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Research Article

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Synthesis, Characterization and Potential Anticancer and Antimicrobial Activities of New Phthalamide Derivatives

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Abstract A series of N^1,N^2-bis [(2-substitutedphenyl)ethyl]phthalamide (compounds **1-10**) and 3-nitro-N^1, N^2-bis [(2-substitutedphenyl)ethyl]phthalamide (compounds **11-20**) were synthesized. Their structures and purity were analyzed by IR, ^{1}H-NMR spectra and elemental analysis. The compounds were evaluated for their *in vitro* cytotoxicity against the MCF7 and Hep3B cancer cell lines. Cytotoxicity screening revealed that N^1,N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (compound **5**), 3-nitro-N^1,N^2-bis[2-(2,4-dichlorophenyl) ethyl]phthalamide (compound **15**) and 3-nitro-N^1,N^2-bis[2-(2-fluorophenyl)ethyl]phthalamide (compound **16**) had the highest activity. The synthesized compounds were also screened for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Among the compounds, N, N'-bis[2-(4-chlorophenyl)ethyl]phthalamide (compound **4**), 3-Nitro-N,N'-bis[2-(2-chlorophenyl) ethyl]phthalamide (compound **12**), 3-Nitro-N,N'-bis[2-(4-chlorophenyl)ethyl] phthalamide (compound **14**), and 3-Nitro-N,N'-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (compound **15**) exhibited good activity. The prediction of drug-likeness, molecular and ADME properties of the compounds were also examined during the study

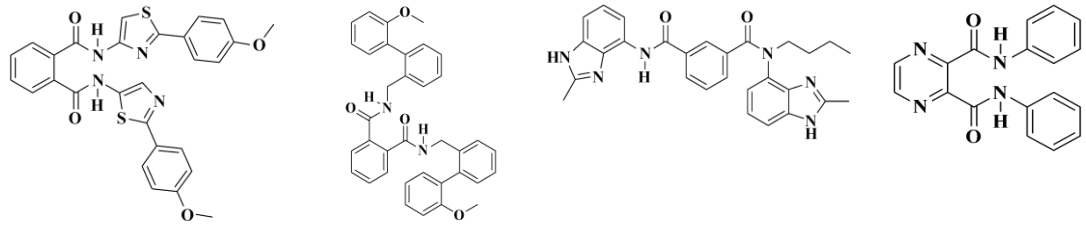
Keywords Phthalamide, Phenylethylamine, Antimicrobial, Anticancer, Cytotoxicity, Insecticide

1. Introduction

Cancer and infectious diseases are the most important health problems of today [1-4]. Although, research has led to a number of new and effective solutions for cancer therapy, there is still a huge need for potent, safe and selective anticancer agents [3]. Development of new safer, potent and resistance-free antimicrobial agents is another important task as pathogens are constantly evolving for multi-drug resistance [5].

Phthalamides are a class of well-known compounds that have long attracted scientists' attention in different fields, particularly in organic synthesis [7-23]. Typically, the most important biological studies that have been reported for phthalamide derivatives are related to their anticancer, antimicrobial and insecticidal activities [5-38]. For example, *N*^{1}-(2-(4-methoxyphenyl)thiazol-4-yl)-*N*^{2}-(2-(4-methoxyphenyl)thiazol-5-yl)phthalimide **(I)** and *N*^{1},*N*^{2}-bis[(2-methoxy-(1,1'-biphenyl)-2-yl)methyl]phthalamide **(II)** compounds showed activity on the MCF7 and HeLa cell lines

with an IC_{50} of 81.46 and 4.37 µM, respectively [35]. In addition, N^1-butyl-N^1,N^3-bis(2-methyl-1H-benzo[d]imidazol-4-yl)isophthalamide **(III)** and N^2,N^3-diphenylpyrazine-2,3-dicarboxamide **(IV)** were reported to be highly potent antibacterial compounds with a MIC of 6 and 3 µg/ml, respectively against *Staphylococcus aureus (S. aureus)* and 4 and 5 µg/ml, respectively against *Escherichia coli (E. Coli)* [31].



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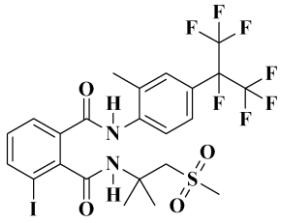
The insecticidal properties of *Ryania speciosa*, a South American plant, were recognized a long time ago. The alkaloid of this species known as ryanodine targets a membrane protein called ryanodine receptors (RyRs), which affect calcium release by locking channels in a partially open state [6].

Some phthalamide derivatives are newly-developed agrochemicals and the first synthetic classes of potent activators of insect RyRs which are ion channels responsible for the release of Ca^{2+} from the endoplasmic reticulum and provide an excellent target for insect control [6]. Flubendiamide (3-iodo-N^2-[(2-methyl-1-(methylsulfonyl)propan-2-yl)-N^1-(2-methyl-4-(perfluoro)propan-2-yl)phenyl]phthalamide) is one of the first synthetic insecticides that control Lepidoptera, which affect RyRs in muscle cells, causing muscle contraction, paralysis, and death [7, 14, 15].



Flubendiamide

In the light of previous studies in the literature, in this study, 20 derivatives of N,N'-bis[(2-substitutedphenyl)ethyl]phthalamide (compounds **1-10**) and 3-nitro-N,N'-bis[(2-substitutedphenyl)ethyl]phthalamide (compounds **11-20**) were synthesized (Figure **1**). The anticancer activity of all compounds was evaluated on the human breast adenocarcinoma (MCF7) and hepatocellular carcinoma (Hep3B) cancer cell lines by MTT assay. Antimicrobial activities were reported against *S. aureus, E. coli*, and *Pseudomonas aeruginosa (P. aeruginosa)* using the conventional agar dilution method.

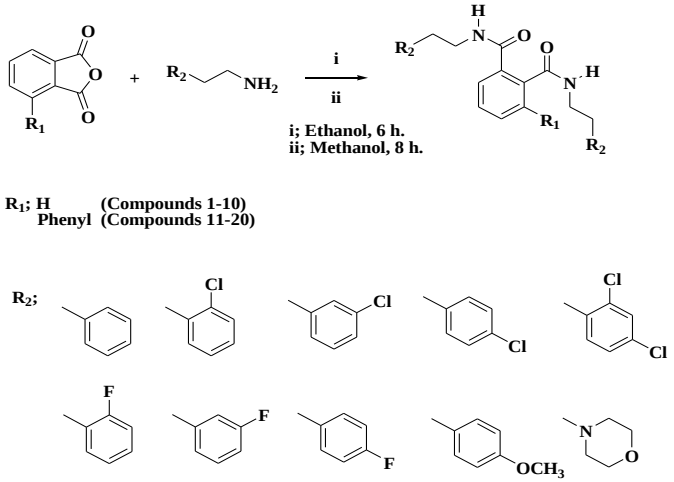


Figure 1: General synthetic pathway of target compounds 1-10 and 11-20

2. Materials and Methods

2.1. Chemistry

Melting points of the compounds were determined by an electro thermal melting point apparatus (Mettler Toledo FP62) in open capillary tubes. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1), and using potassium bromide pellets, the frequencies were expressed in cm^{-1}. The ^{1}H-NMR were recorded in deuterated-dimethyl sulfoxide (DMSO-*d*6) with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane (TMS) as the internal reference. The chemical shifts were reported in parts per million (ppm). Elemental analysis was performed on a LECO 932 CHNS (Leco-932, St. Joseph, MI, USA) instrument.

2.1.1. General procedure A for compounds 1-10

The compounds were prepared through a condensation reaction between 1 mmol phthalic anhydride and 2 mmol phenylethylamine derivatives in 20 ml ethanol under reflux. After six hours, the reaction mixture was evaporated under reduced pressure. The obtained crude products were crystallized in ethanol, and then filtered and dried in vacuum.

N^1, N^2-bis[(2-phenyl)ethyl]phthalamide (1)

(CAS Registry Number: 38229-00-4).

N^1, N^2-bis[2-(2-chlorophenyl)ethyl]phthalamide (2)

0.150 g (1 mmol) phthalic anhydride and 0.233 g (2 mmol) [2-(2-chlorophenyl)ethyl]amine were reacted as described in general procedure A. IR (KBr, V_{max}, cm^{-1}): 3236, 3073, 2930, 1591 and 1571. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.9 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.47 (q, 4H, NH-**CH_2**-CH_2, *J*=7.6 Hz), 7.15-7.5 (m, 12H, Aromatic **CH**), 8.4 (t, 2H, **NH**-C=O, *J*=5.6 Hz). Anal. Calcd. for $C_{24}H_{22}Cl_2N_2O_2$ (441.35 g/mol): C, 65.31; H, 5.02; N, 6.35. Found: C, 65.12; H, 5.08; N, 6.32.

N^1, N^2-bis[2-(3-chlorophenyl)ethyl]phthalamide (3)

(CAS Registry Number: 548439-88-9).

N^1, N^2-bis[2-(4-chlorophenyl)ethyl]phthalamide (4)

0.150 g (1 mmol) phthalic anhydride and 0.233 g (2 mmol) [2-(4-chlorophenyl)ethyl]amine were reacted as described in the general procedure A. IR (KBr, V_{max}, cm^{-1}): 3237, 3075, 2975, 1590 and 1551.^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.81 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.8 Hz), 3.38 (q, 4H, NH-**CH_2**-CH_2, *J*=8.0 Hz), 7.2-7.48 (m, 12H, Aromatic **CH**), 8.35 (t, 2H, **NH**-C=O, *J*=5.6 Hz).Anal. Calcd. for $C_{24}H_{22}Cl_2N_2O_2$ (441.35 g/mol): C, 65.31; H,5.02; N, 6.35. Found: C, 65.87; H, 5.42; N, 6.11.

N^1, N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (5)

0.150 g (1 mmol) phthalic anhydride and 0.285 g (2 mmol) [2-(2,4-dichlorophenyl)ethyl]amine were reacted as described in the general procedure A. IR (KBr, V_{max}, cm^{-1}): 3235, 3081, 2935, 1585 and 1551. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.92 (t, 2H, CH_2-**CH_2**-Ar, *J*=4.0 Hz), 3.03 (t, 2H, CH_2-**CH_2**-Ar, *J*=8.0 Hz), 3.42 (q, 2H, NH-**CH_2**-CH_2, *J*= 8.0 Hz), 3.84 (q, 2H, NH-**CH_2**-CH_2, *J*=8.0 Hz), 7.2-7.84 (m, 10H, Aromatic **CH**), 8.2 (t, 2H, **NH**-C=O, *J*=5.6 Hz). Anal. Calcd. for $C_{24}H_{20}Cl_4N_2O_2$ (441.35 g/mol): C, 64.7; H, 4.92; N, 17.98. Found: C, 65.54; H, 4.69; N, 17.33.

N^1, N^2-bis[2-(2-fluorophenyl)ethyl]phthalamide (6)

0.150 g (1 mmol) phthalic anhydride and 0.208 g (2 mmol) [2-(2-fluorophenyl)ethyl]amine were reacted as described in the general procedure A. IR (KBr, V_{max}, cm^{-1}): 3238, 3079, 2880, 1592 and 1556.^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.85 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.45 (q, 4H, NH-**CH_2**-CH_2, *J*=6.8 Hz), 7.15-

7.5 (m, 12H, Aromatic **CH**), 8.4 (t, 2H, **NH**-C=O, *J*=6.0 Hz). Anal. Calcd. for $C_{24}H_{22}F_2N_2O_2$ (408.44 g/mol): C, 70.57; H, 5.43; N, 6.86. Found: C, 70.52; H, 5.42; N, 6.85.

N^1, N^2-bis[2-(3-fluorophenyl)ethyl]phthalamide (7)

0.150 g (1 mmol) phthalic anhydride and 0.208 g (2 mmol) [2-(3-fluorophenyl)ethyl]amine were reacted as described in the general procedure A. IR (KBr, V_{max}, cm^{-1}): 3236 (N-H), 3080 (C-H, Aromatic), 2878 (C-H, Aliphatic), 1591 and 1554 (C=O, Amide). ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.85 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.45 (q, 4H, NH-**CH_2**-CH_2, *J*=7.0 Hz), 7.1-7.5 (m, 12H, Aromatic **CH**), 8.4 (t, 2H, **NH**-C=O, *J*=6.0 Hz). Anal. Calcd. for $C_{24}H_{22}F_2N_2O_2$ (408.44 g/mol): C, 70.57; H, 5.43; N, 6.86. Found: C, 70.46; H, 5.31; N, 6.73.

N^1, N^2-bis[2-(4-fluorophenyl)ethyl]phthalamide (8)

0.150 g (1 mmol) phthalic anhydride and 0.208 g (2 mmol) [2-(4-fluorophenyl)ethyl]amine were reacted as described in the general procedure A. IR (KBr, V_{max}, cm^{-1}): 3259 (N-H), 3067 (C-H, Aromatic), 2877 (C-H, Aliphatic), 1538 and 1512 (C=O, Amide).^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.85 (t, 4H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 3.45 (q, 4H, NH-**CH_2**-CH_2, *J*=7.2 Hz), 7.25-7.6 (m, 12H, Aromatic **CH**), 8.03 (t, 2H, **NH**-C=O, *J*=6.0 Hz). Anal. Calcd. for $C_{24}H_{22}F_2N_2O_2$ (408.44 g/mol): C, 70.57; H, 5.43; N, 6.86. Found: C, 69.79; H, 5.83; N, 6.83.

N^1, N^2-bis[2-(4-methoxyphenyl)ethyl]phthalamide (9)

(CAS Registry Number: 547711-55-7).

N^1, N^2-bis[2-(4-morpholino)ethyl]phthalamide (10*)*
(CAS Registry Number: 570429-11-7).

2.1.2. General procedure B for compounds 11-20

The compounds were prepared through a condensation reaction between 2 mmol 3-nitrophthalic anhydride and 4 mmol phenylethylamine derivatives in 20 ml methanol under reflux. After eight hours, the reaction mixture was evaporated under reduced pressure. The solid was dissolved in 10 mL $CHCl_3$ and washed with HCl solution (3%), NaOH solution (2%), and H_2O until neutral. The $CHCl_3$ was distilled off. The obtained products were filtered and dried in vacuum.

3-nitro-N^1, N^2-bis[(2-phenyl)ethyl]phthalamide (11)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.363 g (4 mmol) [(2-phenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3070, 2930, 1579 and 1547, 1571 and 1316. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.75 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.6 Hz), 2.82 (t, 2H, CH_2-**CH_2**-Ar, *J*=8.0 Hz), 3.3 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.42 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 7.28-8.05 (m, 13H, Aromatic **CH**), 8.2 and 8.3 (t, 2H, **NH**-C=O, *J*=5.2 Hz and *J*=5.6 Hz). 7.2-8.6 (m, 13H, Aromatic C-H), 8.1 (s, 2H, NH-CH_2). Anal. Calcd. for $C_{24}H_{23}N_3O_4$ (417.46 g/mol): C, 69.05; H, 5.55; N, 10.07. Found: C, 69.00; H, 5.54; N, 10.06.

3-nitro-N^1, N^2-bis[2-(2-chlorophenyl)ethyl]phthalamide (12)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.466 g (4 mmol) [2-(2-chlorophenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3296, 3013, 2937, 1590 and 1564, 1542 and 1330. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.78 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 2.9 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.41 (q, 2H, NH-**CH_2**, *J*=6.0 Hz), 3.8 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 7.25-8.05 (m, 11H, Aromatic **CH**), 8.23 and 8.3 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.6 Hz). Anal. Calcd. for $C_{24}H_{21}Cl_2N_3O_4$ (486.35 g/mol): C, 59.27; H, 4.35; N, 8.64. Found: C, 59.11; H, 4.33; N, 8.61.

3-nitro-N^1, N^2-bis[2-(3-chlorophenyl)ethyl]phthalamide (13)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.466 g (4 mmol) [2-(3-chlorophenyl)ethyl]amine were reacted as described in the general procedure B.

IR (KBr, V_{max}, cm^{-1}): 3329, 3094, 2963, 1586 and 1542, 1540 and 1338. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.72 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 2.83 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.45 (q, 2H, NH-**CH_2**, *J*=6.2 Hz), 3.65 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 7.2-8.05 (m, 11H, Aromatic **CH**), 8.45 and 8.6 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz). Anal. Calcd. for $C_{24}H_{21}Cl_2N_3O_4$ (486.35 g/mol): C, 59.27; H, 4.35; N, 8.64. Found: C, 59.11; H, 4.33; N, 8.61.

3-nitro-N^1, N^2-bis[2-(4-chlorophenyl)ethyl]phthalamide (14)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.466 g (4 mmol) [2-(4-chlorophenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3292, 3060, 2960, 1570 and 1544, 1548 and 1332. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.7 (t, 2H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 2.85 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.0 Hz), 3.48 (q, 2H, NH-**CH_2**, *J*=6.2 Hz), 3.6 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.2-7.7 (m, 11H, Aromatic **CH**), 8.4 and 8.7 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz). Anal. Calcd. for $C_{24}H_{21}Cl_2N_3O_4$ (486.35 g/mol): C, 59.27; H, 4.35; N, 8.64. Found: C, 59.11; H, 4.33; N, 8.61.

3-nitro-N^1, N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (15)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.570 g (4 mmol) [2-(2,4-dichlorophenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3234, 3058, 2937, 1590 and 1564, 1549 and 1335.^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.65 (t, 2H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 2.78 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.35 (q, 2H, NH-**CH_2**, *J*=6.0 Hz), 3.7 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.3-7.9 (m, 9H, Aromatic **CH**), 8.2 and 8.5 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz).Anal. Calcd. for $C_{24}H_{19}Cl_4N_3O_4$ (555.24 g/mol): C, 51.92; H, 3.45; N, 7.57. Found: C, 51.71; H, 3.42; N, 7.53.

3-nitro-N^1, N^2-bis[2-(2-fluorophenyl)ethyl]phthalamide (16)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.417 g (4 mmol) [2-(2-fluorophenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3267, 3092, 2937, 1573 and 1545, 1525 and 1318. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.62 (t, 2H, CH_2-**CH_2**, *J*=7.0 Hz), 2.83 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 3.57 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.7 (q, 2H, NH-**CH_2**, *J*=7.2 Hz) 7.2-8.1 (m, 11H, Aromatic **CH**), 8.3 and 8.6 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz). Anal. Calcd. for $C_{24}H_{21}F_2N_3O_4$ (453.44 g/mol): C, 63.53; H, 4.67; N,9.27. Found: C, 63.57; H, 4.60; N, 9.26.

3-nitro-N^1, N^2-bis[2-(3-fluorophenyl)ethyl]phthalamide (17)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.417 g (4 mmol) [2-(3-fluorophenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3279, 307, 2924, 1586 and 1545, 1522 and 1326. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.76 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 2.85 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 3.43 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.79 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.25-8.05 (m, 11H, Aromatic **CH**), 8.15 and 8.32 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz). Anal. Calcd. for $C_{24}H_{21}F_2N_3O_4$ (453.44 g/mol): C, 63.53; H, 4.67; N, 9.27. Found. C, 63.57, H, 4.6; N, 9.26.

3-nitro-N^1, N^2-bis[2-(4-fluorophenyl)ethyl]phthalamide (18)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.417 g (4 mmol) [2-(4-fluorophenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3288, 3070, 2935, 1579 and 1550, 1519 and 1323. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.64 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 2.82 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 3.47 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.72 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.2-8.1 (m, 11H, Aromatic **CH**), 8.29 and 8.5 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz). Anal. Calcd. for $C_{24}H_{21}F_2N_3O_4$ (453.44 g/mol): C, 63.53; H, 4.67; N, 9.27. Found: C, 63.57; H, 4.6; N, 9.26.

3-nitro-N^1, N^2-bis[2-(4-methoxyphenyl)ethyl]phthalamide (19)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.453 g (4 mmol) [2-(4-methoxyphenyl)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3288, 3070, 2935, 1579 and 1550, 1538 and 1349. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.6 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 2.86 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 3.44 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 3.62 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 3.82 (s, 6H, O-**CH_3**, *J*=7.6 Hz), 7.2-8.1 (m, 11H, Aromatic **CH**), 8.2 and 8.7 (t, 2H, **NH**-C=O, *J*=8.0 Hz and *J*=7.2 Hz). Anal. Calcd. for $C_{26}H_{27}N_3O_6$ (477.51 g/mol): C, 65.40; H, 5.70; N, 8.80. Found: C, 65.35; H, 5.70; N, 8.79.

3-nitro-N^1, N^2-bis[2-(4-morpholino)ethyl]phthalamide (20)

0.400 g (2 mmol) 3-nitrophthalic anhydride and 0.390 g (4 mmol) [2-(4-morpholino)ethyl]amine were reacted as described in the general procedure B. IR (KBr, V_{max}, cm^{-1}): 3256, 3050, 2848, 1789 and 1708, 1498 and 1315. ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.32 (m, 8H, N-**CH_2**, *J*=4.4 Hz), 2.61 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 2.78 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 3.49 (q, 2H, NH-**CH_2**, *J*=6.4 Hz), 3.60 (q, 2H, NH-**CH_2**, *J*=6.4 Hz), 3.65 (t, 8H, O-**CH_2**, *J*=6.8 Hz), 8.0-8.1 (m, 3H, Aromatic **CH**), 8.2 and 8.7 (t, 2H, **NH**-C=O, *J*= 5.6 Hz and *J*=5.2 Hz). Anal. Calcd. for $C_{20}H_{29}N_5O_6$ (435.47 g/mol): C, 55.16; H, 6.71; N, 16.08. Found: C, 55.12; H, 6.70; N, 16.07.

2.2. Pharmacological activities

2.2.1. Anticancer Activity Test

The anticancer activity of compounds was tested against two different cancer cell lines; liver (Hep3B) and breast (MCF7) cells (ATCC, USA), which were maintained in DMEM (Gibco, England) and supplemented with 10% FBS (Gibco, USA), 1% streptomycin and penicillin (Gibco, USA) at 37°C in 5% CO_2. The cells were seeded on 48-well plates at the density of $2x10^4$ cell per mL in a 48-well plate and incubated for 24 hours at 37°C in 5% CO_2. All the compounds were solubilized in DMSO and kept at room temperature, protected from sunlight. As a reference compound, doxorubicin (DOX) was used. The cells were exposed to different concentrations of the compounds and DOX and incubated in 5% CO_2 at 37^{o}C for 72 hours. At the end of the incubation period, the supernatants were discarded, and MTT (3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) was applied as 0.5 µg/mL. After two hours, MTT was discarded from the plate wells and isopropanol was added to dissolve the blue formazan. Absorbance of the MTT formazan was determined at 540 nm by a UV-spectrophotometric plate reader (Thermo Multiscan Spectrum, Finland). Viability was defined as the ratio (expressed as a percentage) of absorbance of the cells exposed to compounds to the cells treated with 0.5% DMSO (as the control). All the measurements were undertaken in triplicate.

2.2.2. Antimicrobial Activity Test

The MICs of the compounds were determined by the conventional agar dilution method. The quinolone antibacterial agent ofloxacin was used as a reference drug. The test compounds (10.0 mg) were dissolved in DMSO (1 mL), and then diluted with distilled water (9 mL) for the preparation of the stock solution. Further progressive two-fold serial dilution with molten sterile Mueller–Hinton agar was performed to obtain the required concentrations of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 µg/mL. The medium containing the test compounds was dispensed into sterile Petri dishes and allowed to solidify. The Petri dishes were inoculated with 1.5×10^4 CFU and incubated at 37 ^{o}C for 18 hours. The MIC was defined as the lowest concentration of the test compound, which resulted in no visible growth.

Statistical analysis

GraphPad Prism 6 was used for the statistical analyses. Data related to anticancer activity were analyzed by using one-way ANOVA following the post-hoc tests by Tukey. Differences were considered as significant a $p < 0.05$.

3. Results and discussion

3.1. Chemistry

The synthetic routes for *N^1*,*N^2*-bis[(2-substitutedphenyl)ethyl]phthalamide (compounds **1-10**) and 3-nitro-*N^1*, *N^2*-bis[(2-substitutedphenyl) ethyl]phthalamide derivatives (compounds **11-20**) are summarized in Figure (**1**). Compounds **1-10** were synthesized by the reaction of phthalic anhydride with the corresponding phenylethylamine

derivatives. The reactions were carried out in ethanol by refluxing for six hours. Compounds **11-20** were synthesized by the reaction of 3-nitrophthalic anhydride with the corresponding phenylethylamine derivatives in methanol under reflux for eight hours. The compounds were obtained in low to moderate yields.

The structures and purities of the compounds were examined by UV, IR, ^{1}H-NMR, elemental analysis. The prediction of drug-likeness, molecular and ADME properties were determined. The physical data of the compounds are given in Table 1.

Table 1: Structures, % yields and melting points of the synthesized compounds

Compound	R_1	R_2	%Yield	Melting Point (^{o}C)
1	H	phenyl	79	163.2
2	H	2-chlorophenyl	35	198.2
3	H	3-chlorophenyl	73	159.5
4	H	4-chlorophenyl	47	143.1
5	H	2,4-dichlorophenyl	70	164.6
6	H	2-fluorophenyl	74	200.7
7	H	3-fluorophenyl	36	131
8	H	4-fluorophenyl	72	123.4
9	H	4-methoxyphenyl	55	139.6
10	H	morpholine	21	119.7
11	NO_2	phenyl	24	177.2
12	NO_2	2-chlorophenyl	38	211.7
13	NO_2	3-chlorophenyl	23	248.4
14	NO_2	4-chlorophenyl	22	208.3
15	NO_2	2,4-dichlorophenyl	9	213.8
16	NO_2	2-fluorophenyl	19	143.2
17	NO_2	3-fluorophenyl	9	139.8
18	NO_2	4-fluorophenyl	16	147.6
19	NO_2	4-methoxyphenyl	10	158.8
20	NO_2	morpholine	7	liquid

3.2. Tumor Cell Growth Inhibition Studies

All the synthesized compounds were examined for *in vitro* cytotoxic activity against the MCF7 and Hep3B cancer cell lines by MTT assay. The results are given in Table 2. The most active compound on the liver (Hep3B) cancer cell line was compound 15 with an IC_{50} value of 46.0 µM.

The most active compound on MCF7 cell line was compound **16**, which had an IC_{50} value of 37.6 µM. Other compounds with IC_{50} values lower than 50 µM were compounds 5, 12 and 15. The cytotoxic activity results of the synthesized compounds are given in Table 2.

Table 2: The IC_{50} values of synthesized compounds 1-20 on the MCF7 and Hep3B cell lines by MTT

Compound	R_2	Cancer Cell Lines (IC_{50}, µM)	
		Hep3B	MCF7
1	phenyl	>100	>100
2	2-chlorophenyl	>100	>100
3	3-chlorophenyl	>100	>100
4	4-chlorophenyl	>100	>100
5	2,4-dichlorophenyl	87.6 ± 5.3*	48.9 ± 5.4**

6	2-fluorophenyl	>100	>100
7	3-fluorophenyl	>100	>100
8	4-fluorophenyl	>100	>100
9	4-methoxyphenyl	>100	>100
10	morpholine	>100	>100
11	phenyl	>100	>100
12	2-chlorophenyl	54.5 ± 7.1**	46.2 ± 6.9**
13	3-chlorophenyl	>100	>100
14	4-chlorophenyl	84.3 ± 2.2*	>100
15	2,4-dichlorophenyl	46.0 ± 2.6**	45.1 ± 2.9**
16	2-fluorophenyl	50.5 ± 3.3**	37.6 ± 4.7**
17	3-fluorophenyl	92.6 ± 5.8*	97.2 ± 0.3*
18	4-fluorophenyl	>100	>100
19	4-methoxyphenyl	>100	>100
20	morpholine	98.2 ±2.5*	>100
Doxorubicin	-	0.035 ± 0.001	0.020 ± 0.001

* p< 0.01 vs medium control; ** p< 0.0001 vs medium control.

In previous studies, the inhibition effect of thalidomide on viability of MCF7 cells was reported as 511.20 µM after 48 h of incubation [39] and 360 µM after 72 h of incubation [40]. As seen in Table 2, especially 12, 14, 15 and 16 seem strong antiproliferative effect against MCF7 cell line. Compounds 12, 15 and 16 were also the most effective molecules against Hep3B cell line even though according to Zahran et al, thalidomide showed no cytotoxic effect against in another hepatocellular carcinoma cell line, HepG2 [39].

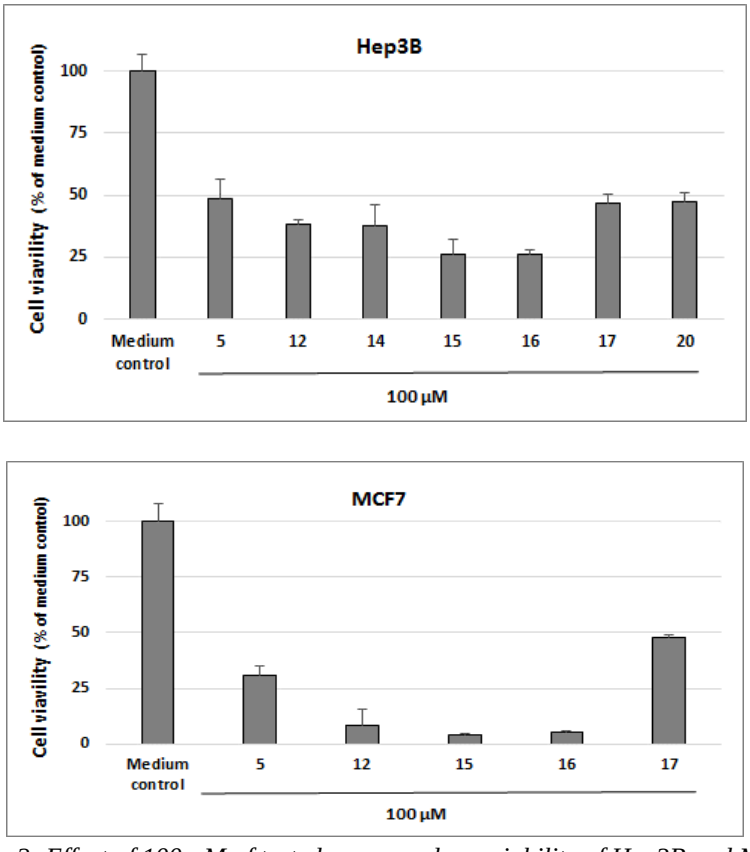


Figure 2: Effect of 100 µM of tested compounds on viability of Hep3B and MCF7

3.3. Antibacterial activity studies

The synthesized compounds were screened against *S. aureus*, *E. coli* and *P. aeruginosa* using the conventional agar dilution method. It was observed that the compounds generally showed moderate activity compared with ofloxacin. Compound **4, 12 and 14** had the highest inhibitory activity against all three bacteria with a MIC of 25 µg/ml. The MICs of compound **15** for *S. aureus*, *P. aeruginosa* and *E. coli* were 25 and 50 µg/ml, respectively. Compounds **1**, **2** and **7** had the lowest activity with a MIC of 100 µg/ml. Compounds **3** and **18** also showed moderate activity with an inhibition value of 50 µg/ml (See Table 3).

Table 3: The MIC of synthesized compounds **1-20** against different bacteria

Compound	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	100	100	100
2	100	100	100
3	50	50	50
4	25	25	25
5	100	100	100
6	-	-	-
7	100	100	100
8	-	-	-
9	-	-	-
10	-	-	-
11	-	-	-
12	25	25	25
13	100	100	100
14	25	25	25
15	25	50	25
16	100	100	100
17	-	-	-
18	50	50	50
19	100	100	100
20	-	-	-
Ofloxacin	0.78	0.78	0.78

It seemed that substitution of halogens (2-chloro, 2, 4-dichloro and 4-chloro) on the aromatic scaffold and 3-nitro moiety on phthalamide increased the anticancer and antimicrobial activity of the compounds.

The physicochemical parameters of the synthesized compounds were generated in order to evaluate their drug-likeness properties using the QikProp module of Schrödinger, and the results are presented in Table 4.

Table 4: Prediction of drug-likeness, molecular and ADME properties

Compound	CNS	logPo/w	$\log_{HERG}$	PCaco	logBB	PMDCK	logKHSA	HOA	%HOA
1	0	4.929	-6.936	2195.086	-0.61	1157.227	0.62	3	100
2	0	5.696	-6.487	2532.264	-0.297	4639.023	0.809	1	100
3	0	6.141	-7.196	2219.098	-0.313	7126.946	0.922	1	100
4	0	6.075	-7.295	1884.733	-0.403	5973.735	0.911	1	100
5	1	7.384	-7.455	3800.66	0.168	10000	1.227	1	100
6	0	5.304	-6.627	2188.257	-0.448	2705.269	0.705	3	100
7	0	5.627	-7.13	2216.44	-0.416	3823.627	0.776	1	100
8	0	5.496	-5.739	2405.958	-0.168	8516.503	0.703	3	100
9	0	5.255	-6.655	3876.44	-0.484	2139.817	0.626	3	100
10	1	0.713	-6.634	164.222	0.274	85.918	-0.644	2	70.769
11	-2	4.781	-7.688	429.856	-1.592	198.617	0.704	1	100
12	-2	5.156	-6.591	462.336	-1.17	705.401	0.762	1	91.876
13	-2	5.512	-7.024	345.539	-1.306	954.766	0.867	1	91.695
14	-2	5.763	-7.463	429.848	-1.302	1208.883	0.933	1	94.862
15	-1	6.135	-6.418	462.413	-0.882	4292.808	0.987	1	84.647
16	-2	4.861	-6.884	427.747	-1.338	428.906	0.688	1	100
17	-1	4.787	-5.988	639.472	-0.968	972.398	0.603	3	100

18	-2	5.201	-7.233	457.368	-1.311	694.464	0.763	1	92.053
19	-2	4.862	-7.307	434.404	-1.753	200.889	0.68	1	100
20	1	0.109	-6.626	28.077	-0.62	12.734	-0.722	2	40.546

Property or Descriptor: Predicted central nervous system (CNS) activity on a scale of –2 (inactive) to +2 (active). **PlogPo/w:** Predicted octanol/water partition coefficient (–2.0 – 6.5). **$\log_{HERG}$:** Predicted IC_{50} value for blockage of HERG K^+ channels (below –5) **logBB:** Predicted brain/blood partition coefficient (–3.0 – 1.2). **$\log K_{HSA}$:** Prediction of binding to human serum albumin (–1.5 – 1.5). **Human Oral Absorption (HOA):** Predicted qualitative human oral absorption measured as 1, 2 or 3 for low. **Percent HOA:** high if >80% and poor if <25%.

The results obtained from this study indicate that the compounds may have an effect on CNS, as well as showing K^+ channel inhibitor activity. The brain/blood partition coefficient and oral absorption values were within the acceptable range of drug-likeness [41].

4. Conclusion

In conclusion, the synthesized compounds generally showed moderate *in vitro* cytotoxic and antibacterial activity against the MCF7 and Hep3B cell lines and *S. aureus, E. coli* and *P. aeruginosa*. Chloro- and nitro-carrying derivatives generally had better IC_{50} values compared to others. In addition, according the ADME properties, K^+ channel inhibition or CNS activities of the compounds can be undertaken in future studies.

Conflict of interest

The authors confirm that they do not have any conflict of interest concerning the content of this article.

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