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Synthesis and Biological Evaluation of 2-(N-substituted)-3H-phthalazin-1, 4-diones and 1-(N- substituted) 2, 4, 5-trihydropyridazin-3, 6-diones as Potent Vasodilators

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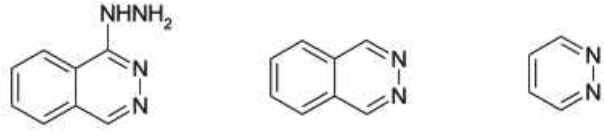
Abstract

*In view of increasing incidents of cardiovascular complications and the subsequent urgent need in the development of therapeutic agents, 2-(N- substituted)-3H-phthalazin-1,4-diones(**3a-d**) and 1-(N- substituted)2,4,5-trihydropyridazin-3,6-diones(**4a-d**) have been synthesized and characterized by spectral data. The title compounds were prepared by condensing substituted aryl hydrazides with either phthalic anhydride or succinic anhydride. The synthesized compounds have been screened for coronary vasodilator activity on the isolated rat heart at 10 µg concentration. Almost all the compounds have exhibited significant activity. The in vitro nitric oxide donor activity by Griess reagent has been studied to understand mechanism of action of these compounds. The compounds released nitric oxide significantly at 100 µmol, indicating the coronary vasodilatation produced by these compounds could be at least, partially due to release of nitric oxide.*

Keywords: *phthalazines, pyridazines, antihypertensive, vasodilator, nitric oxide, Griess reagent*

INTRODUCTION

Cardiovascular diseases remain the most common cause of death in industrialized countries like India, and hypertension is one of the most important treatable causes of morbidity and mortality leading to stroke and end-stage renal disease in elderly.[1] The prevalence of hypertension in 2000 was 26% of the adult population globally and that in 2025 would increase by 24% in developed countries and 80% in developing countries.[2] There are an estimated 1 billion hypertensives in the world; fifty million out of them are in India alone. Hence, there seems to be an urgent requirement to develop newer antihypertensive agents. Vasodilators form an important class of antihypertensive agents and act by dilating the blood vessels. Hydralazine **I**, a phthalazine derivative is a potent vasodilator which acts by an unknown mechanism and clinically prescribed antihypertensive agent.[3] Phthalazine **II** compounds have been synthesized with diverse biological interest and reported for anticonvulsant,[4] antitumor,[5] cytotoxic,[6] antimicrobial[7] and vascular endothelial growth factor receptor II (VEGFR-2) inhibitory[8] activities. Pyridazine **III** (that closely resemble in structure to phthalazine lacking the condensed benzene ring) compounds have also been reported to possess varied biological activities such as anticonvulsant, [9] antibacterial, [10] anti-inflammatory,[11] anticancer[12] and antiplatelet[13] activities. These facts have prompted us to synthesize some novel phthalazine and pyridazine compounds namely, 2-(N-substituted)-3H-phthalazin-1, 4-diones and 1-(N- substituted) 2, 4, 5-trihydropyridazin-3, 6-diones as potent vasodilators.



I **II** **III**

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MATERIALS AND METHODS

General: The chemicals used were of AR grade. The progress of reaction and the purity of products were analyzed by TLC. Melting points were taken in an open capillary tube and are uncorrected. IR spectra were obtained on Shimadzu FTIR-8700 by KBr pellet technique. ^{1}H-NMR spectra were recorded on Burker advance II 400 in DMSO*d6* by using TMS as internal standard. Mass spectra were recorded on Finnigan-Mat 1020 (EI, 70 ev).

Animals: Wister albino rats of either sex weighing 175-200 g, maintained on standard diet water *ad libitum* were used and the usage was permitted by Institutional Animal Ethics Committee (IAEC).

Statistics: The values expressed were mean ± SD of a triplicate result. Data were analyzed by Student 't' test using Graph-pad Prism software. The results were considered significant at $p<0.05$.

Vasodilator activity:[14]

Albino rats were sacrificed and the heart was isolated quickly and placed in a dish containing Krebs-Henseleit buffer solution at 37 ^{0}C. Associated pericardial and lung tissue were removed. The aorta was located and cut just below the point of its division. A glass cannula was introduced into the aorta, tied with two threads and perfusion was started with oxygenated Krebs-Henseleit buffer solution. The heart was transferred to a double walled plexi-glass perfusion apparatus. A small steel hook with a string was attached to the apex of the heart. Heart rate was measured through a chronometer coupled to the physiograph. Cardiac output was measured by a drop counter. Compounds were injected into the perfusion medium just above the aortic cannula. Parameters of normal heart were recorded first and then with barium chloride. The synthesized compounds dissolved in DMSO were introduced at 10 µg concentration. Sodium nitroprusside (SNP) aqueous solution at 10 µg concentration was used as a standard.

Nitric oxide donor property:[15]

Solutions of the synthesized phthalazines and pyridazines in DMSO measuring 100 µmol of substance were added to phosphate buffer pH 7.4 (50mM) containing 5 mM of L-cysteine hydrochloride and the final volume was made up to 2 ml with phosphate buffer pH 7.4. After the reaction mixture stood for 3 hr at 37± 1 ^{o}C, it was added with 500 µL of the Griess reagent and the absorbance was measured at 550 nm after 20minutes; 5-50 µM sodium nitrite standard solutions were used for calibration curve.

EXPERIMENTAL

Preparation of ethyl-N-aryl glycinates (1a-c):

A mixture of substituted aniline (0.1mol), ethyl chloroacetate (0.12mol) anhydrous sodium acetate (0.15mol) in 50ml absolute ethanol was refluxed for 6 hours. The mixture was cooled and left overnight at room temperature. It was poured in to 150 ml cold water. The solid obtained was washed with cold water.

Ethyl–N-phenylglycinate (**1a**): 56%, 57 ^{0}C, Ethyl –N-(*p*-anisyl)glycinate (**1b**): 58%, 50 ^{0}C, Ethyl –N-(3-chloro-4-flurophenyl)glycinate (**1c**): 59%, 89-91 ^{0}C

Preparation of hydrazides (2a-c):

A portion of hydrazine hydrate (80%, 0.1mol) was added drop wise to ethyl-N-arylglycinate (**1a-c)** (0.1mol) with constant stirring. The mixture was gently refluxed for 15 min. Then about 25 ml absolute ethanol was added to produce a clear solution. The reaction mixture was refluxed for 3 hr. The ethanol was distilled off under reduced pressure, cooled. Resulting solid was recrystallized from ethanol.

2a: (87 %, 166 ^{0}C), **2b**: (66%, 110 ^{0}C), **2c**: (85 %, 80 ^{0}C)

Preparation of phthalazine derivatives (3a-d):[16]

A mixture of hydrazide **(2a-c)** (0.01mol), phthalic anhydride (0.01mol) in 5 ml absolute ethanol and glacial acetic acid (0.005mol) was refluxed for 3 hr, cooled. The reaction mixture was poured into crushed ice. The solid obtained was filtered, washed with dilute sodium bicarbonate solution and recrystallized with suitable solvent.

Preparation of pyridazine derivatives (4a-d):

The pyridazine derivatives were prepared in similar fashion with succinic anhydride.

RESULTS AND DISCUSSION

The title compounds, 2-(N-substituted)-3H-phthalazin-1, 4-diones (**3a-d**) and 1-(N- substituted) 2, 4, 5-trihydropyridazin-3, 6-diones (**4a-d**) were synthesized in good yields (**Scheme - 1**). Substituted anilines were condensed with ethylchloroacetate in presence of

anhydrous sodium acetate to give the corresponding ethyl-N-(substituted phenyl) glycinates (**1a-c**), which on treatment with hydrazine hydrate, yielded the corresponding acid hydrazides, N-(substituted phenyl) ethyl carbohydrazides (**2a-c**) which, on condensation with phthalic anhydride and succinic anhydride afforded phthalazine (**3a-c**) and pyridazine (**4a-c**) derivatives respectively. The compounds **3d** and **4d** were synthesized by treating isonicotinic acid hydrazide with phthalic anhydride and succinic anhydride respectively. The intermediates and the final compounds were characterized by IR, ^{1}H-NMR and Mass spectral studies (Table-1). The physical data of **3a-d** and **4a-d** are presented in Table-2.

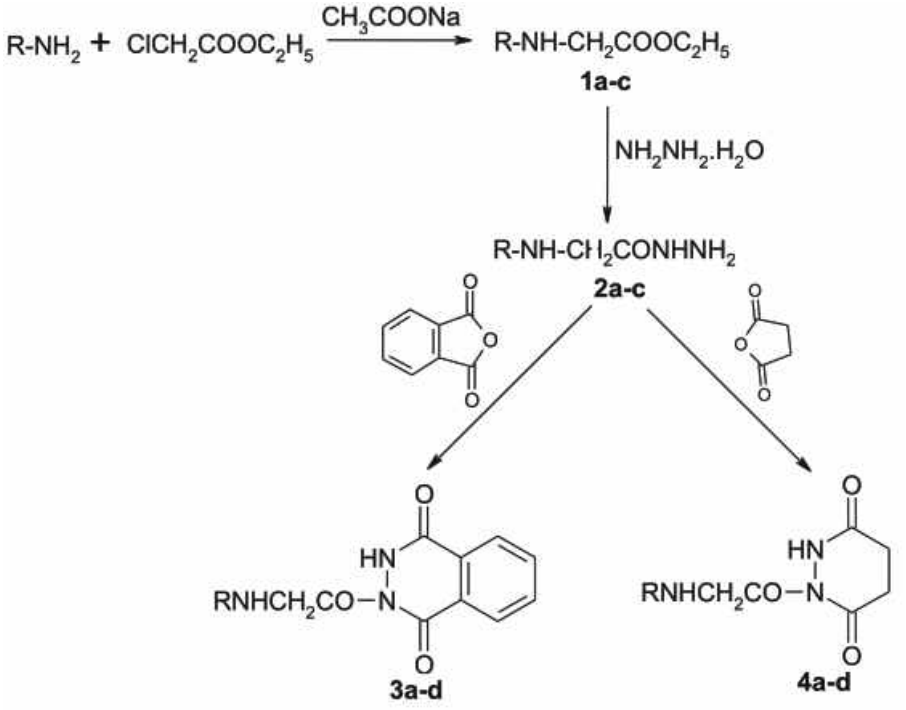
The title compounds were screened for *in vitro* coronary vasodilator activity at 10μg concentration on isolated rat heart by Langendorff-technique. Vasodilatation was measured by cardiac output and stroke volume. Increase in these parameters indicates vasodilatation. Vasoconstriction was induced by Barium chloride as it selectively acts on blood vessels by blocking the K^+ ion channels.[17] Barium chloride decreased the cardiac output and stroke volume. Such a heart when administered with synthesized compounds exhibited increased cardiac output and stroke volume indicating an excellent vasodilator activity by these compounds. Compounds **3a**-**d**, **4a**, **4b** and **4d** increased cardiac out put more than **4c**. In general, phthalazine derivatives increased the stroke volume more than pyridazine derivatives. All the synthesized compounds did not increase the force of contraction (measured by the magnitude of contraction) appreciably, indicating their locus of action on coronary blood vessels rather than the cardiac muscle.

The *in vitro* nitric oxide (NO) donor activity of these compounds by Griess reaction was studied to probe the mechanism of action. The capacity to release NO was expressed as NO_2^-% *i.e.*, the ratio between the number of NO_2^- mol detected and the number of phthalazines/pyridazines mol, multiplied by 100. NO is a potent vasodilator produced in the endothelial cells of blood vessels and any compound that can donate NO endogenously can act as vasodilator.[18] At 100 μm, all the compounds showed highly significant NO donor activity. Phthalazine compounds **3a-d** have released higher amount of NO, compared to pyridazine compounds **4a-d.** Compound **3d** released more NO than the standard, SNP. Compounds **3d** and **4d** have been found to show higher vasodilator activity as well as NO donor activity implying 4-pyridylcarbonyl group when present in the side chain enhances the activity than substituted phenyl ring. The results are shown in Table-3.

CONCLUSION

In the present study, 2-(N-substituted)-3H-phthalazin-1, 4-diones and 1-(N- substituted)-2, 4,5-trihydropyridazin-3,6-diones were synthesized with the presumption of vasodilator activity. Phthalazine derivatives showed better coronary vasodilator activity than pyridazine derivatives on the isolated rat heart. The title compounds have also exhibited good NO donor activity *in vitro*. Thus the vasodilator activity exhibited by these compounds could be mediated at least partially, through the release of NO. However, further work is needed in the form of synthesis and activity of some more compounds before one could conclude any thing definite about the therapeutic potential of these compounds.

SCHEME - 1



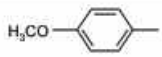
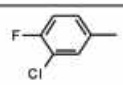
Compound	R
3a, 4a	
3b, 4b	
3c, 4c	
3d, 4d	$RNHCH_2$ = 

Table 1: Spectral data of Synthesized Compounds

Comp	Name	IR, cm^{-1}	^{1}H-NMR, δ ppm	MS, *m/z*
2a	Anilinomethyl carbo hydrazide	3339.11, 3308.94 (NH str), 3018.71 (Ar C-H str), 2903.26 (aliphatic C-H str), 1652.00 (CO amide)	8.70 (s, 1H, CONH), 6.58- 7.17 (m, 5H, Ar), 5.06-5.09 (d, 1H, ArNH), 3.95-3.99 (d, 2H, NH_2), 3.77-3.79 (d, 2H, CH_2CO)	--
2b	*p*-anisylamino methylcarbohydrazide	3348.64, 3298.25 (NH str), 3050.04 (Ar C-H str), 2897.59 (aliphatic C-H str), 1659.59 (CO amide)	6.54 – 6.74 (m, 4H Ar), 8.78 (s, 1H, CONH), 4.9 (s, 1H, ArNH), 3.99 (s, 2H, NH_2), 3.70 (s, 5H, merged signal of OCH_3, CH_2)	--
2c	3-chloro-4-fluoro anilinomethyl carbohydrazide	3474.02, 3411.85 (NH str), 3043.33 (Ar C-H str), 1672.19 (CO amide)	8.92 (s, 1H, CONH), 6.46- 6.96 (m, 3H, Ar), 5.50-5.53 (d, 1H, ArNH), 4.00-4.02 (d, 2H, NH_2), 3.70-3.72 (d, 2H, CH_2CO)	--
3a	2-(N-phenyl glycyl)-3-H-pthalazin-1, 4-dione	3326.54 (NH str), 3039.66 (Ar C-H str), 1748.12, 1664.17 (CO str)	10.34 (s, 1H, ring NH), 6.71-7.91 (m, 9H, Ar), 5.12 (s, 1H, ArNH), 4.03 (s, 2H, CH_2)	M^+ 294.6 (14), 296.3 (100), 297.2 (18)
3b	2-(N-*p*-anisyl glycyl)-3-H-phthalazin-1, 4-dione	3317 (NH str), 3024 (Ar C-H str), 2930.12 (aliphatic C-H str), 1741.31 (CO str)	10.19 (s, 1H, CONH), 6.69-7.90 (m, 8H, Ar), 4.74-4.81 (d, 1H, ArNH), 3.98-4.00 (d, 2H, CH_2), 3.75 (s, 3H, OCH_3)	--
3c	2-[N-(3 chloro-4-fluorophenyl) glycyl]-3-H-phthalazin-1,4-dione	3380.27, 3319.94 (NH str), 1734.98 (CO str)	10.52 (s, 1H, ring NH), 6.56-7.90 (m, 7H, Ar), 5.61-5.62 (d, 1 H, ArNH), 3.97-3.98 (d, 2H, CH_2)	M^++1 348 (100), 350 (33), 349 (18), 346(8), 351(6)
3d	2-N-isonicotinyl-3-H-phthazin-1, 4-dione	3340.15 (NH str), 3185.01 (Ar C-H str), 1734.99 (CO str)	--	--
4a	1-(N-phenyl glycyl)-2, 4, 5-trihydropyridazin -3, 6-dione	3378.43 (NH str), 3036.28 (Ar C-H str), 1721.15 (CO str)	10.48 (s, 1H, ring NH), 6.80-8.10 (m, 5H, Ar), 4.60 (s, 1H, ArNH), 4.02 (s, 2H, CH_2), 2.56-2.63 (m, 4H, ring CH_2)	--
4b	1- (N-*p*-anisyl glycyl)-2, 4, 5-trihydropyridazin -3, 6-dione	3362.81, 3250.41 (NH str), 3034.13 (Ar C-H str), 2925.37 (aliphatic C-H str), 1715.89 (CO str)	--	M^++1 278.2 (20), 124.3 (8), 240.3 (7),264.2(2), 287.3(25), 359.2 (100)
4c	1-[N-(3chloro -4-fluoro phenyl) glycyl]-2, 4, 5-trihydropyridazin -3, 6-dione	3424.04, 3316.35 (NH str), 1735.38 (CO str)	--	--
4d	1-N-isonicotinyl-2, 4, 5-trihydro pyridazin-3, 6-dione	3259.31, 3201.25 (NH str), 3033.83 (Ar C-H str), 1710.13 (CO str)	10.54 (s, 1H, ring NH), 7.80-8.73 (m, 4H, Ar), 2.58-2.64 (m, 4H, ring CH_2)	--

Table 2: Physical data of Synthesized Compounds

Comp	Mol. Formula	Mol.Wt.	Yield %	M.P. ^{0}C	Recrystallization solvent
3a	$C_{16}H_{13}N_3O_3$	295	82	167	Water
3b	$C_{17}H_{15}N_3O_4$	325	71	166	Ethanol
3c	$C_{16}H_{11}ClFN_3O_3$	347	86	223	Water
3d	$C_{14}H_9N_3O_3$	267	70	241	Ethanol
4a	$C_{12}H_{13}N_3O_3$	247	69	201	Ethanol
4b	$C_{13}H_{15}N_3O_4$	277	63	158	Water
4c	$C_{12}H_{11}ClFN_3O_3$	299	58	228	Water
4d	$C_{10}H_9N_3O_3$	219	84	258	Ethanol

Table 3: Vasodilator and Nitric oxide Donor Activities of Synthesized Compounds

Treatment	Heart Rate/min	Cardiac Out put/min (ml)	Stroke Volume (ml)	% NO_2^-
Normal	39.867±0.904	2.500±0.081	0.063±0.002	--
$BaCl_2$	47.4±1.230	1.980±0.094	0.041±0.002	--
3a	43.33±1.528	2.533±0.0573**	0.0580±0.001*	55±5.65***
3b	39.67 ± 1.528	2.467 ± 0.1528*	0.06227 ± 0.0055	38 ± 2.01***
3c	34.67 ± 1.155	2.367 ± 0.0577*	0.06708 ± 0.0005***	49 ± 4.67***
3d	34.33 ± 1.528	2.433 ± 0.0577**	0.06747 ± 0.0043**	83 ± 1.86***
4a	43.33 ± 0.577	2.367 ± 0.0577*	0.0540 ± 0.001	32 ± 1.75***
4b	42.33 ± 0.577	2.600 ± 0.1**	0.06137 ± 0.0024*	23 ± 3.53***
4c	37.00 ± 1.00	2.267 ± 0.0577	0.06123 ± 0.003*	16 ± 1.34***
4d	39.33 ± 1.155	2.467 ± 0.0577**	0.06273±0.0028*	52 ± 2.33***
SNP	40.33 ± 0.577	3.133 ± 0.1155***	0.08173±0.0021***	65 ± 2.17***
DMSO	38.33±1.528	2.167±0.0577	0.05373±0.0021	--

*p<0.05; **p<0.01; ***p<0.001 when compared to control.

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