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Original Research Article

Novel Amides Derived from Fenofibrate and Sulfa Drugs: Synthesis, BioLogical Evaluation, and Molecular Docking Studies

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K E Y W O R D S

Amide Sulfa drug Antioxidant Anti-cancer Breast Molecular docking

Sulfa drugs were chemical compounds used to treat and prevent bacterial infections in humans. This study includes designing and producing a new series of amide derivatives from various sulfa drugs with fenofibrate assessed as antioxidants and anticancer agents *in vitro*. Their structures were definitively confirmed by the study of spectroscopic data, including Infrared spectroscopy (IR), 1H-NMR, 13C-NMR, 2D-NMR, and elemental analysis. All products were screened *in vitro* against cell lines MDA-MB-231. The cytotoxicity assay results revealed that derivatives **5d**-**5f** exhibited good inhibition for MDA-MB-231 with IC_{50} values 117.15, 79.09, and 98.72 μ g/mL, respectively. A molecular docking study of the synthetic compounds confirmed the cytotoxicity test results. In addition, the DPPH investigation revealed good antioxidant activity for the derivatives **5a**, **5d**, and **5e** with inhibition percentages of 97.91, 94.53, and 95.26%, respectively, compared to ascorbic acid.

Introduction

The sulfonamide group, a pharmaceutical compound with a wide range of biologically active properties, has been widely utilized as a precursor for bioactive compound synthesis in the last decade, with its applications in medicinal chemistry [\[1\]](#page-7-0). In our previous work, many substituted amides derived from sulfa drugs were synthesized, and according to studies, amide bonds are extremely common and interesting couplings in both organic synthesis and nature $[2-7]$ $[2-7]$. Some of them possess a wide range of biological activities such as anticancer [\[8\]](#page-7-3), [\[9\]](#page-7-4), antimicrobial $[10-12]$ $[10-12]$, anti-inflammatory [\[13](#page-8-2)[-15\]](#page-8-3), antibacterial $[16-18]$ $[16-18]$, antioxidant $[19]$, and antifungal activity [\[20,](#page-8-7)[21\]](#page-8-8). On the other hand, there are several different biological actions for fenofibrate, including its use in treating severe hypertriglyceridemia and mixed dyslipidemia in patients who have not shown improvement with non-pharmacological treatments [\[22\]](#page-8-9). Fenofibrate is furthermore effective in reducing levels of uric acid in the blood and is used as a complementary therapy for gout $[23]$. It also has pleiotropic effects, including anti-inflammatory, antioxidant, antiatherogenic, and antiviral properties [\[24](#page-9-0)[-26\]](#page-9-1). Here, we reported synthesized new pro-drugs as sulfa drugs and fenofibrate derivatives; some tested as anti-breast cancer, and the anti-oxidant tested for all. These activities were studied theoretically via molecular docking and *in vitro* studies.

Materials and Methods

General information

Merck and Aldrich provide all chemicals; Fourier transform varian spectrometer is used for obtaining the 1H-NMR, 13C-NMR, and 2D-NMR spectra, which operate at a frequency of 300 MHz. The spectra are recorded using a standard internal reference in DMSO-*d⁶* solvent. The FTIR 8400S Shimadzu spectrophotometer (Japan) was employed to record FT-IR spectra throughout the 400-4000 cm-1 wavelength range. We used a micro analytical unit of the EA 300 C.H.N element analyzer to determine the elemental analyses (C.H.N.). The Gallenkamp MFB-600 melting point stuart apparatus was used to ascertain the melting points.

Preparation method of fenofibric acid

A sodium hydroxide solution in water was combined with fenofibrate **1** in ethanol, refluxed at 84 °C for 3 hours, and monitored using thinlayer chromatography (TLC). The mixture was cooled, concentrated, and cooled to room temperature. A residue was obtained, which was then acidified with dilute hydrochloric acid. The solid product **2** was separated by filtration and dried to form a white solid. The yield of the substance was 92%, with a melting point of 180 °C [\[27\]](#page-9-2).

Preparation method of acid chloride

In a fume hood, a round bottom flask of 100 mL, an excess of thionyl chloride was added to (4 g) of fenofibric acid **2**. The combination was heated at 60 °C, ranging from 1 and 30 minutes to 2 hours. After the thionyl chloride was evaporated at low pressure, acid chloride **3** was obtained and used immediately in the subsequent process [\[28\]](#page-9-3).

General procedures for synthesis of amides (5a-5f)

The amine 4 (1 equivalent) was dissolved in 15 mL of anhydrous dichloromethane. If the amine did not dissolve, 0.5 mL of DMF was added. Additionally, 2 equivalents of triethylamine were added. After cooling the mixture to 0° C, the acid chloride 3 (1 eq) solution in DCM was gradually added. The ice bath was removed after the addition. The reaction mixture underwent stirring for a period of 2 to 5 hours. It was

thereafter treated with a 15 mL solution of hydrochloric acid with a concentration of 0.5 N, followed by a water wash. The resulting mixture was then dried using anhydrous MgSO4. The solvent was removed by vacuum evaporation to form a solid, which was then refined using recrystallization from ethanol to get the desired end result **5a**-**5f** (Figure S1) [\[29\]](#page-9-4).

N-(4-(N-Acetylsulfamoyl) phenyl)-2-(4-(4 chlorobenzoyl) phenoxy)-2-methylpropanamide (**5a**)

Light brown crystals, yield 84%. FT-IR (cm−1): *ν* 3358 (Amid N-H), 3035(Ar C-H), 2870 (C-H aliphatic), 1647 (Carbonyl O=CNH), and 1500 (Aromatic C=C). 1H-NMR (DMSO*-d*6, 400 MHz) δ 12.03 (s, 1H), 10.53 (s, 1H), 7.98-7.60 (m, 4H), 6.97 (dd, *J* = 41.3, 8.5 Hz, 2H), 1.89 (s, 3H), 1.65 (s, 6H). Analytical analysis of $C_{25}H_{23}C\cdot C_5$, C_7 59.21; H, 5.17; Cl, 7.54; N, 5.81; O, 17.48; and S, 7.12, m.p.: 122-124 °C.

2-(4-(4-Chlorobenzene) phenoxy)-2-methyl-N-(4- (N-(pyridine-2-yl) sulfamoyl) phenyl) propenamide (5b)

Off-white crystals, yield 87%. FT-IR (cm−1): *ν* 3383 (amid N-H), 3051 (Ar C-H), 2981 (C-H aliphatic), 1645 (carbonyl O=CNH), and 1498 (aromatic C=C). 1H-NMR (DMSO*-d*6, 400 MHz) δ 10.38 (s, 1H), 7.72-7.66 (m, 13H), 6.92 (d, *J* = 6.9 Hz, 4H), and 1.62 (s, 6H).¹³C- NMR (101 MHz, DMSO) δ 193.76, 174.93, 159.99, 142.25, 140.73, 137.52, 136.69, 133.00, 132.32, 131.64, 130.54, 129.75, 129.04, 127.94, 120.54, 118.66, 117.49, 115.80, 114.05, 79.36, 40.45, 40.24, 40.03, 39.82, 39.61, 39.41, 39.20, and 25.53. Analytical analysis of C28H24ClN3O5S: C, 61.96; H, 5.32; Cl, 7.26; N, 7.90; O, 13.89; and S, 6.45, m.p.: 122-124 °C.

2-(4-(4-Chlorobenzoyl) phenoxy)-2-methyl-N-(4- (N-(pyrimidin-2-yl) sulfamoyl) phenyl) propenamide (5c)

Brown crystals, yield 86%. FT-IR (cm−1): *ν* 3375 (amid N-H), 3039 (Ar C-H), 2943 (C-H aliphatic), 1631 (carbonyl O=CNH), 1502 (aromatic C=C). ¹H-NMR (DMSO*-d*6, 400 MHz) δ 10.24 (s, 1H), 8.26 (s, 1H), 7.48 (td, *J* = 9.9, 6.1 Hz, 10H), 6.91- 6.72 (m, 4H), and 1.40 (d, *J* = 12.6 Hz, 6H). ¹³C-NMR (101 MHz, DMSO) δ 193.67, 174.94, 160.02, 158.80, 157.38, 142.91, 137.50, 136.72, 136.54, 135.31, 132.32, 131.95, 130.57, 129.73, 129.04, 120.30, 118.67, 117.48, 116.24, 79.38, 40.54, 40.33, 40.12, 39.92, 39.71, 39.50, 39.29, and 25.56. Analytical analysis of $C_{27}H_{23}C_1N_4O_5S$: C, 59.72; H, 5.06; Cl, 7.37; N, 11.22; O, 13.78; S, 6.22, m.p.: 122-124°C.

2-(4-(4-Chlorobenzoyl) phenoxy)-2-methyl-N-(4- (N-(4-methylpyrimidin-2-yl) sulfamoyl) phenyl) propenamide (5d)

Yellow crystals, yield 91%. FT-IR (cm−1): *ν* 3344 (amid N-H), 3074 (Ar C-H), 2873 (C-H aliphatic), 1651 (carbonyl O=CNH), and 1562 (aromatic C=C). 1H-NMR (DMSO*-d*6, 400 MHz) δ 10.43 (s, 1H), 8.30 (d, *J* = 5.2 Hz, 1H), 7.72–7.54 (m, 11H), 7.05-6.88 (m, 4H), 2.29 (s, 3H), and 1.59 (s, 6H). ¹³C-NMR (101 MHz, DMSO) δ 193.69, 174.91, 162.78, 159.99, 159.37, 156.99, 142.77, 136.73, 136.56, 135.48, 132.37, 132.34, 131.66, 130.57, 129.77, 129.22, 129.06, 120.18, 118.69, 117.49, 79.35, 40.56, 40.35, 40.14, 39.94, 39.73, 39.52, 39.31, 25.55, and 25.16. Anal. Analysis for $C_{28}H_{25}CIN_4O_5S$: C, 60.17; H, 5.19; Cl, 7.08; N, 10.64; O, 13.78; and S, 6.48, m.p.: 122-124 °C.

2-(4-(4-Chlorobenzoyl) phenoxy)-2-methyl-N-(4- (N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) propenamide (5e)

Orange crystals, yield 88%. FT-IR (cm−1): *ν* 3390 (amid N-H), 3151 (Ar C-H), 2985 (C-H aliphatic), 1651 (carbonyl O=CNH), 1516 (aromatic C=C). ¹H NMR (DMSO*-d*6, 400 MHz) δ 11.38 (s, 1H), 10.50 (s, 1H), 7.91 – 7.63 (m, 13H), 6.13 (s, 1H), 2.27 (s, 3H), and 1.63 (s, 6H). ¹³C-NMR (101 MHz, DMSO) δ 193.71, 174.92, 170.76, 162.78, 159.99, 159.34, 157.98, 143.28, 137.57, 136.72, 136.55, 134.30, 132.34, 131.66, 130.61, 129.76, 129.05, 128.24, 120.76, 118.68, 117.48, 95.81, 79.34, 40.55, 40.34, 40.13, 39.92, 39.71, 39.50, 39.29, 25.54, and 12.50. Analytical analysis of $C_{27}H_{24}C_1N_3O_6S$: C, 59.62; H, 5.27; Cl, 5.27; N, 7.14; O, 16.43; and S, 6.22, m.p.: 122-124 °C.

2-(4-(4-Chlorobenzoyl) phenoxy)-2-methyl-N-(4- (N-(thiazol-2-yl) sulfamoyl) phenyl) propenamide (5f)

Light yellow crystals, yield 93%. FT-IR (cm−1): *ν* 3350 (amid N-H), 3095 (Ar C-H), 2989 (C-H aliphatic), 1645 (carbonyl O=CNH), 1527 (aromatic C=C). ¹H NMR (DMSO*-d*6, 400 MHz) δ 10.41 (s, 1H), 7.77-7.66 (m, 13H), and 7.05-6.95 (m, 3H), 1.63 (s, 6H). ¹³C-NMR (101 MHz, DMSO) δ 193.70, 172.86, 169.25, 160.23, 142.11, 137.47, 136.77, 132.28, 131.64, 130.54, 129.53, 129.04, 127.14, 125.25, 120.50, 118.65, 117.45, 108.56, 79.63, 40.54, 40.33, 40.12, 39.91, 39.71, 39.50, 39.29, and 25.47. Analytical analysis of for $C_{26}H_{22}CIN_3O_5S_2$: C, 55.88; H, 4.57; Cl, 7.19; N, 8.08; O, 13.41; and S, 10.21, m.p.: 122-124 °C.

Antioxidant assay

Blois approach was used to determine the presence of antioxidants in a sample. A DPPH solution was prepared, dissolving in methanol as a control and ascorbic acid as a standard. Various concentrations of compound solutions were generated, and 2 mL of DPPH solution was poured onto these samples; the solution was placed in a light-restricted area and allowed to incubate for 2 hours. The sample's absorbance was quantified at a wavelength of 517 nm using a UV-Vis Shimadzu spectrophotometer [\[30\]](#page-9-5). The quantification of the free radical scavenging activity was determined by calculating the percentage of inhibition using a specific Equation 1:

Inhibition%

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=A(control)–A(sample)/A(control)\times100 (1)
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A (control): Absorption of DPPH + solvent (MeOH)

A (sample): Absorption of DPPH + sample (sample test/standard)

Measurement of in vitro anticancer activity

Cell lines and culture

The MDA-MB-231 cell line was used to culture human breast cancer cells from Iranian national cell bank. The cells were grown in RPMI 1640 medium supplemented with FBS and antibiotics, maintained at 37 °C in a humidified atmosphere with 5% CO₂ [\[31](#page-9-6)[,32\]](#page-9-7).

MTT cell viability assay

In this study, the MTT test was used to measure cellular proliferation and viability. The cells were collected, standardized, and placed in 96-well plates. After 24 hours, they were exposed to substances at varying concentrations for 24 hours at 37 \degree C in a CO₂-free environment. After 24 hours, 100 µl of MTT solution was introduced. and the plates were incubated for an additional 4 hours. The cells were then shaken until the crystals were fully dissolved. Cell viability was assessed using an ELISA reader, and the concentration of compounds causing 50% cell death was determined.

Docking studies analysis

Five synthesized compounds were subjected to molecular docking analyses to find putative binding interactions with placental aromatase cytochrome P450, specifically with the ID 3EQM retrieved from the PDB website at https://www.rcsb.org/. The chosen derivatives were shown in a two-dimensional format and then transformed into a three-dimensional representation using molecular mechanics. These three-dimensional structures were then used as ligands. The MOE 2015.10 software was used to

calculate the docking analysis results, namely the binding energy and the configuration of the receptor in the form of 2D interaction poses [\[34\]](#page-9-8).

Results and Discussion

The derivatives were synthesized by reacting several amino derivatives with acid chloride **3** in the presence of triethylamine and CH_2Cl_2 as a reaction medium, then stirring the mixture for 2 hours at room temperature until the end of the reaction after following it up using TLC, as displayed in [Scheme 1.](#page-4-0)

Insilco biological activity

Program MOE 2015 was employed to analyze the capacity of compounds to inhibit breast cancer by coupling them with a single protein

(PDB: 3eqm). [Table 1](#page-5-0) shows that the prepared compounds showed good activity against the studied protein, and the best of them (highlighted in yellow) were selected for in vitro study. [Figure 2](#page-6-0) depicts the method of binding protein to these prepared derivatives.

Cytotoxicity of synthesized compounds

The anticancer effects of **5d**–**5f** derivatives against breast cancer were evaluated *in vitro* using the conventional MTT technique. A cell line obtained from breast cancer tissue MDA-MB-231cell line MDA-MB-231. The percentages of cell viability of compounds **5d**–**5f** are illustrated in [Figure 3.](#page-6-1)

Scheme 1. Synthesis steps of compounds **5a-5f.**

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Table 1. Molecular docking for anti-cancer of prepared derivatives

Figure 2. The interaction mode of compounds **5d**-**5f** with active site amino acids of the protein (PDB 3EQM).

Figure 3. Cell viability percentage of compounds **5d-5f** against cancer cell line MDA-MB-231.

rapic 5. Dr Fir rautal staveliging activity of the synthesized compound							
Compounds	$%$ RSA (radical scavenging activity) at seven different concentrations (μ g/mL)						
	1000	800	750	400	200	50	12.4
5a	97.91	74.42	67.44	58.14	46.51	39.53	30.23
5 _b	70.07	61.65	54.12	42.88	27.19	17.56	16.82
5c	85.11	79.27	70.87	64.88	52.54	48.25	40.92
5d	94.53	88.11	84.39	81.76	73.20	66.68	52.61
5e	95.26	89.73	75.86	67.48	60.24	54.64	42.33
5f	77.63	74.45	62.66	51.76	35.87	28.83	18.49
Ascorbic acid	99.77	98.72	97.33	95.14	93.67	92.35	91.12

Table 3. DPPH radical scavenging activity of the synthesized compound

Antioxidant activity

The DPPH test, a widely used method for assessing antioxidant activity, was used to evaluate synthesized compounds. The test measures the ability of the samples to quench DPPH radicals by donating hydrogen. Antioxidant drugs convert DPPH into a stable diamagnetic molecule, with a change in color indicating increased radical scavenging activity. Based on [Table 3,](#page-6-2) all of the synthesized compounds exhibited significant antioxidant activity compared to the standard ascorbic acid.

Conclusion

To summarize, a group of compounds including fenofibrate and sulfa drugs were synthesized as amide derivatives. The purification, structural characterization, and *in vitro* biological evaluation of these compounds as anticancer and antioxidant agents were conducted. The cytotoxicity test findings suggest that compounds **5d**-**5f** have the potential to be used as antiproliferative agents against MDA-MB-231 cell lines. The findings obtained from the DPPH test demonstrated significant antioxidant activity of these novel amide derivatives.

Conflict of Interest

The authors declares that there is no conflict of interest in this study.

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