Coupling Reactions of Hydralazine with Amino Acids and their Adducts for Antihypertensive Activities.

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Dedicated to the memory of Prof. Hans Zimmer

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Despite the fact that hydralazine (phthalazin-1-yl-hydrazine - Apresoline®) **1** was introduced in hypertension therapy for more than 50 years, the mechanism of its action is still obscure [2]. The hydrazine group being a reactive pharmacophore might actively react with any of the C=O of the biological receptors to exert its effect [3,4].

It is notable that the so called N-acetyl derivative of **1** which was found to be among the major metabolites, was reassigned as the hydrate form of the stable triazolophthalazine system, namely, 3-methyl-s-triazolo[3,4-*a*]phthalazine (3-MeTP) [5-7]. Consequently, an extensive acylation of **1** was studied using a variety of acids and derivatives and attempts were made to fuse six, seven and eight-membered rings to the phthalazine ring system for better understanding of this molecule chemical behavior [8-18]. Furthermore, up to our knowledge no trial was carried out to deliver hydralazine supported on amino acids or peptide moiety as a pro-drug concept for improving its bioavailability and decreasing its undesirable side effects. Spurred by the aforementioned finding, it became interesting enough to assign a synthetic program to obtain such triazolophthalazines supported on amino acid or peptide moiety. The open-chain counter-parts were also not far from our attention.

Results and Discussion.

Chemistry.

The reaction of phthalazin-1-yl-hydrazine hydrochloride **1.HCl** with different *N*-Boc-L-amino acids **2a-2e** in dimethylformamide in the presence of diisopropylethylamine (DIEA) as a base and *N*-[(Dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophsphate *N*-oxide (HATU) as coupling reagent at 0 °C gave smoothly the triazolophthalazine derivatives **3a-3e**. The structures of compounds **3a-3e** were established by mean of ir, ^{1}H nmr, ^{13}C nmr and elemental analyses. The infrared spectra of these compounds showed absorption peak of the urethane carbonyls in the range 1690-1711 cm^{-1} and the absorption peak of the N-H in the range 3234-3359 cm^{-1}. The ^{1}H nmr showed only one exchangeable signal for NH and the structure of **3** was secured by ^{13}C nmr where a peak corresponding to the amide carbonyl was detected and only the urethane carbonyls were observed at 148.10-155.94 ppm.

On the other hand, molecular modeling supported the idea that the introduction of a bulky R group might provide an avenue to stabilize the open intermediate structure by keeping distance between the two active sites (N-2 of the phthalazine ring and the amide carbonyl side chain), with the hope that such designed structure comply with the enhanced characters or pro-drug requirements. Indeed it was the case. Thus, utilizing the *N*-Boc-L-(*O*-benzyl)-tyrosine **2f** (R= CH_2-[4-(OBn)C_6H_4]) afforded compound in the open structure and this was also supported by ir, ^{1}H nmr, ^{13}C nmr and elemental analysis. The infrared spectrum of **4** showed an absorption peak for the amide carbonyl at 1663 cm^{-1}, an urethane carbonyl at 1710 cm^{-1} and the absorption peak of the N-H at 3442 cm^{-1}. The ^{13}C nmr showed also a peak corresponding to the amide carbonyl at 172.16 ppm in addition to the urethane carbonyl peak at 166.88 ppm.

Sterric hindrance may prevented ring closure in compound **4** as the distance between the N-2 nitrogen atom and the amide carbonyl carbon atom is larger. The N-C distance was compared for the intermediate open structure **4** with different R group utilizing PM3/MOPAC [19] (Table 1, Figure 2 and 3).

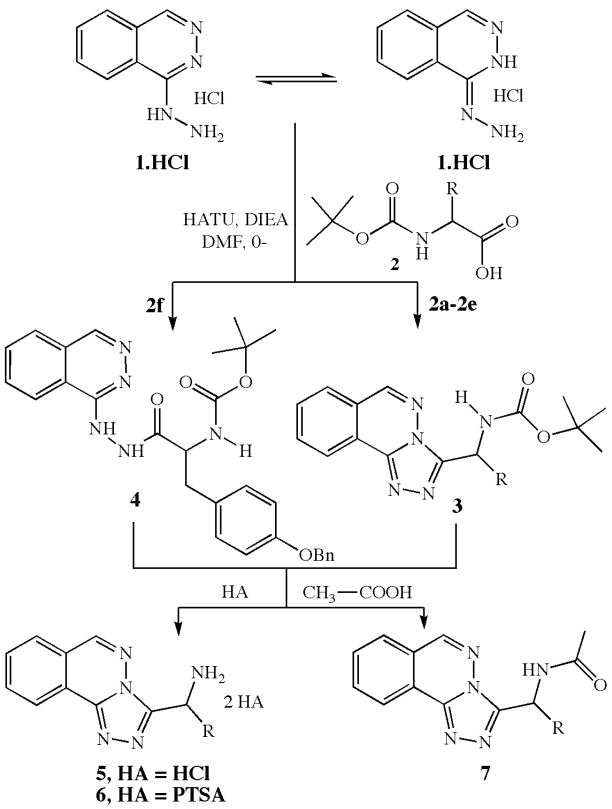
Table 1

The N-C Bond Distance of Compound **4**

R	N-C Bond Distance
CH_3	3.460 A°
$CH(CH_3)_2$	3.503 A°
$CH(CH_3)CH_2$-CH_3	3.546 A°
CH_2-OBn	3.461 A°
CH_2-CH_2-COOBn	3.376 A°
CH_2-[4-(OBn)C_6H_4]	4.035 A°

Unfortunately, it was found that this intermediate was very sensitive to acidic medium. Thus, deprotection of the Boc group by passing HCl gas into a solution of compounds **3a-3e** and compound **4** as well as in methylene chloride led to the formation of the corresponding triazolophthalazine **5** as stable white hydrochloride salts in good yield. Deprotection of the Boc group from compounds **3a-3e** and **4** was carried out with *p*-toluenesulphonic acid (2 equivalent) in acetonitrile, and gave the

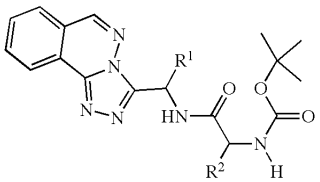
Scheme 1



a, R = CH_3; **b**, R = $CH(CH_3)_2$; **c**, R = $CH(CH_3)CH_2$-CH_3;
d, R = CH_2-OBn; **e**, R = CH_2-CH_2-COOBn; **f**, R = CH_2-[4-(OBn)C_6H_4].

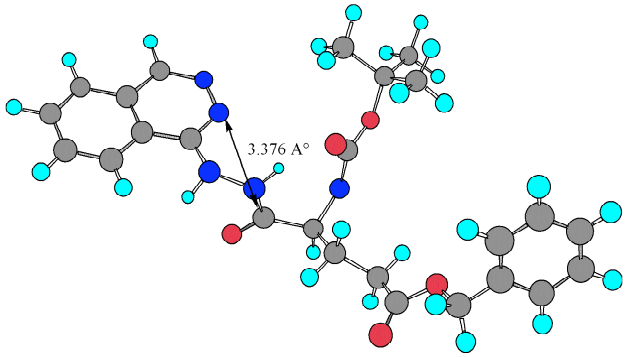
corresponding tosylate as moisture sensitive salts **6**. The structure of compounds **5** and **6** were confirmed by spectroscopic measurements. Our attempt to remove the protecting group (Boc) from compounds **3a-3e** and compound **4** with acetic acid as a weaker acid, led to the formation of the N-acetyl triazolophthalazines **7**. The structure of compounds **7** were established by ir, ^{1}H nmr and ^{13}C nmr as well as elemental analyses.

Our approach to build up a peptide chain on the triazolophthalazine structure **6** using N-Boc-amino acid and HATU as a coupling reagent in the presence of DIEA has met with success and compounds **8a** and **8b** were prepared as a prototype (Figure 1). Further synthetic work is in progress to furnish more compounds for SAR.



8a, R^1 = $CH(CH_3)_2$; R^2 = CH_2-[4-(OBn)C_6H_4].
8b, R^1 = CH_2-[4-(OBn)C_6H_4]; R^2 = CH_3.

Figure 1



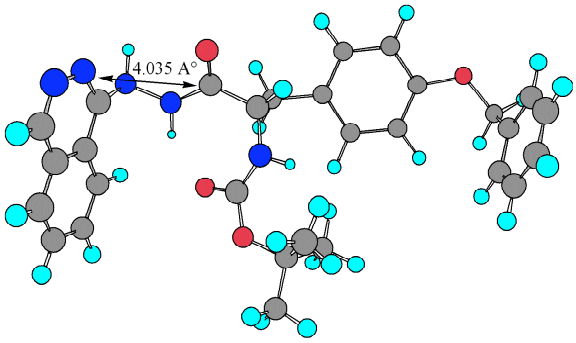
The N-C distance measured for the intermediate of structure **3e** as a model

Figure 2

Biological Results.

Antihypertensive Activity.

Compounds **5b, 5e** and **5f** showed significant hypotensive activity comparable to or higher than that of the reference standard hydralazine, whereas other compounds showed lower activity (Table 2). The Mean change in arterial pressure (MAP) of compound **5b** was -36±2.57 mmHg, while that of compound **5f** was -39±2.32. Hydralazine showed MAP of -32±2.44. Moreover 5e showed hypotensive activity of -31.74±0.96 mmHg. It is noteworthy, that the hypotensive effect of compounds **5b**, **5e** and **5f** were sustained for about 3 hours. This result speculates that the free amino group of 5b, 5e and 5f is essential for their hypotenssive activity.



The N-C distance measured for structure **4**

Figure 3

Acute Toxicity.

Compounds **5b**, **5f** and **5e** were further evaluated for their approximate LD_{50} in male mice using the method described in the literature [20,21]. The results indicated that most of the tested compounds proved to be non-toxic and well tolerated by the experimental animals as evidenced by their LD_{50} values (> 90 mg/Kg). Moreover, these compounds were tested for their toxicity through

parenteral route [22]. The results revealed that all the test compounds were non-toxic up to 50 mg/Kg.

Table 2

Hypotensive Activity of the Test Compounds

Compound	Change in MAP mmHg as mean ± SE
Control	1.0 ± 0.62
Hydralazine	-32±2.44
5b	-36±2.57
5f	-39±2.32
5e	-31.74±0.96
3b	-11.12±1.35
4	-11.85.25±0.67
3d	-16.35±1.36
3c	-12.32±1.56
3a	-7.86±2.39

Post-hoc Gabriel test between control and the rest of the groups P<0.001

EXPERIMENTAL

Chemistry.

Melting points were determined with a Mel-Temp apparatus and are uncorrected. Magnetic resonance spectra (^{1}H nmr and ^{13}C nmr spectra) were recorded on a Brucker 300 MHz spectrometer with chemical shift values reported in δ (part per million) relative to an internal standard (tetramethylsilane). Infrared data were obtained on a Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets. Geometry optimizations were performed initially by PM3/MOPAC in Alchemy 2000 and subsequently the N-C distances were measured. The compounds were named using Chem. Draw Ultra version 6, Cambridgesoft Corporation. The abbreviations: HATU, *N*-[(Dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophsphate *N*-oxide; Boc, *t*-butyloxycarbonyl; DIEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; PTSA, *p*-toluenesulphonic acid were used through out the manuscript. Elemental analyses were performed in the Chemistry Department, Faculty of Science, Cairo University.

General Procedure for the Reaction of N-Boc-L-Amino Acids with Phthalazin-1-yl-hydrazine Hydrochloride (**1.HCl**).

A mixture of (0.5 mmol) of N-Boc-L-amino acid (**2**), 0.19 g (0.5 mmol) of HATU and 0.17 mL (1 mmol) of DIEA was stirred at 0 °C for 3 min in 2 mL dimethylformamide. This solution was then added to a solution of hydralazine hydrochloride (**1.HCl**) (0.098 g, 0.5 mmol) and (0.09 mL, 0.5 mmol) DIEA in 2 mL of dimethylformamide at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour and left overnight at room temperature. The reaction mixture was diluted with 80 mL ethyl acetate, and the mixture was washed with 5% aqueous citric acid solution (2×10 mL), saturated sodium bicarbonate solution (2×10 mL) and saturated sodium chloride solution (2×10 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed *in vacuo*. The crude product was crystallized from methylene chloride-hexane. Only in the case of *N*-Boc L-(*O*-benzyl)-tyrosine (**2f**) the reaction mixture was poured into ice water, filtered, washed with 5% aqueous citric acid solution, saturated sodium bicarbonate solution and finally with water, dried and recrystallized.

(1-[1,2,4]Triazolo[3,4-*a*]phthalazin-3-yl-ethyl)-carbamic Acid *tert*-Butyl Ester (**3a**).

This compound was obtained as yellow crystals (methylene chloride-hexane), 0.13 g (83 %) yield, mp 128-130 °C; ir (KBr): 3353 (NH), 1696 (C=O, urethane) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.37 (s, 9H, 3 CH_3), 1.57 (d, 3H, CH_3), 5.29 (m, 1H, CH), 7.65 (d, 1H, NH), 7.91, 8.03 (2 t, 2H, H_8, H_9 aromatic), 8.19, 8.47 (2d, 2H, H_7, H_{10} aromatic), 9.06 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 19.3, 28.5, 42.2, 76.5, 120.1, 120.6, 120.9, 127.1, 129.0, 132.3, 140.3, 146.1, 149.7, 153.0.

Anal. Calcd. for $C_{16}H_{19}N_5O_2$: C, 61.33; H, 6.11; N, 22.35. Found: C, 61.08; H, 6.21; N, 22.11.

(2-Methyl-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-propyl)-carbamic Acid *tert*-Butyl Ester (**3b**).

This compound was obtained as yellow needles (methylene chloride-hexane), 0.09 g (52.8 %) yield, mp 186-188 °C; ir (KBr): 3234 (NH), 1710 (C=O, urethane) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 0.8, 0.9 (2d, 6H, 2 CH_3), 1.29 (s, 9H, 3 CH_3), 2.31 (m, 1H, CH), 5.08 (m, 1H, CH), 7.50 (d, 1H, NH), 7.70, 7.83 (2 t, 2H, H_8, H_9 aromatic), 7.96, 8.23 (2d, 2H, H_7, H_{10} aromatic), 9.10 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 19.18, 19.44, 28.5, 31.7, 50.14, 76.79, 120.8, 121.4, 121.6, 127.8, 129.7, 133.1, 140.6, 146.8, 149.6, 154.2.

Anal. Calcd for $C_{18}H_{23}N_5O_2$: C, 63.32; H, 6.79; N, 20.51. Found: C, 63.22; H, 6.83; N, 20.55.

(2-Methyl-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-butyl)-carbamic Acid *tert*-Butyl Ester (**3c**).

This compound was obtained as yellow crystals (methylene chloride-hexane), 0.171 g (96.3 %) yield, mp 114-115 °C; ir (KBr): 3269 (NH), 1710 (C=O, urethane) cm^{-1}; ^{1}H nmr ($CDCl_3$): δ 0.95 (m, 6H, 2 CH_3), 1.2, 1.6 (2 m, 2H, CH_2), 1.35 (s, 9H, 3 CH_3), 2.13 (m, 1H, CH), 5.5 (m, 1H, CH), 5.65 (d, 1H, NH), 7.75, 7.90, 8.70 (3m, 5H, aromatic); ^{13}C nmr ($CDCl_3$): δ 11.72, 15.79, 25.33, 28.72, 39.80, 50.94, 80.12, 123.54, 123.70, 123.86, 128.51, 131.28, 134.43, 142.97, 147.98, 151.37, 155.82.

Anal. Calcd. for $C_{19}H_{25}N_5O_2$: C, 64.20; H, 7.09; N, 19.70. Found: C, 64.30; H, 7.15; N, 19.82.

(2-Benzyloxy-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-ethyl)-carbamic Acid *tert*-Butyl Ester (**3d**).

This compound was obtained as yellow crystals (methylene chloride-hexane), 0.17 g (81.2 %) yield, mp 138-139 °C; ir (KBr): 3303 (NH), 1711 (C=O, urethane) cm^{-1}; ^{1}H nmr ($CDCl_3$): δ 1.39 (s, 9H, 3 CH_3), 3.91 (m, 2H, CH_2), 4.46 (m, 2H, CH_2), 5.65 (m, 1H, CH), 6.20 (d, 1H, NH), 7.08 (s, 5H, aromatic), 7.82 (d, 1 H, H_{10} aromatic), 7.90 (t, 2H, H_8, H_9 aromatic), 8.5 (d, 1H, H_7 aromatic) 8.6 (s, 1H, H_6 aromatic); ^{13}C nmr ($CDCl_3$): δ 27.3, 45.6, 69.0, 71.9, 78.8, 121.9, 122.1, 122.2, 126.48, 126.51, 126.75, 127.1, 127.35, 130.0, 133.1, 136.6, 142.0, 146.7, 148.1.

Anal. Calcd. for $C_{23}H_{25}N_5O_3$: C, 65.86; H, 6.01; N, 16.70. Found: C, 65.78; H, 6.03; N, 16.55.

4-*tert*-Butoxycarbonylamino-4-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-butyric Acid Benzyl Ester (**3e**).

This compound was obtained as pale beige crystals (methylene chloride-hexane), 0.17 g (73.8 %) yield, mp 143-144 °C; ir

(KBr): 3359 (NH), 1733 (C=O, ester), 1690 (C=O, urethane), cm^{-1}; ^1H nmr (DMSO-d_6): δ 1.37 (s, 9H, 3 CH_3), 2.06 (m, 2H, CH_2), 3.2 (m, 2H, CH_2-CO), 4.13 (m, 1H, CH), 5.13 (m, 2H, CH_2-O), 7.34 (s, 5H, aromatic), 7.5 (d, 1H, NH), 7.92, 8.08 (2 t, 2H, H_8, H_9 aromatic), 8.21, 8.48 (2 d, 2H, H_7, H_{10} aromatic), 9.05 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 20.8, 27.7, 28.5, 53.4, 66.3, 78.70, 122.38, 123.07, 123.28, 128.05, 128.33, 128.70, 129.40, 131.30, 134.63, 136.27, 142.48, 148.37, 149.90, 155.94, 172.48.

Anal. Calcd. for $C_{25}H_{27}N_5O_4$: C, 65.06; H, 5.90; N, 15.17. Found: C, 65.15; H, 6.03: N, 15.30.

[2-(4-Benzyloxy-phenyl)-1-(*N*'-phthalazin-1-yl-hydrazinocarbonyl)-ethyl]-carbamic Acid *tert*-butyl Ester (**4**).

This compound was obtained as pale yellow crystals (methylene chloride-hexane), 0.23 g (89.5 %) yield, mp 85 °C; ir (KBr): 3442 (NH), 1710 (C=O, urethane), 1663 (C=O, amide) cm^{-1}; ^1H nmr (DMSO-d_6): δ 1.32 (s, 9H, 3 CH_3), 2.8, 3.0 (2 m, 2H, CH_2), 4.15 (m, 1H, CH), 5.07 (2brs, 2H, CH_2-O), 6.92-9.09 (m, 15H, aromatic + NH), 9.89, 11.15 (2 br.d, 2NH); ^{13}C nmr (DMSO-d_6): δ 27.7, 35.99, 47.4, 68.6, 77.50, 113.87, 113.94, 122.32, 125.94, 127.08, 127.13, 127.23, 127.87, 128.58, 129.58, 129.78, 130.55, 130.80, 133.85, 136.72, 136.86, 154.85, 155.08, 156.41, 166.88, 172.16.

Anal. Calcd. for $C_{29}H_{31}N_5O_4$: C, 67.82; H, 6.08; N, 13.64. Found: C, 67.99; H, 6.18; N, 13.49.

2-Methyl-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-propylamine Dihydrochloride Salt (**5b**).

Compound **2b** (0.237 g, 0.69 mmol) was dissolved in a mixture of 4 mL of methylene chloride and 4 mL of ether. Gaseous hydrochloric acid was passed through the solution for 2 hours. A white precipitate was formed, was collected by filtration and washed with ether. The crude product was recrystallized from methyl alcohol-ether to give white crystals, 0.17 g (71.7 %), mp 229 °C; ir (KBr): 3083-2890 (NH); ^1H nmr (DMSO-d_6): δ 0.92, 1.05 (2d, 6H, 2 CH_3), 2.20 (m, 1H, CH), 4.83 (m, 1H, CH), 7.99, 8.10 (2 t, 2H, H_8, H_9 aromatic), 8.25, 8.57 (2d, 2H, H_7, H_{10} aromatic), 9.07 (br s, 3H, 3NH), 9.23 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 18.07, 18.79, 31.02, 50.07, 118.46, 122.45, 123.34, 129.52, 131.73, 134.88, 142.77, 147.00, 149.04.

Anal. Calcd. for $C_{13}H_{15}N_5$ 2HCl 1.5H_2O: C, 45.72; H, 5.86; N, 20.51. Found: C, 45.43; H, 6.14; N, 20.21.

4-Amino-4-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-butyric Acid Benzyl Ester Dihydrochloride Salt (**5e**).

Compound **2e** (0.239 g, 0.52 mmol) was dissolved in a mixture of 4 mL of methylene chloride and 4 mL of ether. Gaseous hydrochloric acid was passed through the solution for 2 hours. A white precipitate was formed, collected by filtration and washed with ether. The crude product was recrystallized from methyl alcohol-ether to give white crystals, 0.186 g (76.2 %), mp 149-150 °C; ir (KBr): 3100-2884 (NH), 1720 (C=O); ^1H nmr (DMSO-d_6): δ 2.49 (m, 2H, CH_2), 3.32 (m, 2H, CH_2-CO), 4.35 (m, 1H, CH), 5.23 (s, 2H, CH_2-O), 7.39 (m, 5H, aromatic), 7.99, 8.10 (2 m, 2H, H_8, H_9 aromatic), 8.28, 8.60 (2 m, 2H, H_7, H_{10} aromatic), 8.93 (br s, 3H, 3NH), 9.19 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 20.20, 26.83, 51.65, 67.58, 122.01, 122.97, 123.73, 128.45, 128.63, 128.77, 129.63, 132.17, 134.93, 135.39, 142.34, 149.14, 149.49, 169.49.

Anal. Calcd. for $C_{20}H_{20}N_5O_2$ 2HCl 2H_2O: C, 50.91; H, 5.52; N, 14.85. Found: C, 51.12; H, 5.26; N, 15.08.

2-Amino-3-(4-benzyloxy-phenyl)-propionic Acid *N*'-Phthalazin-1-yl-hydrazide Dihydrochloride Salt (**5f**).

Compound **2f** (0.094 g (0.18 mmol) was dissolved in a mixture of 4 mL of methylene chloride and 4 mL of ether. Gaseous hydrochloric acid was passed through the solution for 2 hours. A white precipitate was formed, collected by filtration and washed with ether. The crude product was recrystallized from methyl alcohol-ether to give white crystals, 0.07 g (76.1 %), mp 194 °C; ir (KBr): 3031-2892 (br., NH), 1720 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 3.1, 3.27 (2 m, 2H, CH_2), 4.40 (m, 1H, CH), 4.95, 5.1 (2 s, 2H, CH_2-O), 6.81-9.1 (m, 16H, aromatic + 3NH), 9.15 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 37.0, 46.45, 53.65, 69.40, 115.08, 115.28, 122.48, 122.63, 123.39, 127.21, 127.93, 128.05, 128.11, 128.19, 128.69, 128.80, 129.72, 130.69, 131.19, 131,96, 135.12, 137.29, 137.47, 142.97, 147.21, 148.98, 157.72, 157.98.

Anal. Calcd. for $C_{24}H_{21}N_5O$•2HCl•2H_2O: C, 57.10; H, 5.36; N, 13.89. Found: C, 57.34; H, 5.61; N, 14.18.

1-[1,2,4]Triazolo[3,4-*a*]phthalazin-3-yl-ethylamine *p*-Toluenesulphonate Salt (**6a**).

Compound **3a** (0.166 g, 0.4 mmol) was dissolved in 3 mL acetonitrile. *p*-Toluene sulfonic acid 0.154 g (0.8 mmol) was added to the reaction mixture, and the reaction mixture was allowed to be stirred for 3 hours. Then the solvent was evaporated *in vacuo* and the crude product obtained was recrystallized from methyl alcohol-ether to give white crystals, 0.17 g (57.63 %), mp 225-226 °C; ir (KBr): 2951 (NH) cm^{-1}; ^1H nmr ($CDCl_3$): δ1.82 (d, 3H, CH_3), 2.24 (s, 6H, 2CH_3), 5.19 (m, 1H, CH), 7.06, 7.59 (2d, 8H, aromatic), 7.73-8.00 (m, 3H, H_8, H_9, H_7 aromatic), 8.6 (d, 1H, H_{10} aromatic), 8.73 (s, 1H, H_6 aromatic), 9.05 (brs, 3H, 3NH); ^{13}C nmr ($CDCl_3$): δ18.00, 21.56, 42.73, 123.80, 126.30, 129.00, 129.10, 132.30, 134.90, 141.50, 141.90, 149.10.

2-Methyl-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-propylamine *p*-Toluenesulphonate Salt (**6b**).

Compound **3b** (0.137 g, 0.4 mmol) was dissolved in 3 mL acetonitrile. *p*-Toluene sulfonic acid 0.154 g (0.8 mmol) was added to the reaction mixture and the reaction mixture was allowed to be stirred for 3 hours. Then the solvent was evaporated *in vacuo* and the crude product obtained was recrystallized from methyl alcohol-ether to give white crystals, 0.171 g (74.35 %), mp 244-245 °C; ir (KBr): 2923 (NH) cm^{-1}; ^1H nmr (DMSO-d_6): δ 0.93, 1.03 (2d, 6H, 2 CH_3), 2.28 (s, 6H, 2CH_3), 2.50 (m, H, CH), 4.90 (m, H, CH), 7.10, 7.47 (2d, 4H, aromatic), 7.98, 8.12 (2t, 2H, H_8, H_9 aromatic), 8.29, 8.57 (2d, 2H, H_7, H_{10} aromatic), 8.79 (br.s, 3H, 3NH), 9.21 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 18.28, 18.76, 21.13, 31.35, 50.54, 122.68, 122.71, 123.55, 125.82, 128.46, 129.76, 131.97, 135.14, 138.15, 143.15, 145.73, 147.22, 149.33.

2-Methyl-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-butylamine *p*-Toluenesulphonate Salt (**6c**).

Compound **6c** (0.142 g, 0.4 mmol) was dissolved in 3 mL acetonitrile. *p*-Toluene sulfonic acid 0.154 g (0.8 mmol) was added to the reaction mixture. The reaction mixture was allowed to be stirred for 3 hours. Then the solvent was evaporated *in vacuo* and the crude product obtained was recrystallized from methyl alcohol-ether to give white crystals, 0.13 g (54.4 %), mp 228-229 °C; ir (KBr): 2953 (NH) cm^{-1}; ^1H nmr (DMSO-d_6): δ 0.91 (m, 6H, 2CH_3), 1.04 (d, 2H, CH_2), 1.56 (m, 1H, CH), 2.27 (s, 6H, 2CH_3),

5.00 (m, H, CH), 7.11, 7.48 (2d, 8H, aromatic), 8.00, 8.12 (2t, 2H, H_8, H_9 aromatic), 8.28, 8.44 (m, 2H, H_7, H_{10} aromatic), 8.56, 8.64, 8.80 (3br.d, 3H, 3NH), 9.21 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 11.40, 14.90, 21.23, 25.06, 37.60, 49.24, 122.68, 123.54, 124.43, 125.81, 127.92, 128.49, 129.76, 131.96, 135.23, 138.20, 143.80, 145.50, 147.04, 149.32, 168.60.

2-(4-Benzyloxy-phenyl)-1-[1,2,4]Triazolo[3,4-*a*]phthalazin-3-yl-ethylamine *p*-Toluenesulphonate Salt (**6f**).

Compound **4** (0.21 g, 0.4 mmol) was dissolved in 3 mL acetonitrile. *p*-Toluene sulfonic acid 0.154 g (0.8 mmol) was added to the reaction mixture, and the reaction mixture was allowed to be stirred for 3 hours. Then the solvent was evaporated *in vacuo* and the crude product obtained was recrystallized from methyl alcohol-ether to give white crystals, 0.195 g (65 %), mp 180-182 °C; ir (KBr): 2921 (NH) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ2.28 (s, 6H, $2CH_3$), 3.40 (d, 2H, CH_2), 4.90 (s, 2H, CH_2), 5.20(m, 1H, CH), 6.80, 7.00 (2d, 4H, aromatic), 7.1, 7.5 (2d, 8H, aromatic), 7.35 (m, 5H, aromatic), 8.00, 8.10 (2t, 2H, H_8, H_9 aromatic), 8.20, 8.50 (2d, 2H, H_7, H_{10} aromatic), 8.90 (brs, 3H, 3NH), 9.10 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 21.13, 37.15, 46.60, 69.40, 115.10, 122.44, 122.65, 123.4, 125.82, 127.04, 127.92, 128.11, 128.51, 128.70, 129.74, 130.76, 132.00, 135.13, 137.30, 138.30, 143.03, 145.50, 147.21, 149.10, 157.80.

N-(1-[1,2,4]Triazolo[3,4-*a*]phthalazin-3-yl-ethyl)-acetamide (**7a**).

Compound **3a** (0.17 g, 0.41 mmol) was dissolved in 3 mL acetic acid. The reaction mixture was allowed to undergo reflux for 8 hours. The reaction mixture was then poured over a cold solution of saturated sodium carbonate. A white precipitate was formed, which was collected by filtration and washed with water. The crude product was recrystallized from methyl alcohol to give white crystals, 0.125 g (85.03 %), mp 230 °C; ir (KBr): 3274 (NH), 1655 (CO, amide) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.68 (d, 3H, CH_3), 1.91 (s, 3H, CH_3), 5.65 (m, 1H, CH), 7.99, 8.09 (2 t, 2H, H_8, H_9 aromatic), 8.27, 8.53 (2 d, 2H, H_7, H_{10} aromatic), 8.69 (d, 1H, NH)-9.15 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 19.35, 22.78, 40.4, 122.43, 123.19, 129.45, 131.46, 134.72, 142.66, 148.56, 151.72, 169.09.

Anal. Calcd. for $C_{14}H_{17}N_5O$: C, 61.98; H, 6.32; N, 25.81. Found: C, 61.71; H, 6.59; N, 26.09.

N-(2-Benzyloxy-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-ethyl)-acetamide (**7d**).

Compound **3d** (0.1 g, 0.32 mmol) was dissolved in 3 mL acetic acid. The reaction mixture was allowed to undergo reflux for 8 hours. The reaction mixture was then poured over a cold solution of saturated sodium carbonate. A white precipitate was formed, whcih was collected by filtration and washed with water. The crude product was recrystallized from methyl alcohol to give white crystals, 0.078 g (90.5 %), mp 163-164 °C; ir (KBr): 3247 (NH), 1659 (C=O, amide) cm^{-1}; ^{1}H nmr ($CDCl_3$): δ 2.02 (s, 3H, CH_3), 3.94 (dd, 2H, CH_2), 4.42 (m, 2H, CH_2), 6.02 (m, 1H, CH), 7.02 (br.s, 6H, 5H aromatic + NH), 8.64 (m, 5H, aromatic); ^{13}C nmr ($CDCl_3$): δ 23.65, 45.70, 69.97, 73.39, 123.48, 123.62, 123.96, 127.96, 128.05, 128.59, 128.62, 131.69, 134.69, 137.88, 143, 33, 148.28, 149.35, 170.35.

Anal. Calcd for $C_{20}H_{19}N_5O_2$: C, 66.47; H, 5.30; N, 19.38. Found: C, 66.21; H, 5.57; N, 19.62.

N-[2-(4-Benzyloxy-phenyl)-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-ethyl]-acetamide (**7f**).

Compound **4** (0.1 g, 0.19 mmol) was dissolved in 3 mL acetic acid. The reaction mixture was allowed to undergo reflux for 8 hours. The reaction mixture was poured over a cold solution of saturated sodium carbonate. A white precipitate was formed, filtered and washed with water. The crude product was recrystallized from methyl alcohol to give white crystals, 0.074 g (83.8 %), mp 215 °C; ir (KBr): 3291 (NH), 1651 (C=O, amide) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.87 (s, 3H, CH_3), 3.2 (m, 2H, CH_2), 5.03 (s, 2H, CH_2), 5.77 (m, 1H, CH), 6.89, 7.15 (2d, 4H, aromatic), 7.38 (s, 5H, aromatic), 7.95-8.23 (m, 3H, NH + 2H aromatic), 8.51, 8.80 (2 d, 2H, H_7, H_{10} aromatic), 9.07 (s, 1H, H_6 aromatic); ^{13}C nmr (DMSO-d_6): δ 22.75, 38.4, 46.15, 69.4, 114.80, 115.18, 122.41, 122.86, 123.25, 127.98, 128.09, 128.70, 129.43, 129.90, 130.49, 131.44, 134.71, 137.44, 142.44, 148.54, 150.80, 157.33, 169.30.

Anal. Calcd. for $C_{27}H_{27}N_5O_2$: C, 71.50; H, 6.00; N, 15.44. Found: C, 71.32; H, 6.16; N, 15.18.

[2-(4-Benzyloxy-phenyl)-1-(2-methyl-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-propylcarbamoyl)-ethyl]-carbamic Acid *tert*-Butyl Ester (**8a**).

A mixture of *N*-Boc L-(*O*-benzyl)-tyrosine (**2f**) 0.186 g (0.5 mmol), HATU 0.19 g (0.5 mmol) and DIEA 0.17 mL (1 mmol) was stirred in 2 mL dimethylformamide at 0 °C for 3 minutes and then the solution was added to a solution of (**6b**) 0.293 g (0.5 mmol) and 0.09 mL (0.5 mmol) DIEA in 2 mL of dimethylformamide at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour and then overnight at room temperature. The reaction mixture was poured into ice water, the solid was collected by filtration, washed with 5% citric acid solution, saturated sodium bicarbonate solution then with water, dried and then recrystallized. The crude product was recrystallized from methylene chloride-hexane to give white crystals, 0.291 g (97.65 %), mp 84-85 °C; ir (KBr): 3428, 3351 (NH), 1710 (C=O, urethane), 1677 (C=O, amide) cm^{-1}; ^{1}H nmr ($CDCl_3$): δ 0.8, 0.95 (2d, 6H, $2CH_3$), 1.39 (s, 9H, $3CH_3$), 2.25 (m, 1H, CH), 2.8, 2.9 (2m, 2H, CH_2), 4.2 (m, 1H, CH), 4.7 (s, 2H, CH_2), 5.1 (m, 1H, CH), 6.5 (brs, 2H, 2NH), 6.9 (m, 4H, aromatic), 7.2 (m, 5H, aromatic), 7.8-8.00 (m, 3H, H_8, H_9, H_7 aromatic), 8.5 (m, 2H, H_{10}, H_6 aromatic); ^{13}C-NMR ($CDCl_3$): δ 19.08, 19.47, 28.69, 33.24, 38.48, 50.96, 56.97, 69.77, 80.46, 114.78, 115.21, 123.40, 123.73, 127.50, 127.86, 128.11, 128.51, 128.78, 128.94, 129.11, 130.58, 131.33, 134.54, 137.34, 142.99, 147.87, 155.74, 157.60, 171.21.

Anal. Calcd. for $C_{34}H_{38}N_6O_4$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.42; H, 6.15; N, 14.41.

{1-[2-(4-Benzyloxy-phenyl)-1-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-ethylcarbamo-yl]-ethyl}-carbamic Acid *tert*-Butyl Ester (**8b**).

A mixture of N-Boc-L-alanine (**2a**) 0.095 g (0.5 mmol), HATU 0.19 g (0.5 mmol) and DIEA 0.17 mL (1 mmol) was stirred at 0 °C for 3 minutes in 2 mL of dimethylformamide and then the solution was added to a solution of (**6f**) 0.37 g (0.5 mmol) and 0.09 mL (0.5 mmol) DIEA in 2 mL of dimethylformamide at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour and then overnight at room temperature. The reaction mixture was poured into ice water, the solid was collected by filtration, washed with 5% citric acid solution, saturated sodium bicarbonate solution then with water, dried and then recrystallized. The crude product was recrystallized from methylene chloride-hexane to give white

crystals, 0.25 g (87.9 %), mp 90-91 °C; ir (KBr): 3303 (NH), 1710 (C=O, urethane), 1674 (C=O, amide) cm^{-1}; ^1H nmr ($CDCl_3$): δ 1.25 (d, 3H, CH_3), 1.37 (s, 9H, $3CH_3$), 3.40 (m, 2H, CH_2), 4.15 (m, 1H, CH), 4.90 (s, 2H, CH_2), 5.00 (m, 1H, CH), 6.10 (m, 1H, NH), 6.70, 7.00 (2d, 4H, aromatic), 7.3 (m, 5H, aromatic), 7.84 (t, 1H, H_8 aromatic), 7.90-8.03 (m, 2H, H_9, H_7 aromatic), 8.56 (s, 1H, H_6 aromatic), 8.67 (d, 1H, H_{10} aromatic; ^{13}C nmr ($CDCl_3$): δ 18.91, 28.71, 39.69, 46.49, 50.59, 70.25, 80.43, 115.13, 123.53, 123.87, 127.83, 128.30, 128.56, 128.61, 128.93, 130.90, 131.54, 134.60, 137.35, 143.15, 148.14, 150.45, 158.08, 172.53.

Anal. Calcd. for for $C_{32}H_{34}N_6O_4$: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.66; H, 5.81; N, 15.13.

Antihypertensive Activity.

Rats were divided into groups, each of six animals. Each group was separately anaesthesized with thiopental sodium (50 mg/kg, i.p.) and fixed in supine position. Hair of the upper part of the left hind limb was shaved then 1 cm incision was made in the skin of the groin and underlying muscles were cut. The femoral artery and vein were exposed. The femoral artery was tied closed to its connection with the pelvic artery and an open tie was made about 1 cm apart near its connection with the internal iliac artery. An artery clamp was placed in the front of the open tie to prevent hemorrhage, then by means of a fine scissors a very small incision was made in the artery through which a polyethylene catheter filled with heparin (100IU/mL saline) was introduced. Then the artery clamp was removed and catheter was connected to the pressure transducer the arterial blood pressure was displaced on a Grass polygraph. The catheter was tunneled subcutaneously and exteriorized at the back of the neck between the scapulae the each rat was housed separately. Blood pressure was recorded at 0 hour then 2 hours after giving 6 mg of the drug/kg/p.o., suspended in olive oil. The control group received olive oil / p.o. The change in MAP was calculated [23].

Acute Toxicity.

The same biologically significant compounds were further investigated for their approximate LD_{50} in male mice [20,21] (each 20 g, supplied by Medical Research Institute, Alexandria University). Groups of mice each consisting of six animals were used. The compounds were given orally suspended in 1% gum acacia, in doses of 1, 10, 100, 200, 250, 500 mg / kg, respectively. Twenty-four hours later, the % mortality in each group and for each compound was recorded. The LD_{50} values were calculated using the method described by Litchfield and Wilcoxon [21] .

Moreover, these compounds were tested for their toxicity through parenteral route. Groups of mice each consisting of six animals were used [22]. The compounds or their vehicle, propylene glycol (control) were given by intrapretonial injection in doses of 10, 25, 50, 75, 100 mg / kg, respectively. Survival was followed up to 7 days.

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