not be determined using proton NMR spectroscopy since all the protons resonate at the same position in both isomers (12). The racemic mixture obtained was used as such for further studies.

The effects of the compounds SK&F-93741, amrinone, isoprenaline, **2**, **3**, **7**, **8**, **10** and **11** on the amplitude of contraction on electrically driven rat isolated left atria and spontaneously beating rat isolated right atria are summarized in Tables II and III, respectively. The results are expressed as the percentage of control values obtained before incorporation of the compounds. Control value for the isometric contraction force in isolated left atria was 260 ± 75 mg (n = 35). For the right atria, control values for the amplitude of contraction and cardiac frequency were 279 ± 21 mg and 257 ± 14 beats min^{-1}, respectively (n = 35). Table IV presents the effects of the new compounds on the sinus rate in

Table I. Characterization data of pyridazinone derivatives

Compd. No.	Solvent for crystallization	Yield (%)	M.p. (°C)	Mol. formula (M_r)	IR (KBr) (cm^{-1})	^1H NMR ($CDCl_3$/ DMSO-d_6, δ ppm)
2	MeOH	4	236–237	$C_{18}H_{17}N_3O_2$ (307.34)	3320 (N–H) 1690 (C=O) 1650 (C=O)	2.14 (s, 3H, -NHCOC**H**$_3$), 2.76 (t, 2H, 4-C**H**$_2$), 3.10 (t, 2H, 5-C**H**$_2$), 7.26 (d, 1H, J_o = 7.35 Hz, 4′-C**H**-Ar), 7.40 (t, 2H, J_o = 7.80 Hz, 3′, 5′-C**H**-Ar), 7.57 (d, 2H, J_o = 7.96 Hz, 2′, 6′-C**H**-Ar), 7.68 (d, 2H, J_o = 8.78 Hz, 2,6-C**H**-Ar), 7.74 (d, 2H, J_o = 8.68 Hz, 3, 5-C**H**-Ar), 9.74 (s, 1H, -N**H**COCH$_3$, exchanged in D_2O)
3	EtOH	41	239–240	$C_{18}H_{16}FN_3O_2$ (325.33)	3321 (N–H) 1692 (C=O) 1656 (C=O)	2.16 (s, 3H, -NHCOC**H**$_3$), 2.76 (t, 2H, 4-C**H**$_2$), 3.08 (t, 2H, 5-C**H**$_2$), 7.09 (t, 2H, J_o = 8.75 Hz, 3′, 5′-C**H**-Ar), 7.56 (m, 2H, 2′, 6′-C**H**-Ar), 7.68 (d, 2H, J_o = 8.81 Hz, 2,6-C**H**-Ar), 7.73 (d, 2H, J_o = 8.87 Hz, 3,5-C**H**-Ar), 9.44 (s, 1H, -N**H**COCH$_3$, exchanged in D_2O)
4	H_2O	19	227–228	$C_{15}H_{17}N_5O_2H_2O$ (317.34)	3326 (N–H) 1667 (C=O)	2.11 (s, 3H, -NHCOC**H**$_3$), 2.37 (t, 2H, 4-C**H**$_2$), 3.09 (t, 2H, 5-C**H**$_2$), 3.72 (s, 4H, 2 x – C**H**$_2$-, imidazoline), 7.63 (d, 2H, J_o = 8.61 Hz, 2,6-C**H**-Ar), 7.79 (d, 2H, J_o = 8.88 Hz, 3,5-C**H**-Ar), 9.91 (s, 1H, -N**H**COCH$_3$, exchanged in D_2O)
5	MeOH	32	180–181	$C_{12}H_{15}SO_5N$ (271.32)	3280 (N–H) 1720 (C=O)	2.71 (t, 2H, -COCH$_2$C**H**$_2$COOH), 3.02 (s, 3H, -NHSO$_2$C**H**$_3$), 3.24 (t, 2H, -COC**H**$_2$CH$_2$COOH), 7.34 (d, 2H, J_o = 8.69 Hz, 2,6-C**H**-Ar), 7.94 (d, 2H, J_o = 8.12 Hz, 3,5-C**H**-Ar), 9.97 (s, 1H, -N**H**SO$_2$CH$_3$, exchanged in D_2O)
6	MeOH	15	70–80	$C_{13}H_{18}SO_5N$ (285.33)	3250 (N–H) 1720 (C=O)	1.26 (d, 3H, -COCH(C**H**$_3$)CH$_2$COOH), 2.93 (m, 2H, -COCH(CH$_3$)C**H**$_2$COOH), 3.02 (s, 3H, -NHSO$_2$C**H**$_3$), 3.43 (m, 1H, -COC**H**(CH$_3$)CH$_2$-COOH), 7.13 (d, 2H, J_o = 8.56 Hz, 2, 6-C**H**-Ar), 7.92 (d, 2H, J_o = 8.68 Hz, 3,5-C**H**-Ar)
7	MeOH	43	189–190	$C_{17}H_{17}SN_3O_3$ (343.39)	3148 (N–H) 1651 (C=O)	2.78 (t, 2H, 4-C**H**$_2$), 2.98 (s, 3H, -NHSO$_2$C**H**$_3$), 3.06 (t, 2H, 5-C**H**$_2$), 7.29 (m, 3H, 2′,4′,6′ -C**H**-Ar), 7.41 (t, 2H, J_o = 8.97 Hz, 3′,5′-C**H**-Ar), 7.58 (dd, 2H, J_o= 8.97 Hz, J_m= 1.25 Hz, 2,6-C**H**-Ar), 7.76 (dd, 2H, J_o = 8.72 Hz, J_m = 1.82 Hz, 3,5 -C**H**-Ar), 8.88 (brs, 1H, -N**H**SO$_2$CH$_3$, exchanged in D_2O)

8	EtOH	45	178–179	$C_{17}H_{16}SFN_3O_3$ (361.38)	3163 (N–H) 1660 (C=O)	2.77 (t, 2H, 4- C**H**$_2$), 2.98 (s, 3H, -NHSO$_2$C**H**$_3$), 3.08 (t, 2H, 5-C**H**$_2$), 7.09 (t, 2H, J_o = 8.67 Hz, 3′,5′ -C**H**-Ar), 7.34 (d, 2H, J_o = 8.71 Hz, 2, 6-C**H**-Ar), 7.56 (m, 2H, 2′, 6′-C**H**-Ar), 7.76 (dd, 2H, J_o = 8.66 Hz, J_m = 2.29 Hz, 3,5-C**H**-Ar), 9.68 (s, 1H, -N**H**SO$_2$CH$_3$, exchanged in D$_2$O)
9	H_2O	22	232–233	$C_{14}H_{17}N_5O_3SH_2O$ (353.39)	3300 (N–H) 1600 (C=O)	2.63 (m, 2H, 4-C**H**$_2$), 2.90 (t, 2H, 5-C**H**$_2$), 3.02 (s, 3H, -NHSOC**H**$_3$), 3.88 (s, 4H, 2 x -C**H**$_2$-, imidazoline), 7.37 (d, 2H, J_o = 8.38 Hz, 2,6-C**H**-Ar), 7.71 (d, 2H, J_o = 8.66 Hz, 3,5 -C**H**-Ar)
10	EtOH	47	255–256	$C_{11}H_{13}SN_3O_3$ (267.29)	3280 (N–H) 1670 (C=O)	2.54 (t, 2H, 4-C**H**$_2$), 2.94 (t, 2H, 5-C**H**$_2$), 2.96 (s, 3H, -NHSO$_2$C**H**$_3$), 7.30 (dd, 2H, J_o = 8.73 Hz, J_m = 1.83 Hz, 2, 6-C**H**-Ar), 7.67 (dd, 2H, J_o = 9.08 Hz, J_m = 2.34 Hz, 3,5 -C**H**-Ar), 9.65 (s, 1H, -N**H**SO$_2$CH$_3$), 10.12 (s, 1H, -N**H**, exchanged in D$_2$O)
11	MeOH	55	232–233	$C_{12}H_{15}SN_3O_3$ (281.35)	3300 (N–H) 1660 (C=O)	1.35 [d, 3H, 5-CH(C**H**$_3$)], 2.33 (s, 3H, -NHSO$_2$C**H**$_3$), 2.90 (m, 2H, 4-C**H**$_2$), 3.22 [m, 1H, 5-C**H**(CH$_3$)], 7.33 (d, 2H, J_o = 7.47 Hz, 2,6-C**H**-Ar), 7.71 (d, 2H, J_o = 7.83 Hz, 3,5-C**H**-Ar)

spontaneously beating rat isolated right atria. Imidazolinyl derivatives **4** and **9** could not be assessed for cardiotonic activity because of limited solubility.

Of the newly synthesized 6-(4-acylaminophenyl)pyridazinone derivatives **2**, **3**, **7**, **8**, **10** and **11**, tested for inotropic activity, 6-(4-methanesulfonamidophenyl)-2-phenyl-4,5-dihydropyridazin-3(2*H*)-one (**7**) produced a significant concentration dependent positive inotropic effect. Introduction of the phenyl group at position 2 in the case of methanesulphonamido substituted pyridazinone derivative **7** resulted in potent cardiotonic activity in comparison to 2-phenyl substituted acetamido congener **2**. However, substitution of *p*-fluorophenyl moiety, as in **3** and **8**, did not produce any change in cardiac response in either series of compounds. Although the inotropic effect of compound **7** is observed at higher concentrations compared to the standard drug isoprenaline, the increase in contractility does not affect the cardiac frequency, whereas the inotropic effect of standard compound isoprenaline is associated with a great increase in sinus rate (Table IV). The cardiotonic effect of **7** is also in sharp contrast to prototypical compounds SK&F--93741 and amrinone, whose inodilatory effects are believed to be mediated through phosphodiesterase inhibition in higher animals (7, 13). Figure 2 shows the concentration-response effect of **7** on the peak contractile force in spontaneously beating rat isolated left atria, which is significant above 10^{-5} mol L^{-1}. 2-Unsubstituted pyridazinones including the newly synthesized compounds **10**, **11** and representative compounds SK&F-93741 and amrinone did not produce a substantial effect either on myocardial contractility or on cardiac frequency, when tested in rats (Tables II–IV). Interestingly, negative inotropic effects indicating decreased myocardial contractility were observed for the inactive compounds.

When tested for vasodilatory activity, many compounds produced a concentration dependent inhibition of the contractile response of phenylephrine. The IC_{50} value means

Table II. Effects of compounds on peak contractile force in rat isolated left atria electrically driven at a basal rate of 3 Hz[a]

Compd. No.	Concentration (mol L^{-1})						
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	3×10^{-5}	10^{-4}
2	0	0	0	–9.9 ± 1.6[b]	–23.2 ± 2.9[b]	–25.1 ± 3.9[b]	–32.3 ± 1.7[b]
3	0	0	–3.6 ± 0.8[b]	–10.2 ± 2.0[b]	–15.6 ± 2.9[b]	–24.4 ± 3.5[b]	–32.1 ± 2.2
7	0	0	–1.1 ± 1.6	–2.0 ± 2.7	4.5 ± 2.9	33.3 ± 5.8[b]	67.8 ± 6.3[b]
8	0	0	9.2 ± 6.5	2.6 ± 7.3	–3.3 ± 8.8	–5.0 ± 10.4	–8.1 ± 10.4
10	0	0	–6.3 ± 3.6	16.0 ± 0.7[b]	–21.6 ± 2.3[b]	–27.8 ± 2.4[b]	–34 ± 3.6[b]
11	0	0	–4.1 ± 3.2[b]	–15.9 ± 2.7[b]	–22.6 ± 4.0[b]	–32.0 ± 3.8[b]	–39.3 ± 3.8[b]
SK&F-93741	0	0	–1.0 ± 6.8	–3.5 ± 7.5	–0.6 ± 6.7	–5.7 ± 6.3	–5.0 ± 8.7
Isoprenaline	0.6 ± 0.6	2.8 ± 1.1[b]	10.8 ± 3.2[b]	63.0 ± 15.7[b]	85.8 ± 18.8[b]	–	–
Amrinone	0	0	0	–1.6 ± 3	–6.7 ± 2.9	–12.8 ± 3.6	–23.5 ± 6.7[b]

[a] Mean ± SEM, $n = 5$.
[b] Significantly different from control: $p < 0.05$.
All compounds were initially dissolved in DMSO to prepare a 10^{-2} mol L^{-1} stock solution, which was further diluted with Tyrode solution to obtain final concentrations containing < 1% DMSO in the organ bath.

Table III. Effects of compounds on peak contractile force in spontaneously beating rat isolated right atria[a]

Compd. No.	Concentration (mol L^{-1})						
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	3×10^{-5}	10^{-4}
2	0	0	0	0	–6.3 ± 3.3[b]	4.3 ± 13.7[b]	6.3 ± 13.5[b]
3	0	0	0	0	–3.0 ± 1.6[b]	–9.0 ± 1.9[b]	–18.4 ± 1.7[b]
7	0	0	7.2 ± 4.7	1.7 ± 2.6	–9.3 ± 4.7	43.3 ± 9.5[b]	1 12 ± 16.7[b]
8	0	0	0.04 ± 1.4	–1.7 ± 3.2	–3.9 ± 2.8	–5.8 ± 3.7	–5.8 ± 8.3
10	0	0	0	–4.8 ± 2.8[b]	–8.4 ± 5.0[b]	–9.9 ± 4.6[b]	–8.4 ± 4.9[b]
11	0	0	–5.0 ± 1.5[b]	–12.2 ± 1.2[b]	–19.6 ± 0.8[b]	–25.6 ± 1.8[b]	–27.3 ± 1.4[b]
SK&F-93741		0	–1.0 ± 2.8	–4.7 ± 2.4	–3.4 ± 3.5	–4.3 ± 5.2	–7.3 ± 6.7
Isoprenaline	43.9 ± 9.3[b]	101.4 ± 17[b]	106 ± 2.1[b]	67.1 ± 17.5[b]	47.9 ± 16.2[b]	–	–
Amrinone	0	0	0	3.7 ± 1.9[b]	6.1 ± 2.8[b]	3.2 ± 2.6[b]	24.8 ± 6.2[b]

[a] Mean ± SEM, $n = 5$.
[b] Significantly different from control: $p < 0.05$.
All compounds were initially dissolved in DMSO to prepare a 10^{-2} mol L^{-1} stock solution, which was further diluted with Tyrode solution to obtain final concentrations containing < 1% DMSO in the organ bath.

the concentration producing 50% relaxation of maximal control phenylephrine-induced responses of various pyridazinone derivatives **2–4, 7, 8, 10** and **11** (Table V). Methanesulphonamide substituted pyridazinone derivatives **7** and **8** with phenyl and *p*-fluorophenyl groups at position 2 displayed potent vasodilatory activity compared to their acetamide--substituted counterparts **2** and **3**, which did not produce 50% relaxation up to 10^{-5} mol L^{-1}. In the acetamide series, only imidazolinyl derivative **6** displayed intermediate po-

Table IV. Effects of compounds on sinus rate in spontaneously beating rat isolated right atria[a]

Compd. No.	Concentration (mol L^{-1})						
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	3×10^{-5}	10^{-4}
2	0	0	4.5 ± 3.6[b]	–0.7 ± 1.7	–3.8 ± 3.0[b]	–6.9 ± 2.7[b]	–9.3 ± 2.7[b]
3	0	0	–1.7 ± 1.8	–2.6 ± 2.8	0.3 ± 4.1	–2.5 ± 1.4[b]	–0.3 ± 2.6[b]
7	0	0	–0.2 ± 1.5	–3.9 ± 2.7	–2.9 ± 2.0	–2.1 ± 3.1	0.2 ± 3.5
8	0	0	–0.05 ± 1.7	–3.2 ± 1.7	–3.5 ± 1.5	–2.6 ± 2.3	1.2 ± 2.1
10	0	0	–12.3 ± 3.0[b]	–16.0 ± 3.4[b]	–17.5 ± 3.1[b]	–19.6 ± 3.0[b]	–22.7 ± 0.8[b]
11	0	0	0	0	–0.6 ± 0.3[b]	–4.8 ± 1.7[b]	–12.7 ± 0.4[b]
SK&F-93741	0	0	0.7 ± 0.7	0.7 ± 1.9	0.7 ± 2.6	2.9 ± 3.2	6.6 ± 3.6
Isoprenaline	16.6 ± 3.3[b]	44.9 ± 7.0[b]	58.3 ± 7.5[b]	62.1 ± 6.4[b]	62.6 ± 6.2[b]	–	–
Amrinone	0	0	0	5.1 ± 1.3[b]	6.9 ± 1.9[b]	10.4 ± 2.6[b]	32.1 ± 5.0[b]

[a] Mean ± SEM, $n = 5$.
[b] Significantly different from control: $p < 0.05$.
All compounds were initially dissolved in DMSO to prepare a 10^{-2} mol L^{-1} stock solution, which was further diluted with Tyrode solution to obtain final concentrations containing < 1% DMSO in the organ bath.

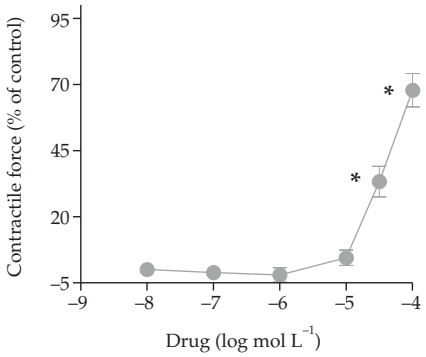


Fig. 2. Concentration-response effects of compound **7** on peak contractile force in Wistar rat isolated left atria. Each value represents the mean of at least five experiments; vertical bars represent the SEM. * Significantly different from control: $p < 0.05$.

tency (IC_{50} = 2.51 μmol L^{-1}). The most active compound **7** (IC_{50} = 0.079 μmol L^{-1}) produced vasorelaxation better than the reference compound hydralazine and prototypical compound SK&F-93741. Imidazolinyl derivative **9** could not be screened for vasodilatory activity because of poor solubility. 2-Unsubstituted derivative **10** possessing methanesulphonamido functionality and compound SK&F-93741 bearing an acetamido group were equipotent in producing vasorelaxation (Table V). It can be said that substitution of methanesulphonamido group at the *para* position of 6-phenylpyridazinones results in potent vasodilatory activity, which is further improved by 2-substitution.

Although earlier research work indicated that an acidic proton adjacent to dipolar moiety in the pyridazinone ring system is essential for optimum inodilating activity through phosphodiesterase inhibition, 2-substitution resulted in good inodilating properties in this study. Further investigations are required to elucidate the exact mecha-

Table V. IC_{50} *values of pyridazinone derivatives to inhibit contractions induced by phenylephrine*[a,b]

Compd. No.	IC_{50} (μmol L^{-1})
2[c]	–
3[c]	–
4	2.51 ± 0.2
7	0.079 ± 0.01
8	3.98 ± 0.3
10	0.199 ± 0.2
11[c]	–
Hydralazine	0.316 ± 0.1
SK&F-93741	0.199 ± 0.2

[a] 10^{-6} mol L^{-1} phenylephrine
[b] Mean ± SEM, n = 5–8.
[c] Compounds which did not produce 50% relaxation up to 10^{-5} mol L^{-1}.
All compounds were initially dissolved in DMSO to prepare a 10^{-2} mol L^{-1} stock solution, which was further diluted with physiological saline solution to obtain final concentrations containing < 1% DMSO in the organ bath.

nism of action of these pyridazinones, which is probably mediated either by combined calcium sensitization and phosphodiesterase 3 inhibition or release of cycloxygenase products or opening of K^+-channels. However, the pharmacological effects observed provide information about the therapeutic interest of pyridazinone derivatives in heart failure and hypertension.

CONCLUSIONS

In the current study, newly synthesized 2-substituted pyridazinones bearing a methanesulphonamido group at *para* position of the 6-phenyl ring exhibited a significant concentration dependent positive inotropic effect and potent vasodilatory activity in rats. This dual inhibition could be of interest for the treatment of congestive heart failure to reduce preload and afterload on the heart.

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S A Ž E T A K

Sinteza i farmakološko vrednovanje 2-supstituiranih-6-fenil--4,5-dihidropiridazin-3(2*H*)-ona kao snažnih srčanih stimulatora

DINESH KUMAR, ROSALIA CARRON, CARMEN DE LA CALLE, DHARAM PAUL JINDAL i RANJU BANSAL

U radu je opisana sinteza i framakološko vrednovanje 2-supstituiranih-6-(4-acilaminofenil)-4,5-dihidropiridazin-3(2*H*)-ona kao snažnih srčanih stimulatora. Spojevi **2–4** i **7–11** sintetizirani su Friedel-Craftsovim acilaranjem odgovarajućeg anilida s anhidridom jantarne ili anhidridom metiljantarne kiseline te ciklizacijom intermedijarnih keto kiselina s različitim derivatima hidrazina. Kardiotonično djelovanje novosintetiziranih derivata piridazinona ispitano je na izoliranim atrijima štakora, a vazodilatirajuće djelovanje na silaznim torakalnim prstenima aorte prethodno kontrahiranim fenilefrinom (10^{-6} mol L^{-1}). 6-(4-Metansulfonamidofenil)-2-fenil-4,5-dihidropiridazin-3(2*H*)-on (**7**) pokazao je značajno stimulativno i vazodilatirajuće djelovanje u nanomolarnim koncentracijama (IC_{50} = 0,08 ± 0,01 μmol L^{-1}).

Ključne riječi: piridazinoni, anilidi, derivati hidrazina, kardiotonično djelovanje, vazodilatacijsko djelovanje

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