Original Article

DOI : 10.26650/IstanbulJPharm.2018.18007

Synthesis, Characterization and Biological Activity Studies on Amide Derivatives

Sevda Türk[1], Kadir Turan[2], Seyhan Ulusoy[3], Sevgi Karakuş[1] and Gülgün Boşgelmez-Tınaz[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, 34668, İstanbul, Turkey

[2]Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Marmara University, 34668, İstanbul, Turkey

[3]Department of Biology, Faculty of Arts and Sciences, Süleyman Demirel University, 32260, Isparta,Turkey

Address for Correspondence

Sevgi Karakuş, e-mail: skarakus@marmara.edu.tr

Cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

ABSTRACT

In this study, with intent to find out novel anti-biofilm and antiviral agents, a series of amide derivatives were synthesized and their structures were elucidated by FT-IR and ^{1}H-NMR and ^{13}C-NMR and MS methods. And also, their purity were proven by TLC, HPLC and elemental analyses. And finally, the synthesized compounds were examined for their biofilm formation and swarming motility inhibitory activities in *P. aeruginosa* PA01. These compounds were found to reduce biofilm formation by 8.7-25.6% and swarming motility by 18.3-33.8% in *P. aeruginosa* PA01 at a concentration of 200 μM. Besides, all of the compounds were evaluated for their antiviral activity against influenza A viruses. The plaque inhibition assays indicated that compound **6** (*N*-(4-{[5-(ethylamino)-1,3,4-thiadiazol-2-yl]methyl}phenyl)-4-fluorobenzamide) has considerable inhibitory effect on influenza A virus plaque formation.

Keywords: Synthesis, amide, anti-biofilm activity, swarming motility, influenza viruses, antiviral activity

INTRODUCTION

Amides are multifunctional groups taking part in many molecules. Not only being used as prodrugs (e.g. salicylamide), but also they possess diverse biological activities such as anticancer (Jung et al. 2009; Xu et al. 2010; Yurttaş et al. 2014; Wang et al. 2014; Huczyński et al. 2015; Mathew et al. 2017), antimalarial (Delarue-Cochin et al. 2008; Kumar et al. 2011), insecticidal (Deng et al. 2016; Yang et al. 2016; Lv et al. 2018); antimicrobial (Huczyński et al. 2012; Soni and Soman 2014; Swapnaja et al. 2016; Wei et al. 2018), anti-inflammatory

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

(Bai et al. 2018), antioxidant (Narender et al. 2011), antinociceptive (Czopek et al. 2016) and anti-thrombotic (Sashidhara et al. 2012), depending on their substituents. Besides, amide carrying compounds were reported for their remarkable antibacterial (Mishra et al. 2008; Cui et al. 2017; Bi et al. 2018) and antifungal (Li et al. 2012; Sun et al. 2015; Yu et al. 2018) activities. Additionally, they had attracted a great deal of attention with their significant anti-biofilm (Ballard et al. 2008; Richards et al. 2009; Rogers et al. 2010; Rane et al. 2012) and antiviral (Hao et al. 2012; Lan et al. 2017) activities.

As is known, the discovery of antibiotics made it possible to treat the infectious diseases that were once untreatable and enabled to save millions of lives by taking many dangerous bacterial infections under control. However, with the occurence of bacterial resistance and in regard to the increasing incidence of multidrug resistance in pathogenic bacteria, the identification of alternative antimicrobial drug targets to develop novel treatment strategies have become a necessity. Recently, it has been regarded that inactivating the quorum sensing (QS) system in bacteria by the use of QS inhibitors holds great promise for the treatment of infectious diseases. QS is a cell-to-cell communication system utilized by a wide variety of Gram (-) and Gram (+) bacteria to control the expression of virulence factors like elastase, extracellular protease, swarming, swimming motility and biofilm formation (de Kievit et al. 2000). Various types of screening have been carried out to find QS inhibitory molecules furanone derivatives, AHL analogs, synthetic compounds and some natural substances have been reported to possess QS inhibitory activity (Bosgelmez-Tinaz et al 2007; Galloway et al. 2011; Miandji et al. 2012).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Viruses have a simple structure and completely depend on the host cells for almost all of their vital functions. This situation makes it difficult to develop antiviral agents, non-toxic for host cell metabolic systems. Antiviral agents that can be used against influenza A viruses, which pose great risks for human health, are also limited with NA inhibitors. These viruses belong to *Orthomixoviridae* family and cause recurrent epidemics and pandemics affecting the all over the world from time to time in human population (Oxford 2000). Recurrent infections of influenza viruses in the human population are largely due to the continual changes occuring in the antigenic properties of virus surface glycoproteins (Laver 1984; Jimenez-Alberto et al. 2013). In particular, the changes of the viral surface antigens enable the virus to avoid the immunological defense of the host organism (Govorkova, 2000). Consequently, the control of the influenza by vaccination is not completely effective. Therefore, a great effort is being spent to develop new drugs and vaccines against influenza A viruses.

Hence, in this study, we synthesized a group of amide molecules with reference to p-aminophenylacetic acid and investigated their effects on biofilm formation and swarming motility in *P. aeruginosa*. Furthermore, antiviral activities of these molecules were examined.

MATERIALS AND METHODS

Chemistry

All of the chemicals, reagents and solvents were purchased from Sigma Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Melting points were determined by Schmelzpunktbestimmer SMP II apparatus. For HPLC studies, an Agilent 1100 series system with a G1311A quaternary HPLC pump, a G1315A DAD detector, a G1379A vacuum

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

degasser and a Kromasil 100 C18 5µm, 250 x 4.6 mm column was used. The Rt (retention time) values were determined by an isocratic HPLC grade acetonitrile/water (60:40 *v/v*) mobile phase at a flow rate of 1 ml/min with DAD detector set at 254 nm. The IR spectra were recorded on a Schimadzu FTIR 8400 S Spectrometer. The NMR spectra were recorded (in DMSO-d_6) with a Bruker spectrometer (Billerica, MA, USA) (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR, decoupled). The chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Electron Spray Ionization (ES) method on LC-MS-Agilent 1100. Elemental analysis was performed on Leco 215 CHNS-932 analyzer.

Synthesis of Amide Derivatives (1-6)

Firstly to obtain the compound **1** p-aminophenylacetic acid (0.012 mol) was reacted with equivalent mole of p-fluorobenzoylchloride in chloroform media, by stirring at room temperature. Secondly, for the compounds **2** and **3,** the amide derivative (0.010 mol) was dissolved in concentrated sulphuric acid/methanol or ethanol media and refluxed. The precipitate was obtained by the neutralization reaction with sodium bicarbonate. Thirdly, to obtain the compound **4** the methyl ester derivative was refluxed with hydrazine hydrate at ethanol media. Fourthly, the compound **5** was obtained by the reaction of hydrazide with ethyl isothiocyanate at ethanol media. And finally, the thiosemicarbazide was reacted with concentrated sulphuric acid by stirring at room temperature for 45 minutes to obtain the compound **6** (Küçükgüzel et al. 2006; Karakuş et al. 2010). All of the compounds were purified with hot ethanol.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

{4-[(4-Fluorobenzoyl)amino]phenyl}acetic acid (1): Cream solid. Yield 75%; m.p. 152 °C; MW: 273.2591 g/mol; *Rt* value: 7.69 min. FT-IR υ_{max}. (cm^{-1}): 3323 (O-H and N-H), 1726 (amide C=O), 1645 (carboxylic acid C=O), 1223 (Ar-F). (CAS Number: 907947-59-5).

Methyl {4-[(4-fluorobenzoyl)amino]phenyl}acetate (2): Cream solid. Yield 79%; m.p. 148 °C; MW: 287.2857 g/mol; *Rt* value: 6.00 min. FT-IR $\upsilon_{max.}$ (cm^{-1}): 3329 (N-H), 1742 (ester C=O), 1651 (amide C=O), 1221 (Ar-F). ^{1}H-NMR (DMSO-d_6/TMS) δ (ppm): 3.62 (3H, s, -CH_3), 3.65 (2H, s, -CH_2-), 7.26 (2H, d, *J:* 8.40 Hz, Ar-H), 7.37 (2H, t, Ar-H), 7.68 (2H, d, *J:* 8.40 Hz, Ar-H), 8.04 (2H, t, Ar-H), 10.27 (1H, s, -CONH-). ^{13}C-NMR (DMSO-d_6/TMS) δ (ppm): 39.57, 51.62, 115.12, 115.41, 120.39, 129.48, 129.60, 130.27, 130.39, 131.29, 131.33, 137.79, 162.37, 164.31 **(**amide C=O**)**, 165.67, 171.67 (C=O). MS (ES m/z): 310 (M^++Na), 180, 179, 101. Elemental analysis for $C_{16}H_{14}FNO_3$ Calculated/Found (%): C: 66.89/66.28, H: 4.91/4.89, N: 4.88/4.72. (CAS Number: 2204929-37-1).

Ethyl {4-[(4-fluorobenzoyl)amino]phenyl}acetate (3): White solid. Yield 65%; m.p. 154-155 °C; MW: 301.3122 g/mol; FT-IR υ_{max}. (cm^{-1}): 3337, 3298 (N-H), 1722 (ester C=O), 1647 (amide C=O), 1229 (Ar-F). ^{1}H-NMR (DMSO-d_6/TMS) δ (ppm): 1.19 (3H, t, -CH_2-CH_3), 3.63 (2H, s, -CH_2-), 4.05-4.12 (2H, q, - CH_2-CH_3), 7.26 (2H, d, *J:* 8.40 Hz, Ar-H), 7.34-7.40 (2H, m, Ar-H), 7.69 (2H, d, *J:* 8.40 Hz, Ar-H), 8.01-8.06 (2H, m, Ar-H), 10.26 (1H, s, -CONH-). ^{13}C-NMR (DMSO-d_6/TMS) δ (ppm): 14.53, 40.26, 60.73, 115.65, 115.94, 120.89, 129.97, 130.23, 130.76, 130.88, 131.74, 131.78, 138.19, 162.87, 164.88 (amide

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

C=O), 166.17, 171.75 (C=O). Elemental analysis for $C_{17}H_{16}FNO_3$ Calculated/Found (%): C: 67.76/68.05, H: 5.35/5.50, N: 4.65/4.53. (CAS Number: 2204959-86-2).

4-Fluoro-*N*-[4-(2-hydrazinyl-2-oxoethyl)phenyl]benzamide (4): White solid. Yield 86%; m.p. 352 °C (decomposed); MW: 287.2890 g/mol; *Rt* value: 2.70 min. FT-IR $\upsilon_{max.}$ (cm^{-1}): 3352, 3295, 3210 (N-H), 1645 (C=O), 1233 (Ar-F). ^{1}H-NMR (DMSO-d_6/TMS) δ (ppm): 3.22 (2H, s, -CH_2-), 4.22 (2H, b.s, -NH-NH_2), 7.25 (2H, d, *J:* 8.70 Hz, Ar-H), 7.36 (2H, t, Ar-H), 7.65 (2H, d, *J:* 8.40 Hz, Ar-H), 8.02 (2H, t, Ar-H), 9.21 (1H, b.s, -N<u>H</u>-NH_2), 10.23 (1H, s, -CON<u>H</u>-). ^{13}C-NMR (DMSO-d_6/TMS) δ (ppm): 39.87, 115.11, 115.40, 120.34, 129.04, 129.48, 130.24, 130.36, 131.28, 131.32, 131.59, 137.17, 162.35, 164.26 (amide C=O), 165.65, 169.66 **(**C=O). MS (ES m/z): 310 (M^++Na), 180, 179, 101. Elemental analysis for $C_{15}H_{14}FN_3O_2$ Calculated/Found (%): C: 62.71/63.37, H: 4.91/4.97, N: 14.63/14.21. (CAS Number: 2214835-31-9).

***N*-(4-{2-[2-(ethylcarbamothioyl)hydrazinyl]-2-oxoethyl}phenyl)-4-fluorobenzamide (5):** White solid. Yield 79%; m.p. 226 °C; MW: 374.4325 g/mol; *Rt* value: 3.63 min. FT-IR $\upsilon_{max.}$ (cm^{-1}): 3314, 3196 (N-H), 1674, 1645 (C=O), 1219 (C=S), 1159 (Ar-F). ^{1}H-NMR (DMSO-d_6/TMS) δ (ppm): 1.07 (3H, t, -CH_2-C<u>H</u>$_3$), 3.45 (4H, s, -C<u>H</u>$_2$-CH_3 and -CH_2-), 7.28 (2H, d, *J*: 8.70 Hz, Ar-H), 7.37 (2H, t, Ar-H), 7.67 (2H, d, *J*: 8.40 Hz, Ar-H); 7.93 (1H, t, N_4H), 8.01-8.06 (2H, m, Ar-H), 9.17 (1H, b.s, N_2H), 9.91 (1H, b.s, N_1H), 10.24 (1H, s, -CON<u>H</u>-). ^{13}C-NMR (DMSO-d_6/TMS) δ (ppm): 14.36, 14.44, 37.13, 115.13, 115.42, 120.11, 120.27, 129.04, 129.40, 129.83, 130.26, 130.38, 130.79, 131.27, 131.31, 131.58, 137.37, 137.49, 162.36, 164.27 (amide C=O), 165.66, 169.65, 169.95 **(**C=O), 181.25 **(**C=S). Elemental analysis for

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

$C_{18}H_{19}FN_4O_2S$ Calculated/Found (%): C: 57.74/58.31, H: 5.11/5.27, N: 14.96/14.94, S: 8.56/7.90.

***N*-(4-{[5-(ethylamino)-1,3,4-thiadiazol-2-yl]methyl}phenyl)-4-fluorobenzamide (6):** Light brown solid. Yield 40%; m.p. 310-311 °C; MW: 365.4248 g/mol; *Rt* value: 4.20 min. FT-IR $\upsilon_{max.}$ (cm^{-1}): 3310, 3188 (O-H and N-H), 1651 (C=O), 1231 (Ar-F), 760 (C-S-C). ^{1}H-NMR (DMSO-d_6/TMS) δ (ppm): 1.13 (3H, t, -CH_2-C$\underline{H}_3$), 3.18-3.27 (2H, m, -C$\underline{H}_2$-CH_3), 4.13 (2H, s, -CH_2-), 7.27 (2H, d, *J:* 8.70 Hz, Ar-H), 7.37 (2H, t, Ar-H), 7.57 (1H, t, -NH-), 7.70 (2H, d, *J:* 8.40 Hz, Ar-H), 8.03 (2H, t, Ar-H), 10.27 (1H, s, -CON$\underline{H}$-). ^{13}C-NMR (DMSO-d_6/TMS) δ (ppm): 14.24, 34.99, 115.13, 115.42, 120.36, 120.61, 128.82, 129.15, 130.27, 130.39, 130.81, 131.27, 131.30, 133.24, 137.60, 137.82, 157.09, 162.37, 164.32, 165.67, 168.78 (amide C=O), 169.69. MS (ES m/z): 357 (M^++1), 189, 182, 179, 101. Elemental analysis for $C_{18}H_{17}FN_4OS.½H_2O$ Calculated/Found (%): C: 59.16/59.34, H: 4.96/4.87, N: 15.33/15.18, S: 8.77/8.74.

Anti-Biofilm Activity

Anti-biofilm capacities of new substituted-amide derivatives was examined by the biofilm assay. Overnight culture of *P. aeruginosa* PA01 strain was diluted to an OD_{600} of 0.02. 1mL aliquots of the diluted cultures were allocated in polystyrene tubes and incubated at 32°C for 10 h. Nonadherent cells were removed. The biofilms were dyed with 1 ml of crystal violet (0.3%) and the absorbance was measured at 570 nm using a spectrophotometer (Truchado et al. 2009).

Swarming Motility Assay

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

The swarming motility was measured as described by Rashid et al. (2000). Five microliters of PA01 cultures was inoculated onto the surface of swarm plates containing Bacto Agar (0.5%), Nutrient Broth and glucose (1%) in the presence and absence of test compounds and incubated overnight at 37°C for 24 h.

Cells and viruses

Madin-Darby canine kidney (MDCK) cells were used for plaque inhibition assays. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (Gibco), penicillin G (100 U/mL) and streptomycin (100 µg/mL), and maintained in a humidified atmosphere containing 5% CO_2 at 37 ºC. Antiviral activity of synthesized compounds were investigated on influenza A virus, strain A/WSN/33 (H1N1). The viruses were grown in the allantoic cavity of 10 day-old chick embryos at 35.5 ºC for 48 h. The allantoic fluid was clarified by centrifugation at 3,000g for 10 minutes, passed through 0.45 µm sterile filter, and the filtrate was stored in small aliquots at 80 ºC.

Plaque inhibition assay

For plaque inhibition assay, confluent monolayer cultures of MDCK cells in 12-well plate were washed twice with DMEM (-), and infected with influenza viruses at the appropriate multiplicity of infection (moi). After adsorption for 30 min at 37 ºC, virus inoculums were completely removed and the cell monolayers were overlaid with maintenance medium (DMEM containing 0.6% agarose, 0.2% Bovine Serum Albumin and 4 µg/mL TPCK-treated trypsin). In test condition, synthesized compounds were added to maintenance medium at defined concentrations. The plates were incubated at 34 ºC for 2-3 days, and plaques were visualized by staining the cells with amido black (Turan et al. 1996; Güveli et al 2018).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

RESULT AND DISCUSSION

In the present study, six amide derivatives (compounds **1-6)** were synthesized from p-aminophenylacetic acid. The synthestic route of compounds is represented in **Scheme 1**.

Their purity were proven by TLC, HPLC and elemental analyses. Also, their structures were elucidated by FT-IR, ^{1}H-NMR, ^{13}C-NMR and MS spectral methods. IR absorbtion bands due to amide C=O and aromatic C-F stretching bands were recorded at 1726-1645 and 1233- 1159 cm^{-1}, respectively. According to the ^{1}H-NMR spectra, the $–CH_2$- and amide N-H peaks were observed at 3.22-4.13 and 10.23-10.27 ppm as singlets, in turn. In addition, the ^{13}C-NMR spectra exhibited resonances at 164.26-168.78 assigned for amide C=O. Besides all these, the elemental analysis and MS spectral data results were in accordance with the compounds structures.

The synthesized compounds' anti-biofilm capacities were confirmed by the biofilm assay. Biofilm formation causes serious problems in medicine and industry. Biofilm-associated bacteria are more resistant to antimicrobials than planktonic cells. We tested effects of compounds **1-6** on biofilm formation of *P. aeruginosa* PA01. According to the results these molecules inhibited biofilm formation by 8.7-25.6% at 200 µM concentration. Among the tested compounds, compound **3** was found to be the most active one with reducing the biofilm formation by 25.6% in *P. aeruginosa* PA01 at a concentration of 200 µM. We also performed swarming motility assay. Swarming motility has an important role in early stages of biofilm development and antibiotic resistance. The swarming motility of *P. aeruginosa* PAO1 was assayed in the presence and absence of test compounds. Swarming plates were supplemented

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

with 200 μM synthesized compounds. The treatment of *P. aeruginosa* PAO1 with these compounds resulted reductions in swarming motility by 18.3-32.2% (**Table 1, Figure 1**).

The antiviral activity of compounds **1-6** were revealed by using plaque inhibition assays on influenza A viruses. Compound **6** among synthesized compounds tested on influenza virus plaque formation showed inhibitory effect on influenza virus plaque formation (**Figure 2**). Plaque formation by influenza A viruses was almost completely inhibited by this compound at 10 μg/mL concentrations. Compounds **6** did not show any cytopathic effect on MDCK cells at 5-20 μg/mL (**Figure 2**).

Influenza viruses are enveloped viruses having negative polarity, segmented RNA genome. Despite the simple structure, they have multi-stage complex replication strategies. Therefore, it is difficult to reach a conclusion about the action mechanism of compound **6** on influenza virus replication based on the results of plaque inhibition assay. The research will continue to elucidate the mode of action of this molecule. Compound **6** differs from the other 5 compounds by thiadiazole group. This group may therefore thought to be important for the antiviral activity (Gan et al. 2017).

CONCLUSION

A series of amide derivatives (compounds **1-6**) were obtained from p-aminophenyl acetic acid, characterized by several spectroscopic methods (IR, NMR, MS) besides elemental analysis. And finally they were evaluated for their anti-biofilm and antiviral effects. The results suggested that compounds **1-6** could be used as antibiofilm agents in combination with conventional antibiotics to increase the efficiency of current antimicrobials. Also, depending

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

on antiviral activity studies, it can be said that compound **6** has a potential as an anti-influenza virus agent. Further studies on this topic are planned to be performed.

Acknowledgements

This study was financially supported by the Marmara University Scientific Research Committee (Project No: SAG-C-DRP-070617-0343).

REFERENCES

Bai R, Sun J, Liang Z, Yoon Y, Salgado E, Feng A, Oum Y, Xie Y, Shim H (2018) Anti-inflammatory hybrids of secondary amines and amide-sulfamide derivatives. *Eur J Med Chem* **150**: 195-205.

Ballard TE, Richards JJ, Wolfe AL, Melander C (2008) Synthesis and antibiofilm activity of a second-generation reverse-amide oroidin library: a structure-activity relationship study. *Chem Eur J* **14**: 10745-10761.

Bi F, Ji S, Venter H, Liu J, Semple SJ, Ma S (2018) Substitution of terminal amide with ^{1}H-1,2,3-triazole: identificaiton of unexpected class of potent antibacterial agents. *Bioorg Med Chem Lett* **28**: 884-891.

Bosgelmez-Tinaz G, Ulusoy S, Ugur A, Ceylan O (2007) Inhibition of QS-regulated behaviors by *Scorzonera sandrasica*. *Curr Microbiol* **55**:114–118.

Cui YJ, Rao XP, Shang SB, Song ZQ, Shen MG, Liu H (2017) Synthesis, structure analysis and antibacterial activity of N-[5-dehydroabietyl-[1,3,4]thiadiazol-2-yl]-aromatic amide derivatives. *J Saudi Chem Soc* **21**: 258-263.

Czopek A, Salat K, Byrtus H, Rychtyk J, Pawlowski M, Siwek A, Soluch J, Mureddu V, Filipek B (2016) Antinociceptive activity of novel amide derivatives of imidazolidine-2,4-dione in a Mouse model of acute pain. *Pharmacol Rep* **68**: 529-535.

De Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. *Infect Immun* **68:** 4839−4849.

Delarue-Cochin S, Grellier P, Maes L, Mouray E, Sergheraert C, Melnyk P (2008) Synthesis and antimalarial activity of carbamate and amide derivatives of 4-anilinoquinoline. *Eur J Med Chem* **43**: 2045-2055.

Deng XL, Zhang L, Hu XP, Yin B, Liang P, Yang XL (2016) Target-based design, synthesis and biological activity of new pyrazole amide derivatives. *Chinese Chem Lett* **27**: 251-255.

Galloway WRJD, Hodgkinson JT, Bowden SD, Welch M, Spring DR (2011) Quorum sensing in gram-negative bacteria: Small molecule modulation of AHL and AI-2 quorum sensing pathways. *Chem Rev* **111**:28–67.

Gan X, Hu D, Chen Z, Wang Y, Song B (2017) Synthesis and antiviral evaluation of novel 1,3,4-oxadiazole/thiadiazole chalcone conjugates. *Bioorg Med Chem Lett* **27**: 4298-4301.

Govorkova EA, Gambaryan AS, Claas EC, Smirnov YA (2000) Amino acid changes in the hemagglutinin and matrix proteins of influenza a (H2) viruses adapted to mice. *Acta Virol* **44**: 241-248.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Güveli S, Turan K, Ülküseven B (2018) Nickel (II)-PPh3 complexes with ONS and ONN chelating thiosemicarbazones: synthesis and inhibition potential on influenza A viruses, *Turk J Chem* **42(2):** 371-384.

Hao LH, Li YP, He WY, Wang HQ, Shan GZ, Jiang JD, Li YH, Li ZR (2012) Synthesis and antiviral activity of substituted bisaryl amide compounds as novel influenza virus inhibitors. *Eur J Med Chem* **55**: 117-124.

Huczyński A, Janczak J, Stefańska J, Antoszczak M, Brzezinski B (2012) Synthesis and antimicrobial activity of amide derivatives of polyether antibiotic-salinomycin. *Bioorg Med Chem Lett* **22**: 4697-4702.

Huczynski A, Klejborowska G, Antoszczak M, Maj E, Wietrzyk J (2015) Anti-proliferative activity of monensin and its tertiary amide derivatives. *Bioorg Med Chem Lett* **25**: 4539-4543.

Jimenez-Alberto A, Alvarado-Facundo E, Ribas-Aparicio RM, Castelan-Vega JA (2013) Analysis of adaptation mutants in the hemagglutinin of the influenza A(H1N1) pdm09 virus. *PLoS One* **8**: 70005.

Jung M, Park N, Moon HI, Lee Y, Chung WY, Park KK (2009) Synthesis and anticancer activity of novel amide derivatives of non-acetal deoxoartemisinin. *Bioorg Med Chem Lett* **19**: 6303-6306.

Karakuş S, Çoruh U, Barlas-Durgun B, Vázquez-López EM, Özbaş-Turan S, Akbuğa J, Rollas S (2010) Synthesis and cytotoxic activity of some 1,2,4-triazoline-3-thione and 2,5-disubstituted-1,3,4-thiadiazole derivatives. *Marmara Pharm J* **14**: 84-90.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Kumar N, Khan SI, Atheaya H, Mamgain R, Rawat DS (2011) Synthesis and in vitro antimalarial activity of tetraoxane-amine/amide conjugates. *Eur J Med Chem* **46**: 2816-2827.

Küçükgüzel G, Kocatepe A, De Clercq E, Şahin F, Güllüce M (2006) Synthesis and biological activity of 4-thiazolidinones, thiosemicarbazides derived from diflunisal hydrazide. *Eur J Med Chem* **41**: 353-359.

Lan X, Xie D, Yin L, Wang Z, Chen J, Zhang A, Song B, Hu D (2017) Novel *α,β*-unsaturated amide derivatives bearing *α*-amino phosphonate moiety as potential antiviral agents. *Bioorg Med Chem Lett* **27**: 4270-7273.

Laver WG (1984) Antigenic variation and the structure of influenza virus glycoproteins. *Microbiol Sci* **1**: 37-43.

Li S, Cui C, Wang MY, Yu SJ, Shi YX, Zhang X, Li ZM, Zhao WG, Li BJ (2012) Synthesis and fungicidal activity of new fluorine-containing mandelic acid amide compounds. *J Fluor Chem* **137**: 108-112.

Lv M, Liu G, Jia M, Xu H (2018) Synthesis of matrinic amide derivatives containing 1,3,4-thiadiazole scaffold as insecticidal/acaricidal agents. *Bioorg Chem* **81**: 88-92.

Mathew B, Hobrath JV, Connelly MC, Guy RK, Reynolds RC (2017) Diverse amide analogs of sulindac for cancer treatment and prevention. *Bioorg Med Chem Lett* **27**: 4614-4621.

Miandji MA, Ulusoy S, Dündar Y, Özgen S, Kaynak Onurdağ F, Boşgelmez-Tınaz G, Noyanalpan N (2012) Synthesis and biological activities of some 1,3-benzoxazol-2(3*H*)-one derivatives as anti-quorum sensing agents. *ARZNEIMITTELFORSCH* **62(7)**: 330-334.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Mishra A, Kaushik NK, Verma AK, Gupta R (2008) Synthesis, characterization and antibacterial activity of cobalt (III) complexes with pyridine-amide ligands. *Eur J Med Chem* **43**: 2189-2196.

Narender T, Rajendar K, Sarkar S, Singh VK, Chaturvedi U, Khanna AK, Bhatia G (2011) Synthesis of novel *N*-(2-oxo-2-*p*-tolylethyl)-amide derivatives and their antidyslipidemic and antioxidant activity. *Bioorg Med Chem Lett* **21**: 6393-6397.

Oxford JS (2000) Influenza A pandemics of the 20th century with special reference to 1918: virology, pathology and epidemiology. *Rev Med Virol* **10:**119-133.

Rane RA, Sahu NU, Shah CP (2012) Synthesis and antibiofilm activity of marine natural product-based 4-thiazolidinones derivatives. *Bioorg Med Chem Lett* **22**: 7131-7134.

Rashid MH, Kornberg A (2000) Inorganic polyphosphate is needed for swimming, swarming and twitching motilities of *P. aeruginosa*. *P Natl Acad Sci USA* **97**: 4885–4890.

Richards JJ, Reyes S, Stowe SD, Tucker AT, Ballard TE, Mathies LD, Cavanagh J, Melander C (2009) Amide isosteres of oroidin: assessment of antibiofilm activity and c. elegans toxicity. *J Med Chem* **52**: 4582-4585.

Rogers SA, Bero JD, Melander C (2010) Chemical synthesis and biological screening of 2-aminoimidazole-based bacterial and fungal antibiofilm agents. *ChemBioChem* **11**: 396-410.

Sashidhara KV, Palnati GR, Avula SR, Singh S, Jain M, Dikshit M (2012) Synthesis and evaluation of anti-thrombotic activity of benzocoumarin amide derivatives. *Bioorg Med Chem Lett* **22**: 3115-3121.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Soni JN, Soman SS (2014) Synthesis and antimicrobial evaluation of amide derivatives of benzodifuran-2-carboxylic acid. *Eur J Med Chem* **75**: 77-81.

Sun M, Yang HH, Tian L, Li JQ, Zhao WG (2015) Design, synthesis, and fungicidal activities of imino diacid anologs of valine amide fungicides. *Bioorg Med Chem Lett* **25**: 5729-5731.

Swapnaja KJM, Yennam S, Chavalli M, Poornachandra Y, Kumar CG, Muthusamy K, Jayaraman VB, Arumugam P, Balasubramanian S, Sriram KK (2016) Design, synthesis and biological evaluation of diaziridinyl quinone isoxazole hybrids. *Eur J Med Chem* **117**: 85-98.

Truchado P, Gil-Izquierdo A, Tomas-Barberan F, Allende A (2009) Inhibition by chestnut honey of n-acyl-l-homoserine lactones and biofilm formation in erwinia carotovora, yersinia enterocolitica, and aeromonas hydrophila. *J Agr Food Chem* **57**: 11186-11193.

Turan K, Nagata K, Kuru A (1996) Antiviral effect of *Sanicula europaea* L. leaves extract on influenza virus-infected cells. *Biochem Biophys Res Commun* **225**: 22-26.

Wang YH, Goto M, Wang LT, Hsieh KY, Morris-Natschke SL, Tang GH, Long CL, Lee KH (2014) Multidrug resistance-selective antiproliferative activity of piperamide alkaloids and synthetic analogues. *Bioorg Med Chem Lett* **24**: 4818-4821.

Wei Q, Wang X, Cheng JH, Zeng G, Sun DW (2018) Synthesis and antimicrobial activities of novel sorbic and benzoic acid amide derivatives. *Food Chem* **268**: 220-232.

Xu Y, Guo ZJ, Wu N (2010) Two new amide alkaloids with anti-leukemia activities from aconitum taipeicum. *FTRPAE* **81**: 1091-1093.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Yang ZB, Hu DY, Zeng S, Song BA (2016) Novel hydrazone derivatives containing pyridine amide moiety: design, synthesis, and insecticidal activity. *Bioorg Med Chem Lett* **26**: 1161-1164.

Yu X, Teng P, Zhang YL, Xu ZJ, Zhang MZ, Zhang WH (2018) Design, synthesis andd antifungal activity evaluation of coumarin-3-carboxamide derivatives. *FTRPAE* **127**: 387-395.

Yurttaş L, Demirayak Ş, Ilgın S, Atlı Ö (2014) In vitro antitumor activity evaluation of some 1,2,4-triazine derivatives bearing piperazine amide moiety against breast cancer cells. *Bioorg Med Chem* **22**: 6313-6323.

Table 1. Effect of compound **1-6** derivatives on the biofilm formation and swarming motility of *P. aeruginosa* PA01 strain. The data represents the averages from the results of three independent experiments.

	Biofilm Formation Inhibition (%)	**Swarming Motility Inhibition (%)**
1	13.9	28.9
2	8.7	22.4
3	25.6	18.3
4	13.0	31.4
5	19.0	32.2
6	17.3	33.8

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Scheme 1. Synthetic route of compounds **1-6**.

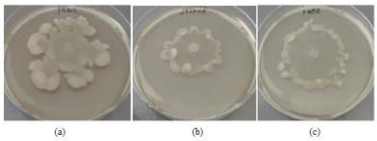
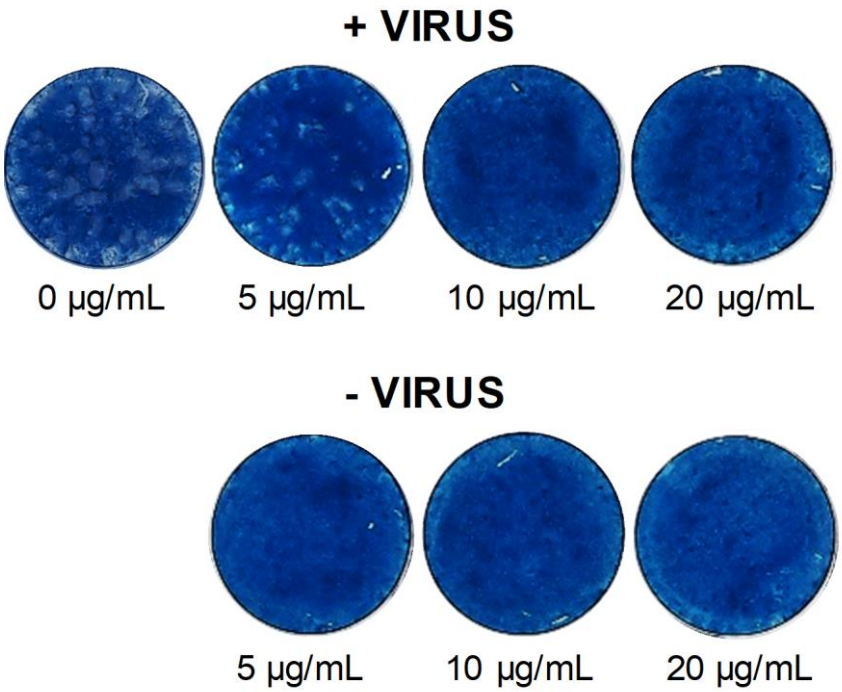


Figure 1. Representative images of inhibition of *P. aeruginosa* PA01 swarming motility. **(a)** Control (no compound), in the presence of compound **6 (b)** and compound **2 (c)**.



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.

Figure 2. The influenza virus plaque formation inhibition of compound **6**. Monolayers of Madin-Darby canine kidney epithelial cells (MDCK) were infected with influenza viruses (A/WSN/33) at the appropriate moi (upper panel). After 30 min of infection, virus inoculums were removed and monolayers were overlaid with 0.6% agarose-maintenance medium, with or without compound **6**. Lower panel shows non-infected MDCK cells monolayers treated with compound **6** at defined concentrations. After 2-3 days of incubation, monolayers were fixed and stained with amido black dye solution.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tıknaz G. Synthesis, Characterization and Biological Activity Studies on Amide Derivatives. Istanbul J Pharm 2018. DOI : 10.26650/IstanbulJPharm.2018.18007.