

Synthesis and antimicrobial activity of some 2-[1-(4-oxo-3,4-dihydrophthalazine-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-dione and their imidoxy derivatives

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Abstract

In this study, a series of 2-[(4-substituted phthalazine-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones **6a-d** and 2-{2-[4-(1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-yl-alkyl)1-oxo phthalazine-2(1*H*)-yl]alkoxy}-1*H*-isoindole-1,3(2*H*)-diones **7a-d** have been synthesized from phthalyl derivatives of amino acids **1a-b** as starting material. The structures of these compounds were confirmed by IR, ¹H NMR and Mass spectral studies. Synthesized compounds **6a-d** and **7a-d** showed significant antimicrobial activity.

Keywords: Phthalazinones, imidoxy, POCl₃/PCl₅, spectral analysis, antibacterial and antifungal activity

Introduction

The diverse biological activities of various functional derivatives of 4-substituted alkyl-1-(2*H*)-phthalazinones are well known. Some of the phthalazinone derivatives have found application in clinical medicine¹ due to their pronounced antipyretic, analgesic and tuberculostatic activity while others have shown interesting vasodialator² and antihypertensive properties.³ Phthalazine-1(2*H*)-ones bearing a substitution at C-4 represent key intermediates in the synthesis of various compounds with highly interesting pharmacological properties, such as the blood platelet aggregation inhibitor MV-5445⁴ [1-(3-chloroanilino)-4-phenylphthalazine] which has been found to be a selective phosphodiesterase V_A inhibitor⁵ or the thromboxane A₂ synthetase inhibitor and bronchodilator, 2-[2-(1-imidazolyl)ethyl]-4-[3-pyridyl]-phthalazine-1[2*H*]-one.⁶ The phthalazinone nucleus has been proved to be a versatile system in medicinal chemistry. Moreover, a number of established drug molecules like *Hydralazine*,⁷ *Budralazine*,⁸ *Azelastine*,⁹ *Ponalrestat*¹⁰ or *Zopolrestat*¹¹ are accessible starting from the corresponding phthalazinones. The development of new and efficient methodologies for the synthesis of such potentially bioactive

phthalazine derivatives is important. Despite the useful nature of phthalazinone, there are very few synthetic approaches in the literature for the formation of 4-phenyl and 4-substituted alkyl-1-(2H)-phthalazinones and its derivatives.¹²⁻¹⁸ Therefore, functionalization of the nucleus continues to be of synthetic interest. In general, most of the structural modifications of the parent system which have been carried out in order to optimize the biological activity of phthalazine-derived drugs can be seen as a variation of the substitution pattern at position 1, 2 and 4, i.e. the substitution pattern of the 1,2-diazine part of the bicyclic system. Considerably less efforts has been devoted in the modification of the benzene part of the phthalazine skeleton.¹⁹

In the present paper we are reporting synthesis of some phthalazinones and their imidoxy derivatives. The newly synthesized compounds have been tested for their biological activity evaluation.

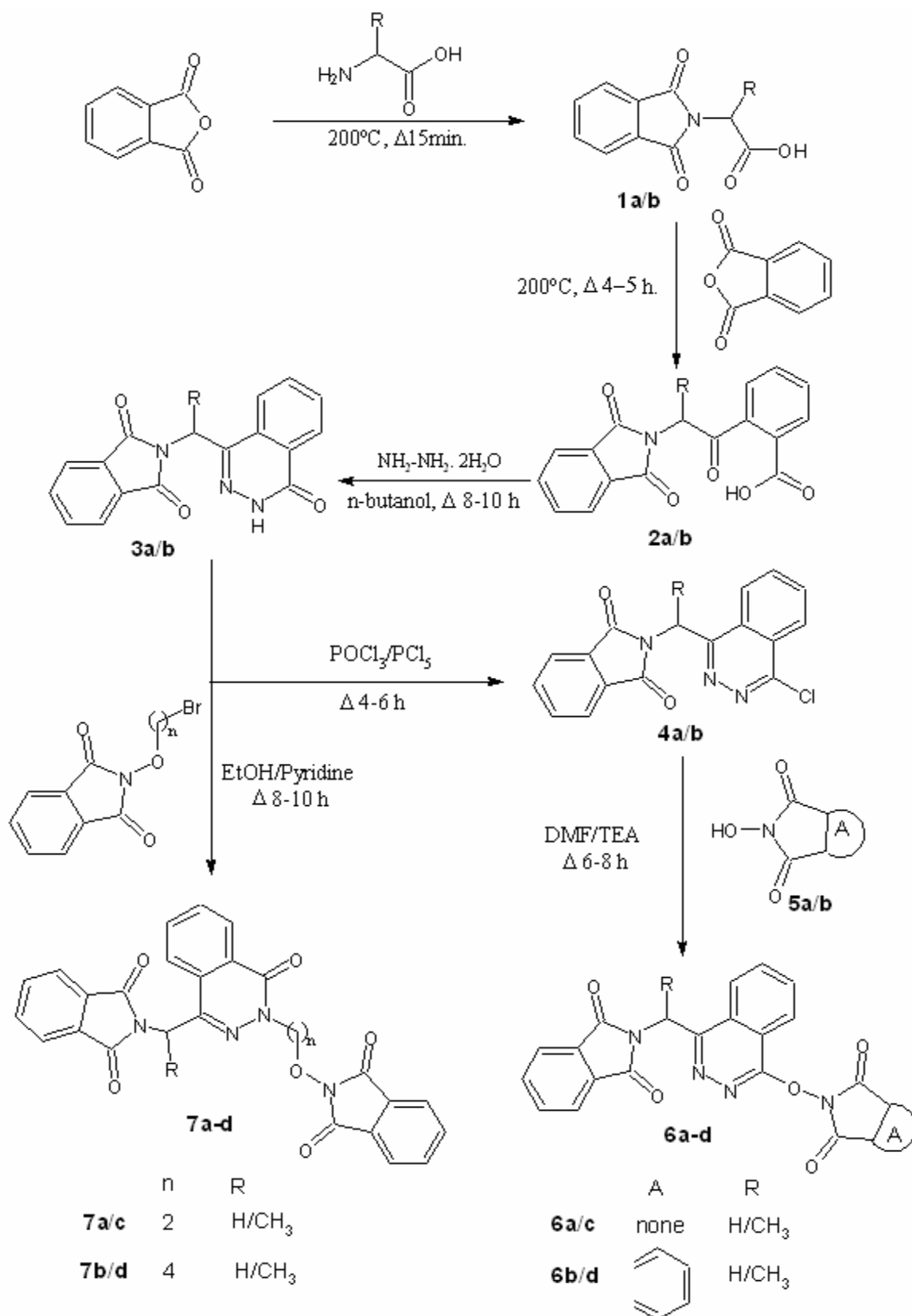
Results and Discussion

Chemistry

In present paper, 2-[1-(4-oxo-3,4-dihydrophthalazine-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-dione **3a-b** were obtained from compounds **1a-b**. Fusion of compounds **1a-b** with phthalic anhydride in the presence of anhydrous CH₃COONa gave acids **2a-b**. The acids **2a-b** readily underwent cyclization giving phthalazinone derivatives **3a-b** when refluxed with hydrazine hydrate in *n*-butanol. Condensation reaction of compounds **3a-b** with ω-bromoalkoxy phthalimide gave compounds 2-{2-[4-(1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-yl-alkyl)1-oxophthalazine-2(1*H*)-yl]alkoxy}-1*H*-isoindole-1,3(2*H*)-dione **7a-d**. On the other hand, treatment of phthalazinone derivatives **3a-b** with a mixture of POCl₃ and PCl₅ yielded **4a-b**, which when reacted with *N*-hydroxy phthalimide/succinimide **5a-b** gave the corresponding 2-[4-substituted phthalazin-1-yl]alkyl]-1*H*-isoindole-1,3(2*H*)-diones **6a-d**. (Scheme 1).

Antimicrobial activity

The antimicrobial activity of the synthesized compounds **6a-d** and **7a-d** was determined *in vitro* against a variety of bacteria and fungi. Comparative studies between the activity of our prepared compounds and standard drug were also carried out. The tests were carried out using disc-diffusion method.²¹ The compounds were dissolved in DMF, and activity mentioned on 1000 ppm. Agar plates were surface inoculated uniformly from fresh broth culture of the gram +ve and gram -ve bacteria and fungi. The Gram +ve bacteria was *Bacillus stablius*; the Gram -ve bacteria were *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella typhi* and the fungi were *Candida albicans* (MTCC 227) and *Aspergillus fumigatus* (MTCC 2550). Flucanazole and Etraconazole were used as standard for antibacterial and antifungal activity respectively. The discs were incubated at 5°C for 1 h. to permit good diffusion and the incubated at 28°C for 24 h, the zones of inhibition were measured in mm.



Scheme 1

From the data presented in **Table 1**, it is clear that compound **6a** and **6d** possess good activity against *Proteus mirabilis*, *Klebsiella pneumoniae*, but show only moderate activity against *Bacillus stablius*, *Salmonella typhi*. Other compounds exhibit low antibacterial activity against all organisms. On the other hand, it was observed that almost all compounds show good activity against both fungal strains as compared to standard Etraconazol. Only compound **6d** shows low antifungal activity. Thus it could be concluded that all the tested compounds exhibited relatively better antifungal activity, but weak activity against Gram +ve bacteria than those of Gram -ve bacteria.

Table 1. Antimicrobial activity of compounds 6a-d and 7a-d. (Zone of inhibition)

Compound	Antibacterial activity				Antifungal activity	
	<i>B. Stablius</i>	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>A. fumigatus</i>
6a	++	+	+++	++	+++	+++
6b	++	+	-	+	++	++
6c	+	+	-	+	+++	+++
6d	++	+++	-	++	+	-
7a	+	-	++	+	+++	++
7b	-	++	-	+	+	++
7c	-	++	-	-	+++	++
7d	+	-	++	+	+++	+++
C ₁	++++	++++	++++	++++	-	-
C ₂	-	-	-	-	++++	++++

Zone of inhibition (in mm): - = <3(no activity), + = 3-5(weak activity), ++ = 5-10(moderate activity), +++ = 10-15(good activity), ++++ = >15(strong activity).

Standards: C₁ = Flucanazole (Zone of inhibition = 18mm for antibacterial activity)

C₂ = Etraconazole (Zone of inhibition = 16mm for antifungal activity)

Experimental Section

General Procedures. All melting points were determined in open capillary tube and are uncorrected. The IR spectra were recorded on Perkin-Elmer spectrophotometer. The ¹H NMR spectra were registered on a DRX-300 MHz. Spectrometer (300 MHz) in DMSO-d₆ using TMS as internal standard and the chemical shifts are expressed in δ ppm. The mass spectra were recorded on Jeol SX-102 (FAB). m-nitrobenzyl alcohol (NBA) was used as matrix. Purity of synthesized compounds was checked by TLC using silica gel-G. Spots were exposed in an iodine chamber. Compounds **1a-b** were prepared according to literature procedure²⁰ and recrystallized prior to use.

Synthesis of 2-[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-yl)acetyl] benzoic acid (2a). A mixture of phthalylglycine **1a** (20.7 g, 0.1 mol), phthalic anhydride (14.8 g, 0.1 mol) and sodium acetate (4 g) was fused at 200°C on an oil bath for 4 h. The solid obtained was acidified with dil. HCl (5%), washed with water and recrystallized from methanol to give **2a**. Yield 80%, m.p. 278-280°C; IR(KBr)cm⁻¹: 1680 (C=O), 3300(OH); ¹H NMR (DMSO d₆): 8.1-8.2 (m, 4H, Ar-H), 7.6-7.7 (m, 4H, Ar-H), 6.4 (s, 2H, -CH₂); MS: m/z: 309 [M]⁺, 265 (100%), 188 (70%), 160 (38%), 146 (13%), 118 (8%), 77 (42%), 65 (31%), 51 (10%); Anal. Calcd. For C₁₇H₁₁NO₅: C, 66.24; H, 3.27; N, 4.54. Found: C, 65.89; H, 3.18; N, 4.51%.

Compound **2b** was also synthesized by a similar method.

2-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindole-2-yl)propanoyl]benzoic acid (2b). Yield 80%, m.p. 205-208°C; IR (KBr) cm⁻¹: 1685 (C=O), 3350 (OH); ¹H NMR (DMSO d₆): 7.9 (m, 4H, Ar-H), 7.6 (m, 4H, Ar-H), 5.6 (q, 1H, -CH), 2.8 (d, 3H, -CH₃) MS: m/z: 323 [M]⁺, 279 (100%), 202 (60%), 174 (30%), 146 (20%), 118 (12%), 77 (48%), 65 (35%), 51 (12%); Anal. Calcd. For C₁₈H₁₃NO₅: C, 66.87; H, 4.05; N, 4.33. Found: C, 66.22; H, 3.86; N, 3.89%.

Synthesis of 2-[1-(4-oxo-3,4-dihydrophthalazine-1-yl)methyl]-1*H*-isoindole-1,3(2*H*)-dione (3a). A mixture of acid **2a** (15.4 g, 0.05 mol) and hydrazine hydrate (2.6 mL, 0.05 mol) in 40ml of n-butanol was refluxed for 10 h. The solid that separated out after concentration and cooling was recrystallized from ethanol. Yield 70%, m.p. 301-304°C; IR (KBr) cm⁻¹: 3364 (N-H), 1672 (C=O, Pyridazinone), 1633 (C=N), 1486 (C=C); ¹H NMR (DMSO d₆): 10.97 (s, 1H, N-H), 8.1-8.2 (m, 4H, Ar-H), 7.52-7.7 (m, 4H, Ar-H), 6.2 (s, 2H, -CH₂); MS: m/z: 305 [M]⁺ (100%), 159 (11%), 146 (68%), 118 (18%), 90 (43%), 77% (15%), 65 (28%), 51 (10%); Anal. Calcd. For C₁₇H₁₁N₃O₃: C, 66.88; H, 3.63; N, 13.76. Found: C, 66.81; H, 3.56; N, 13.71%.

Compound **3b** was synthesized by a similar method.

2-[1-(4-Oxo-3,4-dihydrophthalazine-1-yl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione (3b). Yield 60%, m.p. 290-292°C; IR (KBr) cm⁻¹: 3286 (N-H), 1692 (C=O), 1632 (C=N), 1395 (C=C); ¹H NMR (DMSO d₆): 11.0 (s, 1H, N-H), 5.9 (q, 1H, -CH), 2.7 (d, 3H, -CH₃), 8.0-8.1 (m, 4H, Ar-H), 7.6-7.5 (m, 4H, Ar-H); MS: m/z: 319 [M]⁺ (100%), 173 (65%), 146 (40%), 118 (26%), 77 (15%), 65 (20%), 51 (15%). Anal. Calcd. For C₁₈H₁₃N₃O₃: C, 67.71; H, 4.10; N, 13.16. Found: C, 67.66; H, 4.05; N, 13.09%.

Synthesis of 2-[1-(4-chlorophthalazine-1-yl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (4a). A suspension of **3a** (3.0 g, 0.01 mol), PCl₅ (3.1 g, 0.015 mol) and POCl₃ (5 mL) was heated on steam bath for 4 h and then poured slowly into ice-cold dil. HCl (5%). The separated solid was filtered, washed with water and recrystallized from acetic acid. Yield 65%, m.p. 238-240°C; IR (KBr) cm⁻¹: 1715 (C=O), 1635, 1615 (C=N), 710 (C-Cl); ¹H NMR (DMSO d₆): 8.1 (m, 4H, Ar-H), 7.5 (m, 4H, Ar-H), 6.73 (s, 2H, -CH₂-); MS: m/z: 325 [M+2], 323 [M]⁺ (100%), 177 (56%), 146 (70%), 118 (26%), 90 (52%), 77 (15%), 65 (28%), 51 (18%). Anal. Calcd. For C₁₇H₁₀ClN₃O₂: C, 63.07; H, 3.11; N, 10.95. Found: C, 63.01; H, 3.02; N, 10.22%.

Compound **4b** was synthesized by a similar method by minor modification e.g., reflux time (3 h), crystallization solvent (ethanol).

2-[1-(4-Chlorophthalazine-1-yl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione (4b). Yield 68%, m.p. 170-172°C; IR (KBr) cm^{-1} : 1710 (C=O), 1610, 1590 (C=N), 720 (C-Cl); ^1H NMR (DMSO d_6): 7.9 (m, 4H, Ar-H), 7.4 (m, 4H, Ar-H), 6.52 (q, 1H, -CH-), 2.6 (d, 3H, -CH₃); MS: m/z: 339 [M+2], 337 [M]⁺ (100%), 191 (45%), 146 (78%), 118 (15%), 77 (14%), 65 (22%). Anal. Calcd. For C₁₈H₁₂ClN₃O₂: C, 64.01; H, 3.58; N, 10.95. Found: C, 63.88; H, 3.52; N, 10.89%.

Synthesis of 2-({4-[(2,5-dioxopyrrolidin-1-yl)oxy]phthalazine-1-yl}methyl)-1*H*-isoindole-1,3(2*H*)-dione (6a). Compounds **4a** (3.2 g, 0.05 mol) and **5a** (1.1 g, 0.05 mol) were dissolved in 20 mL DMF and then TEA (2 mL) was added dropwise. The reaction mixture was refluxed for 8 h. Excess of solvent was distilled and the resulting product was filtered, washed with water and recrystallized in ethanol. Yield 65%, m.p. 287-289°C; IR (KBr) cm^{-1} : 1705 (C=O), 1690 (C=O, succinimidoxy), 1625, 1605 (C=N), 1395 (N-O); ^1H NMR (DMSO d_6): 7.8 (m, 4H, Ar-H), 7.3 (m, 4H, Ar-H), 6.72 (s, 2H, -CH₂-), 2.5 (s, 4H, -CH₂-CH₂-). MS: m/z: 402 [M]⁺, 350, 326, 262, 242, 216, 114, 84, 56. Anal. Calcd. For C₂₁H₁₄N₄O₅: C, 62.69; H, 3.51; N, 13.92. Found: C, 62.61; H, 3.41; N, 13.87%.

Compounds **6b-d** were synthesized by a similar method by minor modification e.g., reflux time (8-12 h), crystallization solvent (ethanol, methanol).

2-(-1{4-[(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phthalazine-1-yl}oxy)-1*H*-isoindole-1,3(2*H*)-dione (6b). Yield 60%, m.p. 310-312°C; IR (KBr) cm^{-1} : 1715 (C=O), 1695 (C=O, phthalimidoxy), 1615, 1595 (C=N), 1390 (N-O); ^1H NMR (DMSO d_6): 8.1(m, 4H, Ar-H), 7.6 (m, 4H, Ar-H), 7.2 (m, 4H, Ar-H), 6.61 (s, 2H, -CH₂-). MS: m/z: 450 [M]⁺, 398, 374, 290, 264, 262, 162, 132, 104, 76. Anal. Calcd. For C₂₅H₁₄N₄O₅: C, 66.67; H, 3.13; N, 12.44. Found: C, 66.58; H, 3.08; N, 12.36%.

2-(1-{4-[2,5-Dioxopyrrolidine-1-yl]oxy}phthalazine-1-yl}ethyl)-1*H*-isoindole-1,3(2*H*)-dione (6c). Yield 70%, m.p. 270-272°C; IR (KBr) cm^{-1} : 1705 (C=O), 1685 (C=O, succinimidoxy), 1620, 1613 (C=N), 1380 (N-O), ^1H NMR (DMSO d_6): 8.0(m, 4H, Ar-H), 7.3 (m, 4H, Ar-H), 6.8 (q, 1H, -CH-), 2.6 (s, 4H, -CH₂-CH₂-), 2.3 (d, 3H, -CH₃). MS: m/z: 416 [M]⁺, 364, 340, 276, 242, 216, 114, 84, 56. Anal. Calcd. For C₂₂H₁₆N₄O₅: C, 63.46; H, 3.87; N, 13.46. Found: C, 63.40; H, 3.82; N, 13.39%.

2-(1-{4-[(1,3-Dioxo-1,3-dihydro-2*H*-isoindole-2-yl)ethyl]phthalazine-1-yl}oxy)-1*H*-isoindole-1,3(2*H*)-dione (6d). Yield 65%, m.p. 285-287°C; IR (KBr) cm^{-1} : 1700 (C=O), 1690 (C=O, phthalimidoxy), 1628, 1615 (C=N), 1368 (N-O), ^1H NMR (DMSO d_6): 7.9 (m, 4H, Ar-H), 7.6 (m, 4H, Ar-H), 7.1 (m, 4H, Ar-H), 6.76 (q, 1H, -CH-), 2.5 (d, 3H, -CH₃). MS: m/z: 464 [M]⁺, 412, 388, 290, 276, 264, 162, 132, 76. Anal. Calcd. For C₂₆H₁₆N₄O₅: C, 67.24; H, 3.47; N, 12.06. Found: C, 67.20; H, 3.41; N, 11.91%.

Synthesis of 2-{2-[4-(1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-yl-methyl)-1-oxo-phthalazine-2(1*H*)-yl]ethoxy}-1*H*-isoindole-1,3(2*H*)-dione (7a). Compd. **3a** (3.0 g, 0.01 mol) and ω -bromomethoxy phthalimide (2.6 g, 0.01 mol) were dissolved in 30 mL ethanol and then pyridine (2 mL) was added to it as a base. The reaction mixture was heated to reflux for 10 h. After completion of reaction, excess of solvent was distilled off under reduced pressure. Resulting mixture is cooled and the solid so obtained was filtered, dried and recrystallized from ethanol.

Yield 65%, m.p. 320-322°C; IR (KBr) cm^{-1} : 1700, 1695, 1680 (C=O), 1620, 1608 (C=N), 1370 (N-O), ^1H NMR (DMSO d_6): 8.1 (m, 4H, Ar-H), 7.7 (m, 4H, Ar-H), 7.2 (m, 4H, Ar-H), 6.6 (s, 2H, $-\text{CH}_2-$), 3.7 (t, 2H, $\text{CH}_2\text{-O}$), 3.2 (t, 2H, $\text{CH}_2\text{-N}$); MS: m/z : 494 $[\text{M}]^+$, 418, 334, 318, 308, 190, 176, 162, 132, 104; Anal. Calcd. For $\text{C}_{27}\text{H}_{18}\text{N}_4\text{O}_6$: C, 65.59; H, 3.67; N, 11.33. Found: C, 65.54; H, 3.61; N, 11.27%.

Compounds **7b-d** were synthesized by a similar method by minor modification e.g., reflux time (10-14 h), crystallization solvent (ethanol, methanol).

2-{2-[4-(1,3-Dioxo-1,3-dihydro-2H-isoindole-2-yl-methyl)-1-oxo-phthalazine-2(1H)-yl]butoxy}-1H-isoindole-1,3(2H)-dione (7b). Yield 73%, m.p. 296-298°C; IR (KBr) cm^{-1} : 1710, 1698, 1685 (C=O), 1615, 1605 (C=N), 1365 (N-O), ^1H NMR (DMSO d_6): 8.0 (m, 4H, Ar-H), 7.8 (m, 4H, Ar-H), 7.4 (m, 4H, Ar-H), 6.7 (s, 2H, $-\text{CH}_2-$), 3.0 (t, 2H, $-\text{CH}_2\text{-O}$), 2.8 (t, 2H, $-\text{CH}_2\text{-N}$), 2.4 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-O}$), 2.2 (m, 2H, $-\text{CH}_2\text{-CH}_2\text{-N}$); MS: m/z : 508 $[\text{M}]^+$, 432, 334, 332, 320, 190, 176, 174, 162, 132, 104, 76; Anal. Calcd. For $\text{C}_{27}\text{H}_{18}\text{N}_4\text{O}_6$: C, 66.66; H, 4.24; N, 10.72. Found: C, 66.60; H, 4.17; N, 10.64%.

2-{2-[4-(1,3-Dioxo-1,3-dihydro-2H-isoindole-2-yl-ethyl)-1-oxo-phthalazine-2(1H)-yl]ethoxy}-1H-isoindole-1,3(2H)-dione (7c). Yield 68%, m.p. 305-307°C; IR (KBr) cm^{-1} : 1703, 1691 (C=O), 1625, 1610 (C=N), 1360 (N-O), ^1H NMR (DMSO d_6): 8.2 (m, 4H, Ar-H), 7.8 (m, 4H, Ar-H), 7.4 (m, 4H, Ar-H), 6.8 (q, 1H, $-\text{CH}$), 3.1 (t, 2H, $-\text{CH}_2\text{-O}$), 2.6 (d, 3H, $-\text{CH}_3$), 2.3 (m, 2H, $\text{CH}_2\text{-N}$); MS: m/z : 522 $[\text{M}]^+$, 494, 362, 346, 336, 218, 190, 176, 162, 160, 132, 104, 76; Anal. Calcd. For $\text{C}_{28}\text{H}_{20}\text{N}_4\text{O}_6$: C, 66.14; H, 3.96; N, 11.02. Found: C, 66.07; H, 3.86; N, 11.01%.

2-{2-[4-(1,3-Dioxo-1,3-dihydro-2H-isoindole-2-yl-ethyl)-1-oxo-phthalazine-2(1H)-yl]butoxy}-1H-isoindole-1,3(2H)-dione (7d). Yield 60%, m.p. 265-267°C; IR (KBr) cm^{-1} : 1708, 1695 (C=O), 1614, 1605 (C=N), 1365 (N-O), ^1H NMR (DMSO d_6): 8.1 (m, 4H, Ar-H), 7.7 (m, 4H, Ar-H), 7.3 (m, 4H, Ar-H), 6.7 (q, 1H, $-\text{CH}$), 3.2 (t, 2H, $-\text{CH}_2\text{-O}$), 2.7 (d, 3H, $-\text{CH}_3$), 2.5 (t, 2H, $-\text{CH}_2\text{-N}$), 2.3 (m, 2H, $-\text{CH}_2\text{-CH}_2\text{-O}$), 2.1 (m, 2H, $-\text{CH}_2\text{-CH}_2\text{-N}$); MS: m/z : 536 $[\text{M}]^+$, 510, 362, 360, 336, 218, 190, 176, 174, 162, 132, 104, 76; Anal. Calcd. For $\text{C}_{30}\text{H}_{24}\text{N}_4\text{O}_6$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.01; H, 4.47; N, 10.40%.

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References

1. (a) Marishita, H.; Nakano, S.; Yoshida, N.; Mori, K. *Chem. Abstr.* **1960**, *54*, 15411. (b) Bavin, E. M.; Drain, D. J., Seiler, M.; Seymour, D. E. *J. Pharm.Pharmacol.* **1952**, *4*, 844.
2. Demirayak, S.; Karaburun, A. C.; Kayagil, I.; Erol, K.; Sirmagul, B. *Archives of Pharmacol. Res.* **2004**, *27*, 13.
3. Vega, A. M.; Aldana, I.; Alvarez E. F. *Eur. J. Med. Chem.* **1978**, *13*, 573.
4. Hagiwara, M.; Endo, T.; Kanayama T.; Hidaka, H. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 467.
5. Murray, K. J. *Drug News and Perspectives* **1993**, *6*, 150.
6. Yamaguchi, M.; Kamei, K.; Koga, T.; Akima, M.; Kuroki, T.; Ohi, N. *J. Med. Chem.* **1993**, *36*, 4052.
7. Kleemann, A.; Engel, J. *Pharmazeutische Wirkstoffe Synthesen Patente Anwendungen*, Thieme, Stuttgart, 1982, Vol. 5.
8. Ueno, K.; Moroi, R.; Kitagawa, M.; Asano, K.; Miyazaki, S. *Chem. Pharm. Bull.* **1976**, *24*, 1068.
9. Scheffler, G.; Engel, J.; Kutscher, B.; sheldrick, W. S.; Bell, P. *Arch. Pharm.* **1988**, *321*, 205.
10. Laudadio, C.; Sima, A. A. F. *Journal of Diabetes and its Complications* **1998**, *12*, 121.
11. Beyer, M. A.; Diecke, F. P. J.; Mistry, K.; Cruze, E. *Pharmacology* **1997**, *54*, 76.
12. Marcaccini, S.; Pepino, R.; Polo, C.; Pazo, M. C. *Synthesis* **2001**, 85.
13. Epszajn, J.; Malinowski, Z.; Brzezinski, J. Z.; Karazatka, M. *Synthesis* **2000**, *14*, 2085.
14. Saito, Y.; Sakamoto, T.; Kikugawa, Y. *Synthesis* **2001**, *2*, 221.
15. Abdel-Khalik, M. M.; Agamy, M. S.; Elnagdi, M. H. *Synthesis* **2001**, *12*, 1861.
16. Castle, N. R.; *Condensed Pyridazines Including Cinnolines and Phthalazines*, R N Ed., Wiley-Interscience: New York, 1973; p 383.
17. Horn, H.; Univerferth, K.; Koerner, B.; Lohmann, D.; Laban, G. *German Patent 274218*, 1989; *Chem. Abstr.* **1990**, *113*, 23938.
18. Horn, H.; Morgenstern, E.; Univerferth, K. *Pharmazie* **1990**, *45*, 724.
19. (a) Eguchi, Y.; Sasaki, F.; Takashima, Y.; Nakajima, M.; Ishikawa M. *Chem. Pharm. Bull.* **1991**, *39*, 795. (b) Sugimoto, A.; Ito, S.; Eguchi, Y.; Tanaka, H.; Takashima, Y.; Ishikawa, M. *Eur. J. Med. Chem.* **1984**, *19*, 223. (c) Cherkez, S.; Herzig, J.; Yellin, H. *J. Med. Chem.* **1986**, *29*, 947.
20. Rajpurohit, S.; Sah, P. *Asian J. Chem.* **2005**, *17*, 949.
21. Collee, G. J.; Fraser, G. A.; Marmion, P. B.; Simmons, A. *Practical Medical Microbiology*, 14th Edn., Churchill Livingstone: Edinburgh, 1996; Vol. 11, p 163.