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Original article

Synthesis and vasodilatory activity of some amide derivatives of 6-(4-carboxymethyloxyphenyl)-4,5-dihydro-3(2*H*)-pyridazinone

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ABSTRACT

Synthesis and vasodilatory activity of some amide derivatives of 6-(4-carboxymethyloxyphenyl)-4,5-dihydro-3(2*H*)-pyridazinone are reported. An effect of substitution at 2-position of pyridazinone ring on vasodilatory potential has also been explored. The most active compound 6-[4-(2-oxo-2-pyrrolidin-1-yl-ethoxy)phenyl]-2-(4-fluorophenyl)-4,5-dihydropyridazin-3(2*H*)-one (**11**) exhibited vasodilating activity in nanomolar range ($IC_{50} = 0.051$ μM).

1. Introduction

Hypertension is the most common cardiovascular disease and is the major risk factor for coronary artery disease leading to myocardial infarction and sudden cardiac death, heart failure, stroke and renal failure [1,2]. Great efforts have been made on obtaining novel antihypertensive agents acting on different mechanisms to control blood pressure [3]. The studies on the hydralazine group drugs led to the synthesis of many pyridazinone and phthalazinone derivatives with a wide activity spectrum on cardiovascular system [4].

Pharmacological activity of 4,5-dihydro-6-phenyl-3(2*H*)-pyridazinones has been actively studied since long and are known for their cardiovascular effects [5–7]. Considering that the 6-arylpyridazinone structure is essential for the activity on cardiovascular system, a number of studies have been performed on different substitutions on both pyridazinone and aryl residues [8,9]. These studies indicate that compounds having amino, acylamino, cyano and halogen substituents on phenyl ring possess interesting antihypertensive activity, e.g., levosimendan (**1**) [9] (Fig 1). This action persisted for a longer duration in compounds possessing a 5-methyl substituent on pyridazinone core. However RS-1893 (**2**) (Fig 1), a 6-phenoxypyridazinone, has also been reported as an orally active pyridazinone, which is about 10 times more active venous and arterial vasodilator in comparison with milrinone [10]. Encouraged by these reports, we thought of synthesizing a new series of 6-phenoxypyridazinones in which heteroatom oxygen is attached directly to the 4-position of phenyl ring along with an extended 4-substituent that placed heteroatom nitrogen further away from ring to improve upon the vasodilatory activity.

Also, from medicinal chemistry research point of view, pyridazinones offer a vulnerable ring system to researchers because of easy functionalization at various ring positions. As the position 2 remained relatively unexplored in context with the antihypertensive effects, several 2-substituted products have also been prepared. Herein we report the synthesis and vasodilatory effects of a new series of pyridazinones, in which carbamoyl derivatives of 6-phenoxypyridazinones have been prepared along with 2-substitution.

2. Chemistry

Various new pyridazinone derivatives have been synthesized according to Schemes 1–3. Compounds **10**–**17** were synthesized by fusing phenoxyacetic acid methyl ester (**3**) [11] with heterocyclic amines such as pyrrolidine and piperidine to obtain **4** [12] and **5** [13] in the first step. The subsequent Friedel–Crafts acylation reaction [14] and cyclocondensation of obtained γ-keto acids **6**–**9** with various hydrazine derivatives afforded corresponding pyridazinones **10**–**17** as shown in Scheme 1. In the NMR spectra of all

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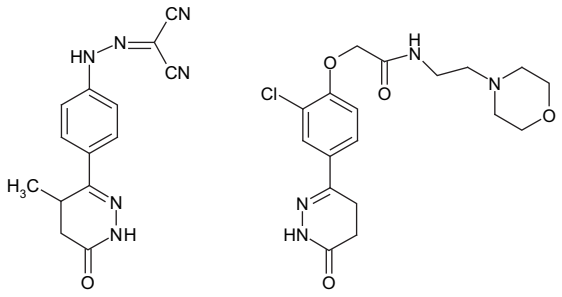
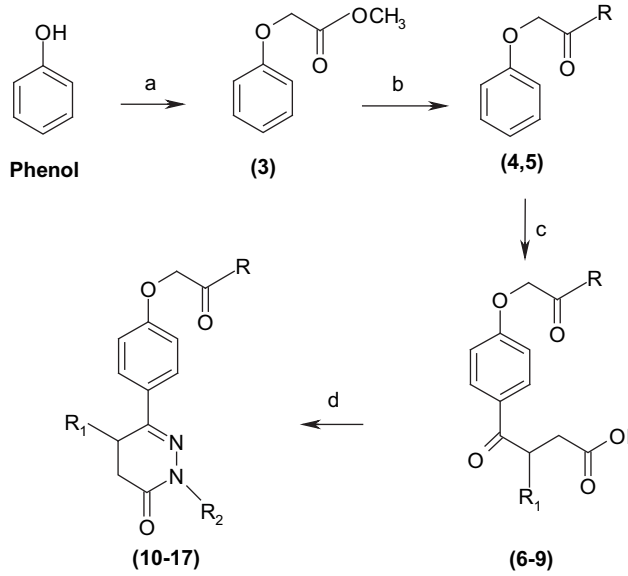


Fig. 1. Structures of levosimendan (**1**) and RS-1893 (**2**).

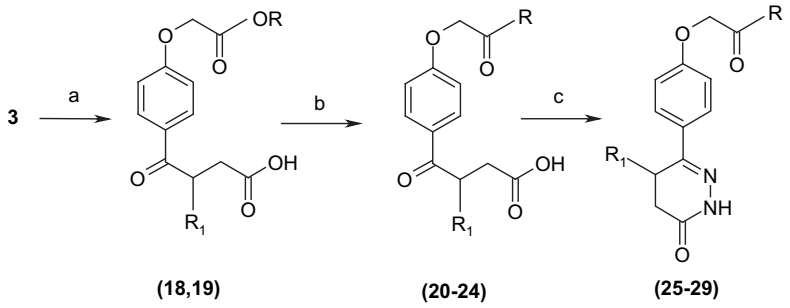
these pyridazinone ring-bearing compounds, 4-CH_2 and 5-CH_2 protons characteristically resonated at their expected positions. Methylene protons of 4-CH_2 were quite downfield at $\delta \sim 2.80$ in case of 2-substituted derivatives **11**, **12**, **15** and **16** in comparison to the 2-unsubstituted ones **10** and **14** at δ 2.59 ppm. Efforts to incorporate 2-phenyl group in pyrrolidinyl and 2-*p*-fluorophenyl moiety in piperidinyl series were unsuccessful.

An alternate route was adopted for the preparation of pyridazinones **25**–**29**, which are substituted with a heterocyclic amine bearing an additional heteroatom. Contrary to Scheme 1, Friedel–Crafts acylation of the phenol ester **3** was carried out instead of its amine substituted derivative to obtain γ-keto acids **18** and **19** as outlined in Scheme 2. Interestingly, a singlet for methyl protons of –OCH_2COOCH_3 was absent in case of β-methyl-γ-keto acid **19** in ^{1}H NMR spectrum. It seems that during Friedel–Crafts acylation, when methylsuccinic anhydride was used, ester group at 4-position of phenyl ring got hydrolyzed to an acid. The structure of compound **19** was further confirmed using ^{13}C NMR spectroscopy. Thermal fusion of **18** and **19** with morpholine, powdered imidazole, a versatile pharmacophore possessing a variety of pharmacological and enzymatic actions including inotropic and vasodilatory activities [15] and *N*-methylpiperazine and further cyclization reaction



Compd No.	R	R_1	R_2	Compd No.	R	R_1	R_2
4		--	--	**11**		H	
5		--	--	**12**		H	
6		H	--	**13**		CH_3	H
7		CH_3	--	**14**		H	H
8		H	--	**15**		H	
9		CH_3	--	**16**		H	
10		H	H	**17**		CH_3	H

Scheme 1. Synthetic routes to compounds **3**–**17**. Reagents and conditions: (a) methyl chloroacetate; (b) pyrrolidine/piperidine; (c) aluminium chloride, succinic anhydride/methyl succinic anhydride; and (d) hydrazine hydrate/phenylhydrazine hydrochloride/*p*-fluoro phenylhydrazine hydrochloride/2-hydrazine-2-imidazoline hydrobromide, absolute ethanol.

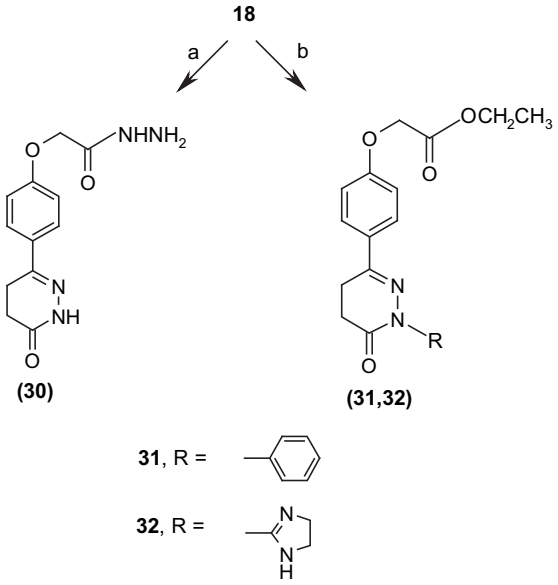


Compd No.	18	19	20,25	21,26	22,27	23,28	24,29
R	CH_3	H					
R_1	H	CH_3	H	CH_3	H	CH_3	H

Scheme 2. Synthetic route to compounds **18**–**29**. Reagents and conditions: (a) aluminium chloride, succinic anhydride/methyl succinic anhydride; (b) morpholine/imidazole/N-methylpiperazine, 70 °C; and (c) hydrazine hydrate, ethanol.

with hydrazine hydrate in aldehyde free ethanol gave the target products **25**–**29**. Efforts to synthesize 2-substituted derivatives of these pyridazinones were unsuccessful and impure products were obtained every time.

Attempts to directly cyclize γ-keto acid **18** using hydrazine derivatives resulted in the formation of some unusual compounds **30**–**32** as depicted in Scheme 3. A hydrazide pyridazinone **30** was obtained on treatment of **18** with hydrazine hydrate, which is in anology with the earlier literature reports regarding the formation of hydrazides from phenyl esters [14]. The infrared peaks in the region 3300–3100 and a broad band at 1680 cm^{-1} represented the N–H stretching and bending modes, respectively. Two broad singlets for protons of hydrazino functionality were prominently present at δ 4.06 (s, 2H, –NH_2) and 8.71 (–N*H*, disappeared on deuterium exchange) in addition to another broad singlet at 10.06 ppm for –N*H* proton of parent pyridazinone nucleus in NMR spectrum of **30**. A singlet of methyl ester was also absent. The reaction of **18** with phenylhydrazine hydrochloride and 2-hydrazino-2-imidazoline hydrobromide in absolute ethanol resulted into base catalyzed transesterification to form ethyl esters **31** and **32**. In NMR spectra a singlet for protons of methyl ester was conspicuously absent, and instead two peaks at ~δ 1.35 (t, 3H, -OCH_2CH_3) and ~4.3 (q, 2H, –OCH_2CH_3) were present, which indicated the transesterification of methyl ester to ethyl ester [16]. It seems that highly reactive hydrazine hydrate immediately formed hydrazide in case of compound **30**, while for **31** and **32**, less reactive phenylhydrazine and 2-hydrazino-2-imidazoline salts were not able to form hydrazides and transesterification took place instead.



Scheme 3. Synthetic route to compounds **30**–**32**. Reagents and conditions: (a) hydrazine hydrate, ethanol and (b) phenylhydrazine hydrochloride/2-hydrazine-2-imidazoline hydrobromide, absolute ethanol.

3. Pharmacology

The newly synthesized pyridazinone derivatives **10**, **11**, **13**–**15**, **17**, **25**–**29**; standard drug hydralazine and prototypical compound SK&F 93741 were screened for vasodilatory activity as reported earlier [17], using rat thoracic aortic rings, precontracted with phenylephrine (10^{-6} M). Imidazolinyl derivatives **12** and **16** could not be screened for vasodilatory activity because of poor solubility profile.

The results are expressed as percentage of the maximal control phenylephrine-induced responses. Maximal relaxation (Emax) and pD_2 ($-\log IC_{50}$) values of various compounds to inhibit the contractions induced by phenylephrine (10^{-6} M) are shown in Table 1.

Many compounds produced a concentration dependent inhibition of the contractile response of phenylephrine and some produced vasorelaxation better than the standard drugs hydralazine and reference compound SK&F 93741 (Fig 2). Most active compound **11** ($IC_{50} = 0.051$ μM) exhibited vasodilating activity in nanomolar range.

4. Results and discussion

In general, pyrrolidinyl substituted derivatives **10** and **11** exhibited potent vasorelaxant activity as shown in Table 1. 2-*p*-Fluorophenyl substituted derivative **11** with $pD_2 = 7.2 \pm 0.1$ ($IC_{50} = 0.051$ μM) showed appreciable vasodilatory activity in

Table 1
Maximal response (Emax) and pD2 ($-\log IC_{50}$) values to inhibit the contractions induced by phenylephrine (10^{-6} M). Values are expressed as means ± SEM, $n = 4$–8.

Compound (code)	Emax	pD_2
10 (DPJ-RG-1137)	52.2 ± 3.4[a]	5.9 ± 0.1
11 (DPJ-RG-1169)	61.8 ± 4.6[a]	7.2 ± 0.1
13[b] (DPJ-RG-1274)	21.7 ± 11.0	–
14 (DPJ-RG-1189)	61.6 ± 8.0[a]	5.9 ± 0.2
15[b] (DPJ-RG-1278)	16.2 ± 5.9	–
17[b] (DPJ-RG-1276)	29.8 ± 12.8[a]	–
25[b] (DPJ-RG-1281)	41.8 ± 2.8[a]	–
26[b] (DPJ-RG-1288)	17.6 ± 12.5	–
27[b] (DPJ-RG-1285)	3.7 ± 3.5	–
28[b] (DPJ-RG-1287)	32.0 ± 8.2	–
29[b] (DPJ-RG-1284)	23.9 ± 14.2	–
Hydralazine	55.0 ± 5.8	6.6 ± 0.2
SK&F-93741	61.1 ± 4.1	7.0 ± 0.2

[a] $p < 0.05$ versus DMSO.
[b] The compounds, which did not produce 50% relaxation upto 10^{-5} M.

nanomolar range and was found 4.5 times and 1.7 times more potent in comparison to standard drug hydralazine and reference compound SK&F 93741, respectively. However 2-unsubstituted compound **10** with $pD_2 = 5.9 \pm 0.1$ ($IC_{50} = 1.025$ μM) was less effective than standard and reference compounds in producing vasorelaxation. Replacement of pyrrolidine with piperidine as in **14** with $pD_2 = 5.9 \pm 0.1$ ($IC_{50} = 0.91$ μM) did not affect the vasodilatory properties, whereas substitution of phenyl ring at 2-position in this series resulted in substantial loss of vasodilatory activity for compound **15**, which produced only 16% maximal relaxation (Table 1). Introduction of heterocyclic systems such as morpholine, imidazole and *N*-methylpiperazine with an additional heteroatom resulted in decreased vasodilatory activity in compounds **25**, **27** and **29**. Substitution of 5-methyl group in the compounds **13**, **17**, **26** and **28** also led to loss of activity.

Further investigations are required to elucidate the exact mechanism of action of these pyridazinones, which probably is mediated by phosphodiesterase 3 inhibition. However, the pharmacological effects observed contribute to give information about therapeutic interest of pyridazinone derivatives in hypertension.

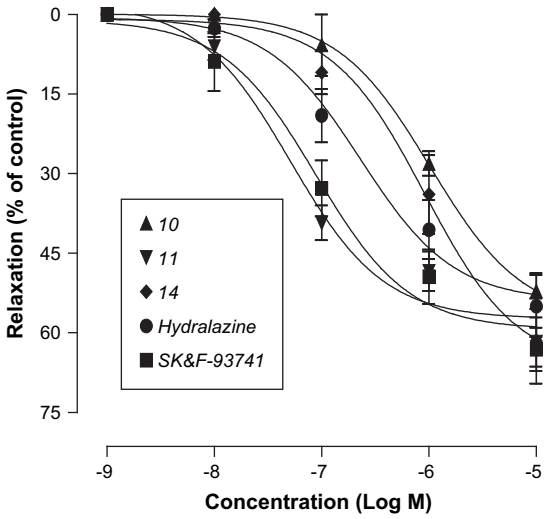


Fig. 2. Concentration–response curves for relaxation activity of compounds **10**, **11**, **14**, hydralazine and SK&F93741 on aortic rings obtained from Wistar rats. Each data point represents the mean ± SEM from four or eight experiments.

5. Experimental

5.1. Chemistry

The m.p.s reported are uncorrected, ^{1}H NMR spectra were recorded on Brucker AC-300F, 300 MHz instrument using Me_4Si (TMS) as the internal standard (chemical shifts in δ, ppm). The IR spectra were recorded on a Perkin–Elmer 882 and Perkin–Elmer spectrum RX 1, FT-IR spectrophotometer models using potassium bromide pellets (υ_{max} in cm^{-1}). The purity of compounds was established by thin layer chromatography and elemental analyses (C, H, N). Elemental analyses were carried out on a Perkin–Elmer-2400 model CHN analyzer. Plates for TLC were prepared with silica gel G according to Stahl's method (E. Merck) using EtOAc as solvent (activated at 110 °C for 30 min) and were visualized by exposure to iodine vapours. Anhydrous sodium sulfate was used as drying agent. All solvents were dried and freshly distilled prior to use according to standard procedures.

5.1.1. General procedure for the synthesis of pyrrolidinyl/piperidinyl substituted γ-keto acids **6**–**9**

A mixture of amide derivatives (**4**/**5**) (1.5 g, 8 mmol) and requisite anhydride succinic anhydride (1.25 g, 12 mmol)/methyl-succinic anhydride (1.0 g, 8 mmol) was added to a stirred solution of aluminium chloride (5 g) in nitrobenzene (10 ml). The reaction mixture was stirred manually under the anhydrous conditions for 20 min and then allowed to stand for 48 h at room temperature. The mixture was decomposed with crushed ice and steam distilled to remove nitrobenzene. The resultant hot solution was filtered and then cooled in ice for precipitation. The solid product obtained was filtered and washed thoroughly with distilled water. The solid residue was dissolved in 10% aqueous sodium bicarbonate solution and filtered off insoluble part. Acidification with concentrated hydrochloric acid gave precipitate, which was filtered, washed with water and recrystallized from methanol to afford the corresponding γ-keto acids **6**–**9**.

*5.1.1.1. 4-Oxo-4-[4-(2-oxo-2-pyrrolidin-1-yl-ethoxy)phenyl]butyric acid (**6**).* Yield: 39.17%; m.p. 196–198 °C. IR: 2967, 1732, 1667, 1633, 1259, 1169, 925; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): 1.86 (t, 2H, –C*H*$_2$–, pyrrolidine), 2.0 (t, 2H, –C*H*$_2$–, pyrrolidine), 2.62 (t, 2H, –COCH$_2$C*H*$_2$COOH), 3.20 (t, 2H, –COC*H*$_2$CH$_2$COOH), 3.44 (t, 2H, N–C*H*$_2$–, pyrrolidine), 3.51 (t, 2H, N–C*H*$_2$–, pyrrolidine), 4.77 (s, 2H, –OC*H*$_2$–), 6.99 (d, 2H, C*H*, arom, $J_o = 8.31$ Hz), 7.93 (d, 2H, C*H*, arom, $J_o = 8.49$ Hz) and 11.93 ppm (brs, 1H, –COCH$_2$CH$_2$COO*H*, disappeared on deuterium exchange).

*5.1.1.2. 3-Methyl-4-oxo-4-[4-(2-oxo-2-pyrrolidin-1-yl-ethoxy)phenyl] butyric acid (**7**).* Yield: 31.06%; m.p. 138–139 °C. IR: 2976, 1737, 1672, 1629, 1599, 1263, 1174, 832; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.31 (d, 3H, –COCH(C*H*$_3$)CH$_2$COOH), 1.87 (s, 2H, –C*H*$_2$–, pyrrolidine), 1.99 (p, 2H, –C*H*$_2$–, pyrrolidine), 3.01 [m, 1H, –COCH(CH$_3$)–C*H*(H)COOH], 3.14 [m, 1H, –COCH(CH$_3$)CH(*H*)COOH], 3.41 [m, 1H, –COC*H*(CH$_3$)–CH$_2$COOH], 3.52 (t, 4H, N–(C*H*$_2$)$_2$–, pyrrolidine], 4.71 (s, 2H, –OC*H*$_2$–), 6.99 (d, 2H, C*H*, arom, $J_o = 8.59$ Hz) and 7.96 ppm (d, 2H, C*H*, arom, $J_o = 8.49$ Hz).

*5.1.1.3. 4-Oxo-4-[4-(2-oxo-2-piperidin-1-yl-ethoxy)phenyl]butyric-acid (**8**).* Yield: 32.96%; m.p. 153–155 °C. IR: 2932, 1722, 1677, 1627, 1508, 1249, 842; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.61 (m, 6H, 3 × –C*H*$_2$–, piperidine), 2.71 (t, 2H, –COCH$_2$C*H*$_2$COOH), 3.25 (t, 2H, –COC*H*$_2$CH$_2$COOH), 3.47 (t, 2H, N–C*H*$_2$–, piperidine), 3.55 (t, 2H, N–C*H*$_2$–, piperidine), 4.79 (s, 2H, –OC*H*$_2$–), 6.98 (d, 2H, C*H*, arom, $J_o = 8.76$ Hz) and 7.96 ppm (d, 2H, C*H*, arom, $J_o = 8.66$ Hz).

*5.1.1.4. 3-Methyl-4-oxo-4-[4-(2-oxo-2-piperidin-1-yl-ethoxy)phenyl]-butyric-acid (**9**).* Yield: 36.18%; m.p. 134–135 °C. IR: 2926, 1719, 1677, 1629, 1442, 1252, 1186, 835; ^{1}H NMR ($CDCl_3$): δ 1.30 [d, 3H, –COCH(CH_3)CH_2COOH)], 1.64 (m, 6H, 3 × –CH_2–, piperidine), 2.98 [m, 1H, –COCH(CH_3)C*H*(H)COOH], 3.15 [m, 1H, –COCH–(CH_3)CH(*H*)COOH], 3.38 [m, 1H, –COC*H*(CH_3)CH_2COOH], 3.47 (t, 2H, N–CH_2–, piperidine), 3.56 [t, 2H, N–CH_2–, piperidine], 4.77 (s, 2H, –OCH_2–), 6.99 (d, 2H, C*H*, arom, J_o = 8.88 Hz) and 7.95 ppm (d, 2H, C*H*, arom, J_o = 8.76 Hz).

*5.1.2. General procedure for the synthesis of pyrrolidine/piperidine substituted 6-(4-carboxymethyloxyphenyl)-4,5-dihydro-3(2H)-pyridazinone **10**–**17***

Requisite hydrazine derivative (1 mmol) was added to a stirred and refluxing solution of substituted γ-keto acids **6**–**9** (1 mmol) in aldehyde free ethanol (50 ml). The reaction mixture was further refluxed for 8 h with continuous stirring (72 h reflux in case of 2-hydrazino-2-imidazoline hydrobromide). The completion of the reaction was monitored by TLC. The solvent was removed under reduced pressure to obtain a solid residue. Ice-cold water was added to it and the precipitate obtained was filtered off, washed with ice-cold water, dried and recrystallized from appropriate solvent to afford corresponding pyridazinone **10**–**17**.

*5.1.2.1. 6-[4-(2-Oxo-2-pyrrolidin-1-yl-ethoxy)phenyl]-4,5-dihydropyridazin-3(2H)-one (**10**).* Yield: 66.49%; m.p. 178–179 °C (methanol). IR: 3193, 3068, 1666, 1609, 1514, 1349, 1249, 827; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.86 (p, 2H, –CH_2–, pyrrolidine), 1.98 (p, 2H, –CH_2–, pyrrolidine), 2.59 (t, 2H, 4-CH_2), 2.95 (t, 2H, 5-CH_2), 3.52 [t, 4H, N–(CH_2)$_2$–, pyrrolidine], 4.67 (s, 2H, –OCH_2–), 6.98 (dd, 2H, C*H*, arom, J_o = 7.24 Hz, J_m = 1.95 Hz), 7.66 (dd, 2H, C*H*, arom, J_o = 6.78 Hz, J_m = 2.05 Hz) and 8.75 ppm (s, 1H, –N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.35; N, 13.94%. Found: C, 63.59; H, 6.17; N, 14.19%.

*5.1.2.2. 6-[4-(2-Oxo-2-pyrrolidin-1-yl-ethoxy)phenyl]-2-(4-fluorophenyl)-4,5-dihydropyridazin-3(2H)-one (**11**).* Yield: 58.68%; m.p. 178–179 °C (methanol). IR: 3190, 2879, 1660, 1645, 1510, 1332, 1257, 1184, 830; ^{1}H NMR ($CDCl_3$): δ 1.86 (p, 2H, –CH_2–, pyrrolidine), 1.98 (p, 2H, –CH_2–, pyrrolidine), 2.76 (t, 2H, 4-CH_2), 3.06 (t, 2H, 5-CH_2), 3.52 [t, 4H, N–(CH_2)$_2$–, pyrrolidine], 4.67 (s, 2H, –OCH_2–), 6.99 (d, 2H, C*H*, arom, J_o = 8.83 Hz), 7.09 (t, 2H, 3-C*H* and 5-C*H*, arom, *N*-*p*-fluorophenyl, J_o = 7.23 Hz), 7.55 ppm (m, 2H, 2-C*H* and 6-C*H*, arom, *N*-*p*-fluorophenyl) and 7.74 ppm (d, 2H, C*H*, arom, J_o = 8.78 Hz). Anal. Calc. for $C_{22}H_{22}N_3O_3$ F: C, 66.82; H, 5.61; N, 10.63%. Found: C, 66.68; H, 5.40; N, 10.86%.

*5.1.2.3. 2-[4,5-Dihydro-1H-imidazol-2-yl]-6-{4-(2-oxo-2-pyrrolidin-1-yl-ethoxy)phenyl}-4,5-dihydropyridazin-3(2H)-one (**12**).* Yield: 30.99%; m.p. 226–228 °C (water). IR: 3350, 2910, 1660, 1560, 1410, 1250, 840; ^{1}H NMR ($CDCl_3$ + CF_3COOD): δ 2.02 (p, 2H, –CH_2–, pyrrolidine), 2.13 (p, 2H,–CH_2, pyrrolidine), 2.80 (t, 2H, 4-CH_2), 2.97 (t, 2H, 5-CH_2), 3.63 [m, 4H, N–(CH_2)$_2$, pyrrolidine], 3.89 (s, 4H, 2 × –CH_2–, imidazoline), 4.83 (s, 2H, –OCH_2–), 6.98 (d, 2H, C*H*, arom, J_o = 8.09 Hz), 7.17 (d, 2H, C*H*, arom, J_o = 7.53 Hz) and 7.70 ppm (brs, 1H, –N*H*, imidazoline). Anal. Calc. for $C_{19}H_{23}N_5O_3 \cdot H_2O$: C, 58.90; H, 6.50; N, 18.08%. Found: C, 59.02; H, 6.33; N, 17.82%.

*5.1.2.4. 5-Methyl-6-[4-(2-oxo-2-pyrrolidin-1-yl-ethoxy)phenyl]-4,5-dihydropyridazin-3(2H)-one (**13**).* Yield: 63.29%; m.p. 158–159 °C (methanol). IR: 3213, 2927, 1680, 1655, 1514, 1453, 1347, 1256, 829; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.30 [d, 3H, 5-CH(CH_3)], 1.86 (p, 2H, –CH_2–, pyrrolidine), 1.97 (p, 2H, –CH_2–, pyrrolidine), 2.63 (m, 2H, 4-CH_2), 3.04 [m, 1H, 5-C*H*(CH_3)], 3.52 (t, 4H, N–(CH_2)$_2$–, pyrrolidine], 4.67 (s, 2H, –OCH_2–), 6.99 (dd, 2H, C*H*, arom, J_o = 7.28 Hz, J_m = 1.75 Hz), 7.67 (dd, 2H, C*H*, arom, J_o = 6.78 Hz, J_m = 2.02 Hz) and 8.55 ppm (s, 1H, –N*H*, pyridazinone, disappeared on deuterium exchange). Anal. Calc. for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32%. Found: C, 64.94; H, 6.74; N, 12.95%.

*5.1.2.5. 6-[4-(2-Oxo-2-piperidin-1-yl-ethoxy)phenyl]-4,5-dihydropyridazin-3(2H)-one (**14**).* Yield: 59.84%; m.p. 220–221 °C (acetone/ether). IR: 3240, 3200, 3020, 1680, 1630, 1320, 1235 820; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.58 (m, 6H, 3 × –CH_2–, piperidine), 2.59 (t, 2H, 4-CH_2), 2.96 (t, 2H, 5-CH_2), 3.49 (t, 2H, N–CH_2–, piperidine), 3.56 (t, 2H, N–CH_2–, piperidine), 4.73 (s, 2H, –OCH_2–), 6.98 (dd, 2H, C*H*, arom, J_o = 7.16 Hz, J_m = 1.79 Hz), 7.66 (dd, 2H, C*H*, arom, J_o = 7.22 Hz, J_m = 1.97 Hz) and 8.51 ppm (s, 1H, –N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32%. Found: C, 64.97; H, 6.53; N, 13.44%.

*5.1.2.6. 6-[4-(2-Oxo-2-piperidin-1-yl-ethoxy)phenyl]-2-phenyl-4,5-dihydropyridazin-3(2H)-one (**15**).* Yield: 48.95%; m.p. 140–142 °C (ether). IR: 2923, 1671, 1602, 1514, 1333, 1242, 1012, 844. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.60 (m, 6H, 3 × –CH_2–, piperidine), 2.77 (t, 2H, 4-CH_2), 3.06 (t, 2H, 5-CH_2), 3.52 [m, 4H, N–(CH_2)$_2$–, piperidine], 4.73 (s, 2H, –OCH_2), 6.98 (d, 2H, C*H*, arom, J_o = 8.83 Hz), 7.26 (m, 1H, 4-C*H*, *N*-phenyl), 7.42 (t, 2H, 3-C*H* and 5-C*H*, arom, *N*-phenyl, J_o = 7.79 Hz), 7.59 (d, 2H, 2-C*H* and 6-C*H*, arom, *N*-phenyl, J_o = 8.50 Hz) and 7.77 ppm (d, 2H, C*H*, arom, J_o = 8.81 Hz). Anal. Calc. for $C_{23}H_{25}N_3O_3$: C, 70.56; H, 6.44; N, 10.73%. Found: C, 70.36; H, 6.23; N, 10.50%.

*5.1.2.7. 2-[4,5-Dihydro-1H-imidazol-2-yl]-6-{4-(2-oxo-2-piperidin-1-yl-ethoxy)phenyl}-4,5-dihydropyridazin-3(2H)-one (**16**).* Yield: 44.42%; m.p. 215–216 °C (water). IR: 2925, 1657, 1387, 1221; ^{1}H NMR ($CDCl_3$ + CF_3COOD): δ 1.80 (brs, 6H, 3 × –CH_2–, piperidine), 2.84 (brs, 2H, 4-CH_2), 2.99 (brs, 2H, 5-CH_2), 3.63 (brs, 2H, N–CH_2–, piperidine), 3.77 (brs, 2H, N–CH_2–, piperidine), 3.91 (s, 4H, 2 × –CH_2–, imidazoline), 4.99 (s, 2H, –OCH_2), 7.03 (d, 2H, C*H*, arom, J_o = 7.34 Hz) and 7.20 ppm (brs, 2H, C*H*, arom). Anal. Calc. for $C_{20}H_{25}N_5O_3 \cdot H_2O$: C, 59.83; H, 6.78; N, 17.45%. Found: C, 59.80; H, 6.76; N, 17.72%.

*5.1.2.8. 5-Methyl-6-[4-(2-oxo-2-piperidin-1-yl-ethoxy)phenyl]-4,5-dihydropyridazin-3(2H)-one (**17**).* Yield: 65.06%; m.p. 164–165 °C (acetone/ether). IR: 3209, 3093.8, 2939, 1646, 1510, 1344, 1239, 1048, 829; ^{1}H NMR ($CDCl_3$): δ 1.31 [d, 2H, 5-CH(CH_3)], 1.64 (m, 6H, 3 × –CH_2–, piperidine), 2.61 (m, 1H, 4-CH_2), 3.04 [m, 1H, 5-C*H*(CH_3)], 3.49 (s, 2H, N–CH_2–, piperidine), 3.56 (s, 2H, N–CH_2–, piperidine), 4.73 (s, 2H, –OCH_2–), 6.99 (d, 2H, C*H*, arom, J_o = 8.72 Hz), 7.67 (d, 2H, C*H*, arom, J_o = 8.95 Hz) and 8.48 ppm (s, 1H, –N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76%. Found: C, 65.69; H, 6.69; N, 12.72%.

*5.1.3. General procedure for the synthesis 4-(4-(methoxycarbonylmethoxyphenyl)-4-oxobutyric acids **18**, **19***

A mixture of phenoxyacetic acid methyl ester **3** (1.0 g, 6.02 mmol) and succinic anhydride (1.0 g, 9.99 mmol)/methyl-succinic anhydride (1.0 g, 8.76 mmol) was added to a stirred solution of aluminium chloride (3 g) in nitrobenzene (6 ml). The reaction mixture was stirred manually under the anhydrous conditions for 20 min and was allowed to stand for 48 h at room temperature. The reaction contents were decomposed with crushed ice and steam distilled to remove nitrobenzene. Hot solution was filtered and filtrate cooled in ice for complete precipitation. The precipitate obtained was filtered and washed thoroughly with distilled water. The resulting solid was dissolved in 10% aqueous sodium bicarbonate solution and filtered off insoluble

part. Acidification of the filtrate with concentrated hydrochloric acid gave precipitate, which was filtered, washed with water, dried and recrystallized from methanol to afford the corresponding γ-keto acids **18**, **19**.

*5.1.3.1. 4-(4-(Methoxycarbonylmethoxyphenyl)-4-oxobutyric acid (**18**).* Yield: 49.93%; m.p. 124–126 °C. IR: 3252, 1735, 1669, 1603, 1509, 1244, 1175, 1084, 847, 815; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.26 (t, 2H, –COCH$_2$C*H*$_2$COOH), 3.26 (t, 2H, –COC*H*$_2$CH$_2$COOH), 3.82 (s, 3H, –COOC*H*$_3$), 4.72 (s, 2H, –OC*H*$_2$–), 6.95 (d, 2H, C*H*, arom, J_o = 8.74 Hz) and 7.97 ppm (d, 2H, C*H*, arom, J_o = 8.74 Hz).

*5.1.3.2. 4-(4-Carboxymethoxyphenyl)-3-methyl-4-oxobutyric acid (**19**).* Yield: 34.61%; m.p. 178–179 °C. IR: 2918, 1720, 1706, 1673, 1597, 1428, 1256, 1174, 1078, 844; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.25 [d, 3H, –COCH(C*H*$_3$)CH$_2$COOH], 2.98 [m, 2H, –COCH(CH$_3$)C*H*$_2$COOH], 3.40 (m, 1H, –COC*H*(CH$_3$)CH$_2$COOH), 4.68 (s, 2H, –OC*H*$_2$–), 6.97 (dd, 2H, C*H*, arom, J_o = 8.96 Hz, J_m = 1.76 Hz) and 7.95 ppm (dd, 2H, C*H*, arom, J_o = 7.26 Hz, J_m = 1.64 Hz). ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 17.03 [–CH(**C**H$_3$)], 34.59 (**C**H$_2$), 41.31 (**C**H), 64.64 (–O**C**H$_2$–), 114.07 (2 × –**C**H–, arom), 129.88 (**C** and 2 × **C**H, arom), 161.45 (**C**, arom), 169.69 (-**C**OOH), 177.32 (–**C**OOH) and 196.30 ppm (C=O).

*5.1.4. General procedure for the synthesis of morpholine/imidazole/N-methylpiperazine substituted γ-keto acids **20**–**24***

A mixture of requisite γ-keto acid **18**/**19** (0.3 g, 1.13 mmol) and morpholine (0.3 ml)/imidazole (0.5 g, 7.34 mmol)/1-methylpiperazine (0.3 ml) was fused together at 70 °C for 5 h. To the fused reaction mixture ice-cold water was added to remove any excess amine and contents were further cooled for complete precipitation. The solid thus obtained in case of compounds **20** and **22** was filtered, washed with water, dried and recrystallized from methanol to afford the corresponding substituted γ-keto acid. However, 3-methyl γ-keto acids **21**, **23** and *N*-methylpiperazine substituted γ-keto acid **24** were obtained as a sticky mass after washing with water and were used as such for further cyclization.

*5.1.4.1. 4-[4-(2-Morpholin-4-yl-2-oxoethoxy)phenyl]-4-oxobutyric acid (**20**).* Yield: 35.91%; m.p. 85–88 °C. IR: 3269, 2921, 1684, 1607, 1436, 1237, 1110, 850; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.70 (t, 2H, –COCH$_2$C*H*$_2$COOH), 3.10 [t, 4H, N–(C*H*$_2$)$_2$–, morpholine], 3.23 (t, 2H, –COC*H*$_2$CH$_2$COOH), 3.85 [t, 4H, O–(C*H*$_2$)$_2$–, morpholine], 4.58 (s, 2H, –OC*H*$_2$–), 6.97 (d, 2H, C*H*, arom, J_o = 8.57 Hz) and 7.94 ppm (d, 2H, C*H*, arom, J_o = 8.76 Hz).

*5.1.4.2. 4-[4-(2-Imidazol-1-yl-2-oxoethoxy)phenyl]-4-oxobutyric acid (**22**).* Yield: 71.34% m.p. 172–173 °C. IR: 3145, 1705, 1670, 1600, 1418, 1343, 1244, 1160, 1052, 825; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.92 (t, 2H, –COCH$_2$C*H*$_2$COOH), 3.45 (t, 2H, –COC*H*$_2$CH$_2$COOH), 4.88 (s, 2H, –OC*H*$_2$–), 7.04 (d, 2H, C*H*, arom, J_o = 8.51 Hz), 7.45 (s, 3H, imidazole), 8.03 (d, 2H, C*H*, arom, J_o = 8.59 Hz) and 8.78 ppm (brs, 1H, –N*H*, disappeared on deuterium exchange).

*5.1.5. General procedure for the synthesis of morpholine/imidazole/N-methylpiperazine substituted derivatives of 6-(4-carboxymethyloxyphenyl)-4,5-dihydro-3(2H)-pyridazinone **25**–**29***

Hydrazine hydrate (1 ml, 99%) was added to a stirred and refluxing solution of required substituted γ-keto acids **20**–**24** (1.5 mmol) in aldehyde free ethanol (50 ml). The reaction mixture was further heated under reflux for 10 h with continuous stirring. The completion of the reaction was monitored by TLC. The solvent was removed under reduced pressure to obtain a solid residue. Ice-cold water was added to it and the precipitate obtained was filtered off, washed with ice-cold water, dried and recrystallized from appropriate solvent to afford corresponding pyridazinones **25**–**29**.

*5.1.5.1. 6-[4-(2-Morpholin-4-yl-2-oxoethoxy)phenyl]-4,5-dihydropyridazin-3(2H)-one (**25**).* Yield: 70.88%; m.p. 191–192 °C (acetone). IR: 3269, 2916, 1681, 1605, 1335, 1237, 1110, 849; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.60 (t, 2H, 4-C*H*$_2$), 2.96 (t, 2H, 5-C*H*$_2$), 3.64 (m, 8H, N–(C*H*$_2$)$_2$– and O–(C*H*$_2$)$_2$–, morpholine), 4.74 (s, 2H, –OC*H*$_2$–), 6.98 (d, 2H, C*H*, arom, J_o = 8.72 Hz), 7.67 (d, 2H, C*H*, arom, J_o = 8.68 Hz) and 8.44 ppm (s, 1H, –N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{16}H_{19}N_3O_4$: C, 60.56; H, 6.03; N, 13.24%. Found: C, 60.43; H, 5.90; N, 12.90%.

*5.1.5.2. 5-Methyl-6-[4-(2-morpholin-4-yl-2-oxoethoxy)phenyl]-4,5-dihydropyridazin-3(2H)-one (**26**).* Yield: 43.19%; m.p. 220–221 °C (ethanol). IR: 3198, 2927, 1677, 1612, 1514, 1424, 1337, 1247, 1107, 832. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.28 [d, 3H, 5-CH(C*H*$_3$)], 2.57 (m, 2H, 4-C*H*$_2$), 3.05 [m, 5H, 5-C*H*(CH$_3$) and N–(C*H*$_2$)$_2$–, morpholine], 3.84 [t, 4H, O–(C*H*$_2$)$_2$–, morpholine], 4.55 (s, 2H, –OC*H*$_2$–), 6.94 (d, 2H, C*H*, arom, J_o = 8.93 Hz), 7.66 (d, 2H, C*H*, arom, J_o = 8.78 Hz) and 9.70 ppm (s, 1H, –N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68%. Found: C; 61.95; H, 6.55; N, 12.47%.

*5.1.5.3. 6-[4-(2-Imidazol-1-yl-2-oxoethoxy)phenyl]-4,5-dihydropyridazin-3(2H)-one (**27**).* Yield: 64.85%; m.p. 235–236 °C (acetone). IR: 3212, 3120, 2928, 1672, 1615, 1429, 1344, 1244, 1061, 824; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.55 (t, 2H, 4-C*H*$_2$), 2.94 (t, 2H, 5-C*H*$_2$), 4.62 (s, 2H, –OC*H*$_2$–), 6.93 (d, 2H, C*H*, arom, J_o = 8.64 Hz), 7.08 (s, 2H, 4-C*H* and 5-C*H*, imidazole), 7.68 (d, 2H, C*H*, arom, J_o = 8.22 Hz), 7.82 (s, 1H, 2-C*H*, imidazole) and 10.37 ppm (s, 1H, –N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{15}H_{14}N_4O_3$: C, 60.39; H, 4.73; N, 18.78%. Found: C, 60.11; H, 4.62; N, 18.57%.

*5.1.5.4. 6-[4-(2-Imidazol-1-yl-2-oxoethoxy)phenyl]-5-methyl-4,5-dihydropyridazin-3(2H)-one (**28**).* Yield: 35.80%; m.p. 232–233 °C (ethanol). IR: 3220, 3115, 2929, 1673, 1610, 1342, 1244, 1061, 878; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.25 [d, 3H, 5-CH(C*H*$_3$)], 2.56 (m, 2H, 4-C*H*$_2$), 3.05 [m, 1H, 5-C*H*(CH$_3$)], 4.61 (s, 2H, –OC*H*$_2$–), 6.93 (d, 2H, C*H*, arom, J_o = 8.58 Hz), 7.07 (s, 2H, 4-C*H* and 5-C*H*, imidazole), 7.67 (d, 2H, C*H*, arom, J_o = 8.82 Hz), 7.80 (s, 1H, 2-C*H*, imidazole) and 10.25 ppm (s, 1H,–N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{16}H_{16}N_4O_3$:C, 61.53; H, 5.16; N, 17.94%. Found: C, 61.27; H, 4.99; N, 17.91%.

*5.1.5.5. 6-[4-[2-(4-Methylpiperazin-1-yl)-2-oxoethoxy]phenyl}-4,5-dihydropyridazin-3(2H)-one (**29**).* Yield: 40.29%; m.p. 160–161 °C (acetone). IR: 3049, 2916, 1660, 1612, 1338, 1219, 1130, 838; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.30 (t, 3H, N-C*H*$_3$), 2.41 (m, 4H, CH$_3$–N–(C*H*$_2$)$_2$–, *N*-methylpiperazine), 2.59 (t, 2H, 4-C*H*$_2$), 2.96 (t, 2H, 5-C*H*$_2$), 3.62 (m, 8H, N–(C*H*$_2$)$_2$–, *N*-methylpiperazine), 4.74 (s, 2H, –OC*H*$_2$–), 6.98 (dd, 2H, C*H*, arom, J_o = 9.60 Hz, J_m = 2.89 Hz), 7.67 (dd, 2H, C*H*, arom, J_o = 9.70 Hz, J_m = 2.90 Hz) and 8.64 ppm (s, 1H, –N*H*, disappeared on deuterium exchange). Anal. Calc. for $C_{17}H_{22}N_4O_3$:C, 61.80; H, 6.71; N, 16.96%. Found: C, 61.55; H, 6.41; N, 16.81%.

*5.1.6. [4-(6-Oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenoxy]-acetohydrazide (**30**).* Hydrazine hydrate (1.0 ml, 99%) was added to a stirred and refluxing solution of **18** (0.6 g, 2.25 mmol) in aldehyde free ethanol (50 ml). The reaction mixture was further refluxed for 2 h with continuous stirring. The reaction was monitored with TLC for the disappearance of starting material. Afterwards, the reaction mixture was concentrated to half of the volume and left overnight in refrigerator for crystallization. The crystals obtained were

filtered off, washed with cold ethanol, dried and recrystallized from ethanol to afford **30**. Yield: 68.33%; m.p. 208–210 °C. IR: 3240, 3200, 1680, 1510, 1340, 1245, 1160, 750; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.55 (t, 2H, 4-C*H*$_2$), 2.94 (t, 2H, 5-C*H*$_2$), 4.06 (s, 2H, –N*H*$_2$), 4.58 (s, 2H, –OC*H*$_2$–), 6.96 (d, 2H, C*H*, arom, $J_o = 8.49$ Hz), 7.66 (d, 2H, C*H*, arom, $J_o = 7.66$ Hz), 8.71 (s, 1H, –N*H*, disappeared on deuterium exchange) and 10.06 ppm (s, 1H, –N*H*, pyridazinone, disappeared on deuterium exchange). Anal. Calc. for $C_{12}H_{12}N_4O_3$: C, 54.95; H, 5.38; N, 21.36%. Found: C, 54.75; H, 5.56; N, 21.58%.

5.1.7. *[4-(6-Oxo-1-substituted-1,4,5,6-tetrahydropyridazin-3-yl)phenoxy]-acetic acid ethyl ester **31**, **32***

Requisite hydrazine derivative (2 mmol) was added to a stirred and refluxing solution of **18** (0.5 g, 1.88 mmol) in aldehyde free ethanol (50 ml). The reaction mixture was further refluxed for 12 h with continuous stirring (72 h reflux in case of 2-hydrazino-2-imidazoline hydrobromide). The reaction was monitored with TLC for the disappearance of starting material. Afterwards, the solvent was removed under reduced pressure to obtain a solid residue. Ice-cold water was added to it and the precipitate obtained was filtered off, washed with ice-cold water, dried and recrystallized from an appropriate solvent to afford corresponding pyridazinones **31**, **32**.

*5.1.7.1. [4-(6-Oxo-1-phenyl-1,4,5,6-tetrahydropyridazin-3-yl)phenoxy]-acetic acid ethyl ester (**31**).* Yield: 46.84%; m.p. 94–95 °C (ether). IR: 2911, 1756, 1728, 1675, 1597, 1513, 1331, 1245, 1179, 1074, 832; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.30 (t, 3H, –OCH$_2$C*H*$_3$), 2.72 (t, 2H, 4-C*H*$_2$), 3.06 (t, 2H, 5-C*H*$_2$), 4.28 (q, 2H, –OC*H*$_2$CH$_3$), 4.66 (s, 2H, –OC*H*$_2$–), 6.94 (d, 2H, C*H*, arom, $J_o = 8.81$ Hz), 7.27 (m, 1H, 4-C*H*, arom, *N*-phenyl), 7.42 (t, 2H, 3-C*H* and 5-C*H*, arom, *N*-phenyl, $J_o = 7.80$ Hz), 7.58 (d, 2H, 2-C*H* and 6-C*H*, arom, *N*-phenyl, $J_o = 8.80$ Hz) and 7.77 ppm (d, 2H, C*H*, arom, $J_o = 8.86$ Hz). Anal. Calc. for $C_{20}H_{20}N_2O_4$: C, 68.16; H, 5.72; N, 7.95%. Found: C, 67.93; H, 5.58; N, 7.66%.

*5.1.7.2. {4-[1-(4,5-Dihydro-1H-imidazol-2-yl)-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl]phenoxy}acetic acid ethyl ester (**32**).* Yield: 25.13%; m.p. 215–216 °C (water). IR: 2920, 1738, 1667, 1508, 1406, 1236, 1086, 703; ^{1}H NMR ($CDCl_3$ + CF_3COOD): δ 1.37 (t, 3H, –OCH$_2$C*H*$_3$), 2.82 (s, 2H, 4-C*H*$_2$), 2.99 (s, 2H, 5-C*H*$_2$), 3.91 (s, 4H, 2 × –C*H*$_2$–, imidazoline), 4.39 (q, 2H, –OC*H*$_2$CH$_3$), 4.76 (s, 2H, –OC*H*$_2$–), 6.97 (m, 2H, C*H*, arom, $J_o = 7.65$ Hz) and 7.19 ppm (brs, 2H, C*H*, arom). Anal. Calc. for $C_{17}H_{20}N_4O_4 \cdot H_2O$: C, 59.28; H, 5.85; N, 16.27%. Found: C, 59.34; H, 6.12; N, 15.96%.

5.2. Vasodilatory activity

Vasodilatory activity of newly synthesized compounds was studied using descending thoracic aortic rings of wistar rats pre-contracted with phenylephrine (10^{-6} M) [17]. 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 mM), KCl (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 of fat and connective tissue was removed, the aorta were cut into rings (4–5 mm in length), mounted under the 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 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 single concentration of phenylephrine (10^{-6} M). When the contractions were 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 dimethyl sulfoxide (DMSO) to prepare a 10^{-2} M stock solution. Further solutions were made in PSS.

All the results are expressed as means ± the standard error of the mean (SEM). The response of the aortic rings to all compounds 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 Emax expressed as negative log molar) and Emax value (the maximal effect).

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