Synthesis and Antimicrobial Evaluation of Some New 2-(5,6-Dihydro-4H-1,2,4-triazolo[4,3-a]benz[F]azepin-1-yl)methyl)-4-substituted Phthalazin-1(2H)-ones

IBRAHIM E. EL-SHAMY^{1,*}, A.M. ABDEL-MOHSEN², MOUSTAFA M.G. FOUDA³,
SALEM S. AL-DEYAB³, AHMED ABDEL-MEGEED^{4,5} and MAHER A. EL-HASHASH⁶

¹Department of Chemistry, Faculty of Science, Fayoum University, Fayoum, Egypt

²Central European Institute of Technology (CEITEC), Brno University of Technology, Brno, Czech Republic

³Petrochemical Research Chair, Chemistry Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

⁴Plant Protection Department, Faculty of Agriculture, (Saba Basha), Alexandria University, Alexandria, Egypt

⁵Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455-Riyadh 11451, Saudi Arabia

⁶Department of Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt

*Corresponding author: E-mail: iei00@fayoum.edu.eg

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Starting from 4-substituted-phthalazin-1(2H)-one (1), A series of new 2-(5,6-dihydro-4H-1,2,4-triazolo[4,3-a]benz[f]azepin-1-yl)methyl)-4-substituted phthalazin-1(2H)-ones derivatives (5) have been synthesized. The structure of synthesized new compounds was established by spectral data and screened for their antimicrobial activities against various bacteria and fungi strains.

Keywords: Triazole derivatives, Phthalazin, Antibacterial activity.

INTRODUCTION

Nitrogen-containing heterocyclic compounds are widespread in nature and their applications to biologically active pharmaceuticals, agrochemicals and functional materials are becoming more and more important¹. Phthalazin-1(2H)-ones are important building blocks in the construction of new molecular systems for biologically active molecules²⁻⁴. The development of new and efficient methodologies for synthesis of potentially bioactive phthalazin-1(2H)-one derivatives is important. Phthalazin-1(2H)-ones are of considerable interest due to their antidiabetic⁵, antiallergic⁶, Vasorelaxant⁷, PDE4 inhibitors⁸, VEGF (vascular endothelial growth factor) receptor tyrosine kinases for the treatment of cancer^{9,10}, antiasthmatic agents with dual activities of thromboxane A2 (TXA2) synthetase inhibition and bronchodilation¹¹, herbicidal¹², like activities. A number of established drug molecules like hydralazine^{13,14}, budralazine^{15,16}, azelastine^{17,18}, ponalrestat¹⁹ and zopolrestat²⁰ are prepared from the corresponding phthalazinones. Several ways have been reported in the literature for the synthesis of phthalazinones^{21,22}. Generally, phthalazines are synthesized from either phthalic anhydride derivatives, 2-aryl-3-hydroxy-inden-1-ones or β -diketones *via* condensation with hydrazine hydrate by either heating²³ or using microwave irradiation²⁴. In view of the aforementioned facts, it seemed most interesting

to synthesize new phthalazinone derivatives with the aim to evaluate their antimicrobial activities.

EXPERIMENTAL

Melting points were determined on a MEL-TEMP II melting point apparatus in open glass capillaries and are uncorrected. The infrared spectra were recorded for potassium bromide discs on a Pye-Unicam SP1025 spectrophotometer. NMR spectra were carried out at ambient temperature (about 25 °C) with a Bruker AC-250 spectrometer or with a Varian Gemini 200 spectrometer at 250 MHz using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were performed on a Hewlett-Packard 5995 gas chromatography-mass spectrometer system or on a Shimadzu GCMS-QP 1000 EX mass spectrometer. Homogeneity of the products and follow up of the reactions were checked by ascending thin-layer chromatography TLC on plates precoated with silica gel G (E. Merck; layer thickness 0.25 mm), used without pretreatment.

General procedure for preparation [4-substituted-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2a-j): A mixture of phthalazinone **1a-j** (0.01 mol), 5 g ethyl bromoacetate (0.03 mol) and 4.1 g potassium carbonate (0.03 mol) in 30 mL dry acetone was heated under reflux for 30 h, cooled at room temperature and poured into water. The obtained solid was filtered off and crystallized from appropriate solvent to give **2a-j**.

[4-Phenyl-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2a): m.p. 101-102 °C; yield 66.4 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1733, 1660 (2CO); MS (70 eV) *m/z* (%): 308 (M⁺, 10). Anal. calcd. (%) for C₁₈H₁₆N₂O₃: C, 70.12; H, 5.23; N, 9.09; found (%) C, 70.19; H, 5.20; N, 9.03.

[4-(4-Methoxyphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2b): m.p. 111-112 °C; yield 60 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1731, 1658 (2CO); MS (70 eV) *m/z* (%): 338 (M⁺, 9). Anal. calcd. (%) for C₁₉H₁₈N₂O₄: C, 67.44; H, 5.36; N, 8.28; found (%) C, 67.40; H, 5.39; N, 8.20.

[4-(4-Chlorophenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2c): m.p. 99-100 °C; yield 61%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1740, 1664 (2CO); MS (70 eV) *m/z* (%): 342 (M⁺, 12). Anal. calcd. (%) for C₁₈H₁₅N₂O₃Cl: C, 63.07; H, 4.41; Cl, 10.34; N, 8.17; found (%) C, 63.02; H, 4.45; Cl, 10.30; N, 8.14.

[4-(3,4-Dichlorophenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2d): m.p. 145-146 °C; yield 70 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1744, 1666 (2CO); MS (70 eV) *m/z* (%): 377 (M⁺, 10). Anal. calcd. (%) for C₁₈H₁₄N₂O₃Cl₂: C, 57.31; H, 3.74; Cl, 18.80; N, 7.43; found (%) C, 57.35; H, 3.70; Cl, 18.77; N, 7.40.

[4-(4-(4-Chlorobenzyl)phenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2e): m.p. 105-106 °C; yield 67%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1736, 1659 (2CO); MS (70 eV) *m/z* (%): 432 (M⁺, 18). Anal. calcd. (%) for C₂₅H₂₁N₂O₃Cl: C, 69.36; H, 4.89; Cl, 8.19; N, 6.47; found (%) C, 69.39; H, 4.93; Cl, 8.18; N, 6.40.

[4-(3-Chloro-4-methylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2f): m.p. 107-108 °C; yield 65%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1732, 1660 (2CO); MS (70 eV) *m/z* (%): 356 (M⁺, 14). Anal. calcd. (%) for C₁₉H₁₇N₂O₃Cl: C, 63.96; H, 4.80; Cl, 9.94; N, 7.85; found (%) C, 63.90; H, 4.84; Cl, 9.90; N, 7.81.

[4-Mesityl-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2g): m.p. 124-125 °C; yield 66%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1731, 1662 (2CO); MS (70 eV) *m/z* (%): 350 (M⁺, 7). Anal. calcd. (%) for C₂₁H₂₂N₂O₃: C, 71.98; H, 6.33; N, 7.99; found (%) C, 71.90; H, 6.37; N, 8.03.

[4-(4-Benzylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2h): m.p. 76-78 °C; yield 71 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1735, 1664 (2CO); MS (70 eV) *m/z* (%): 398 (M⁺, 19). Anal. calcd. (%) for C₂₅H₂₂N₂O₃: C, 75.36; H, 5.57; N, 7.03; found (%) C, 75.30; H, 5.62; N, 7.07.

[4-(Pyridin-4-ylmethyl)phenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2i): m.p. 117-118 °C; yield 62 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1741, 1665 (2CO); MS (70 eV) *m/z* (%): 399 (M⁺, 10). Anal. calcd. (%) for C₂₄H₂₁N₃O₃: C, 72.16; H, 5.30; N, 10.52; found (%) C, 72.10; H, 5.34; N, 10.54.

[4-(Biphenyl-4-yl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (2j): m.p. 119-120 °C; yield 60%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1731, 1658 (2CO); MS (70 eV) *m/z* (%): 384 (M⁺, 30). Anal. calcd. (%) for C₂₄H₂₀N₂O₃: C, 74.98; H, 5.24; N, 7.29; found (%) C, 74.96; H, 5.26; N, 7.33.

General procedure for preparation [4-substituted-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3a-j): A mixture of ester **2** (0.01 mol) and 2 mL hydrazine hydrate in 50 mL absolute ethanol was refluxed for 2 h and cooled at room temperature. The resultant solid was filtered and crystallized from appropriate solvent to give **3**.

[4-Phenyl-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3a): m.p. 210-211 °C; yield 71%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3451, 3211 (NHNH₂), 1660 (CO); MS (70 eV) *m/z* (%): 294 (M⁺, 43). Anal. calcd. (%) for C₁₆H₁₄N₄O₂: C, 65.30; H, 4.79; N, 19.04; found (%) C, 65.34; H, 4.78; N, 19.08.

[4-(4-Methoxyphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3b): m.p. 235-236 °C; yield 61%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3433, 3220 (NHNH₂), 1663 (CO); MS (70 eV) *m/z* (%): 324 (M⁺, 10). Anal. calcd. (%) for C₁₇H₁₆N₄O₃: C, 62.95; H, 4.97; N, 17.27; found (%) C, 62.90; H, 4.99; N, 17.20.

[4-(4-Chlorophenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3c): m.p. 254-255 °C; yield 63%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3450, 3220 (NHNH₂), 1664 (CO); MS (70 eV) *m/z* (%): 328 (M⁺, 15). Anal. calcd. (%) for C₁₆H₁₃N₄O₂Cl: C, 58.45; H, 3.99; Cl, 10.78; N, 17.04; found (%) C, 58.40; H, 3.94; Cl, 10.79; N, 17.08.

[4-(3,4-Dichlorophenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3d): m.p. 260-261 °C; yield 68%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3444, 3231 (NHNH₂), 1667 (CO); MS (70 eV) *m/z* (%): 363 (M⁺, 11). Anal. calcd. (%) for C₁₆H₁₂N₄O₂Cl₂: C, 52.91; H, 3.33; Cl, 19.52; N, 15.43; found (%) C, 52.98; H, 3.30; Cl, 19.55; N, 15.40.

[4-(4-(4-Chlorobenzyl)phenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3e): m.p. 244-245 °C; yield 66%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3459, 3210 (NHNH₂), 1661 (CO); MS (70 eV) *m/z* (%): 418 (M⁺, 10). Anal. calcd. (%) for C₂₃H₁₉N₄O₂Cl: C, 65.95; H, 4.57; Cl, 8.46; N, 13.38; found (%) C, 65.90; H, 4.59; Cl, 8.46; N, 13.41.

[4-(3-Chloro-4-methylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3f): m.p. 271-272 °C; yield 70%; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3450, 3238 (NHNH₂), 1658 (CO); MS (70 eV) *m/z* (%): 342 (M⁺, 12). Anal. calcd. (%) for C₁₇H₁₅N₄O₂Cl: C, 59.57; H, 4.41; Cl, 10.34; N, 16.34; found (%) C, 59.50; H, 4.45; Cl, 10.38; N, 16.39.

[4-Mesityl-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3g): m.p. 227-228 °C; yield 69 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3466, 3219 (NHNH₂), 1662 (CO); MS (70 eV) *m/z* (%): 336 (M⁺, 20). Anal. calcd. (%) for C₁₉H₂₀N₄O₂: C, 67.84; H, 5.99; N, 16.66; found (%) C, 67.88; H, 5.94; N, 16.60.

[4-(4-Benzylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3h): m.p. 130-132 °C; yield 65 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3467, 3243 (NHNH₂), 1665 (CO); MS (70 eV) *m/z* (%): 384 (M⁺, 13). Anal. calcd. (%) for C₂₃H₂₀N₄O₂: C, 71.86; H, 5.24; N, 14.57; found (%) C, 71.80; H, 5.29; N, 14.53.

[4-(Pyridin-4-ylmethyl)phenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3i): m.p. 130-131 °C; yield 62 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3461, 3210 (NHNH₂), 1660 (CO); MS (70 eV) *m/z* (%): 385 (M⁺, 18). Anal. calcd. (%) for C₂₂H₁₉N₅O₂: C, 68.56; H, 4.97; N, 18.17; found (%) C, 68.59; H, 4.90; N, 18.19.

[4-(Biphenyl-4-yl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (3j): m.p. 250-251 °C; yield 77 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 3450, 3221 (NHNH₂), 1660 (CO); MS (70 eV) *m/z* (%): 370 (M⁺, 29). Anal. calcd. (%) for C₂₂H₁₈N₄O₂: C, 71.34; H, 4.90; N, 15.13; found (%) C, 71.34; H, 4.90; N, 15.13.

General procedure for preparation 2-((5,6-dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-substituted-phthalazin-1(2H)-one (5a-j): A mixture of hydrazide **3** (0.01 mol) and seven-membered lactim ether **4** (0.01 mol) in 40 mL absolute ethanol was refluxed for 5 h and cooled at room temperature. The resultant solid was filtered and crystallized from appropriate solvent to give **5a-j**.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-phenyl-phthalazin-1(2H)-ones (5a): m.p. 149-150 °C; yield 61 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1660 (CO); MS (70 eV) *m/z* (%): 419 (M⁺, 19). Anal. calcd. (%) for C₂₆H₂₁N₅O: C, 74.44; H, 5.05; N, 16.70; found (%) C, 74.48; H, 5.00; N, 16.73.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(4-methoxyphenyl)phthalazin-1(2H)-ones (5b): m.p. 130-131 °C; yield 55 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1658 (CO); MS (70 eV) *m/z* (%): 449 (M⁺, 10). Anal. calcd. (%) for C₂₇H₂₃N₅O₂: C, 72.14; H, 5.16; N, 15.58; found (%) C, 72.10; H, 5.15; N, 15.60.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(4-chlorophenyl)phthalazin-1(2H)-ones (5c): m.p. 119-120 °C; yield 56 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1664 (CO); MS (70 eV) *m/z* (%): 453 (M⁺, 11). Anal. calcd. (%) for C₂₆H₂₀N₅OCl: C, 68.80; H, 4.44; Cl, 7.81; N, 15.43; found (%) C, 68.82; H, 4.40; Cl, 7.85; N, 15.40.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(3,4-dichlorophenyl)phthalazin-1(2H)-ones (5d): m.p. 177-178 °C; yield 60 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1666 (CO); MS (70 eV) *m/z* (%): 488 (M⁺, 14). Anal. calcd. (%) for C₂₆H₁₉N₅OCl₂: C, 63.94; H, 3.92; Cl, 14.52; N, 14.34; found (%) C, 63.90; H, 3.94; Cl, 14.52; N, 14.30.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(4-(4-chlorobenzyl)phenyl)-phthalazin-1(2H)-ones (5e): m.p. 120-121 °C; yield 54 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H);

IR (KBr, $\nu_{\max}$, cm⁻¹): 1660 (CO); MS (70 eV) *m/z* (%): 543 (M⁺, 9). Anal. calcd. (%) for C₃₃H₂₆N₅OCl: C, 72.85; H, 4.82; Cl, 6.52; N, 12.87; found (%) C, 72.80; H, 4.85; Cl, 6.50; N, 12.89.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(3-chloro-4-methyl phenyl)phthalazin-1(2H)-ones (5f): m.p. 157-158 °C; yield 56 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1665 (CO); MS (70 eV) *m/z* (%): 467 (M⁺, 8). Anal. calcd. (%) for C₂₇H₂₂N₅OCl: C, 69.30; H, 4.74; Cl, 7.58; N, 14.97; found (%) C, 69.39; H, 4.70; Cl, 7.57; N, 14.90.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-mesityl-phthalazin-1(2H)-ones (5g): m.p. 123-124 °C; yield 56 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1663 (CO); MS (70 eV) *m/z* (%): 461 (M⁺, 12). Anal. calcd. (%) for C₂₉H₂₇N₅O: C, 75.46; H, 5.90; N, 15.17; found (%) C, 75.49; H, 5.93; N, 15.19.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(4-benzylphenyl)phthalazin-1(2H)-ones (5h): m.p. 100-101 °C; yield 61 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1657 (CO); MS (70 eV) *m/z* (%): 509 (M⁺, 15). Anal. calcd. (%) for C₃₃H₂₇N₅O: C, 77.78; H, 5.34; N, 13.74; found (%) C, 77.81; H, 5.34; N, 13.78.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(pyridin-4-ylmethyl)phenyl)phthalazin-1(2H)-ones (5i): m.p. 144-145 °C; yield 55 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1661 (CO); MS (70 eV) *m/z* (%): 510 (M⁺, 14). Anal. calcd. (%) for C₃₃H₂₆N₆O: C, 75.27; H, 5.13; N, 16.46; found (%) C, 75.20; H, 5.18; N, 16.48.

2-((5,6-Dihydro-4H-1,2,4-triazolo[4,3-*a*]-benz[*f*]azepin-1-yl) methyl)-4-(biphenyl-4-yl)phthalazin-1(2H)-ones (5j): m.p. 153-154 °C; yield 60 %; ¹H NMR (DMSO-*d*₆) δ: 4.91 (s, 2H, CH₂), 7.01-8.23 (m, 14H, Ar-H); IR (KBr, $\nu_{\max}$, cm⁻¹): 1666 (CO); MS (70 eV) *m/z* (%): 495 (M⁺, 10). Anal. calcd. (%) for C₃₂H₂₅N₅O: C, 77.56; H, 5.08; N, 14.13; found (%) C, 77.50; H, 5.09; N, 14.18.

Antibacterial: The biological activity of the newly synthesized compounds **5a-j** were assessed against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* bacterial strains and *Aspergillums niger* and *Candida albicans* fungal strains in term of disk diffusion method. As described in previous work²⁵. Amoxicillin and ketoconazole were utilized as standard drugs for the bacteria and fungi, respectively. Initially, a screening of phthalazine-derivatives and standard drugs was accomplished at definite concentrations; 500 µg/mL. The results was documented by calculating the inhibition zone diameter at the end of 24 h for bacteria incubation and 72 h for fungi as well. Each trial was repeated twice. Based on the obtained results, the minimum inhibitory concentration (MIC) of compounds; **5a-j** against all bacterial and fungal strains was further investigated by liquid dilution method as described by El-Hashash *et al.*²⁵, in which “stock solutions of the target compounds of 500, 250, 200, 100, 62.5, 50, 25 and 12.5 µg mL⁻¹ concentrations were mixed with DMSO as solvent”. Solutions of amoxicillin and ketoconazole, which considered as standard drugs were arranged at the same concentrations. Cultures of both bacteria and fungi were also prepared prior

to the main test. Then, in a test tube series; 1 mL of phthalazine compound solution; different concentrations together with 0.2 mL of bacteria/fungi was added. To the last solutions, 3.8 mL of sterile distilled water was added to each of the previous test tubes. All tubes were left for incubation at 37 °C for 24 h. The observed turbidity obtained is representing the growth of both bacteria and fungi. This technique was repeated, for comparison by altering the phthalazine compounds with amoxicillin and ketoconazole. The minimum inhibitory concentration, at which there is no growth, was detected as the MIC value (Table-1). The comparison between the MICs (in µg/mL) for the powerful compounds and standard drugs against all tested microorganisms are represented in the Table-1.

RESULTS AND DISCUSSION

The target compound [4-substituted-1(2H)-oxo-phthalazin-2-yl] acetic acid hydrazide (**3**) in the present study was readily obtained by the reaction of phthalazine acetic acid ethyl ester **2** with hydrazine hydrate, which in turn prepared from 4-substituted-phthalazin-1(2H)-one (**1**).

The structure of compounds **2a-j** was confirmed on the basis of their elemental analysis and spectral data. The IR spectrum showed a characteristic absorption band at $\nu = 1742\text{--}1733\text{ cm}^{-1}$ corresponding to CO of ester, CO of cyclic amide at $\nu = 1665\text{--}1658\text{ cm}^{-1}$ and devoid any band for NH. The ^1H NMR spectrum of compound **2a-j** showed a triplet signal at δ 1.33-1.48 assigned for CH_3CH_2 , a quartet signal at 4.13-4.22 assigned for CH_2CH_3 , a singlet at 4.70-4.82 assigned for CH_2CO . The hydrazide derivative **3a-j** revealed absorption bands at $\nu = 3188\text{--}3162$ and $3458\text{--}3308\text{ cm}^{-1}$ corresponding to NHNH_2 groups. The $^1\text{HNMR}$ spectrum of compound **5c** showed $\delta = 4.43$ (s, 2H, NH_2 exchangeable with D_2O), 4.74 (s, 2H, CH_2CO), 9.3 (s, 1H, NH exchangeable with D_2O) ppm and aromatic protons.

Cyclocondensation of hydrazide derivatives **3a-j** with seven-membered lactim ether **4** afforded the corresponding 5,6-dihydro-4H-1,2,4-triazolo[4,3-a]benz[f]azepine derivatives **5a-j**. The structure of the tricycles **5** was confirmed on the basis of their elemental analysis and spectral data. The IR spectrum revealed no absorption for NHNH_2 groups. The features of the ^1H NMR spectrum include very broad signals

for the methylene protons in the seven-membered ring: 2.05-2.18 (2H, 5-H); 2.30-2.39 ppm (2H, 4-H) and two equal intensity, broad signals at 2.66-2.76 and 2.95-3.11 ppm (each 1H, 6-H). The ^{13}C NMR spectrum of compound **5a-j** agrees well with the proposed structure.

Conclusion

We reported here the successful synthesis of different 2-((5,6-dihydro-4H-1,2,4-triazolo[4,3-a]benz[f]azepin-1-yl)methyl)-4-substituted-phthalazin-1(2H)-ones. Most of the newly synthesized compounds were tested for their antimicrobial and antiinflammatory activity. The antimicrobial activity study revealed that all the compounds tested showed moderate to good antibacterial and antifungal activities against pathogenic strains. In case of antiinflammatory activity, compounds showed good activity among the tested compounds.

TABLE-1
ANTIMICROBIAL ACTIVITY OF COMPOUNDS **5a-j**

Compounds	Minimum inhibitory concentration (MIC) in µg/mL					
	Bacterial strains				Fungal strains	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. typhi</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albican</i>
5a	250	250	500	500	—	500
5b	—	500	—	—	500	—
5c	100	50	50	100	250	500
5d	25	25	25	25	125	62.5
5e	25	25	25	50	62.5	62.5
5f	25	50	50	50	125	125
5g	25	50	25	25	62.5	125
5h	250	—	—	500	250	—
5i	50	25	25	25	62.5	125
5j	500	500	—	200	—	500
Amoxicillin	6.25	6.25	6.25	6.25	—	—
Ketoconazole	—	—	—	—	31.25	31.25

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REFERENCES

1. (a) E.C. Franklin, *Chem. Rev.*, **16**, 305 (1935); (b) F.W. Bergstrom, *Chem. Rev.*, **35**, 77 (1944); (c) F.W. Lichtenthaler, *Acc. Chem. Res.*, **35**, 728 (2002); (d) V.P. Litvinov, *Russ. Chem. Rev.*, **72**, 69 (2003); (e) Q.-X. Guo and Y. Xu, *Heterocycles*, **63**, 903 (2004).
2. B. Abd El-Fattah, M.I. Al-Ashmawi, S. El-Feky and E. Roder, *Egypt. J. Pharm. Sci.*, **29**, 259 (1988).
3. B.E. Bayoumy, S.A. El-Feky and M. El-Mobayed, *Egypt. J. Chem.*, **33**, 267 (1991).
4. M.A. Khalil, S.M. El-Khawss and M.G. Kassem, *Sci. Pharm.*, **48**, 344 (1980).
5. O.M. Boland, C.C. Blackwell, B.F. Clarke and D.J. Ewing, *Diabetes*, **42**, 336 (1993).
6. Y. Hamamoto, K. Nagai, M. Muto and C. Asagami, *Exp. Dermatol.*, **2**, 231 (1993).
7. E. Olmo, B. Barboza, M.I. Ybarra, J.L. López-Pérez, R. Carrón, M.A. Sevilla, C. Boselli and A.S. Feliciano, *Bioorg. Med. Chem. Lett.*, **16**, 2786 (2006).
8. M. Napoletano, G. Norcini, F. Pellacini, F. Marchini, G. Morazzoni, P. Ferlenga and L. Pradella, *Bioorg. Med. Chem. Lett.*, **10**, 2235 (2000).
9. G. Bold, K.-H. Altmann, J. Frei, M. Lang, P.W. Manley, P. Traxler, B. Wietfeld, J. Brügggen, E. Buchdunger, R. Cozens, S. Ferrari, P. Furet, F. Hofmann, G. Martiny-Baron, J. Mestan, J. Rösel, M. Sills, D. Stover, F. Acemoglu, E. Boss, R. Emmenegger, L. Ldsrer, E. Masso, R. Roth, C. Schlachter, W. Vetterli, D. Wyss and J.M. Wood, *J. Med. Chem.*, **43**, 2310 (2000).
10. J.M. Arif, M. Kunhi, A.A. Bekhit, M.P. Subramanian and K. Al-Hussein, H.Y. Aboul-Enein Fahad and M. Al-Khodairy, *Asian Pac. J. Cancer Prev.*, **7**, 249 (2006).
11. M. Yamaguchi, K. Kamei, T. Koga, M. Akima, T. Kuroki and N. Ohi, *J. Med. Chem.*, **36**, 4052 (1993).
12. Y.X. Li, Y.P. Luo, Z. Xi, C.W. Niu, Y.Z. He and G.F. Yang, *J. Agric. Food Chem.*, **54**, 9135 (2006).
13. F.H.H. Leenen, D.L. Smith, R.M. Farkas, R.A. Reeves and A. Marquez-Julio, *Am. J. Med.*, **82**, 969 (1987).
14. J.M. Leiro, E. Alvarez, J.A. Arranz, E. Cano and F. Orallo, *Int. Immunopharmacol.*, **4**, 163 (2004).
15. S. Tanaka, M. Tanaka and A. Akashi, *Stroke*, **20**, 1724 (1989).
16. R. Moroi, K. Ono, T. Saito, T. Akimoto and M. Sano, *Chem. Pharm. Bull. (Tokyo)*, **25**, 830 (1977).
17. J.P. Kemp, E.O. Meltzer, H.A. Orgel, M.J. Welch, G.A. Bucholtz, E. Middleton Jr., S.L. Spector, J.J. Newton and J.L. Perhach Jr., *J. Allergy Clin. Immunol.*, **79**, 893 (1987).
18. G. Scheffler, J. Engel, B. Kutscher, W.S. Sheldrick and P. Bell, *Arch. Pharmazie*, **321**, 205 (1988).
19. P.F. Kador, J.H. Kinoshita and N.E. Sharpless, *J. Med. Chem.*, **28**, 841 (1985).
20. B.L. Mylari, E.R. Larson, T.A. Beyer, W.J. Zembrowski, C.E. Aldinger, M.F. Dee, T.W. Siegel and D.H. Singleton, *J. Med. Chem.*, **34**, 108 (1991).
21. S. Grasso, G. De Sarro, A. De Sarro, N. Micale, M. Zappalà, G. Puja, M. Baraldi and C. De Micheli, *J. Med. Chem.*, **43**, 2851 (2000).
22. D. Viña, E. del Olmo, J.L. Lopez-Pérez and A. San Feliciano, *Tetrahedron*, **65**, 1574 (2009).
23. F.F. Blicke and R.D. Swisher, *J. Am. Chem. Soc.*, **56**, 923 (1934).
24. E. Olmo, B. Barboza, M.I. Ybarra, J.L. López-Pérez, R. Carrón, M.A. Sevilla, C. Boselli and A.S. Feliciano, *Bioorg. Med. Chem. Lett.*, **16**, 2786 (2006).