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Synthesis of New 4,5-3(2H)pyridazinone Derivatives and Their Cardiotonic, Hypotensive, and Platelet Aggregation Inhibition Activities

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4,5-dihydro-3(2H)pyridazinones such as CI-914, CI-930 and pimobendan along with tetrahydropyridopyridazine (endralazine) and perhydropyridazinodiazepine (cilazopril) have been used as potent positive inotropes, antihypertensives as well as platelet aggregation inhibitors. Accordingly, the present work involves the synthesis of 24 target compounds; 4,5-dihydro-3(2H)pyridazinones in addition to seven reported intermediates. The chemical structures of the new compounds were assigned by microanalysis, IR, ^{1}H-NMR spectral analysis and some representatives by mass spectrometry. The positive inotropic effect of the final compounds and the intermediates **12a-12d** as well as the reported intermediate compound **10** was determined in-vitro on isolated rabbit heart in comparison to digoxin. Data obtained revealed that twelve of the test compounds exhibited higher effective response than digoxin, nine compounds elicited comparable effects to digoxin and eight compounds were less active than digoxin. In addition, four compounds approved marked significant hypotensive effect better than that of the previously reported compound **10**. Moreover, two compounds induced complete platelet aggregation inhibition. The last two compounds were also subjected to determination of their LD_{50} and they showed no signs of toxicity up to the dose level 300 mg/kg (i.p.), while the reported oral LD_{50} of digoxin is 17.78 mg/kg. Correlation of cardiotonic and hypotensive activities with structures of compounds was tried and pharmacophore models were computed to get useful insight onto the essential structural features required for inhibiting phosphodiesterase-III in the heart muscles and blood vessels.

Key words: Positive inotropic (Cardiotonic), 4,5-dihydropyridazinones, Hypotensive, Platelet aggregation inhibition, Acute toxicity, Pharmacophore models

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INTRODUCTION

Cardiac glycosides (digoxin, digitoxin) and sympathomimetic agents such as dopamine and dobutamine are well known positive inotropes and have been used for the treatment of congestive heart failure. However, these agents have got a limited therapeutic use due to arrhythmogenic and chronotropic liabilities respectively. In addition, sympathomimetic agents lack oral efficacy (Erhardt, 1987; Hansen, 1984; Forest et al., 1992)

Also, a series of pyridazine and pyridazinone derivatives were introduced into clinical practice as positive inotropes and vasodilators such as imazodan **1** (Erhardt, 1987; Forest et al., 1992; Bristol et al., 1984; Mertens et al., 1990; Sircar et al., 1985; Weishaar et al., 1985; Moss et al., 1987). CI-930 **2** (Erhardt, 1987; Sircar et al., 1985; Moss et al., 1987), pimobendan **3** (Sweetman, 2002; O' Neil et al., 2006; Rüegg et al., 1984; Rüegg, 1986; Fujino et al., 1988; Von Meel, 1985), endralazine **4** (Sweetman, 2002; O' Neil et al., 2006) and cilazopril **5** (Sweetman, 2002; O' Neil et al., 2006; Calam, 2001) (Chart 1). In addition, (Thyes et

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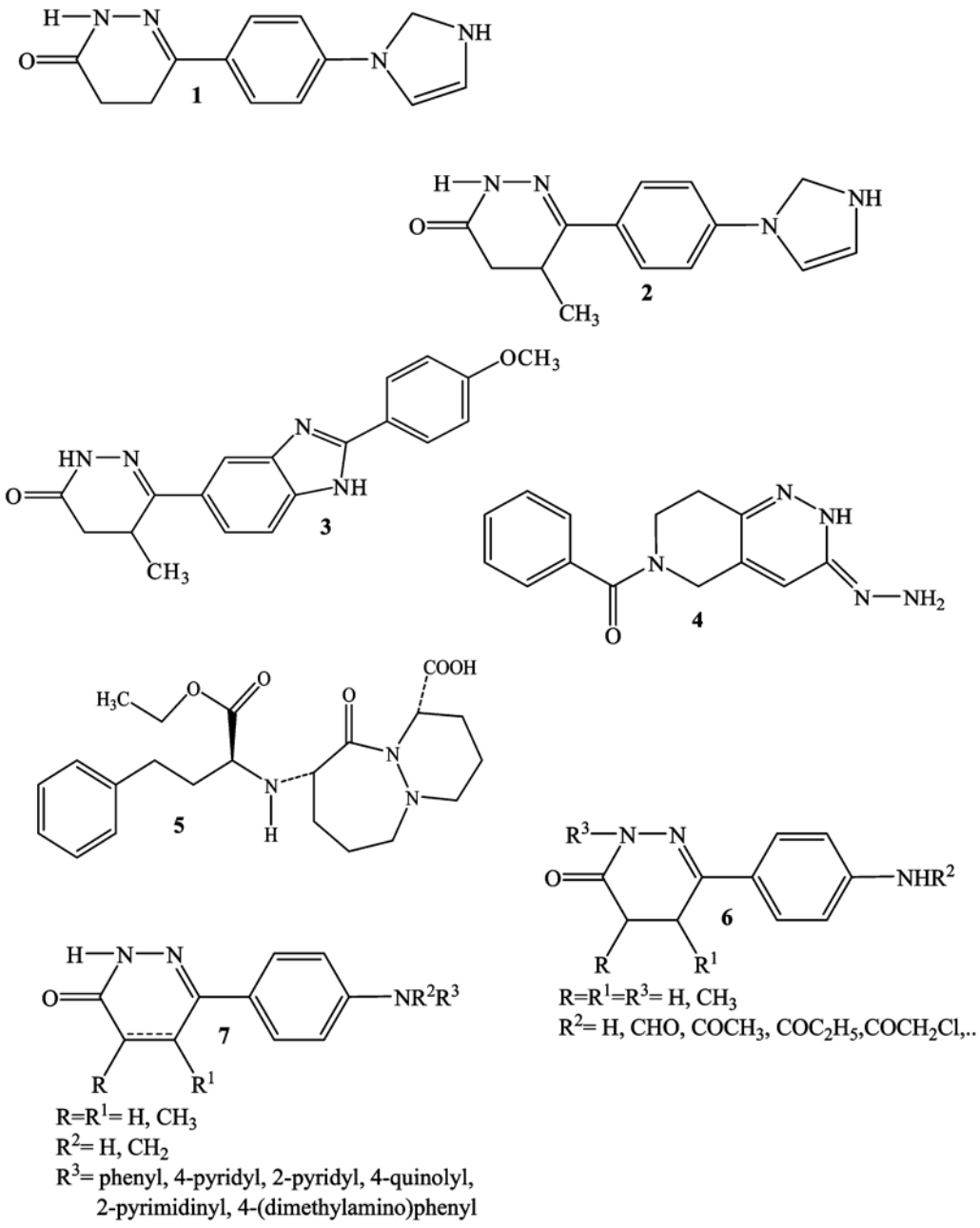

Chart 1. Structures of different pyridazine and pyridazinone positive inotropic agents

al., 1983) reported some 6-aryl-4,5-dihydro-3(2H) pyridazinones **6** with platelet aggregation inhibition and hypotensive activities. Moreover, (Okushima et al., 1987) reported the synthesis of a class of 6-[4-(substituted-amino)phenyl]-4,5-dihydro-3(2H)-pyridazinones **7** with extremely potent positive inotropic activity along with vasodilating effect. In addition, compounds **12a-d** were reported in an EP-patent (Haikala et al., 1990) as positive inotropes in dogs and guinea pigs heart muscle.

Although compounds **12a-d** were reported as cardiotonics on dogs, we undertook their synthesis to characterize their physicochemical and spectroscopic properties since no data are available about them in this aspect. These compounds were, also, tested for their cardiotonic effect on isolated rabbit heart, hypotensive activity on anesthetized normotensive rabbits and platelet aggregation inhibition activity in-vitro on human blood. Activity of these compounds was also taken as references for our new target compounds.

Accordingly, the present work aims at the synthesis of 6-[4-(2-substituted hydrazino) phenyl]-4,5-dihydro-3(2H)-pyridazinones to be assessed as positive inotropes, vasodilators, and platelet aggregation inhibitors.

MATERIALS AND METHODS

The starting materials and solvents required in this paper are commercially available**.** Anhydrous aluminum chloride was purchased from Sigma-Aldrich. Melting points were determined on an electrothermal Stuart Scientific SMPI and were uncorrected. Precoated silica gel plates Kieselgel 0.25 mm 60G F254 (Merck) were used for monitoring the reactions using (ethyl acetate: hexane) (9:1) as a mobile phase unless otherwise stated. Visualization was effected by ultraviolet lamp Spectroline ENF-240C/F (model CM-10) at short wavelength ($\lambda = 254$ nm) and/or iodine stain. All chemical yields are unoptimized and generally represent a single experiment. The IR spectra were recorded on a Shimadzu 200-91527 spectrophotometer as potassium bromide discs. ^{1}H-NMR spectra were scanned on a Varian EM-360 L NMR spectrophotometer (60 MHz). Chemical shifts are expressed in δ-values (ppm) relative to tetramethylsilane (TMS) as an internal standard, using $CDCl_3$ as a solvent unless otherwise specified, and deuterium oxide was used for the detection of the exchangeable protons. Electron-Impact mass were done on a JEOL JMS600 mass spectrometer. The microanalysis for C, H and N were performed on a Perkin Elmer 240 elemental analyzer.

Compounds **10** (Thyes et al., 1983) and **12a-d** (Haikala et al., 1990) were prepared according to reported procedures. The physical and spectral data for compounds **12a-d** are not found in the available literatures, while that of compound **10** is compatible with the reported (Thyes et al., 1983). Positive inotropic, hypotensive activities and toxicity determinations were done at the department of Pharmacology, Faculty of Medicine, Assiut University. The platelet aggregation inhibition activity was performed at the department of Clinical Pathology, Assiut University Hospital.

All the animals used in this work were obtained from the house of laboratory animals, Faculty of Medicine, Assiut University.

Preparation of 6-(4-Aminophenyl)-4,5-dihydro-3(2H)–pyridazinone (10)

A mixture of 4-(4-aminophenyl)-4-oxobutanoic acid compound **9** (2 g, 0.01 mole) and hydrazine hydrate 98% (0.75 g, 0.015 mole) in absolute ethanol was refluxed for 24 h. After cooling, the faint brown crystals produced were filtered off, washed with ethanol and dried. Physical and spectral data are compatible with the reported, yield (92%), m.p. 236-238ºC with decomposition as reported (Thyes et al., 1983).

Preparation of 6-{4-[2-(Disubstitutedmethylidene)hydrazino]-phenyl}-4,5-dihydro-3(2H)-pyridazinones (12a-d)

Sodium nitrite (0.393 g, 0.0057 mole) was added, little at a time in the course of 30 min to the ice cooled solution of compound **10** (1 g, 0.0053 mole) in conc. hydrochloric acid/water mixture (2:1). (Solution A). Appropriate amount from the active methylene derivatives **11a-d** (0.0053 mole) was added to an ice-cooled solution of sodium acetate (1.195 g, 0.015 mole) in 2.7 mL of water and 2.7 mL of absolute ethanol. (Solution B). Solution A was added, portionwise, with stirring to solution B during 1 h with cooling. The precipitate formed was left under stirring in the ice bath for 15 min and then, 2.7 mL of ethanol and 1.3 mL of water were added. The precipitate obtained was filtered, washed with water and dried. Compound **12a** was purified by column chromatography using chloroform as mobile phase. Compounds **12b** and **12c** were crystallized from aqueous ethanol and compound **12d** was crystallized from ethyl acetate. Physical, micro-analytical and spectral data are listed in Table I.

Preparation of 6-{4-[2-(3-Methyl-5-oxo-1(un)substituted-4-pyrazolinylidene)-hydrazino]phenyl}-4,5-dihydro-3(2H)-pyridazinones (13a-c)

A mixture of compound **12a** (1 g, 0.003 mole) and the appropriate hydrazine derivative (0.03 or 0.003 mole) was heated under reflux in ethanol for 26 h. The reactions were monitored by TLC using the system of ($CHCl_3$:CH_3OH) (9:1). The reaction mixtures were cooled and the solids obtained were filtered and washed with water. Compound **13a** was purified by column chromatography using $CHCl_3$:CH_3OH (9.25: 0.75), **13b** was crystallized from aqueous ethanol, and **13c** was crystallized from glacial acetic acid. Physical, micro-analytical and spectral data are listed in Table II.

Preparation of 6-{4-[3, 5-Dimethyl-1(un)substituted-4-pyrazolylazo]phenyl}-4,5-dihydro-3(2H)-pyridazinones (14a, b)

Compound **12c** (1 g, 0.003 mole) was stirred with the appropriate hydrazine derivative (0.03 or 0.003 mole) in absolute ethanol for 24 or 10h respectively to give **14a** or **14b**. Reactions were monitored by TLC using ($CHCl_3$:CH_3OH) (9:1). The reaction mixtures were cooled and the solids obtained were filtered, washed with water and dried. Compound **14a** was purified by column chromatography and compound **14b** was crystallized from ethyl acetate. Physical, micro-analytical and spectral data are listed in Table II.

Table I. Physicochemical and spectral data of compounds **12a-d**

Comp. No.	Yield %	M.p. °C	Molecular formula (M. wt.)	Microanalysis	Calc. %	Found %	^{1}H-NMR (DMSO-d_6, δ ppm)*	IR Spectra (KBr, cm^{-1})**
*** 12a	95	178 - 180	$C_{16}H_{18}N_4O_4$ (330.30)	C H N	58.17 5.49 16.95	58.35 5.49 16.65	1.37 (3H, t, *J* = 7.5 Hz, $CH_2C\underline{H}_3$); 2.73 (7H, m, $COC\underline{H}_3$, $COC\underline{H}_2C\underline{H}_2C$=N); 4.50 (3H, br q, *J* = 7.4 Hz, $C_6H_5N\underline{H}$, $C\underline{H}_2CH_3$); 7.67 (2H, d, *J* = 8.7 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.10 (2H, d, *J* = 8.7 Hz, C'3<u>H</u>, C'5<u>H</u>) and 11.37 (1H, s, CON<u>H</u>N).	1720 (υ C=O ester) and 1697 (υ C=O ketone).
12b	80	180 - 182	$C_{17}H_{20}N_4O_5$ $^1/_2$ H_2O (369.34)	C H N	55.28 5.73 15.16	54.99 5.26 14.96	1.33 (6H, t, *J* = 7.5 Hz, $2CH_2C\underline{H}_3$); 2.77 (4H, m, $COC\underline{H}_2C\underline{H}_2C$=N); 3.70 (1H, s, N<u>H</u>N=C); 4.45 (4H, q, *J* = 7.5 Hz, $2C\underline{H}_2CH_3$); 7.60 (2H, d, *J* = 8.5 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.10 (2H, d, *J* = 8.5 Hz, C'3<u>H</u>, C'5<u>H</u>) and 11.18 (1H, s, CON<u>H</u>N).	1689 (υ C=O ester).
12c	92	228 - 230	$C_{15}H_{16}N_4O_3$ (300.28)	C H N	59.99 5.37 18.64	59.85 5.13 18.58	2.60 (6H, s, $2COC\underline{H}_3$); 2.68 (2H, t, *J* = 8.0 Hz, $COCH_2C\underline{H}_2C$=N); 3.10 (3H, m, $COC\underline{H}_2$ CH_2 C=N and N<u>H</u>N=C); 7.78 (2H, d, *J* = 8.4 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.03 (2H, d, *J* = 8.4 Hz, C'3<u>H</u>, C'5<u>H</u>); 11.28 (1H, s, CON<u>H</u>N).	1680 (υ C=O ketone).
12d	94	242 - 243	$C_{15}H_{15}N_5O_3$ (313.27)	C H	57.50 4.83	57.35 4.68	1.38 (3H, t, *J* = 7.5 Hz, $CH_2C\underline{H}_3$); 2.53 (2H, t, *J* = 7.8 Hz, $COCH_2C\underline{H}_2$ C=N); 3.10 (3H, m, $COC\underline{H}_2CH_2$ C=N and N<u>H</u>N=C)); 4.43 (2H, q, *J* = 7.5 Hz, $COOC\underline{H}_2CH_3$); 7.70 (2H, d, *J* = 8.6 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.08 (2H, d, *J* = 8.6 Hz, C'3<u>H</u>, C'5<u>H</u>); and 11.20 (1H, s, CON<u>H</u>N).	2185 (υ C≡N) and 1700 (υ C=O ester).

*****Protons of NH groups are exchangeable by D_2O.

**IR spectra (KBr, cm^{-1}) showed the following common bands: 3445 (υ NH), 1665 (υ C=O amide), 1614, 1556 (υ C=N), 1223-1188 (υ C-N), 1129, 1101 (υ C-O) and 800-840 (δ *para* disubstituted aromatic).

***EI-Mass Spectrum of Compound 12a m/z (%): 54.58 (14.7), 91.07 (24.6), 117.05 (29.0), 145.11(14.0), 187.07(80.0), 188.07 (60.2), 214.08(100.0) {base peak}, 215.09 (23.2), 330.07 (87.0) {Molecular ion peak $M^{\bullet+}$} and 331.82 (20.5) {$M^{\bullet+}$+1}.

Preparation of 6-{4-[2-(3-Amino-5 oxo-pyrazolin-4-ylidene)hydrazino]phenyl}-4,5-dihydro-3(2H)-pyridazinone (15a)

A mixture of compound **12d** (1 g, 0.003 mole) and hydrazine hydrate (98%) (0.25 g, 0.005 mole) was refluxed in absolute ethanol for 24 h and the reaction was monitored by TLC. The reaction mixture was cooled and the precipitate obtained was filtered, washed with water and dried; yield 73%. The product was crystallized from glacial acetic acid to give dark reddish brown crystals. Physical, micro-analytical and spectral data are listed in Table II.

Preparation of 6-{4-[2-(1-N-methylcarbamoyl-1-{1-(N-methylimino)ethyl}-methylidene)-hydrazino]phenyl}-4,5-dihydro-3(2H)-pyridazinone (16a)

Compound **12a** (0.5 g, 0.002 mole) was refluxed with methylamine 30% (1.7 mL, 0.05 mole) for two days as monitored by TLC. The reaction mixture was cooled and the precipitate formed was filtered, washed with water and dried. The product obtained was crystallized from chloroform. Physical, micro-analytical and spectral data are listed in Table III.

Preparation of amides or amide/imine derivatives (16c, 16e, 16f and 16h)

Compound **12a** (0.5 g, 0.002 mole) was refluxed with either propylamine (0.05 mole) or cyclohexylamine (0.5 g, 0.005 mole) or isopropylamine (1.18 g, 0.02 mole) or 2-phenethylamine (0.005 mole) for 15 min then absolute ethanol (10-20 mL) was added. The reactions were refluxed for five days as monitored by TLC. The reaction mixtures were cooled and the precipitates formed were filtered, washed with water and dried. Compounds **16c** and **16h** were purified by column chromatography. Compounds **16e** and **16f** were crystallized from aqueous ethanol. Physical, micro-analytical and spectral data are listed in Table III.

Preparation of 6-{4-[2-(1-Acetyl-1-N-n-butylcarbamoylmethylidene)hydrazino]-phenyl}-4,5-dihydro-3(2H)-pyridazinone (16d)

Compound **12a** (0.5 g, 0.002 mole) was dissolved in 3 mL hot dimethylformamide and n-butylamine (0.37 g, 0.005 mole) was added. The reaction mixture was refluxed with stirring for a week where the reaction

Table II. Physicochemical and spectral data of compounds **13a-c; 14a-b; 15a**

Comp. No.	Yield %	M.p. °C	Molecular formula (M. wt.)	Microanalysis	Calc. %	Found %	^{1}H-NMR (DMSO-d_6, δ ppm)*	IR Spectra (KBr, cm^{-1})
13a	66	290 - 292	$C_{14}H_{14}N_6O_2$ $^1/_2$ H_2O (307.26)	C H	54.72 4.92	54.21 5.00	2.23 (3H, s, C<u>H</u>$_3$C=N); 2.53 (3H, t, *J* = 7.0 Hz, COCH$_2$C<u>H</u>$_2$C=N and N<u>H</u>N=C); 3.00 (2H, t, *J* = 7.0 Hz, COC<u>H</u>$_2$CH$_2$C=N); 7.67 (2H, d, *J* = 8.5 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.03 (2H, d, *J* = 8.5 Hz,C'3<u>H</u>, C'5<u>H</u>); 10.90 (1H, s, CON<u>H</u>N) and 11.50 (1H, br. s, CON<u>H</u>N).	3500-3450 (υ NH); 1665 (υ C=O amide) and 800-840 (δ para-disubstituted aromatic)
13b	88	293 - 295	$C_{20}H_{18}N_6O_2$ $^1/_2$ H_2O (383.33)	C H N	62.65 4.99 21.90	62.41 4.94 21.63	2.23 (3H, s, C<u>H</u>$_3$C=N); 2.53 (3H, br. t, *J* = 7.0 Hz, COCH$_2$C<u>H</u>$_2$C=N and N<u>H</u>N=C); 3.00 (2H, t, *J* = 7.0 Hz, COC<u>H</u>$_2$CH$_2$C=N); 7.8 (9H, m, C$_6$<u>H</u>$_4$ and C$_6$<u>H</u>$_5$) and 11.20 (1H, s, CON<u>H</u>N).	The same data as 13a in addition to: 697, 770 (δ mono-substituted aromatic).
13c	80	300 - 302	$C_{20}H_{16}N_8O_6$ (464.33)	C H	51.71 3.47	51.70 4.15	**^{1}H-NMR (CF_3COOD, δ ppm)** 3.10 (7H, m, C<u>H</u>$_3$C=N, COC<u>H</u>$_2$C<u>H</u>$_2$C=N) and 7.50 – 8.70 (8H, m, C$_6$<u>H</u>$_4$, C$_6$<u>H</u>$_3$ and N<u>H</u>N=C).	The same data as 13a in addition to: 1570, 1380 (υ NO_2) and 900 & 800 (δ 1, 2, 4-tri-substituted aromatic).
14a	81	162 - 164	$C_{15}H_{16}N_6O$ 1moleH_2O (314.28)	C HN	57.32 5.77 26.73	57.54 5.68 26.43	**^{1}H-NMR (CF_3COOD, δ ppm)** 3.45 (10H, m, 2C<u>H</u>$_3$C=N and COC<u>H</u>$_2$ C<u>H</u>$_2$C=N) and 8.62 (6H, m, C$_6$<u>H</u>$_4$; CON<u>H</u>N and N<u>H</u>N=C).	3500-3450 (υ NH); 1665 (υ C=O amide); 1614, 1556 (υ C=N); 1223-1188 (υ C-N); 1129, 1101 (υ C-O) and 800-840 (δ para-disubstituted aromatic).
14b	80	238 - 240	$C_{21}H_{20}N_6O$ $HCl.2H_2O$ (444.87)	C H N	56.69 5.66 18.88	56.83 5.66 19.02	**^{1}H-NMR ($CDCL_3$, δ ppm)** 2.95 (10H, m, 2COC<u>H</u>$_3$ and COC<u>H</u>$_2$ C<u>H</u>$_2$C=N); 7.60 (5H, s, C$_6$<u>H</u>$_5$); 8.00 (4H, s, C$_6$<u>H</u>$_4$) and 9.23 (1H, s, CON<u>H</u>N).	3500-3450 (υ NH); 2460 (υ N^+); 1665 (C=O amide); 800-840 (δ para-disubstituted aromatic) and 691, 760 (δ mono-substituted aromatic).
15a	73	308 - 310	$C_{13}H_{13}N_7O_2$ $^1/_2$ H_2O (308.23)	C H	50.65 4.58	50.33 4.22	2.68 (2H, t, *J* = 7.5 Hz, COCH$_2$CH$_2$C=N); 3.30 (2H, t, *J* = 7.5 Hz, COCH$_2$CH$_2$C=N); 3.82 (1H, br s, NHNC); 6.28 (2H, br s, NH$_2$); 8.16 (2H, d, *J* = 8.0 Hz,C'2<u>H</u>, C'6<u>H</u>); 8.50 (2H, d, *J* = 8.0 Hz, C'3<u>H</u>, C'5<u>H</u>); 11.40 (1H, br s, CONHN) and 11.76 (1H, s, CONHN).	3500-3450 (υ NH), 1665 (υ C=O amide), and 800-840 (δ para-disubstituted aromatic).

*Protons of NH groups are exchangeable with D_2O.

was monitored by TLC. The warm mixture was poured onto 50 gm crushed ice. The formed precipitate was filtered, washed thoroughly with water and dried. The product was purified by column chromatography. Physical, micro-analytical and spectral data are listed in Table III.

Preparation of 6-{4-[2-(1, 1-Bis-N-methyl (ethyl) carbamoylmethylidene)-hydrazino]phenyl}-4,5-dihydro-3(2H) – pyridazinones (17a, b)

Compound **12b** (0.5 g, 0.002 mole) was dissolved in 3mL hot dimethylformamide and methylamine 30% (1.8 mL, 0.05 mole) or ethylamine 70% (1.3 mL, 0.02 mole) was added respectively. The reaction mixture was refluxed with stirring for a week as been monitored by TLC. The warm mixture was poured onto 50 gm crushed ice. The precipitates formed were filtered, washed thoroughly with water and dried. Compound **17a** was crystallized from chloroform and compound **17b** was purified by column chromatography. Physical, micro-analytical and spectral data are listed in Table IV.

Preparation of diamides or amide/ester; compounds (17c and 17e-h)

Compound **12b** (0.5 g, 0.002 mole) was fused with the appropriate amine (0.04 mole of n-propyl and isopropyl amines and 0.005 mole of other amines) for 15 min. Absolute ethanol (10-20 mL) was added and the mixtures were refluxed for the appropriate time ranges from 4 to ten days. The reactions were monitored by TLC using the usual system in all compounds except

Table III. Physicochemical and spectral data of compounds **16a, 16c-f, 16h**

Comp. No.	Yield %	M.p. °C	Molecular formula (M. wt.)	Microanalysis	Calc. %	Found %	^{1}H-NMR (DMSO-d_6, δ ppm)*	IR Spectra (KBr, cm^{-1})**
16a	73	244 - 246	$C_{16}H_{20}N_6O_2$ (328.32)	C H	58.52 6.14	58.32 6.30	3.14 (14H, m, C<u>H</u>$_3$N=C, C<u>H</u>$_3$NH, COC<u>H</u>$_2$, C<u>H</u>$_2$C=N, C<u>H</u>$_3$C=N and N<u>H</u>N=C); 8.13 (2H, d, *J* = 8.5 Hz,C'2<u>H</u>, C'6<u>H</u>); 8.43 (2H, d, *J* = 8.5 Hz, C'3<u>H</u>, C'5<u>H</u>); 9.48 (1H, s, CON<u>H</u>N) and 12.13 (1H, br s, CON<u>H</u>CH$_3$).	
16c	72	202 - 204	$C_{20}H_{28}N_6O_2$ $^1/_4$ H_2O (388.92)	C H	61.76 7.38	61.72 6.97	**^{1}H-NMR (DMSO-d_6, δ ppm)** 1.33 (14H, m, NHC<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_3$ and NC<u>H</u>$_2$C<u>H</u>$_2$ C<u>H</u>$_3$); 3.18 (7H, m, C<u>H</u>$_3$C=N and COC<u>H</u>$_2$C<u>H</u>$_2$ C=N); 7.68 (2H, d, *J* = 8.4 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.00 (2H, d, *J* = 8.4 Hz, C'3<u>H</u>, C'5<u>H</u>); 8.5 (2H, m, CON<u>H</u>N and N<u>H</u>N=C) and 11.1 (1H, br s, CON<u>H</u>CH$_2$CH$_2$).	
16d	92	152 - 154	$C_{18}H_{23}N_5O_3$ (357.36)	C H N	60.49 6.48 19.58	60.90 6.48 19.07	1.03 (3H, t, *J* = 7.5 Hz, CH$_2$C<u>H</u>$_3$); 1.77 (6H, m, C<u>H</u>$_2$-C<u>H</u>$_2$C<u>H</u>$_2$CH$_3$); 3.32 (8H, m, C<u>H</u>$_3$C<u>O</u>, COC<u>H</u>$_2$C<u>H</u>$_2$C=N and N<u>H</u>N=C); 8.00 (2H, d, *J* = 8.6 Hz,C'2<u>H</u>, C'6<u>H</u>); 8.48 (2H, d, *J* = 8.6 Hz, C'3<u>H</u>, C'5<u>H</u>); 9.63 (1H, s, CON<u>H</u>N) and 10.18 (1H, br s, N<u>H</u>CH$_2$CH$_2$).	1697 (υ C=O ketone).
*** **16e**	95	216 - 218	$C_{20}H_{25}N_5O_3$ (383.39)	C H	62.65 6.57	62.95 6.82	1.68 (10H, m, 5C<u>H</u>$_2$ cyclohexyl); 3.10 (7H, m, C<u>H</u>$_3$CO, COC<u>H</u>$_2$C<u>H</u>$_2$C=N); 4.18 (2H, m, N<u>H</u>N=C and NHC<u>H</u> cyclohexyl); 7.68 (2H, d, *J* = 8.0 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.58 (2H, d, *J* = 8.0 Hz, C'3<u>H</u>, C'5<u>H</u>); 10.00 (2H, m, CON<u>H</u>CH cyclohexyl and CON<u>H</u>N).	1697 υ C=O ketone).
16f	77	200 - 202	$C_{17}H_{21}N_5O_3$ $1^1/_4$ H_2O (365.84)	C H N	55.81 6.47 19.13	55.90 5.61 18.78	**^{1}H-NMR (DMSO-d_6, δ ppm)** 1.20 (6H, d, *J* = 8.0 Hz, CH (C<u>H</u>$_3$)$_2$); 2.82 (9H, m, C<u>H</u>$_3$CO, COC<u>H</u>$_2$C<u>H</u>$_2$C=N, C<u>H</u> (CH$_3$)$_2$ and N<u>H</u>N=C); 7.84 (5H, m, C$_6$<u>H</u>$_4$ and CON<u>H</u>N) and 11.38 (1H, d, *J* = 6.0 Hz, CON<u>H</u>CH (CH$_3$)$_2$).	1697 (υ C=O ketone).
16h	84	194 - 196	$C_{30}H_{32}N_6O_2$ 1 mole H_2O (526.55)	C H N	68.43 6.51 15.95	68.85 6.11 16.37	2.95 (12H, m, 2C<u>H</u>$_2$C$_6$H$_5$ C<u>H</u>$_3$C=N, COC<u>H</u>$_2$ C<u>H</u>$_2$C=N and N<u>H</u>N=C); 3.9 (4H, m, 2C<u>H</u>$_2$ C$_6$H$_5$); 8.10 (15H, m, C$_6$<u>H</u>$_4$, 2C$_6$<u>H</u>$_5$ and CON<u>H</u>N) and 9.4 (1H, br s, CON<u>H</u>CH$_2$).	743 and 694 (δ monosubstituted aromatic).

*Protons of NH groups are exchangeable by D_2O.

**IR spectra (KBr, cm^{-1}) showed the following common bands: 3500-3450 (υ NH), 1665 (υ C=O amide), 1614, 1556 (υ C=N), 1223-1188 (υ C-N), 1129, 1101 (υ C-O) and 800-840 (δ *para*-disubstituted aromatic)

***EI-Mass Spectrum of *Compound 16e* m/z (%): 54.57 (31.9), 98.06 (14.6), 116.99 (9.2), 187.01 (15.6), 195.07 (9.1), 214.01 (29.6), 383.00 (100.0) {M$^{\cdot+}$ and base peak} and 383.99 (26.1) {M$^{\cdot+}$ + 1}.

in case of compound **17h** where CH_3Cl:CH_3OH (9:1) was used. Reaction mixtures were cooled and the precipitates formed were filtered, washed thoroughly with water and dried. Compounds **17c** and **17e** were crystallized from ethyl acetate and compounds **17g** and **17h** were purified by column chromatography. Physical, micro-analytical and spectral data are listed in Table IV.

Preparation of 6-{4-[2-(1-Acetyl-1-[1-N-alkyl(aryl) iminoethyl]methylidene)-hydrazino]-phenyl}-4,5-dihydro-3(2H)-pyridazinones (18d-h)

Compound **12c** (0.5 g, 0.002 mole) was dissolved in absolute ethanol. Two drops of glacial acetic acid were added and the reaction mixtures were refluxed for 15 min then the appropriate amine (1.18 g, 0.02 mole isopropylamine or 0.004 mole of other amines) was added and the reaction mixtures were refluxed with stirring for the appropriate time ranging from four to ten days as monitored by TLC. Reaction mixtures were cooled and the precipitates formed were filtered, washed thoroughly with water and dried. Compounds **18d**, **18f** and **18g** were crystallized from ethyl acetate, chloroform and aqueous ethanol respectively. Compounds **18e** and **18h** were purified by column chromatography. Physical, micro-analytical and spectral data are listed in Table V.

Positive inotropic activity

Experiments were done on the isolated hearts of rabbits. The isolated hearts were prepared for perfusion according to Langendorff (Abdel-Rahman, 1989).

Table IV. Physicochemical and spectral data of compounds **17a-c, 17e-h**

Comp. No.	Yield %	M.p. °C	Molecular formula (M. wt.)	Microanalysis	Calc. %	Found %	^{1}H-NMR (DMSO-d_6, δ ppm)*	IR Spectra (KBr, cm^{-1})**
17a	65	294 - 296	$C_{15}H_{18}N_6O_3$ (330.2922)	C H	54.54 5.49	54.28 5.37	**^{1}H-NMR (DMSO-d_6, δ ppm)** 2.75 (4H, m, $COC\underline{H}_2C\underline{H}_2C$=N); 3.40 (7H, m, $2NHC\underline{H}_3$ and N<u>H</u>N=C); 8.30 (5H, m, $C_6\underline{H}_4$ and CON<u>H</u>N); 10.40 (1H, m, $CON\underline{H}CH_3$) and 11.00 (1H, m, $CON\underline{H}CH_3$).	
17b	70	272 - 274	$C_{17}H_{22}N_6O_3$ (358.34)	C H N	56.98 6.18 23.44	56.34 6.00 23.30	1.30 (6H, t, J = 7.5 Hz, 2 $CH_2C\underline{H}_3$); 3.03 (5H, m, $COC\underline{H}_2C\underline{H}_2C$=N and N<u>H</u>N); 3.64 (4H, q, J = 7.5 Hz, $2C\underline{H}_2CH_3$); 7.70 (1H, s, CON<u>H</u>N); 7.68 (2H, d, J = 8.0 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.35 (2H, d, J = 8.0 Hz,C'3<u>H</u>, C'5<u>H</u>); 9.43 (1H, br s, $CON\underline{H}CH_2$) and 10.76 (1H, br s, $N\underline{H}CH_2$).	
17c	95	239 - 241	$C_{19}H_{26}N_6O_3$ (386.39)	C H N	59.05 6.78 21.73	58.93 6.73 21.82	1.00 (6H, t, J = 7.5 Hz, $CH_2CH_2C\underline{H}_3$); 1.80 (4H, m, $CH_2C\underline{H}_2CH_3$); 3.09 (8H, m, $COC\underline{H}_2$ $C\underline{H}_2C$=N and 2 $NC\underline{H}_2CH_2CH_3$); 8.05 (6H, m, $C_6\underline{H}_4$, N<u>H</u>N=C and CON<u>H</u>N); 9.90 (1H, br s, CON<u>H</u> pr.) and 10.90 (1H, br s, N<u>H</u> pr.).	
17e	62	198 - 200	$C_{25}H_{34}N_6O_3$ (466.52)	C H	64.35 7.34	64.33 7.67	**^{1}H-NMR (DMSO-d_6, δ ppm)** 1.58 (20H, m, 2(5$C\underline{H}_2$) cyclohexyl); 2.80 (2H, t, J = 8.0 Hz, $COCH_2C\underline{H}_2C$=N); 3.23 (2H, t, J = 8.0 Hz, $COC\underline{H}_2CH_2C$=N); 4.96 (3H, m, 2C<u>H</u> of cyclohexyl and N<u>H</u>N=C); 8.15 (5H, m, $C_6\underline{H}_4$ and CON<u>H</u>N) and 11.52 (2H, d, J = 6.5 Hz, 2 CON<u>H</u>CH cyclohexyl).	
17f	41	170 - 172	$C_{18}H_{23}N_5O_4$ $^1/_4$ H_2O (377.86)	C H N	57.21 6.27 18.53	57.02 5.86 18.08	1.40 (9H, m, CH ($C\underline{H}_3$)$_2$ and $CH_2C\underline{H}_3$); 2.80 (5H, m, $COC\underline{H}_2C\underline{H}_2C$=N and C<u>H</u> $(CH_3)_2$); 4.63 (3H, m, $COOC\underline{H}_2CH_3$ and NHN=C); 8.00 (2H, d, J = 8.4 Hz, C'2<u>H</u>, C'6<u>H</u>); 8.40 (2H, d, J = 8.4 Hz, C'3<u>H</u>, C'5<u>H</u>); 9.58 (1H, d, J = 6.0 Hz, CON<u>H</u>CH $(CH_3)_2$ and 9.88 (1H, s, CON<u>H</u>N).	1710 (υ C=O ester).
17g	75	214 - 216	$C_{27}H_{26}N_6O_3$ (482.48)	C H N	67.21 5.43 17.41	66.82 5.15 17.31	3.00 (4H, m, $COC\underline{H}_2C\underline{H}_2C$=N); 4.98 (4H, d, J = 8.0 Hz, $2C\underline{H}_2C_6H_5$); 8.29 (16H, m, $C_6\underline{H}_4$; $2C_6\underline{H}_5$; N<u>H</u>N=C and CON<u>H</u>N); 9.70 (1H, t, J = 6.5 Hz, $N\underline{H}CH_2$) and 11.13 (1H, t, J = 6.5 Hz, $N\underline{H}CH_2$).	795 and 693 (δ monosubstituted aromatic).
17h	67	206 - 208	$C_{29}H_{30}N_6O_3$ $^1/_2$ H_2O (519.53)	C H N	67.04 6.02 16.17	66.93 6.32 15.65	3.30 (13H, m, $COC\underline{H}_2C\underline{H}_2C$=N); $2C\underline{H}_2C\underline{H}_2$ C_6H_5 and N<u>H</u>N=C); 7.93 (14H, m, $C_6\underline{H}_4$ and $2C_6\underline{H}_5$); 9.60 (2H, br s, $2N\underline{H}CH_2$) and 10.10 (1H, br s, CON<u>H</u>N).	795 and 693 (δ monosubstituted aromatic).

*Protons of NH groups are exchangeable with D_2O.

**IR spectra (KBr, cm^{-1}) showed the following common bands: 3500-3450 (υ NH), 1665 (υ C=O amide), 1614, 1556 (υ C=N), 1223-1188 (υ C-N), 1129, 1101 (υ C-O) and 800-840 (δ *para*-disubstituted aromatic).

Rabbits of either sex weighing (1-1.2 Kg) were sacrified. The heart with at least 1cm of the aorta attached was removed as quickly as possible and placed in a dish containing Lock-Ringer solution at 37°C. The heart was gently squeezed several times in the solution so as to remove as much blood as possible. The aorta afterwards was located and dissected free and all other vessels connected to the heart were trimmed away. The aorta was cut just below the point where it divides and the heart was transferred to the perfusion apparatus that contains a bottle filled with perfusion fluid that is an oxygenated Lock-ringer solution warmed to 37°C by means of water jacket adjusted at 38°C. The rate of flow of the perfusion fluid was kept constant at 3 mL/minute by adjusting the pressure exerted on the fluid by means of a mercury manometer connected to the perfusion system. The aorta was then tied onto the rubber tip of the glass cannula. Care must be taken to see that air bubbles do not enter the aorta and any bubbles which have been formed in the cannula should be removed. A thread was attached to the ventricle by a hook and the other end of the thread was passed over pulley wheels and was attached to the displacement transducer connected to oscillograph (400 MD 2C Bioscience). The contractions of the perfused heart were then

Table V. Physicochemical and spectral data of compounds **18d-h**

Comp. No.	Yield %	M.p. °C	Molecular formula (M. wt.)	Microanalysis	Calc. %	Found %	^{1}H-NMR (DMSO-d_6, δ ppm)*	IR Spectra (KBr, cm^{-1})**
18d	81	176 - 178	$C_{19}H_{25}N_5O_2$ $^1/_4$ H_2O (359.89)	C H N	63.40 7.14 19.45	63.42 7.25 18.94	1.57 (7H, m, C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_3$); 3.03 (10H, m, C$\underline{H}_3$C=N, C$\underline{H}_3$C=O, COC$\underline{H}_2$C$\underline{H}_2$C=N); 3.97 (3H, m, N$\underline{H}$N=C and C=NC$\underline{H}_2$); 8.10 (2H, d, *J* = 8.0 Hz, C'2$\underline{H}$, C'6$\underline{H}$); 8.43 (2H, d, *J* = 8.0 Hz, C'3$\underline{H}$, C'5$\underline{H}$) and 9.63 (1H, s, CON$\underline{H}$N).	
18e	79	210 - 212	$C_{21}H_{27}N_5O_2$ $^1/_2$ H_2O (390.42)	C H N	64.59 7.23 17.93	64.74 6.70 17.78	2.12 (20H, m, 5C$\underline{H}_2$ cyclohexyl, C$\underline{H}_3$C=N, C$\underline{H}_3$C=O and COC$\underline{H}_2$C$\underline{H}_2$C=N); 3.91 (2H, m, N$\underline{H}$N=C and =NC$\underline{H}$ cyclohexyl); 7.73 (2H, d, *J* = 8.4 Hz, C'2$\underline{H}$, C'6$\underline{H}$); 8.10 (2H, d, *J* = 8.4 Hz, C'3$\underline{H}$, C'5$\underline{H}$) and 9.18 (1H, s, CON$\underline{H}$N).	
18f	70	186 - 188	$C_{18}H_{23}N_5O_2$ $^1/_4$ H_2O (345.86)	C H N	62.50 6.85 20.24	62.45 6.57 19.77	1.28 (6H, d, *J* = 8.0 Hz, C$\underline{H}_3$CHC$\underline{H}_3$; 2.76 (11H, m, COC$\underline{H}_2$C$\underline{H}_2$C=N, N$\underline{H}$N=C, C$\underline{H}_3$C=N and C$\underline{H}_3$C=O); 4.17 (1H, m, CH$_3$C$\underline{H}$CH$_3$); 7.88 (2H, d, *J* = 8.5 Hz, C'2$\underline{H}$, C'6$\underline{H}$); 8.50 (2H, d, *J* = 8.5 Hz, C'3$\underline{H}$, C'5$\underline{H}$) and 9.50 (1H, s, CON$\underline{H}$N).	
18g	46	200 - 202	$C_{22}H_{23}N_5O_2$ (389.40)	C H N	67.85 5.95 17.98	67.31 5.76 17.62	2.96 (11H, m, C$\underline{H}_3$C=N, C$\underline{H}_3$C=O, COC$\underline{H}_2$C$\underline{H}_2$C=N and N$\underline{H}$N=C); 5.03 (2H, s, C=NC$\underline{H}_2$C$_6$H$_5$); 8.14 (9H, m, C$_6\underline{H}_4$ and C$_6\underline{H}_5$) and 9.60 (1H, s, CON$\underline{H}$N).	731 and 691 (δ monosubstituted aromatic).
18h	52	204 - 206	$C_{23}H_{25}N_5O_2$ $^1/_2$ H_2O (412.43)	N	16.97	16.86	3.03 (12H, m, C$\underline{H}_3$C=N, C$\underline{H}_3$C=O, COC$\underline{H}_2$C$\underline{H}_2$C=N and C=NC$\underline{H}_2$CH$_2$C$_6$H$_5$); 4.2 (2H, t, *J* = 8.0 Hz, CH$_2$C$\underline{H}_2$C$_6$H$_5$); 8.15 (9H, m, C$_6$H$_4$ and C$_6\underline{H}_5$); 9.2 (1H, s, N$\underline{H}$N=C) and 9.8 (1H, s, CON$\underline{H}$N).	731 and 691 (δ monosubstituted aromatic).

*****Protons of NH groups are exchangeable by D_2O.

**IR spectra (KBr, cm^{-1}) showed the following common bands: 3500-3450 (υ NH), 1680 (υ C=O ketone), 1665 (υ C=O amide), 1614, 1556 (υ C=N), 1223-1188 (υ C-N), 1129, 1101 (υ C-O) and 800-840 (δ *para*-disubstituted aromatic).

recorded at a constant chart speed (Abdel-Rahman, 1989).

Normal contraction was recorded during a period of 5 min until it was stabilized and become consistent. Thereafter, the definite concentration (0.5, 1.5, 2.5, 5 and 10 μM) of the test compounds was then injected in the rubber tip of the cannula and myocardial contractility was recorded for a definite time interval. The percentage changes in the amplitude representing the force of contraction and the percentage changes in frequency of myocardial contractility representing the heart rate from the normal contractility were calculated. Each experiment was done 5 times and the results were expressed as a mean change in percent. The test compounds and digoxin were used as solutions or suspensions in saline or saline/NaCMC (0.5% in water) mixture.

Hypotensive activity

Adult healthy rabbits of either sex weighing (1.2-1.5 Kg) were anaesthetized with an intraperitoneal (i.p.) injection of urethane in a dose of 1.6 g/kg (6 mL) (Hui et al., 2001). Arterial blood pressure was recorded via the carotid artery; the latter was cannulated to Burden blood pressure transducer. Heparin was placed in the tip of the cannula to prevent clotting. Blood pressure was recorded using an oscillograph (400 MD 2C Bioscience). The transducer was calibrated and the test compounds were injected i.v. in the ear vein. Blood pressure was recorded before and after administration of the test compounds over a period of 30 min and the results were given as a mean change in percent. Screening of the effects of the test com- pounds on blood pressure was done on groups of rabbits; each of five animals. Test compounds were injected i.v. in the rabbit's ear veins as solutions in saline/NaCMC (0.5% aqueous solution) mixture at doses of 0.1 and 0.3 mg/kg.

Acute toxicity studies

Groups of adult albino mice (either sex), each of five animals weighing (20-30 g) were used. Graded doses starting from 10 mg/kg of each of the test compounds were injected i.p. and the toxic effects were noticed for 72 h latter to injection. Both compounds **12a** and **16d** were injected (i.p.) as solutions in a mixture of saline/NaCMC (0.5% in water).

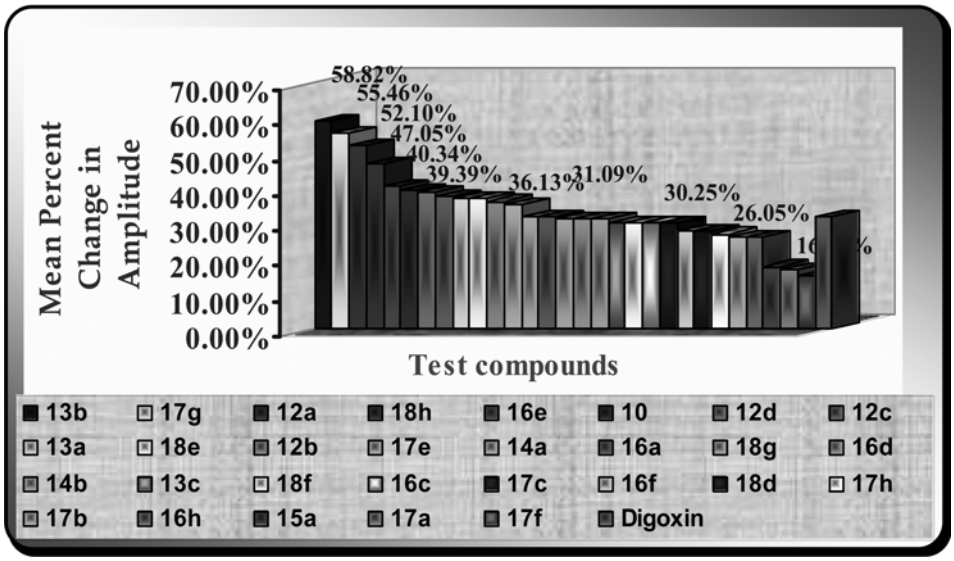

Fig. 1. Cardiotonic activity of all test compounds in descending order according to their mean percent change in amplitude of contractility in comparison to digoxin

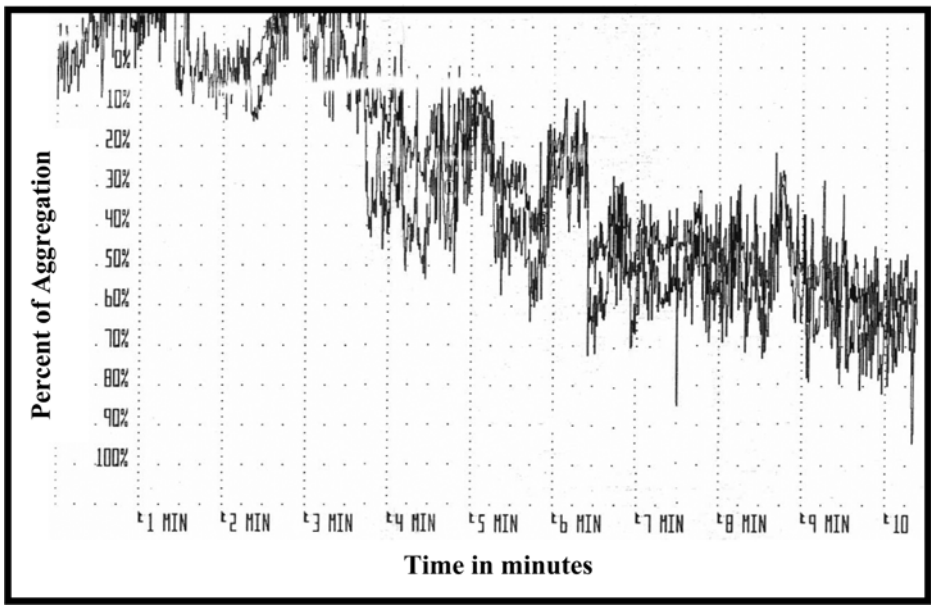

Fig. 2. Platelet aggregation after ADP (PAF) addition

Platelet aggregation inhibition activity

Screening of the effects of the test compounds on platelet aggregation was done on human platelet-rich plasma that were prepared from whole blood, freshly drawn from healthy volunteers who had not ingested aspirin or other non steroidal anti-inflammatory drugs within the preceding two weeks and who were fasting for 9 h before blood draw (Sircar et al., 1989) Aggregation was measured in a 4-channel PAP4 Biodata aggregometer. Aliquots, at a final of 450 μL and 480 μL platelets were equilibrated to 37ºC before being placed in the sample chamber. They were then incubated for 5 min with different concentrations from the test compounds. The reported derivatives (Sircar et al., 1989) were used as 300-0.001 μM. So, at first, compound **12a** was used in 0.6 μM/20 μL and 1.5 μM/ 50 μL respectively, then compound **16d** was tested using smaller concentrations 0.1 μM/50 μL and 0.04 μM/20 μL respectively. After the addition of 2.5 μM adenosine diphosphate (ADP) used as platelet activating factor (PAF), aggregation was determined, before and after addition of the test compounds, by the change in absorbance monitored for 4 min (Murray et al., 1992). Experiments were repeated on three samples

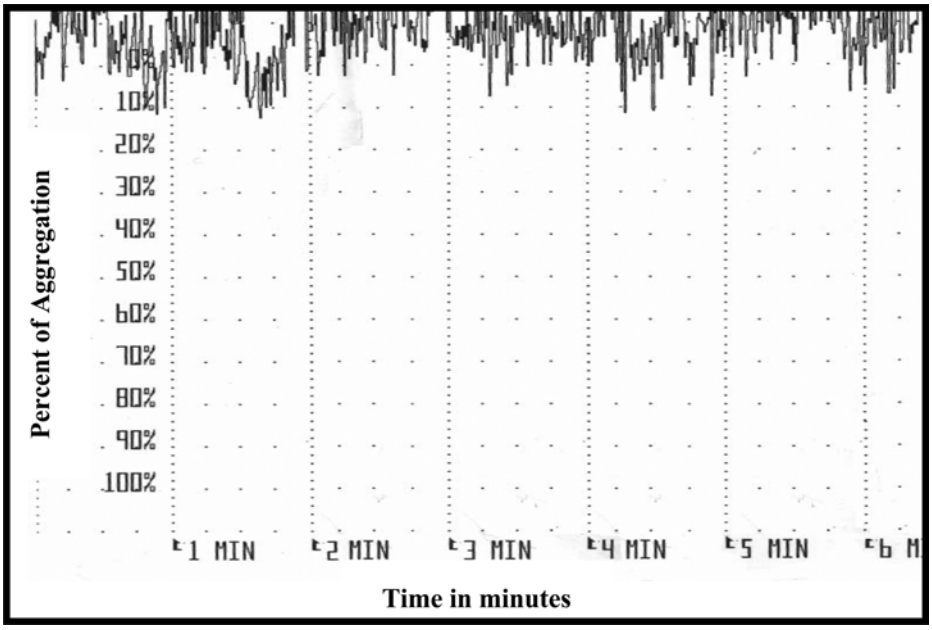

Fig. 3. Inhibition of platelet aggregation after addition of 0.6 μM/20 μL from compound **12a**

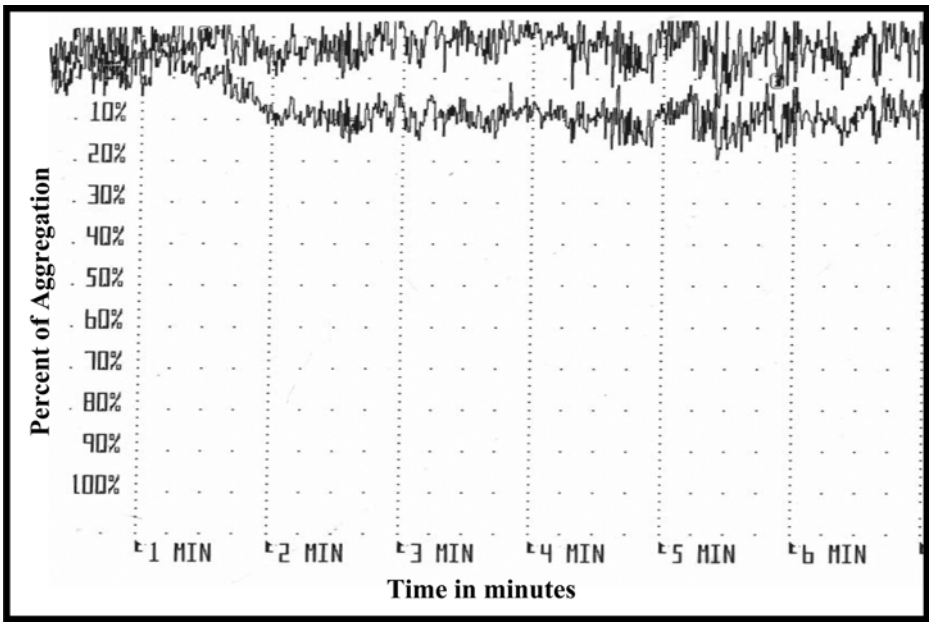

Fig. 4. Addition of 0.04 μM/20 μL from compound **16d** showing 5 & 10% platelet aggregation for two volunteer's samples

from three volunteers for each compound.

Pharmacophore building and pharmacophore identifications

All the computational works were carried out at the Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt. Receptor building and pharmacophore identification were performed on Molecular Operating Enviornment (MOE) version 2007.09, Chemical Computing Group Inc. 1010 Sherbooke St. West, Suite 910, Montreal, Quebec, H3A 2R7, Canada. The program operated under "Microsoft Windows XP" operating system installed on an Intel Pentium IV PC with a 2.8 GHz processor and 512 Mb of RAM. Two Pharmacophore models were constructed, one for the cardiotonic activity and the other for the hypotensive one. Construction of the models was based on the reported active compounds (Thyes et al., 1983; Okushiima et al., 1987). The aim of this approach is to gain useful insights into ligand

receptor interactions, to identify pharmacophoric structural features of the active ligands, and to use this model for searching molecular databases in order to find new structural categories, a process known as virtual screening.

Eight reported active ligands compounds **10** and **19a-g** (Fig. 5) in addition to pimobendan **3** were selected as the training set for cardiotonic activity, to build up the hypothetical model using molecular builder of MOE program. Each structure was subjected to energy minimization up to gradient of 0.01 kcal/mol A^{o} using MMffyn force field. The training set molecules were aligned using MOE's flexible alignment. Alignment had the lowest strain energy, U, and the highest S value was selected to build the pharmacophore query.

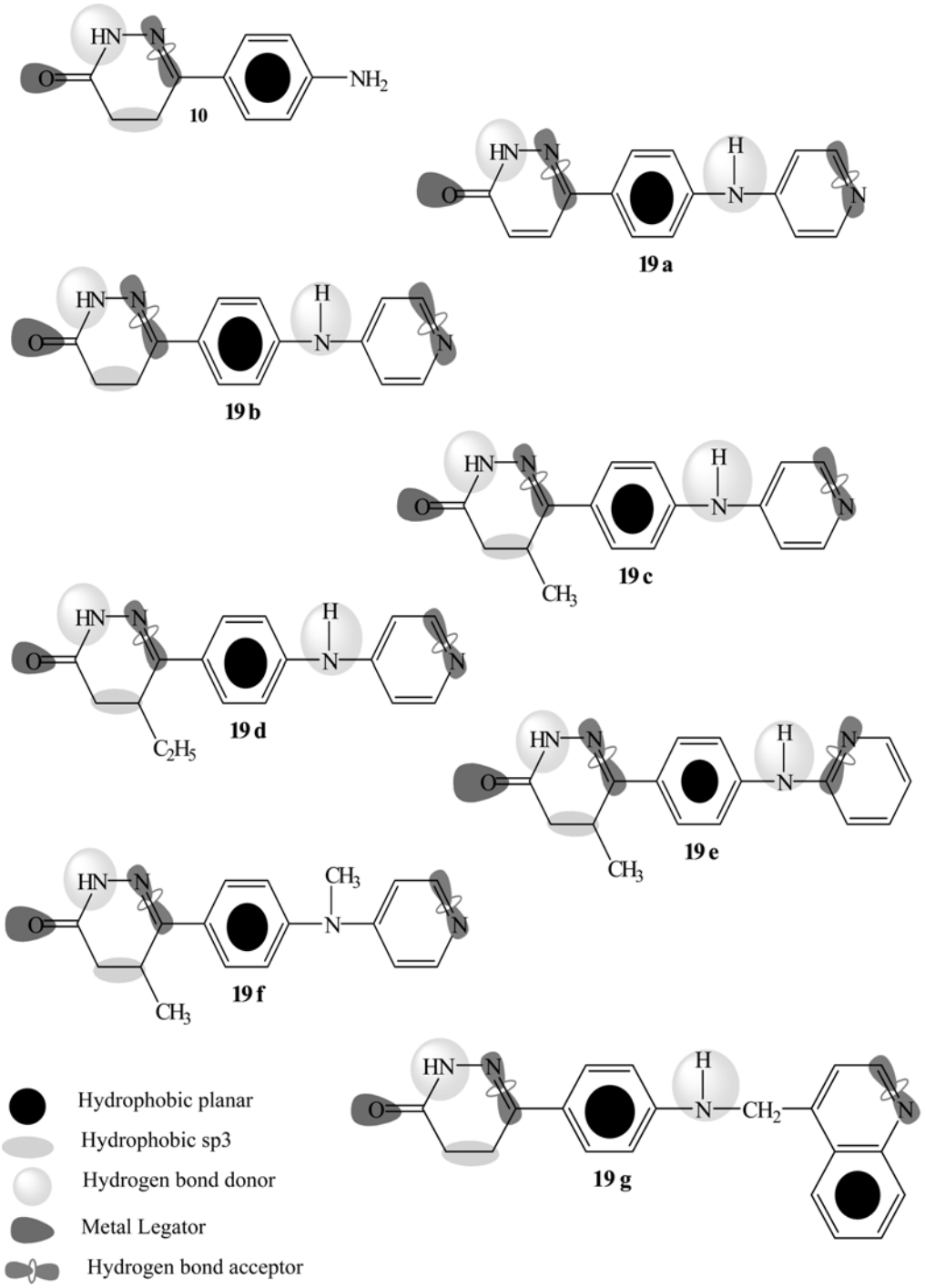

Fig. 5. Training set used in building the cardiotonic hypothetical model, compounds **10** and **19a-g**

A pharmacophore query was created with the pharmacophore query editor of MOE, using PPCH-All (Planar-Polarity-Charge-Hydrophobicity) scheme. The pharmacophore query was composed of five common chemical features (F1-F5) that would be considered in the generation of the hypothetical model within the training set.

The pharmacophoric features and distances between them in A° are shown in Fig. 6. Under this scheme, ML denotes metal ligator. Hyd S denotes non planar hydrophobic region (sp3). Hyd P denotes planar hydrophobic region (sp2). Acc P denotes planar H-bond acceptor (sp2). Don P denotes planar H-bond donor (sp2).

The performance of the obtained hypothetical model was evaluated by fitting of all the target compounds

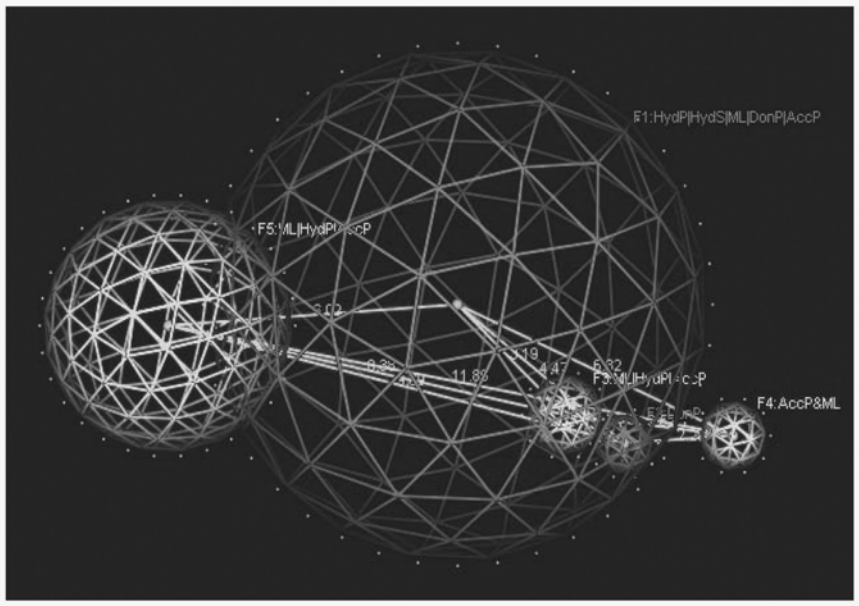

Fig. 6. Cardiotonic pharmacophore features and distances

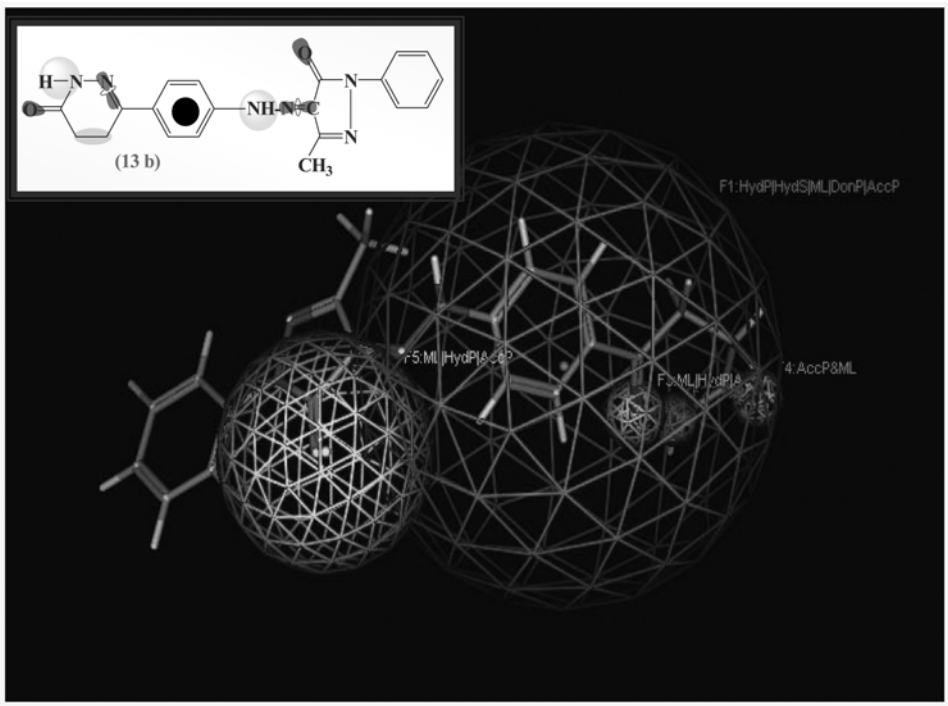

Fig. 7. Mapping of compound **13b** onto the cardiotonic pharmacophore

12a-d; 13a-c; 14a, b; 15a; 16a, c-f, h; 17a-c, e-h and 18d-h. Fitting operations to the hypothetical model were accomplished through pharmacophore output contains RMSD, i.e., the root mean square distance between the query features and their corresponding ligand target points. The smaller the RMSD, the better fitting the query compound has.

Another pharmacophoric model was constructed for the hypotensive activity using ten reported compounds (Thyes et al., 1983) as the training set **20a-i** Fig. 8. The same procedures used for building the cardiotonic activity pharmacophore model was followed. The pharmacophore query composed of five common chemical features and the pharmacophore search was done for the synthesized target compounds.

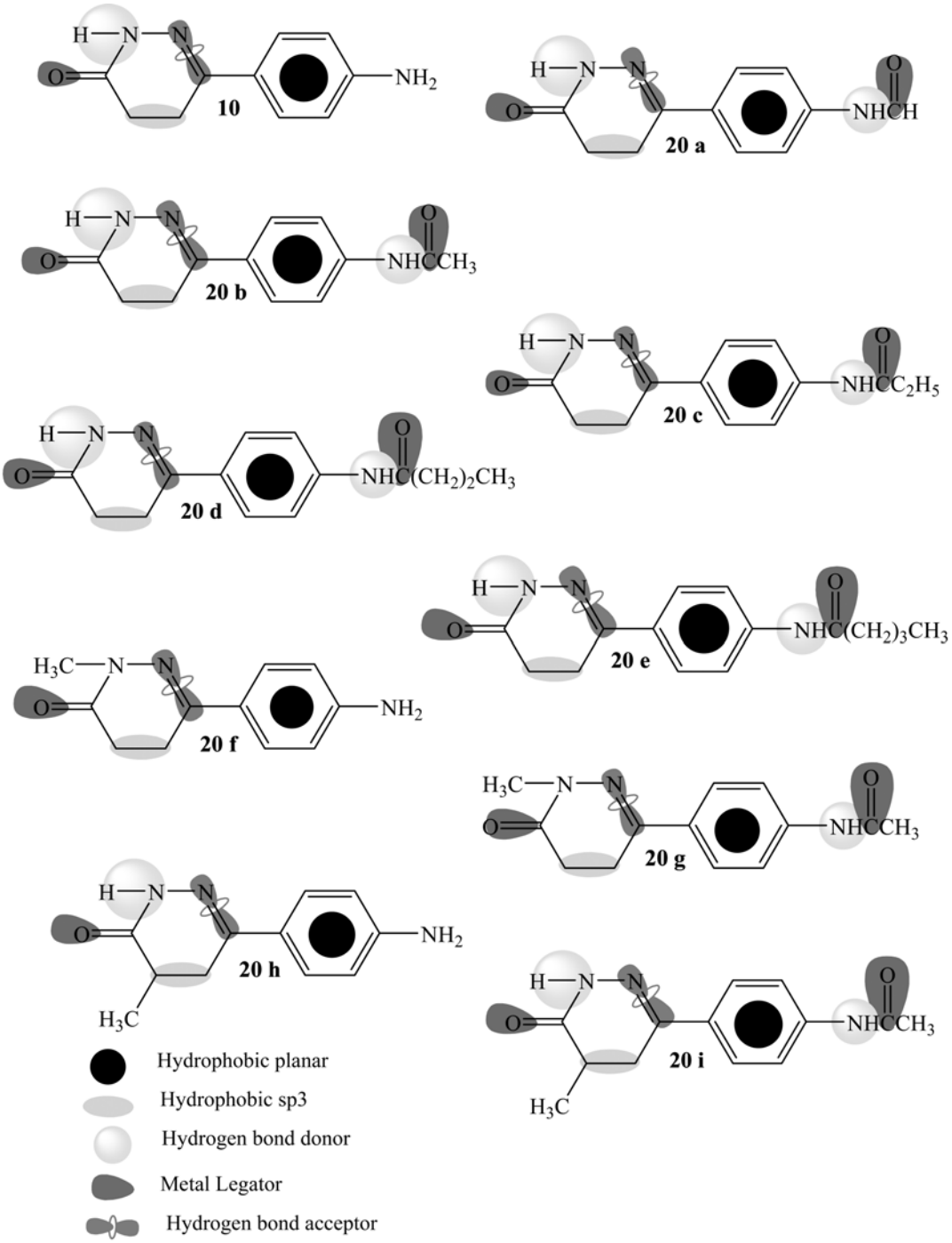

Fig. 8. Training set used in building the hypotensive hypothetical model, compounds **10** and **20a-i**

RESULTS AND DISCUSSION

Chemistry

The key intermediates **12a-d** were prepared by coupling the diazonium chloride of compound **10** with the different active methylene containing compounds **11a-d** in presence of sodium acetate (Chart 2). The formed intermediates **12a-d** are reported compounds (Haikala et al., 1990) but their physical and spectral data are not available so their structures have been established by IR, ^{1}H-NMR, and mass spectrometry in addition to elemental analysis.

The target compounds **13a-c** were prepared by cyclization of compound **12a** with hydrazine hydrate, phenylhydrazine hydrochloride or 2,4-dinitrophenyl-hydrazine by reflux in absolute ethanol for about 26 h (Chart 3a).

On the other hand, pyrazolopyridazinones **14a** and **b** were prepared by cyclization of compound **12c** with either hydrazine hydrate (24%) or phenylhydrazine hydrochloride in absolute ethanol by refluxing for 24 h or 10 h, respectively (Chart 3a). Trials to cyclise **12c** with 98% hydrazine hydrate, didn't afford the required product **14a**, and reduction of the azo bond took place to give the starting compound **10** as has been identified by ^{1}H-NMR.

Also, aminopyrazolinonopyridazinone **15a** was prepared by cyclization of compound **12d** with hydrazine hydrate in absolute ethanol by reflux for 24 h (Chart 3a).

Moreover, reaction of compound **12a** with alkyl or aralkyl amines (Chart 3b) afforded the target amide/imine derivatives **16a, c** and **h** only on using methyl-, n-propyl-, and phenethyl-amines, respectively. However, on using other bulky or branched amines such as n-butyl-, cyclohexyl- or isopropyl- amines, aminolysis of the ester group of **12a** only occurred leaving the keto group of the acetyl moiety intact to afford compounds **16d**, **16e** and **16f** respectively. This finding may be attributed to the steric hindrance induced by such bulky or branched amines at the site of the reaction.

Also, reaction of the di-ester intermediate **12b** with alkyl- or aralkyl amines (Chart 3b) in DMF or ethanol afforded the target di-amides **17a-c, e, g, h** except compound **17f** where only one molecule of isopropylamine was involved in the aminolysis of only one

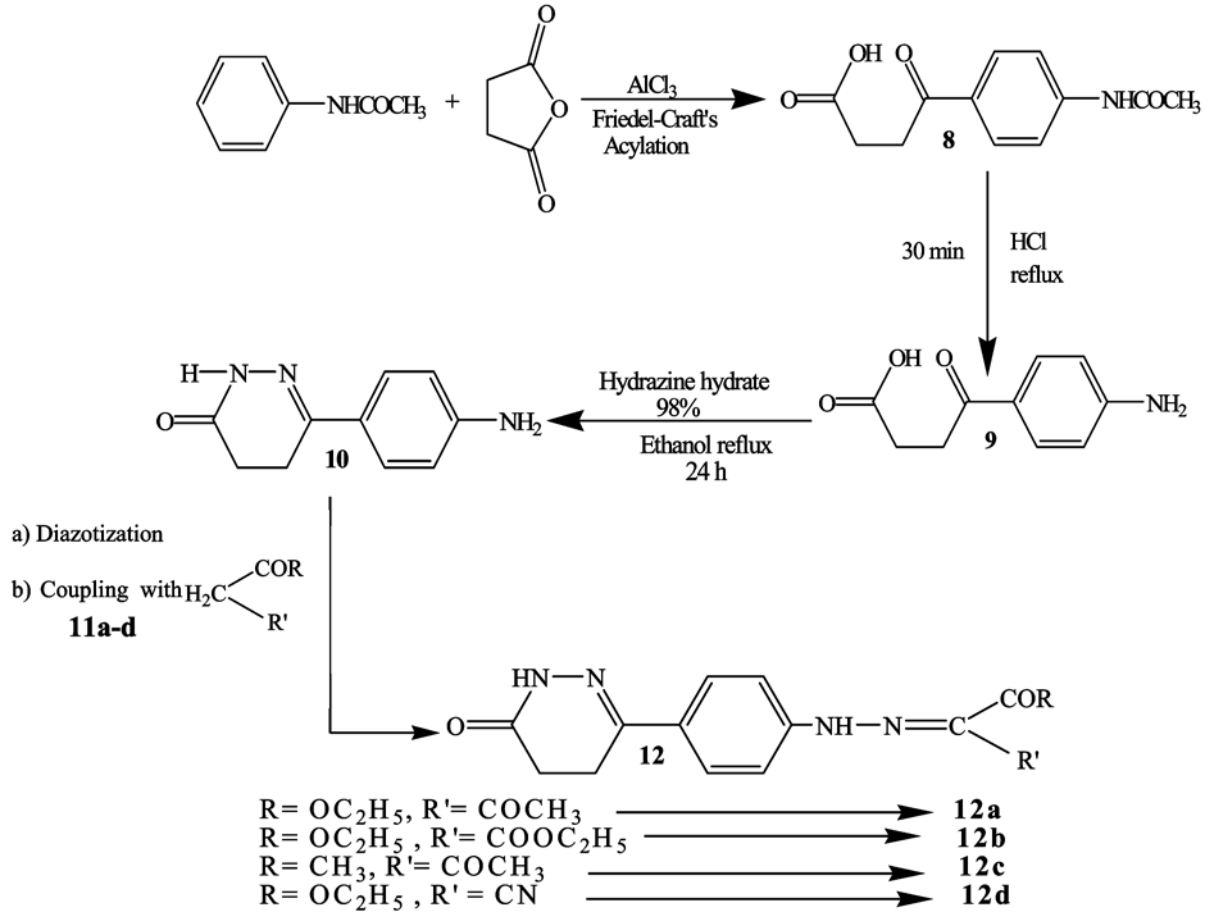

Chart 2. Synthetic pathway for the preparation of compounds **10** and **12a-d**

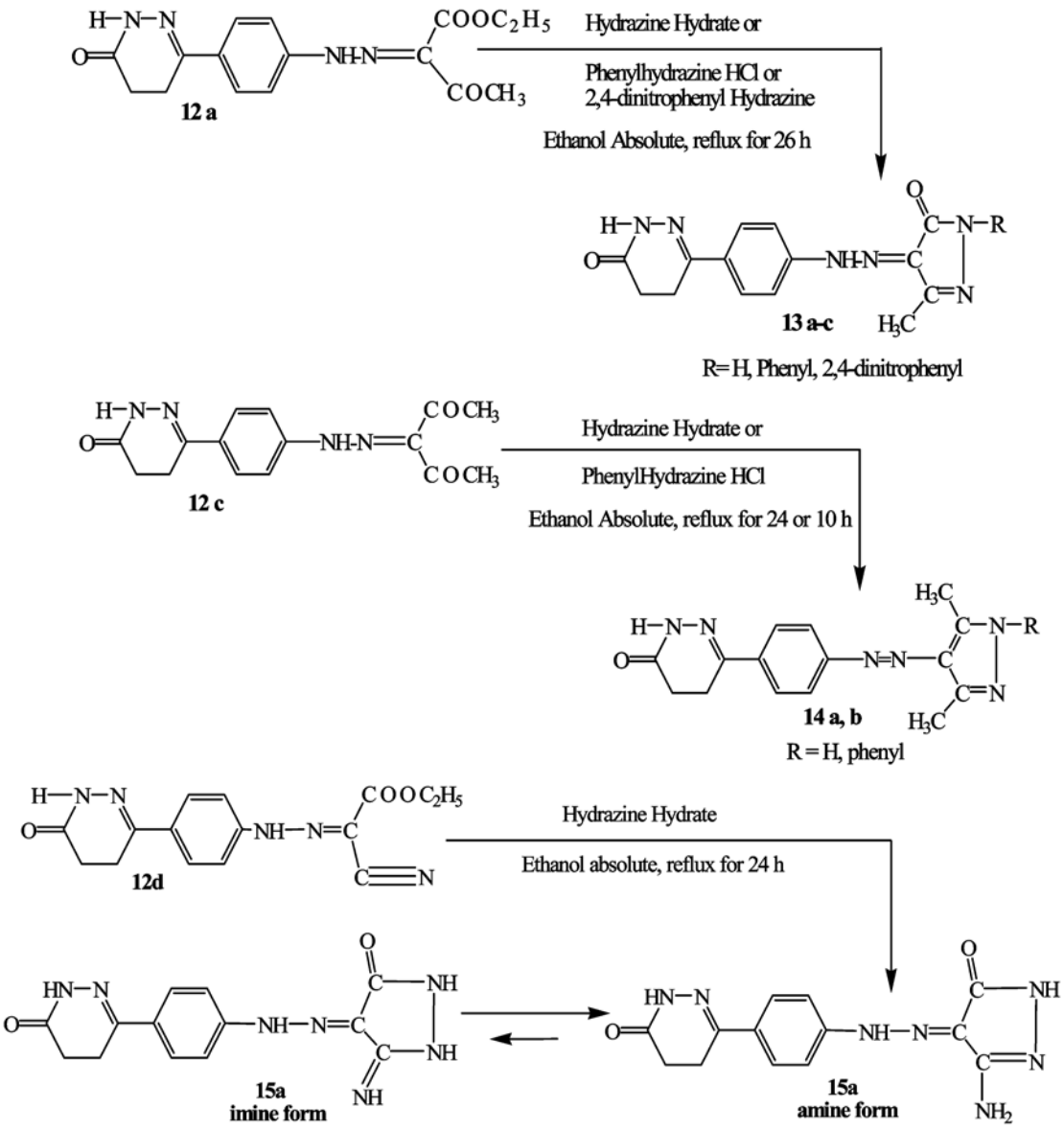

Chart 3a. Synthetic pathway for the preparation of compounds **13a-c; 14a, b; 15a**

ester group of **12b** due to branching. Decoupling of the benzyl methylene of compound **17g** was done in order to notice the change in the nearby NH- splitting and it showed change in the splitting from triplet to singlet at (9.70 ppm) and to hump at (11.13) ppm. The two methylene groups of the benzyl moieties are not equivalent thus the nearby NH groups differ in their appearance. This is confirmed from the pharmacophoric studies where one of the two amidic chains is nearby the ($C_6H_5N\underline{H}N$), thus forms an intramolecular hydrogen bond and consequently appears more downfield than the other.

Reaction of the diketopyridazinone derivative **12c** with amines in absolute ethanol under reflux (Chart 3b) yielded the corresponding iminoketones **18d-h** and only one molecule of the used amines attack only one ketonic group of **12c** as confirmed by analytical techniques.

Several attempts were carried out to form imines from **12c** with methyl-, ethyl-, and n-propyl- amines but they were unsuccessful. This might be attributed to the aqueous nature of the first two amines and the volatilization of the n-propyl one (B.P. 48°C). All the obtained compounds are crystalline solids and their structures were confirmed by IR, ^{1}H-NMR and mass spectrometry along with microanalysis.

Pharmacological Screening

Positive Inotropic Effect: Twenty four new compounds **13a-c; 14a, b; 15a; 16a, c-f & h; 17a-c, e-h**

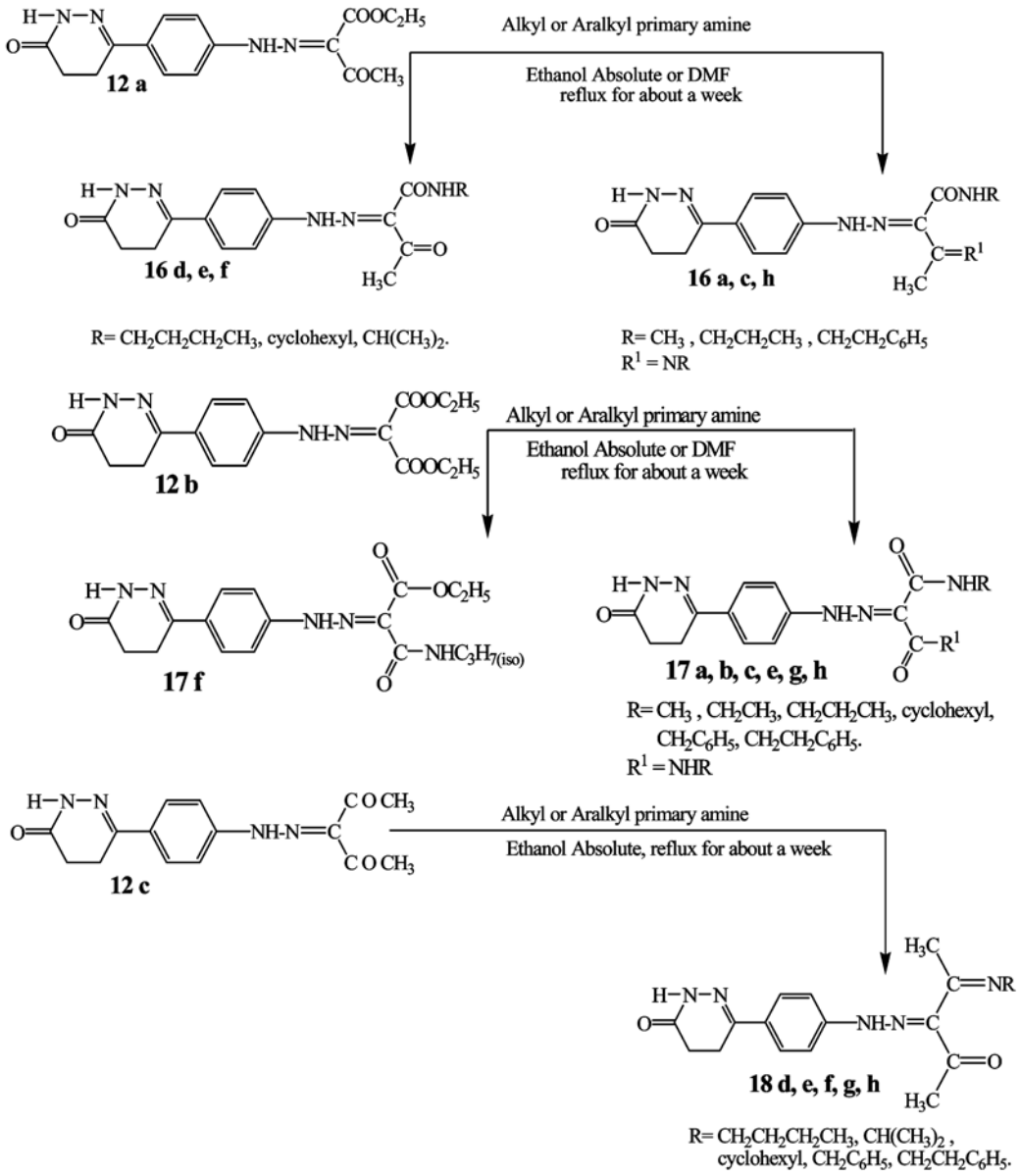

Chart 3b. Synthetic pathway for the preparation of compounds **16a, c-f, h; 17a-c, e-h; 18d-h**

and 18d-h in addition to the five reported intermediates compounds **10** and **12a-d** were evaluated for their positive inotropic effect (Abdel-Rahman, 1989) in comparison to digoxin[1]. It was found that the first effective dose was 5 μM, thus was used thereafter. Results are listed in Fig. 1 and Tables VI-IX.

Study of results listed in Tables VI-IX reveals that the inotropic activity of the tested intermediates **12a-d** surpassed that of digoxin and more or less similar to that of the reported compound **10**.

It is noteworthy to mention that the inotropic effect of compound **12a** was the best of the tested intermediates, since it exceeds that of both digoxin and **10**. This finding indicates that presence of an acetyl and ester moieties at the methylidene carbon atom linked to the hydrazine substituent at the para position of the 6-phenyl seemed to be crucial for the inotropic effect of this class of pyridazinones.

It is also of interest to mention that cyclization of the intermediate **12a** into pyrazolinone derivatives **13a-c** (Table VIII) resulted in either elevation **13b** or reduction **13a** and **13c** of the activity. Superiority of the inotropic activity of **13b** than its congeners **13a** and **13c** could be explained by the presence of a

1) Pimobendan, Imazodan or any phosphodiesterase acting drugs are not available in the middle-east area to be used as positive inotropic reference thus digoxin was used instead in comparison.

Table VI. Cardiotonic activity of compounds **10; 12a-d; 13a-c; 14a, b; 15a** and digoxin at 5 μM concentration

No.	Mean change in amplitude (cm) [a]	% mean change in amplitude (cm)	Mean change in frequency/ (min)	% mean change in frequency
Normal	2.38 ± 0	···	30.4	···
10	3.32 ± 0.057**	39.49	25.0 ± 0.54	-17.76[b]
12a	3.62 ± 0.100***	52.10	35.4 ± 0.75	16.45
12b	3.24 ± 0.057**	36.13	33.4 ± 0.75	9.87
12c	3.28 ± 0.112**	37.81	36.2 ± 0.92	19.07
12d	3.30 ± 0.185**	38.65	25.0 ± 0.54	17.76
13a	3.26 ± 0.150**	36.97	35.8 ± 0.96	17.76
13c	3.14 ± 0.003**	31.93	36.8 ± 0.96	21.05
14a	3.14 ± 0.043**	31.93	37.2 ± 0.92	22.37
14b	3.12 ± 0.049**	31.09	35.8 ± 0.96	17.76
15a	2.80 ± 0.144*	17.64	33.4 ± 0.75	9.87
Digoxin	3.14 ± 0.011**	31.93	16.2 ± 0.375	-46.71[b]

[a]Each result represents the mean of five observations ± S.E.M.
[b](-) Negative chronotropic effect (i.e. decrease in heart rate).
*Significant at $p \leq 0.05$ (degree of 95% confidence limits) compared to control.
**Significant at $p \leq 0.01$ (degree of 99% confidence limits) compared to control.
***Significant at $p \leq 0.005$ (degree of 99.5% confidence limits) compared to control.

Table VII. Cardiotonic activity of compounds **16a, c-f, h; 17a-c, e-h; 18d-h** and digoxin using 5 μM concentration

Comp. No.	Mean change in amplitude (cm) [a]	% mean change in amplitude	Mean change in frequency/ (min)	% mean change in frequency
16a	3.14 ± 0.095**	31.93	34.8 ± 0.96	14.47
16c	3.10 ± 0.025**	30.25	27.4 ± 0.75	-9.87[b]
16d	3.12 ± 0.025**	31.09	33.4 ± 0.75	9.87
16e	3.34 ± 0.020**	40.34	39.8 ± 0.96	30.92
16f	3.04 ± 0.134*	27.73	31.6 ± 0.59	3.95
16h	3.00 ± 0.0190**	26.05	34.8 ± 0.96	14.47
17a	2.78 ± 0.057*	16.80	30.4 ± 0.75	0
17b	3.00 ± 0.055**	26.05	26.0 ± 0.54	-14.47[b]
17c	3.10 ± 0.191*	30.25	30.4 ± 0.75	0
17e	3.22 ± 0.104**	35.29	31.8 ± 0.96	4.61
17f	2.74 ± 0.023*	15.12	26.0 ± 0.54	-14.47[b]
17g	3.70 ± 0.095***	55.46	32.4 ± 0.75	6.57
17h	3.02 ± 0.120*	26.89	35.8 ± 0.96	17.76
18d	3.04 ± 0.091**	27.73	36.2 ± 2.05	19.07
18e	3.26 ± 0.07**	36.97	38.2 ± 2.05	25.65
18f	3.10 ± 0.028**	30.25	32.4 ± 0.75	6.57
18g	3.12 ± 0.085**	31.09	39.8 ± 0.96	30.92
18h	3.50 ± 0.185**	47.05	35.8 ± 0.96	17.76
Digoxin	3.14 ± 0.011**	31.93	16.2 ± 0.38	-46.71[b]

[a]Each result represents the mean of five observations ± S.E.
[b](-) Negative chronotropic effect (i.e. decrease in heart rate).
*Significant at $p \leq 0.05$ (degree of 95% confidence limits) compared to control.
**Significant at $p \leq 0.01$ (degree of 99% confidence limits) compared to control.
***Significant at $p \leq 0.005$ (degree of 99.5% confidence limits) compared to control.

phenyl substituent at N^1 of the pyrazolinone ring rather than hydrogen **13a** which might be attributed to the best fitting of **13b** with the appropriate receptors. Further increase in bulkiness led to steric hindrance of the resulted N^1-(2,4-dinitrophenyl)pyrazolinone derivative **13c** at the receptors and hence bad fitting and decrease of the observed inotropic effect.

On the other hand, either amide formation **16d, 16e and 16f** (Table III) or amide and imine formation **16a, 16c** and **16h** (Table VIII) of the intermediate **12a** resulted in marked decrease of activity in comparison to the parent **12a**.

Also, results of Table VIII show that the activity of the intermediate **12b** is 1.13 times as active as digoxin which is almost comparable to that of the reported compound **10**. Compound **12b** contains di-ester moieties at the methylidene carbon atom linked to the hydrazino group at the p-position of the phenyl ring at position 6 of the pyridazinone ring system. Substitution of the di-ester moieties by di-amide ones **17a, 17b, 17c, 17e, 17g and 17h** (Table VIII) resulted in diminishing of activity compared to the parent compound **12b** except compound **17g** which exhibited 1.72 fold as active as digoxin.

Study of the observed activities of the amide series **17a–h** reveals that activity increases by the increase of bulkiness of the alkyl moieties on the amide nitrogen atoms with a noticeable drop of activity of the isopropyl containing amide **17f** than its analog **17c**, although the former is a monosubstituted amide. This observation may be attributed to the steric hindrance which might be produced at the receptor due to branching of the propyl moiety **17f** and in turn bad fitting with the receptor.

It is worth noting to discuss the activity of compounds **17g** and **17h** in comparison to **12b.** It is clear that substitution of the methyl group of the ethyl moiety at the amide nitrogen by a phenyl one **17g** augmented the activity to more than the double, while substitution of such methyl by a benzyl one **17h** retains the same activity of **12b**. Also, it is of interest to mention that presence of benzyl substituents at the amide nitrogens **17g** rather than phenethyl ones **17h** might lead to better fitting on the receptor of the former than the latter, which indicates the importance

Table VIII. % mean changes in amplitude of contractility of isolated cardiac muscles of all test compounds in comparison to digoxin and **10** at 5μM concentration

Comp. No.	% mean change in amplitude of contractility	Activity in folds in comparison to digoxin
Digoxin	31.93	···
10	39.39	1.23
12a	52.00	1.63
12b	36.00	1.13
12c	38.00	1.20
12d	39.00	1.22
13a	37.00	1.16
14a	31.93	1.00
14b	31.09	0.97
15a	17.64	0.55
16a	31.93	1.00
16c	30.25	0.95
16d	31.09	0.97
16e	40.00	1.25
16f	27.73	0.87
16h	26.05	0.82
17a	16.80	0.53
17b	26.05	0.82
17c	30.25	0.95
17e	35.00	1.10
17f	15.12	0.47
17g	55.00	1.72
17h	26.89	0.84
18d	27.73	0.87
18e	37.00	1.16
18f	30.25	0.95
18g	31.09	0.97
18h	47.00	1.47

of the presence of a phenyl group at just a distance of one methylene group rather than two from the NH of the amide moiety. This finding was verified by the obtained data of the corresponding rmsd values of these compounds (Table IX).

Results cited in Table VIII show that the intermediate **12c** is 1.2 times as active as digoxin and also comparable to **10**. Transformation of **12c** into keto imines resulted in final compounds **18d–h** which revealed inotropic effect ranging from 0.95 to 1.47 as active as digoxin. These results indicate that increase of bulkiness of the substituent at the imine nitrogen from alkyl to cyclohexyl or phenethyl exerted a prompt effect on the activity of these compounds (c.f. **18e** and **18h**). Superiority of cardiotonic activity of compound **18h** over **18g**, also, was in a good agreement with the determined rmsd values of these compounds (Table IX).

Table IX. Rmsd values of the synthesized compounds and their positive inotropic activity

Comp. No.	% increase in contractility of cardiac muscle	RMSD value *
12a	52	0.1992
12b	36	0.2818
12d	39	0.2730
13a	37	0.2349
13b	59	0.2144
16e	40	0.2855
17g	56	0.2643
18h	47	0.2589

***Rmsd values of the most aligned eight compounds are listed.

In addition, cyclization of the diketo intermediate **12c** into pyrazoles **14a** and **14b** didn't show a marked decrease of activity than the parent **12c.**

Also, inspection of results listed in Table VIII indicates that intermediate **12d** which embody a cyano group and an ester moiety at the methylidene carbon, exhibited a comparable inotropic effect as **10** and slightly exceeds that of digoxin. Cyclization of **12d** into pyrazolinone **15a** resulted in 50% decrease of activity.

Hypotensive activity

Thirteen selected new pyridazinones **13a-c; 16a, c-f, h; 17f, g, h; and 18d** in addition to the intermediates **10** and **12a-d** were tested for their effect on blood pressure of normotensive rabbits.

It was noticed that there are fluctuations of the effect of the test compounds on blood pressure as there are two competing forces, the cardiotonic and the vasodilator effects, and the net result was due to the stronger effect. Results are listed in Tables X-XII.

Study of results listed in Tables X and XI indicates that the maximum decrease in blood pressure induced by this series of compounds appears at 15 min after i.v. injection. It was, also, noticed that the decrease of BP of compounds **16d** and **17g** remains up to 30 min, while the effect of most of the other compounds showed a significant drop at 30 min. On the other hand, compounds **12b, 16a, 16e, 16f** and **16h** showed a noticeable increase in BP at 30 min interval from injection, the latter observation may be explained by the dominance of their inotropic effect.

Also, it is especially worth noting that the hypotensive effect of compounds **16c, 16d, 16e** and **17g** at 15 min in comparison to the reported compound **10** ranges from 1.07 to 2.38 as active as **10**.

These data also show that an acetyl and an ester substituents at the methylidene carbon linked to the hydrazino moiety at the para position of the phenyl

Table X. % mean changes in BP of test compounds in comparison to **10**

Comp. No. Time (min)	% mean change in BP at 0.1 mg/kg dose[a]			
	D'	5'	15'	30'
10	-8.00 ± 0.080**	-9.00 ± 0.070***	-13.00 ± 0.070	-28.00 ± 0.070
12a	-5.57 ± 0.051*	-1.52 ± 0.084*	-9.96 ± 0.040*	-4.79 ± 0.038*
12b	1.66 ± 0.090	4.03 ± 0.050*	2.00 ± 0.04**	5.00 ± 0.040
12c	3.00 ± 0.094	9.00 ± 0.080**	no change	no change
12d	-2.00 ± 0.100*	-4.20 ± 0.040	-4.00 ± 0.070	0
13a	-3.50 ± 0.060**	-4.00 ± 0.040**	no change	no change
13b	-2.00 ± 0.100*	-3.00 ± 0.060	coagulation	coagulation
13c	-9.00 ± 0.090***	-11.00 ± 0.090***	coagulation	coagulation
16a	-3.60 ± 0.036**	1.00 ± 0.050	4.00 ± 0.037***	4.00 ± 0.037***
16c	2.55 ± 0.069*	-13.77 ± 0.020***	-14.54 ± 0***	-2.55 ± 0.029*
16d	3.66 ± 0.042**	-16.09 ± 0.077**	-31.46 ± 0.055***	-22.19 ± 0.02***
16e	8.91 ± 0.058*	6.05 ± 0.038*	-14.85 ± 0.052**	7.88 ± 0.064*
16f	2.30 ± 0.050	-3.80 ± 0.100	2.00 ± 0.096	2.00 ± 0.096
16h	-4.00 ± 0.037***	1.20 ± 0.050	2.00 ± 0.050	2.00 ± 0.048
17f	-3.00 ± 0.088	-4.00 ± 0.084	3.00 ± 0.084	-7.00 ± 0.074**
17g	4.04 ± 0.051*	4.04 ± 0.059*	-13.93 ± 0.034*	-20.09 ± 0.06**
17h	-5.00 ± 0.090*	2.00 ± 0.070*	3.00 ± 0.060*	-7.00 ± 0.06***
18d	-1.50 ± 0.040	3.40 ± 0.140	-5.00 ± 0.150	-8.00 ± 0.11**

[a]Each result represents the mean of five observations ± S.E.
D: Direct after injection.
***Significant at $p \leq 0.05$(degree of 95% confidence limits).
****Significant at $p \leq 0.01$(degree of 99% confidence limits).
*****Significant at $p \leq 0.005$(degree of 99.5% confidence limits).
N.B.: (-) negative sign means decrease in blood pressure.

group induced the highest hypotensive effect (c.f. **12a, 12b, 12c** and **12d)**.

Moreover, comparison of the activity of the intermediates **12a** and **12b** with their corresponding amides (final compounds) **16c–16e** and **17g** respectively show that amide formation improves the hypotensive activity of this series of pyridazinones.

The results obtained comply with the pharmacophoric studies rmsd values (Table XII).

Platelet aggregation inhibition

Since compounds **12a** and **16d** are the most significant cardiotonic and hypotensive, respectively. Therefore, these two compounds were, also, tested as platelet aggregation inhibitors at different concentrations. The results observed for compound **12a** were complete inhibition to the previously aggregated platelets in both cases nevertheless how much the percent of aggregation was (i.e. whether the percent was 68, 74 or 80% aggregation) for different volunteers. The normal percent of aggregation after addition of PAF should be more than 50%.

On the other hand, results for compound **16d** revealed complete inhibition with the higher concentration and possessed only 5 and 10% aggregation

Table XI. % mean changes in BP after injection of all test compounds at 15 min and 30 min in comparison to 10 at 0.1 mg/kg

Comp. No.	% mean change in BP	
	15 min	30 min
10	-13	-28
12a	-10	-5
12b	2	5
12c	no change	no change
12d	-4	0
13a	no change	no change
13b	coagulation	coagulation
13c	coagulation	coagulation
16a	4	4
16c	-15	-3
16d	-31	-22
16e	-15	8
16f	2	2
16h	2	2
17f	3	-7
17g	-14	-20
17h	4	-3
18d	-5	-8

N.B.: the negative sign means decrease in blood pressure.

Table XII. Rmsd values of the most aligned synthesized compounds onto the hypotensive pharmacophore

Comp. no.	RMSD value
12a	0.4651
12d	0.4477
16c	0.4686
16e	0.3879
17g	0.4262
18g	0.4114

with the smaller one, which is also considered as a neglected percent. Test was done as described in the experimental protocol using **ADP** as platelet activating factor PAF on platelet rich plasma (Murray et al., 1992) of human volunteers. It is worth noting that increasing concentration of PAF from 2.5 μM to 5 μM showed no effect on platelet aggregation inhibition percent observed with the test compounds.

Results are shown in Fig. 2-4 which indicate complete inhibition of platelet aggregation at all concentrations.

Toxicity studies

No signs of toxicity were noticed up to the dose level 300 mg/kg concentration for both test compounds, while the reported oral LD_{50} of digoxin is 17.78 mg/kg (Roche, through Internet).

Pharmacophore building and pharmacophore identifications

Study of the results (Table IX) revealed that **12a** was the most active compound according to its rmsd and this also was confirmed from the percent increase in contractility that decreased by increasing the rmsd value.

Comparing the cyclized derivatives **13a** and **13b**, it is clear that compound **13b** was more active than **13a** and this also was in accordance with their rmsd values. Also, comparing the amide derivative **16e** with its parent **12a**, the parent was more active due to the smaller rmsd value that makes better fitting to the hypothetical model. Similarly, compounds **17g** and **18h** showed better fitting than their parent **12b** and **12c** respectively.

Mapping of compound **13b** onto the pharmacophore model is shown in Fig. 7. It can be seen that all the chemical features of the hypothesis are matched by the chemical groups of the molecule: **F1:** *p*-phenyl substituted, CH_2-CH_2 of the pyridazinone ring and C_6H_5-NHN=C; **F2:** NH of the pyridazinone ring; **F3:** C=NNH of the pyridazinone ring; **F4:** oxygen of the carbonyl group of the pyridazinone ring; and **F5:** carbonyl in the pyrazolinone ring.

As for the hypotensive pharmacophore, it was interesting to find that almost all compounds showed well fitting onto the pharmacophore hypothesis with RMSD values range between (0.3879-0.4686). Mapping of compound **17g** onto the pharmacophore model is

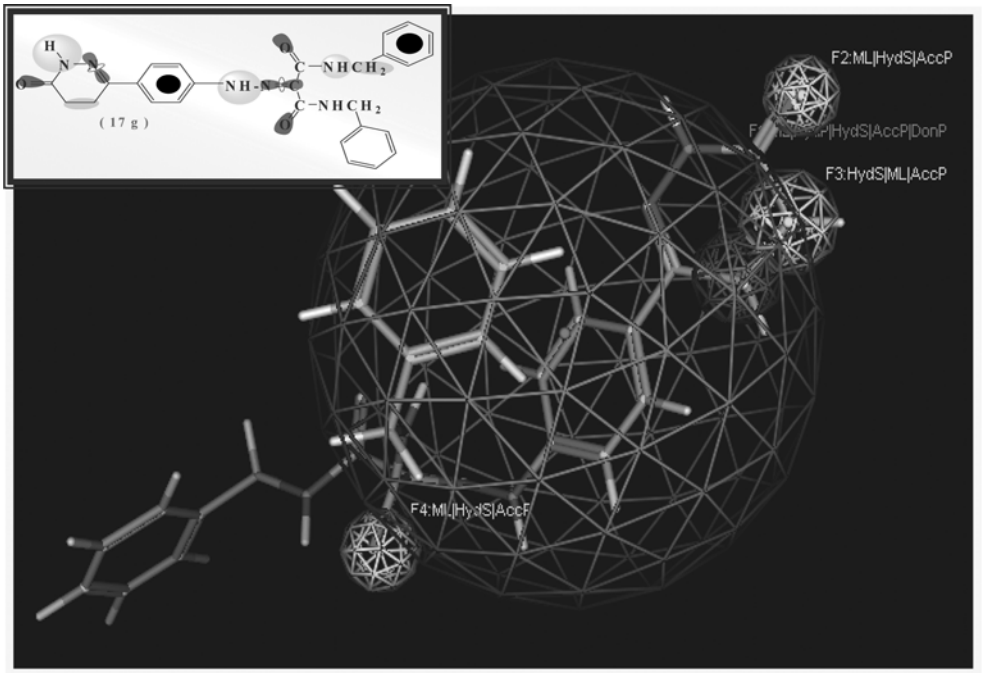

Fig. 9. Mapping of compound **17g** onto the hypotensive pharmacophore

shown in Fig. 9. Also, here it was noticed that all the chemical functionalities of the hypothesis are matched by the chemical groups of the synthesized compounds as follows: **F1:** *p*-phenyl-NHN, NHR (different amides) and NHN of the pyridazinone ring; **F2:** oxygen of the carbonyl group of the pyridazinone ring; **F3:** $CO\underline{CH_2}$ of the pyridazinone ring; **F4:** carbonyl group of the ester moiety; and **F5:** $N=C\underline{CH_2}$ of the pyridazinone ring.

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