Novel Sulfonamide−Triazine Hybrid Derivatives: Docking, Synthesis, and Biological Evaluation as Anticancer Agents

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ABSTRACT: The biological benefits of trisubstituted 1,3,5-triazine derivatives include their ability to reduce inflammation and fight cancer. A unique series of sulfonamide−triazine hybrid molecules were produced chemically by synthesizing triazine derivatives utilizing the usual nucleophilic aromatic substitution of cyanuric chloride via the solvent-free/neat fusion method. Fourier-transform infrared spectroscopy (FTIR), ^{1}H NMR, and ^{13}C NMR spectroscopic analyses were used to identify novel trisubstituted synthetic compounds. The synthesized compounds have a moderate inhibition percentage when tested at 100 μM against the phosphoinositol 3-kinases (PI3Kα) enzyme; compounds **20** and **34** showed 46 and 68% anti-PI3Kα activity, respectively. To comprehend the anticipated interactions, the most successful compounds were subsequently docked into a PI3Kα protein's binding site (PDB code: 6OAC, resolution: 3.15 Å). The final synthetic compounds' anticancer activity was tested on the breast (MCF-7) and lung (A549) cancer cell lines at doses of 100 and 50 μM for additional evaluation of anticancer characteristics. The IC$_{50}$ values for the sulfaguanidine−triazine derivatives **27**, **28**, **29**, **31**, and **35** ranged from 14.8 to 33.2 μM, showing that compounds containing sulfaguanidine and diethylamine in their structures significantly inhibited the activity. Compound **34** could be a promising lead compound for developing new target-selected anticancer compounds with low toxicity and high selectivity.
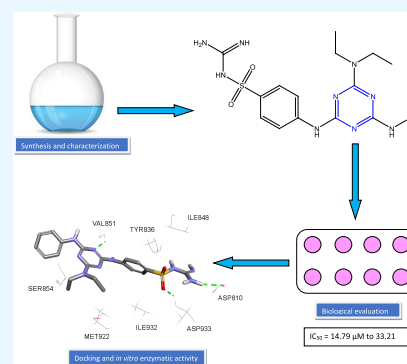
1. INTRODUCTION

Cancer is a term that refers to a condition in which abnormal cells proliferate uncontrollably and have the potential to spread to other areas of the body.[1] The second leading cause of mortality worldwide is cancer.[2] Breast cancer is an uncontrolled expansion of epithelial cells that begins in the ducts or lobules of the breast. The most prevalent malignancy in women is breast cancer, which has become a major worldwide health concern in recent years.[3] Besides breast cancer, lung cancer is one of the most aggressive cancers with the highest mortality rate among all of the cancer types worldwide.[4] The integration of signals from cytokines and growth factors is carried out by phosphoinositol 3-kinases (PI3Kα), which are lipid kinases. After that, the integrated signals create intracellular signals to control various pathways. Cell growth, survival, metabolism, and proliferation are only a few of the physiological and cellular activities that these signaling pathways regulate.[5−7] One of the most prevalent mechanisms seen in human cancer cells is the overactivation of the PI3K pathway. Genetic and epigenetic changes are the cause of this enzyme's overexpression.[8,9] One of the most crucial targets for cancer treatment is the PI3K isozyme. Numerous substances, including triazine compounds, act as PI3Kα inhibitors with varying scaffolds.[10] Three nitrogen atoms are arranged with three additional carbon atoms in 1,3,5-triazines, which have a six-membered ring structure.[11] This system is a highly reactive reactant in addition and substitution processes involving

nucleophiles because it contains three electronegative nitrogen atoms.[12]

Triazine isomers and tetrazines have several applications due to their chemistry as reactants, making symmetric triazines the most researched isomer.[13] Additionally, 1,3,5-triazines were extensively investigated as shown in Figure 1, triazines have multiple biological actions, including antimicrobial activity,[14−16] antifungal activity,[14−16] antiviral activity,[17] anti-inflammatory activity,[18] anti-diabetic activity,[19] and anticancer activity with multiple targets.[20] The chloride atom was replaced by aromatic nucleophilic substitution with the proper nucleophile.[21] It is commercially available and inexpensive, making cyanuric chloride the most crucial starting material in the synthesis of triazine.[22] A series of synthetic chemicals known as sulfonamides are employed as an antibacterial agent and share structural similarities with para-aminobenzoic acid.[23] Sulfonamides have a wide range of pharmacological effects, including anticancer like belinostat and amsacrine;[24] PI3Kα inhibitors such as pictilisib;[25] anticarbonic anhydrase activity like acetazolamide;[26] antibacterial like sulfathiazole and
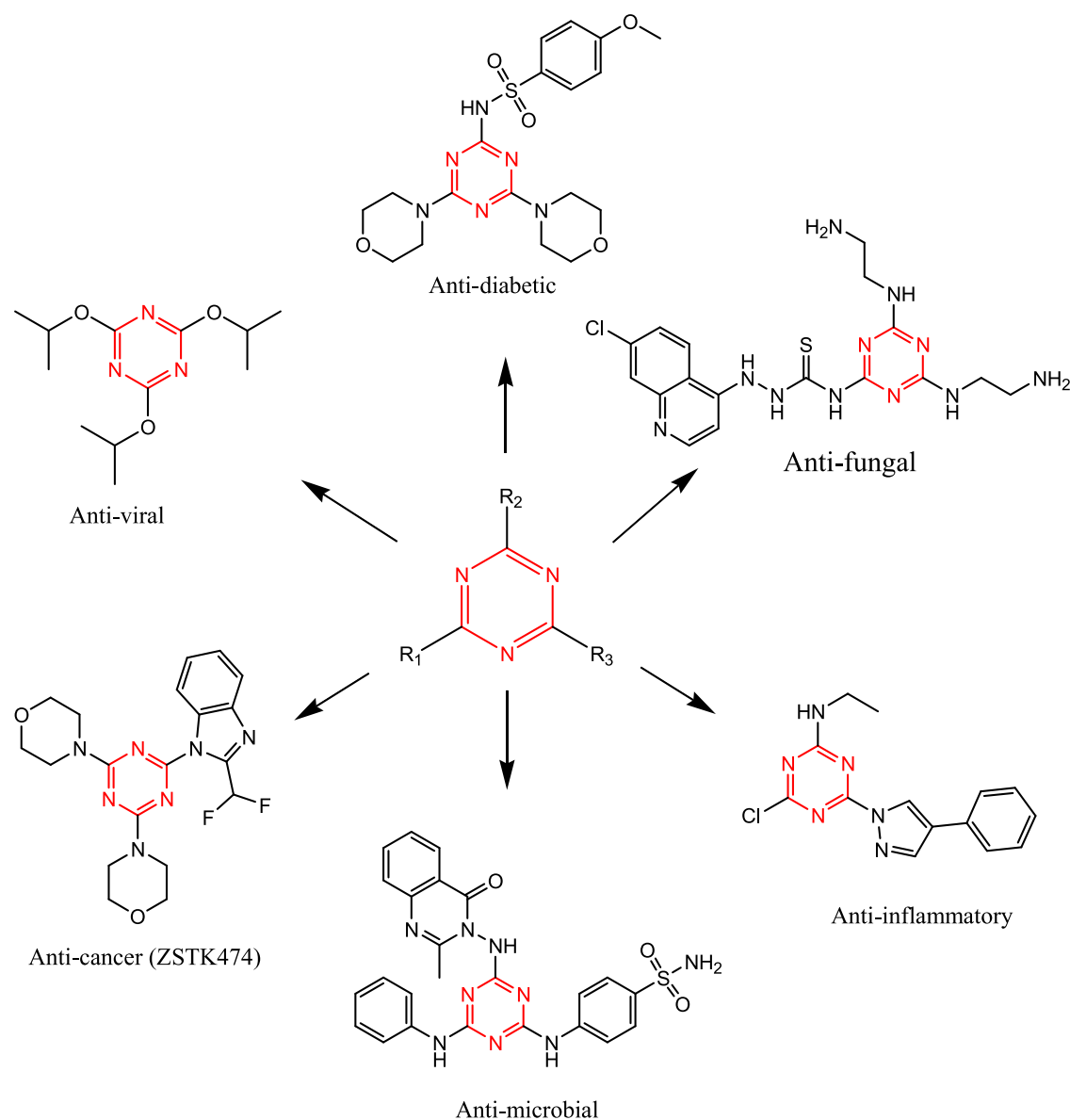
Figure 1. Different biologically active triazines.

sulfaguanidine;[27] hypoglycemic like sulfonyl urea derivatives, tolbutamide and chlorpropamide;[28] and diuretic effect like torsemide, furosemide.[29] This study involved synthesizing, characterization, docking, and evaluating a novel family of sulfapyridine and sulfaguanidine−triazine hybrid compounds.
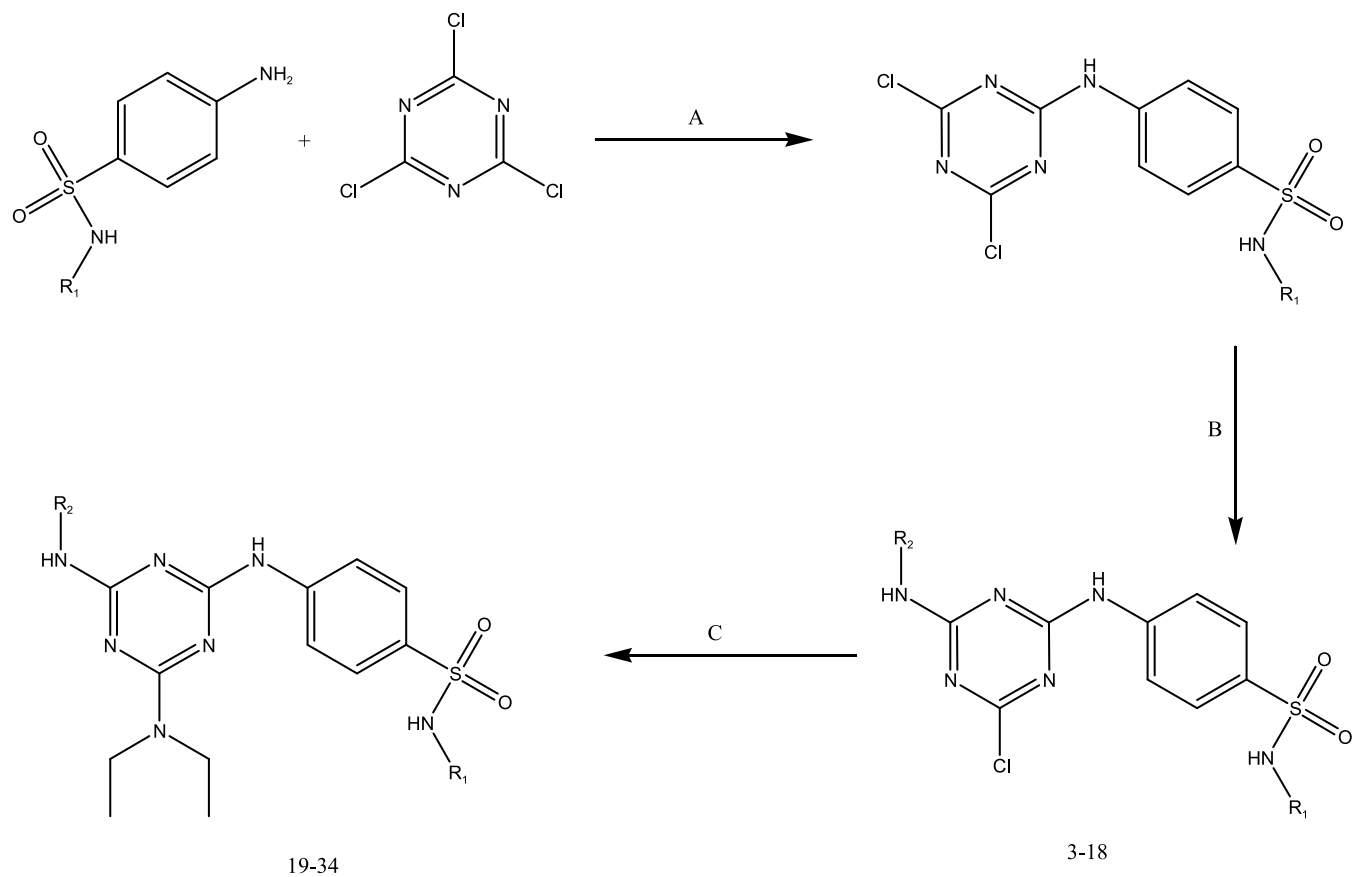
2. METHODS AND MATERIALS

2.1. Materials. All of the reagents and chemicals were of analytical grade and purchased from commercial suppliers. Sulfapyridine (NENTECH, U.K., 99%), sulfaguanidine (Tokyo Chemical Industry, Japan, 98%), cyanuric chloride (Acros Organics, China, 99%), aniline (GCC, U.K., 99.5%), benzylamine (LobaChemi, India, 99%), cyclopropylamine (Sigma-Aldrich, 98%), cyclohexyl amine (Sigma-Aldrich, 99%), diethylamine (GCC, U.K., 98%), m-toluidine (Sigma-Aldrich, 99%), 3,4-dimethylaniline (GCC, U.K., 98%), 4-chloroaniline (Sigma-Aldrich, 98%), 4-bromoaniline (Sigma-Aldrich, 90%), 4-fluoroaniline (Sigma-Aldrich, 99%), 4-nitro-aniline (Janssen, Belgium, 98%), morpholine (Tokyo Chemical Industry, Japan, 99%), acetone (Carlo Erba, France, 99%),
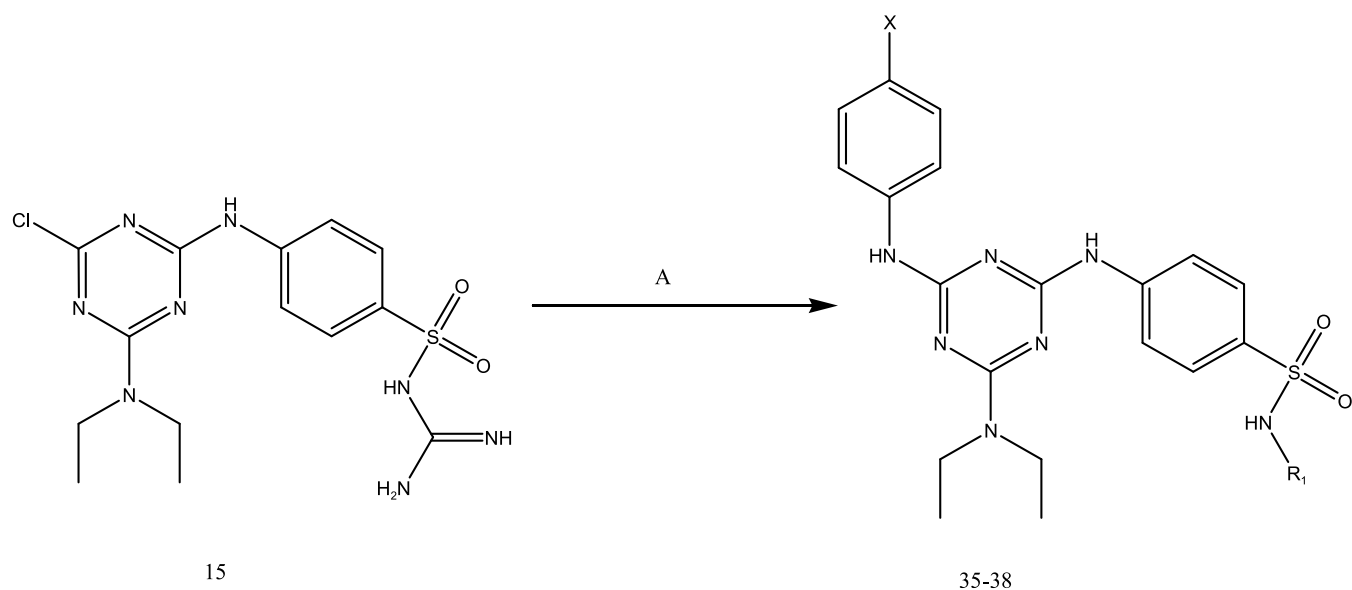
ethanol (Carlo Erba, France, 98%), dimethylformamide (DMF) (Carlo Erba, France, 99.9%), n-hexane (Carlo Erba, France, 95%), sodium bicarbonate (NaHCO$_3$) (GCC, U.K., 99.5%), sodium hydroxide (NaOH) (GCC, U.K., 99.5%), triethylamine (TEA) (TEDIA, 99%), chloroform (Carlo Erba, France, 99.9%) and dimethyl sulfoxide (DMSO) (Carlo Erba, France, 99%).

2.2. Apparatus and Equipment. The following apparatus and equipment were used in this study:

Hei-Tec heating magnetic stirrer (Heidolph, Germany), HR-100A analytical balance (A&D), MZ 2C NT vacuum pump (Vacuubrand, Germany), Benchtop centrifuge type Z 326 (HermleLabortechnik, Germany), heating mantle (Electro-thermal Engineering, U.K.), vacuum oven 3608 (Thermo Fisher Scientific), black-box-type UV analyzer WFH-203B-(China), inverted microscope (OPTIKA, Italy), CO$_2$ incubator, Luna-FL Fluorescence Cell Counter L20001 (Korea), precoated thin-layer chromatography (TLC)-sheets ALU-GRAM Xtra SIL G/UV$_{254}$ (Germany), Promega GloMax Multi + detection system.

Scheme 1. Reagents and Conditions[a]

[a](A) Acetone, (0−5) °C, 4−5 H, NaHCO$_3$; (B) different amine, DMF, (0−25) °C, 12 H, TEA; (C) diethyl amine, NaOH, reflux (70−80) °C, 2 H.

Scheme 2. Reagents and Conditions[a]

[a](A) Solvent-free/neat fusion, arylamine (160−170) °C, (7−10) min.

2.3. Experimental Part. According to Schemes 1 and 2, the chlorine atoms in cyanuric chloride were nucleophilically substituted with various amines to create the final compounds (**19−34** and **35−38**).

KRÜSS Optronic Melting Point Meter M3000 was used to measure the melting points of the synthesized compounds, including the intermediates. A PerkinElmer Spectrum Two FTIR Spectrometer (Diamond ATR FTIR) (4000−400/4

cm^{-1} Spectral Resolution) was used to record the FTIR Spectra. The final trisubstituted compounds' ^{1}H NMR and ^{13}C NMR spectra were captured at 300 and 75 MHz using a Bruker spectrometer (Al-Albayt University, Jordan). The chemical shift in ^{1}H NMR spectra was reported in ppm, and tetramethylsilane (TMS) was utilized as a reference. As a solvent, deuterated DMSO was employed. For ^{1}H NMR and ^{13}C NMR, the run times were 2 min and 24 h, respectively. The evolution of the reactions was seen using precoated TLC-sheets ALUGRAM Xtra SIL G/UV254 (Germany), with various ratios of (n-hexane/acetone) mixture as the mobile phase (see the Supporting Information), and liquid chromatography−mass spectrometry (LC−MS/MS) triple quad 8040 Shimadzu (Japan) was used to confirm the identity and purity of the compounds above 95%.

2.3.1. Synthesis of Compounds 1 and 2. Sulfaguanidine or sulfapyridine (1 equiv) was gradually added over a period of 2 min to a solution of cyanuric chloride in dry acetone that had been magnetically agitated and cooled with ice. Until TLC (n-hexane/acetone, 2:3) indicated that the initial reactants had completely been consumed, the reaction mixture was agitated at a temperature of 0−5 °C. Then, the HCl acid that had been produced was neutralized by progressively adding NaHCO$_3$ solution (1 equiv) in little amounts. The reaction was stirred for 10 min. In the end, crushed ice (100 mL) was placed on top of the reaction mixture. The product that had precipitated was gathered by suction filtration and vacuum-dried overnight at 30−40 °C. Melting point and FTIR measurements were measured for products **1** and **2** (see the Supporting Information).

2.3.1.1. 4-[(Dichloro-1,3,5-triazin-2-yl)amino]-N-(pyridin-2-yl)benzene-1-sulfonamide (1). Compound (**1**) was prepared by reacting sulfapyridine (5 g, 20 mmol) with cyanuric chloride (3.7 g, 20 mmol) in acetone (100 mL) to give a pale yellowish white solid powder (7.58 g, 95.2%): mp > 360 °C; FTIR (ATR): v = 1528, 1563, 3444 cm^{-1}.

2.3.1.2. 1-{4-[(Dichloro-1,3,5-triazin-2-yl)amino]benzene-sulfonyl}guanidine (2). Compound (**2**) was prepared by reacting sulfaguanidine (5 g, 23.3 mmol) with cyanuric chloride (4.3 g, 23.3 mmol) in acetone (100 mL) to give a white solid powder (7.88 g, 93.2%): mp > 360 °C; FTIR (ATR): v = 1528, 1626, 3324 cm^{-1}.

2.3.2. Synthesis of Compounds 3−18. Triethylamine (TEA) (1.05 equiv) was added to a magnetically agitated, ice-bathed solution of compound **1** or **2** in dimethylformamide (DMF) (25 mL) to begin the reaction at 0 to 5 °C. After 3 min, a chosen amine (1 equiv) was added to the reaction mixture. The reaction started at 0 °C and then continued at room temperature. The reaction mixture was cooled after 12 h and then added to the ice-water solution (100 mL). The precipitated crude product was then collected by suction filtering and vacuum-dried overnight at 30 °C. Melting point and FTIR were used to describe products **3−18** (see the Supporting Information).

2.3.2.1. 4-{[4-Chloro-6-(phenylamino)-1,3,5-triazin-2-yl]-amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (3). Compound (**3**) was prepared by reacting compound (**1**) (1 g, 2.5 mmol) with aniline (0.235 g, 0.23 mL, 2.5 mmol) in DMF (25 mL) to give a white solid powder (1.01 g, 88.6%): mp 251−252 °C; FTIR (ATR): v = 1353, 1492, 1542, 3301 cm^{-1}.

2.3.2.2. 4-{[4-(Benzylamino)-6-chloro-1,3,5-triazin-2-yl]-amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (4). Compound (**4**) was prepared by reacting compound (**1**) (1 g, 2.5

mmol) with benzylamine (0.27 g, 0.275 mL, 2.5 mmol) in DMF (25 mL) to give a yellowish white solid powder (1.03 g, 87.3%): mp 254−256 °C; FTIR (ATR): v = 1388, 1515, 1572 cm^{-1}.

2.3.2.3. 4-{[4-Chloro-6-(cyclohexyl-amino)-1,3,5-triazin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (5). Compound (**5**) was prepared by reacting compound (**1**) (1 g, 2.5 mmol) with cyclohexyl amine (0.25 g, 0.29 mL, 2.5 mmol) in DMF (25 mL) to give a white solid powder (0.87 g, 75.2%): mp 237−239 °C; FTIR (ATR): v = 1414, 1494, 1561 cm^{-1}.

2.3.2.4. 4-{[4-Chloro-6-(cyclopropyl-amino)-1,3,5-triazin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (6). Compound (**6**) was prepared by reacting compound (**1**) (1 g, 2.5 mmol) with cyclopropyl amine (0.144 g, 0.175 mL, 2.5 mmol) in DMF (25 mL) to give a white solid powder (0.94 g, 89.5%): mp 211−212 °C; FTIR (ATR): v = 1383, 1512, 1573, 3264 cm^{-1}.

2.3.2.5. 4-{[4-Chloro-6-(diethylamino)-1,3,5-triazin-2-yl]-amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (7). Compound (**7**) was prepared by reacting compound (**1**) (1 g, 2.5 mmol) with diethyl amine (0.185 g, 0.27 mL, 2.5 mmol) in DMF (25 mL) to give a white solid powder (0.92 g, 84.3%): mp 222−223 °C; FTIR (ATR): v = 1392, 1526, 1576 cm^{-1}.

2.3.2.6. 4-({4-Chloro-6-[(3-methylphenl)amino]-1,3,5-tria-zin-2-yl}amino)-N-(pyridin-2-yl)benzene-1-sulfonamide (8). Compound (**8**) was prepared by reacting compound (**1**) (1 g, 2.5 mmol) with m-toluidine (0.27 g, 0.27 mL, 2.5 mmol) in DMF (25 mL) to give a white solid powder (0.97 g, 82.5%): mp 174−175 °C; FTIR (ATR): v = 1386, 1486, 1512, 3281 cm^{-1}.

2.3.2.7. 4-({4-Chloro-6-[(3,4-dimethylphenyl)amino]-1,3,5-triazin-2-yl}amino)-N-(pyridin-2-yl)benzene-1-sulfona-mide (9). Compound (**9**) was prepared by reacting compound (**1**) (1 g, 2.5 mmol) with 3,4-dimethylaniline (0.31 g, 2.5 mmol) in DMF (25 mL) to give a white solid powder (1.05 g, 86.3%): mp 112−113 °C; FTIR (ATR): v = 1390, 1496, 1596 cm^{-1}.

2.3.2.8. 4-{[4-(Diethylamino)-6-(morpholin-4-yl)-1,3,5-tri-azin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (10). Compound (**10**) was prepared by reacting compound (**1**) (1 g, 2.5 mmol) with morpholine (0.22 g, 0.22 mL, 2.5 mmol) in DMF (25 mL) to give a white solid powder (0.93 g, 82.5%): mp 240−243 °C; FTIR (ATR): v = 1503, 1526, 2859 cm^{-1}.

2.3.2.9. 1-(4-{[4-Chloro-6-(phenylamino)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (11). The compound was prepared by reacting compound (**2**) (1 g, 2.76 mmol) with aniline (0.26 g, 0.26 mL, 2.76 mmol) in DMF (25 mL) to give a white solid powder (1.04 g, 89.6%): mp 178−179 °C; FTIR (ATR): v = 1412, 1492, 1509, 3334 cm^{-1}.

2.3.2.10. 1-(4-{[4-(Benzylamino)-6-chloro-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (12). Compound (**12**) was prepared by reacting compound (**2**) (1 g, 2.76 mmol) with benzylamine (0.3 g, 0.3 mL, 2.76 mmol) in DMF (25 mL) to give a white solid powder (1.07 g, 89.5%): mp 249−251 °C; FTIR (ATR): v = 1495, 1519, 3364 cm^{-1}.

2.3.2.11. 1-(4-{[4-Chloro-6-(cyclohexyl-amino)-1,3,5-tria-zin-2-yl]amino}benzene-sulfonyl)guanidine (13). Compound (**13**) was prepared by reacting compound (**2**) (1 g, 2.76 mmol) with cyclohexyl amine (0.28 g, 0.32 mL, 2.76 mmol) in DMF (25 mL) to give a white solid powder (1.09 g, 92.6%): mp 222−225 °C; FTIR (ATR): v = 1494, 1532, 1577, 3317 cm^{-1}.

2.3.2.12. 1-(4-{[4-Chloro-6-(cyclopropylamino)-1,3,5-tria-zin-2-yl]amino}benzene-sulfonyl)guanidine (14). Compound (**14**) was prepared by reacting compound (**2**) (1 g, 2.76 mmol) with cyclopropyl amine (0.16 g, 0.19 mL, 2.76 mmol) in DMF (25 mL) to give a white solid powder (0.99 g, 93.75%): mp 155−157 °C; FTIR (ATR): ν = 1529, 1575, 1507, 3447 cm^{-1}.

2.3.2.13. 1-(4-{[4-Chloro-6-(diethylamino)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (15). Compound (**15**) was prepared by reacting compound (**2**) (5 g, 13.8 mmol) with diethylamine (1 g, 1.44 mL, 13.8 mmol) in DMF (25 mL) to give a white solid powder (4.2 g, 76.3%): mp 271−273 °C; FTIR (ATR): ν = 1490, 1520, 3311 cm^{-1}.

2.3.2.14. 1-[4-({4-Chloro-6-[(3-methylphenyl)amino]-1,3,5-triazin-2-yl}amino)benzene-sulfonyl]guanidine (16). Compound (**16**) was prepared by reacting compound (**2**) (1 g, 2.76 mmol) with *m*-toluidine (0.3 g, 0.3 mL, 2.76 mmol) in DMF (25 mL) to give a white solid powder (1.08 g, 90.4%): mp 256−257 °C; FTIR (ATR): ν = 1489, 1510, 3334 cm^{-1}.

2.3.2.15. 1-[4-({4-Chloro-6-[(3,4-dimethylphenyl)amino]-1,3,5-triazin-2-yl}amino)benzene-sulfonyl]guanidine (17). Compound (**17**) was prepared by reacting compound (**2**) (1 g, 2.76 mmol) with 3,4-dimethylaniline (0.33 g, 2.76 mmol) in DMF (25 mL) to give a white solid powder (1.07 g, 86.4%): mp 272−274 °C; FTIR (ATR): ν = 1408, 1496, 1542, 3321, 3441 cm^{-1}.

2.3.2.16. 1-(4-{[4-Chloro-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (18). Compound (**18**) was prepared by reacting compound (**2**) (1 g, 2.76 mmol) with morpholine (0.24 g, 0.24 mL, 2.76 mmol) in DMF (25 mL) to give a white solid powder (0.96 g, 84.3%): mp 175−178 °C; FTIR (ATR): ν = 1501, 1527, 3205, 3313 cm^{-1}.

2.3.3. Synthetic Procedure for Compounds 19−34. NaOH (1 equiv) was added as an acid scavenger to a magnetically agitated solution of compound (**3−18**) (1 equiv) in diethylamine (DEA) (15 mL). The reaction mixture was stirred for 10 min at room temperature. The reaction was then refluxed at 70−80 °C for 2 h. The reaction was completed, allowed to cool to ambient temperature, and then poured onto the ice-water mixture (10 mL). The precipitated product was removed by suction filtration, chloroform washing, and recrystallization using an acetone−water antisolvent solution. The crystalline products were then recovered using suction filtration and dried overnight at 40 °C under vacuum. Melting point, FTIR, ^{1}H NMR, and ^{13}C NMR analyses of the final compounds were performed (see the Supporting Information).

2.3.3.1. 4-{[4-(Diethylamino)-6-(phenylamino)-1,3,5-tria-zin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (19). Compound (**19**) was prepared by reacting compound (**3**) (1 g, 2.2 mmol) with diethyl amine (5 mL) and recrystallized from acetone−water mixture (10 mL) to give brown crystals (0.67 g, 62%): mp 211−212 °C; FTIR (ATR): ν = 1403, 1432, 1493, 3334 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.54 (s, 1H, NH-SO$_2$), 9.22 (s, 1H, NH-triazine), 8.06 (d, J = 5.4 Hz, 1H, H-C5‴′), 7.99 (d, J = 8.6 Hz, 1H, H-C2‴′), 7.84−7.74 (m, 4H, H-C2′/3′/5′/6′), 7.73−7.63 (m, 2H, H-C2″/6″), 7.27 (t, J = 7.8 Hz, 2H, H-C3″/5″), 7.17 (d, J = 8.5 Hz, 1H, H-C4‴′), 6.96 (t, J = 7.4 Hz, 1H, H-C4″), 6.87 (t, J = 6.3 Hz, 1H, H-C3‴′), 3.57 (q, J = 7.2 Hz, 4H, H-C1‴), 1.14 ppm (t, J = 7.0 Hz, 6H, H-C2‴); ^{13}C NMR (75 MHz, DMSO) δ 164.0 (C2,C4,C6), 152.7 (C1‴′), 144.4 (C3‴′,C5‴′), 140.1 (C1″), 139.6 (C1′), 133.1 (C4′), 128.3 (C3″, C5″), 127.5 (C3′, C5′), 121.7 (C4″), 119.8 (C2″, C6″),

118.7 (C2′, C6′), 116.2 (C4‴′), 113.1 (C2‴′), 40.8 (2X C1‴), 13.2 ppm (2X C2‴), LC−MS/MS calculated 491.58 found MH$^+$ = 491.39.

2.3.3.2. 4-{[4-(Benzylamino)-6-(diethylamino)-1,3,5-tria-zin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (20). Compound (**20**) was prepared by reacting compound (**4**) (1 g, 2.14 mmol) with diethyl amine (5 mL) and recrystallized from acetone−water mixture (10 mL) to give a white solid powder (0.73 g, 67.7%): mp 259−260 °C; FTIR (ATR): ν = 1438, 1488, 1508, 1597, 3339 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.32 (d, J = 8.1 Hz, 2H, NH-SO$_2$ and NH-triazine), 8.04 (d, J = 4.4 Hz, 1H, H-C5‴′), 7.92 (d, J = 8.1 Hz, 1H, H-C2‴′), 7.72 (dd, J = 19.2, 11.4 Hz, 4H, H-C2′/3′/5′/6′), 7.30 (d, J = 5.3 Hz, 4H, H-C2″/3″/5″/6″), 7.20 (br s, 1H, H-C4″), 7.11 (d, J = 8.5 Hz, 1H, H-C4‴′), 6.87 (t, 1H, H-C3‴′), 4.46 (s, 2H, H-C7″), 3.52 (s, 4H, H-C1‴), 1.11 (s, 6H, H-C2‴), 1.00 ppm (s, 1H, NH-BA); ^{13}C NMR (75 MHz, DMSO) δ = 165.41 (C2), 163.9 (C4,C6), 152.9 (C1‴′), 144.72 (C5‴′), 144.6 (C3‴′), 144.4 (C1″), 140.6 (C4″), 139.5 (C1′), 132.7 (C4′), 128.1 (C3′, C5′), 127.3 (C3″,C5″), 126.8 (C2″), 126.4 (C6″), 118.2 (C2′, C6′), 116.2 (C4‴′), 113.1 (C2‴′), 43.6 (C7″), 40.7 (2X C1‴), 13.2 ppm (2X C2‴) LC−MS/MS: calculated 505.60 found MH$^+$ = 505.41.

2.3.3.3. 4-{[4-(Cyclohexyl-amino)-6-(diethylamino)-1,3,5-triazin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (21). Compound (**21**) was prepared by reacting compound (**5**) (1 g, 2.17 mmol) with diethyl amine (5 mL) and recrystallized from acetone−water mixture (10 mL) to give white crystals (0.5 g, 46.9%): mp 220−221 °C; FTIR (ATR): ν = 1435, 1493, 1548, 1578, 2927, 3339 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ 9.26 (d, J = 44.4 Hz, 3H, NH-SO$_2$ and NH-triazine), 8.04 (d, J = 4.0 Hz, 1H, H-C5‴′), 7.93 (d, J = 8.2 Hz, 1H, H-C2‴′), 7.69 (d, J = 16.1 Hz, 4H, H-C2′/3′/5′/6′), 7.13 (d, J = 8.6 Hz, 1H, H-C4‴′), 6.88 (t, 1H, H-C3‴′), 3.70 (s, 1H, H-C1″), 3.53 (s, 4H, H-C1‴), 2.05−1.50 (m, 4H, H-C2″/6″), 1.21 (d, J = 17.4 Hz, 6H, H-C3″/4″/5″), 1.11 ppm (s, 6H, H-C2‴); ^{13}C NMR (75 MHz, DMSO) δ = 164.7 (C2), 163.9 (C4,C6), 152.7 (C1‴′), 144.7 (C3‴′,C5‴′), 139.5 (C1′), 132.5 (C4′), 127.4 (C3′, C5′), 118.1 (C2′,C6′), 116.3 (C4‴′), 113.1 (C2‴′), 49.1 (2X C1‴), 40.7 (C1″), 32.5 (C2″,C6″), 25.1 (C3″,C4″,C5″), 13.3 ppm (2X C2‴), LC−MS/MS: calculated 497.62 found MH$^+$ = 497.40.

2.3.3.4. 4-{[4-(Cyclopropyl-amino)-6-(diethylamino)-1,3,5-triazin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (22). Compound (**22**) was prepared by reacting compound (**6**) (1 g, 2.4 mmol) with diethyl amine (5 mL) and recrystallized using an acetone−water mixture (10 mL) to give white crystals (0.63 g, 58%): mp 226−227 °C; FTIR (ATR): ν = 1326, 1419, 1518, 1571, 2966, 3395 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ 11.55 (s, 1H, NH of CPA), 9.36 (d, J = 49.9 Hz, 1H, NH-SO$_2$), 8.09−7.89 (m, 2H, H-C5‴′/2‴′), 7.83−7.63 (m, 4H, H-C2′/3′/5′/6′), 7.13 (d, J = 8.6 Hz, 1H, H-C4‴′), 6.88 (t, 1H, H-C3‴′), 3.57−3.49 (m, 4H, H-C1‴), 2.73 (tt, J = 7.5, 3.9 Hz, 1H, H-C1″), 1.11 (t, J = 7.3 Hz, 6H, H-C2‴), 0.75−0.37 ppm (m, 4H, H-C2″/3″). ^{13}C NMR (75 MHz, DMSO) δ = 166.8 (C2), 163.9 (C4,C6), 152.6 (C1‴′), 144.7 (C3‴′, C5‴′), 139.6 (C1′), 132.5(C4′), 127.4 (C3′, C5′), 118.2(C2′,C6′), 116.3 (C4‴′), 113.0 (C2‴′), 40.3 (2X C1‴), 23.4 (C1″), 13.3 (2X C2‴), 6.2 ppm(C2″,C3″), LC−MS/MS: calculated 455.55 found MH$^+$ = 455.32.

2.3.3.5. 4-{[Bis(diethylamino)-1,3,5-triazin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (23). Compound (**23**) was prepared by reacting compound (**7**) (1 g, 2.3 mmol) with

diethyl amine (5 mL) and recrystallized using acetone–water mixture (10 mL) to give a white solid powder (0.79 g, 72.9%): mp 216–217 °C; FTIR (ATR): ν = 1496, 1511, 3339 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.31 (s, 2H, H-NH-SO$_2$ and NH-triazine), 8.04 (d, J = 4.0 Hz, 1H, H-C5′′′), 7.91 (d, J = 8.5 Hz, 1H, H-C2′′′), 7.70 (dd, J = 19.7, 7.9 Hz, 4H, H-C2′/3′/5′/6′), 7.12 (d, J = 8.5 Hz, 1H, H-C4′′′), 6.87 (t, 1H, H-C3′′′), 3.53 (s, 8H, H-C1′′), 1.12 ppm (t, J = 6.6 Hz, 12H, H-C2′′); ^{13}C NMR (75 MHz, DMSO) δ = 163.8 (C2, C4, C6), 152.9 (C1′′′), 144.6 (C3′′′, C5′′′), 139.6 (C1′), 132.7 (C4′), 127.4 (C3′, C5′), 118.0 (C2′,C6′), 116.1 (C4′′′), 113.1 (C2′′′), 40.7 (4X C1′′), 13.2 ppm (4X C2′′), LC–MS/MS: calculated 470.59 found MH$^+$ = 470.22.

2.3.3.6. 4-{[4-(Diethylamino)-6-[(3-methylphenyl)amino]-1,3,5-triazin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (24). Compound (**24**) was prepared by reacting compound (**8**) (1 g, 2.14 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give white crystals (0.85 g, 79%): mp 224–225 °C; FTIR (ATR): ν = 1416, 1524, 1543, 2976, 3417 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.49 (s, 1H, H-NH-SO$_2$), 9.12 (s, 2H, NH-triazine), 8.05 (d, J = 6.1 Hz, 1H, H-C5′′′), 7.96 (d, J = 8.6 Hz, 1H, H-C2′′′), 7.78–7.72 (m, 4H, H-C2′/3′/5′/6′), 7.68 (d, J = 6.7 Hz, 1H, H-C6′′), 7.47 (d, J = 8.2 Hz, 1H, H-C5′′), 7.15 (d, 1H, H-C2′′), 7.12 (d, J = 4.2 Hz, 1H, H-C4′′′), 6.88 (t, 1H, H-C3′′′), 6.79 (d, J = 7.5 Hz, 1H, H-C4′′), 3.59 (q, J = 7.1 Hz, 4H, H-C1′′′), 2.27 (s, 3H, H-C7′′), 1.22–1.11 ppm (m, 6H, H-C2′′′); ^{13}C NMR (75 MHz, DMSO) δ = 164.0 (C2, C4, C6), 152.7 (C1′′′′), 144.2 (C3′′′′, C5′′′′), 140.0 (C1′′), 139.6 (C1′), 137.4 (C3′′), 133.0 (C4′), 128.1 (C5′′), 127.4 (C3′, C5′), 122.5 (C4′′), 120.4 (C6′′), 118.6 (C2′,C6′), 117.0 (C2′′), 116.3 (C4′′′′), 113.1 (C2′′′), 40.8 (2X C1′′′), 21.3 (C7′′), 13.2 ppm (2X C2′′′) LC–MS/MS: calculated 455.55 found MH$^+$ = 455.32.

2.3.3.7. 4-{[4-(Diethylamino)-6-[(3,4-dimethylphenyl)-amino]-1,3,5-triazin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (25). Compound (**25**) was prepared by reacting compound (**9**) (1 g, 2.07 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give a white solid powder (0.81 g, 75.3%): mp 231–232 °C; FTIR (ATR): ν = 1430, 1497, 2973, 3326 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.45 (s, 1H, H-NH-SO$_2$), 9.01 (s, 1H, NH-triazine), 8.05 (d, J = 5.4 Hz, 1H, H-C5′′′), 7.95 (d, J = 8.6 Hz, 1H, H-C2′′′), 7.80–7.68 (m, 4H, H-C2′/3′/5′/6′), 7.64 (d, J = 15.8 Hz, 1H, H-C6′′), 7.38 (d, J = 8.3 Hz, 1H, H-C2′′), 7.14 (d, J = 8.6 Hz, 1H, H-C5′′), 7.01 (d, J = 8.2 Hz, 1H, 4′′′), 6.89 (t, J = 6.3 Hz, 1H, H-C3′′′), 3.58 (q, J = 7.0 Hz, 4H, H-C1′′′), 2.17 (d, J = 5.0 Hz, 6H, H-C7′′/8′′), 1.16 ppm (t, J = 7.0 Hz, 6H, H-C2′′′); ^{13}C NMR (75 MHz, DMSO) δ 163.9 (C2, C4, C6), 152.7 (C1′′′′), 144.3 (C3′′′′, C5′′′′), 140.1 (C1′′), 139.6 (C1′), 137.7 (C3′′), 135.8 (C4′′), 132.9 (C4′), 129.2 (C2′′, C5′′), 127.4 (C3′, C5′), 121.2 (C6′′), 118.5 (C2′,C6′), 116.2 (C4′′′′), 113.0 (C2′′′), 40.7 (2X C1′′′), 19.7 (C7′′), 18.7 (C8′′), 13.2 ppm (2X C2′′′), LC–MS/MS: calculated 471.59 found MH$^+$ = 471.47.

2.3.3.8. 4-{[4-(Diethylamino)-6-(morpholin-4-yl)-1,3,5-tri-azin-2-yl]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (26). Compound (**26**) was prepared by reacting compound (**10**) (1 g, mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give a white solid powder (g, 66.5%): mp 124–127 °C; FTIR (ATR): ν = 1393, 1494, 2970 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.46 (s, 2H, NH-SO$_2$ and NH-triazine), 8.04 (d, J = 5.4 Hz, 1H, H-

C5′′′), 7.86 (d, J = 8.6 Hz, 1H, H-C2′′′), 7.79–7.63 (m, 4H, H-C2′/3′/5′/6′), 7.12 (d, J = 8.7 Hz, 1H, H-C4′′′), 6.87 (t, J = 6.4 Hz, 1H, H-C3′′′), 3.75–3.58 (m, 8H, H-C1′′/2′′), 3.58–3.47 (m, 4H, H-C1′′′), 1.11 ppm (q, J = 6.0 Hz, 6H, H-C2′′′); ^{13}C NMR (75 MHz, DMSO) δ = 164.6 (C2), 163.9 (C4, C6), 152.7 (C1′′′),144.3 (C3′′′, C5′′′), 139.6 (C1′), 132.9 (C4′), 127.5 (C3′, C5′), 118.2 (C2′, C6′), 116.2 (C4′′′), 113.1 (C2′′), 65.9 (2X C2′′), 43.3 (2X C1′′), 40.7 (2X C1′′′), 13.1 ppm (2X C2′′′), LC–MS/MS: calculated 505.60 found MH$^+$ = 505.43.

2.3.3.9. 1-(4-{[4-(Diethylamino)-6-(phenylamino)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (27). Compound (**27**) was prepared by reacting compound (**11**) (1 g, 2.38 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give white crystals (0.81 g, 74.8%): mp 169–171 °C; FTIR (ATR): ν = 1414, 1488, 1541, 3338 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.41 (s, 1H-NH-SO$_2$), 9.16 (s, 1H, NH-aniline), 7.91 (d, J = 8.6 Hz, 2H, H-C3′/5′), 7.78 (d, J = 7.9 Hz, 2H, H-C2′′′/6′′′), 7.63 (d, J = 8.7 Hz, 2H, H-C2′/6′), 7.28 (t, J = 7.8 Hz, 2H, H-C3′′′/5′′′), 6.97 (t, J = 7.3 Hz, 1H, H-C4′′′), 6.67 (s, 4H, NH-guanidine), 3.60 (d, J = 6.9 Hz, 4H, H-C1′′), 1.17 ppm (t, J = 6.8 Hz, 6H, H-C2′′); ^{13}C NMR (75 MHz, DMSO) δ 163.8 (C2, C4, C6), 158.0 (C7′), 143.0 (C1′), 140.1 (C1′′′), 136.9 (C4′), 128.3 (C3′′′,C5′′′), 126.1 (C3′,C5′), 121.7 (C4′′′), 119.8 (C2′′′,C6′′′), 118.6 (C2′,C6′), 40.8 (2X C1′′), 13.2 ppm (2X C2′′), LC–MS/MS: calculated 456.53 found MH$^+$ = 456.31.

2.3.3.10. 1-(4-{[4-(Benzylamino)-6-(diethylamino)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (28). Compound (**28**) was prepared by reacting compound (**12**) (1 g, 2.3 mmol) with diethyl amine (5 mL) and recrystallized using acetone–water mixture (10 mL) to give a white solid powder (0.5 g, 46.3%): mp 235–237 °C; FTIR (ATR): ν = 1511, 1578, 3347 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.23 (s, 1H, H-NH-SO$_2$), 7.87 (d, J = 7.3 Hz, 2H, H-C3′/5′), 7.58 (d, J = 8.0 Hz, 2H, H-C2′/6′), 7.32 (d, J = 2.5 Hz, 4H, H-C2′′′/3′′′/5′′′/6′′′), 7.22 (d, J = 5.7 Hz, 1H, H-C4′′′), 6.65 (br s, 4H, H-guanidine), 4.46 (s, 2H, H-C7′′′), 3.53 (s, 4H, H-C1′′), 1.13 (s, 6H, H-C2′′), 1.02 ppm (s, 1H, NH-BA); ^{13}C NMR (75 MHz, DMSO) δ = 165.5 (C2), 163.9 (C4, C6), 158.0 (C7′), 143.4 (C1′), 140.7 (C4′′), 136.4 (C4′), 128.1 (C3′′′,C5′′′), 127.2 (C2′′′), 126.8 (C6′′), 126.4 (C1′′′), 126.1 (C3′,C5′), 118.2 (C2′,C6′), 43.6 (C7′′′), 40.7 (2X C1′′), 13.3 ppm (2X C2′′), LC–MS/MS: calculated 470.56 found MH$^+$ = 470.22.

2.3.3.11. 1-(4-{[4-(Cyclohexylamino)-6-(diethylamino)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (29). Compound (**29**) was prepared by reacting compound (**13**) (1 g, 2.35 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give white crystals (0.63 g, 58.1%): mp 155–157 °C; FTIR (ATR): ν = 1486, 1543, 2929, 3449 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.18 (d, J = 42.1 Hz, 2H, NH-SO$_2$ and NH-triazine), 7.89 (d, J = 8.6 Hz, 2H, H-C3′/5′), 7.59 (d, J = 8.4 Hz, 2H, H-C2′/6′), 6.64 (br s, 4H, H-guanidine), 3.73 (s, 1H, H-C1′′′), 3.54 (s, 4H, H-C1′′), 1.95–1.47 (m, 4H, H-C2′′′/6′′′), 1.47–1.18 (m, 6H, H-C3′′′/4′′′/5′′′), 1.13 ppm (s, 6H, H-C2′′); ^{13}C NMR (75 MHz, DMSO) δ = 164.4 (C2), 163.9 (C4,C6), 157.9 (C7′), 143.5 (C1′), 136.3 (C4′), 126.1 (C3′,C5′), 118.1 (C2′,C6′), 49.1 (C1′′′), 40.7 (2X C1′′), 32.6 (C2′′′, C6′′′), 25.1 (C3′′′,C4′′′,C5′′′), 13.32 ppm (2X C2′′), LC–MS/MS: calculated 462.58 found MH$^+$ = 462.41.

2.3.3.12. 1-(4-{[4-(Cyclopropylamino)-6-(diethylamino)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (30).

Compound (**30**) was prepared by reacting compound (**14**) (1 g, 2.6 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give a white solid powder (0.45 g, 41.5%): mp 247–248 °C; FTIR (ATR): v = 1488, 2973, 3159 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.31 (s, 1H, NH-SO$_2$), 7.94 (br s, 2H, H-C3′/5′), 7.59 (d, J = 8.4 Hz, 2H, H-C2′/6′), 7.07 (s, 1H, NH-CPA), 6.65 (br s, 4H, H-guanidine), 3.56 (s, 4H, H-C1″), 2.91–2.68 (m, 1H, H-C1‴), 1.12 (s, 6H, H-C2″), 0.85–0.19 ppm (m, 4H, H-C2‴/3‴); ^{13}C NMR (75 MHz, DMSO) δ = 166.8 (C2), 163.9 (C4, C6), 157.9 (C7′), 143.5 (C1′), 136.3 (C4′), 126.1 (C3′,C5′), 118.1 (C2′,C6′), 41.6 (2X C1″), 23.4 (C1‴), 13.3 (2X C2″), 6.2 ppm (C2‴,C3‴), LC–MS/MS: calculated 420.50 found MH$^+$ = 420.34.

2.3.3.13. 1-(4-{[Bis(diethylamino)-1,3,5-triazin-2-yl]-amino}benzene-sulfonyl)guanidine (31). Compound (**31**) was prepared by reacting compound (**15**) (1 g, 2.5 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water (10 mL) mixture to give a white solid powder (0.99 g, 90.7%): mp 154–156 °C; FTIR (ATR): v = 1494, 2972, 3339 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.24 (s, 1H, NH-SO$_2$), 7.88 (d, J = 8.3 Hz, 2H, H-C3′/5′), 7.60 (d, J = 8.4 Hz, 2H, H-C2′/6′), 6.65 (br s, 4H, H-guanidine), 3.45 (d, J = 56.3 Hz, 8H, H-C1″), 1.13 ppm (s, 12H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 163.8 (C2, C4, C6), 157.9 (C7′), 143.5 (C1′), 136.2 (C4′), 126.1 (C3′, C5′), 117.9 (C2′, C6′), 40.7 (4X C1″), 13.3 ppm (4X C2″), LC–MS/MS: calculated 436.54 found MH$^+$ = 436.30.

2.3.3.14. 1-(4-{[4-(Diethylamino)-6-[(3-methylphenyl)-amino]-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)-guanidine (32). Compound (**32**) was prepared by reacting compound (**16**) (1 g, 2.31 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give colorless crystals (0.8 g, 74.2%): mp 239–240 °C; FTIR (ATR): v = 1435, 1514, 1542, 3327 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.41 (s, 1H, NH-SO$_2$), 9.11 (s, 1H, NH-triazine), 7.91 (d, J = 8.5 Hz, 2H, H-C3′/5′), 7.70 (s, 1H, H-C6‴), 7.62 (d, J = 8.5 Hz, 2H, H-C2′/6′), 7.49 (d, J = 8.3 Hz, 1H, H-C5‴), 7.15 (t, J = 7.8 Hz, 1H, H-C2‴), 6.78 (d, J = 7.5 Hz, 1H, H-C4‴), 6.68 (s, 4H, guanidine), 3.60 (q, J = 7.0 Hz, 4H, H-C1″), 2.28 (s, 3H, H-C7‴), 1.17 ppm (t, J = 7.1 Hz, 6H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 163.9 (C2, C4, C6), 158.0 (C7′), 143.1 (C1′), 140.1 (C1‴), 137.4 (C3‴), 136.8 (C4′), 128.1 (C5‴), 126.1 (C3′,C5′), 122.4 (C4‴), 120.3 (C6‴), 118.5 (C2′,C6′), 116.9 (C2‴), 40.1 (2X C1″), 21.3 (C7‴), 13.2 ppm (2X C2″), LC–MS/MS: calculated 470.56 found MH$^+$ = 470.39.

2.3.3.15. 1-(4-{[4-(Diethylamino)-6-[(3,4-dimethylphenyl)-amino]-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)-guanidine (33). Compound (**33**) was prepared by reacting compound (**17**) (1 g, 2.24 mmol) with diethyl amine (5 mL) and recrystallized from acetone–water mixture (10 mL) to give white crystals (0.61 g, 56.2%): mp 174–175 °C; FTIR (ATR): v = 1416, 1504, 1533, 3361 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.38 (s, 1H, NH-SO$_2$), 9.02 (s, 1H, NH-triazine), 7.92 (d, J = 8.6 Hz, 2H, H-C3′/5′), 7.62 (d, J = 9.0 Hz, 3H, H-C2′/6′/6‴), 7.40 (d, J = 8.1 Hz, 1H, H-C2‴), 7.02 (d, J = 8.3 Hz, 1H, H-C5‴), 6.69 (s, 4H, guanidine), 3.59 (q, J = 7.0 Hz, 4H, H-C1″), 2.18 (d, J = 8.9 Hz, 6H, H-C7‴/8‴), 1.17 ppm (t, J = 7.0 Hz, 6H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 163.9 (C2, C4, C6), 158.0 (C7′), 143.1 (C1′), 140.1 (C1‴), 137.8 (C3‴), 136.7 (C4′), 135.8 (C4‴), 129.2 (C2‴, C5‴), 126.1 (C3′,C5′), 121.1 (C6‴), 118.5 (C2′,C6′),

40.5 (2X C1″), 19.8 (C7‴), 18.7 (C8‴), 13.3 ppm (2X C2″), LC–MS/MS: calculated 484.59 found MH$^+$ = 484.37.

2.3.3.16. 1-(4-{[4-(Diethylamino)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (34). Compound (**34**) was prepared by reacting compound (**18**) (g, mmol) with diethyl amine (5 mL) and recrystallized from the acetone–water mixture (10 mL) to give a white solid powder (0.4 g, 71.3%): mp 251–253 °C; FTIR (ATR): v = 1432, 1494, 1531, 3343 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.36 (s, 1H, NH-SO$_2$), 7.81 (d, J = 8.5 Hz, 2H, H-C3′/5′), 7.60 (d, J = 8.6 Hz, 2H, H-C2′/6′), 6.65 (s, 4H, guanidine), 3.73–3.48 (m, 8H, H-C1‴/2‴), 3.34 (s, 4H, H-C1″), 1.11 ppm (d, J = 6.8 Hz, 6H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 169.9 (C2), 169.1 (C4, C6), 163.2 (C7′), 148.5 (C1′), 141.8 (C4′), 131.5 (C3′, C5′), 123.4 (C2′, C6′), 71.2 (2X C2‴), 48.5 (2X C1‴), 45.9 (2X C1″), 18.5 (2X C2″), LC–MS/MS: calculated 450.53 found MH$^+$ = 450.42.

2.3.4. Synthetic Procedure for Compounds 35–38. Starting from compound (**15**), the final compounds (**35–38**) were prepared by neat fusion. Five equivalents of substituted aniline (*p*-Br-aniline, *p*-Cl-aniline, *p*-F-aniline, *p*-nitro-aniline), respectively, were melted down into a mortar in a hot plate at (160–170) °C. One equivalent of compound (**15**) was added to the melted substituted aniline, and the reaction mixture was stirred by a glass rod at the same temperature for 7–10 min. After that, the reaction mixture was allowed to cool to room temperature. The product was washed several times with acetone to remove the excess of unreacted substituted aniline. The final compounds were recrystallized from ethanol using the hot filtration recrystallization method. The final compounds were analyzed by melting point, FTIR, ^{1}H NMR, and ^{13}C NMR analyses (see the Supporting Information), and LC–MS/MS triple quad analyses confirmed the identity, purity above 95%, and molecular weight of the final synthetic compounds.

2.3.4.1. 1-[4-({4-[(4-Bromophenyl)amino]-6-(diethylami-no)-1,3,5-triazin-2-yl}amino)benzene-sulfonyl]guanidine (35). Compound (**35**) was prepared by reacting compound (**15**) (0.5 g, 1.25 mmol) with melted 4-bromoaniline (1.08 g, 6.27 mmol) and recrystallized from ethanol to give a white solid powder (0.58 g, 86.6%): mp 187–190 °C; FTIR (ATR): v = 1555, 1582, 1618, 3339 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ 10.19 (d, J = 43.3 Hz, 2H, NH-SO$_2$ and NH-triazine), 7.85 (d, J = 8.4 Hz, 2H, H-C3′/5′), 7.71 (d, J = 8.8 Hz, 4H, H-C2‴/6‴/2′/6′), 7.51 (d, J = 8.7 Hz, 2H, H-C3‴/5‴), 6.88 (s, 4H, guanidine), 3.62 (d, J = 7.7 Hz, 4H, H-C1″), 1.18 ppm (t, J = 7.0 Hz, 6H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 164.5 (C2, C4, C6), 157.7 (C7′), 141.6 (C1‴),140.4 (C1′), 137.9 (C4′), 131.3 (C3‴, C5‴), 126.5 (C3′, C5′), 122.3 (C4‴), 119.5 (C2‴, C6‴), 118.1 (C2′, C6′), 41.6 (2X C1″), 13.0 ppm (2X C2″), LC–MS/MS: calculated 535.43 found MH$^+$ = 535.16.

2.3.4.2. 1-[4-({4-[(4-Chlorophenyl)amino]-6-(diethylami-no)-1,3,5-triazin-2-yl}amino)benzene-sulfonyl]guanidine (36). Compound (**36**) was prepared by reacting compound (**15**) (0.5 g, 1.25 mmol) with melted 4-chloroaniline (0.8 g, 6.27 mmol) and recrystallized from ethanol to give a white solid powder (0.52 g, 84.2%): mp: 196–198 °C; FTIR (ATR): v = 1541, 1582, 1620, 3351 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ = 9.90 (d, J = 43.2 Hz, 2H, NH-SO$_2$ and NH-triazine), 7.87 (d, J = 8.5 Hz, 2H, H-C3′/5′), 7.78 (d, J = 8.5 Hz, 2H, H-C2‴/6‴), 7.68 (d, J = 8.5 Hz, 2H, H-C2′/6′), 7.36 (d, J = 8.9 Hz, 2H, H-C3‴/5‴), 6.81 (s, 4H, guanidine), 3.61
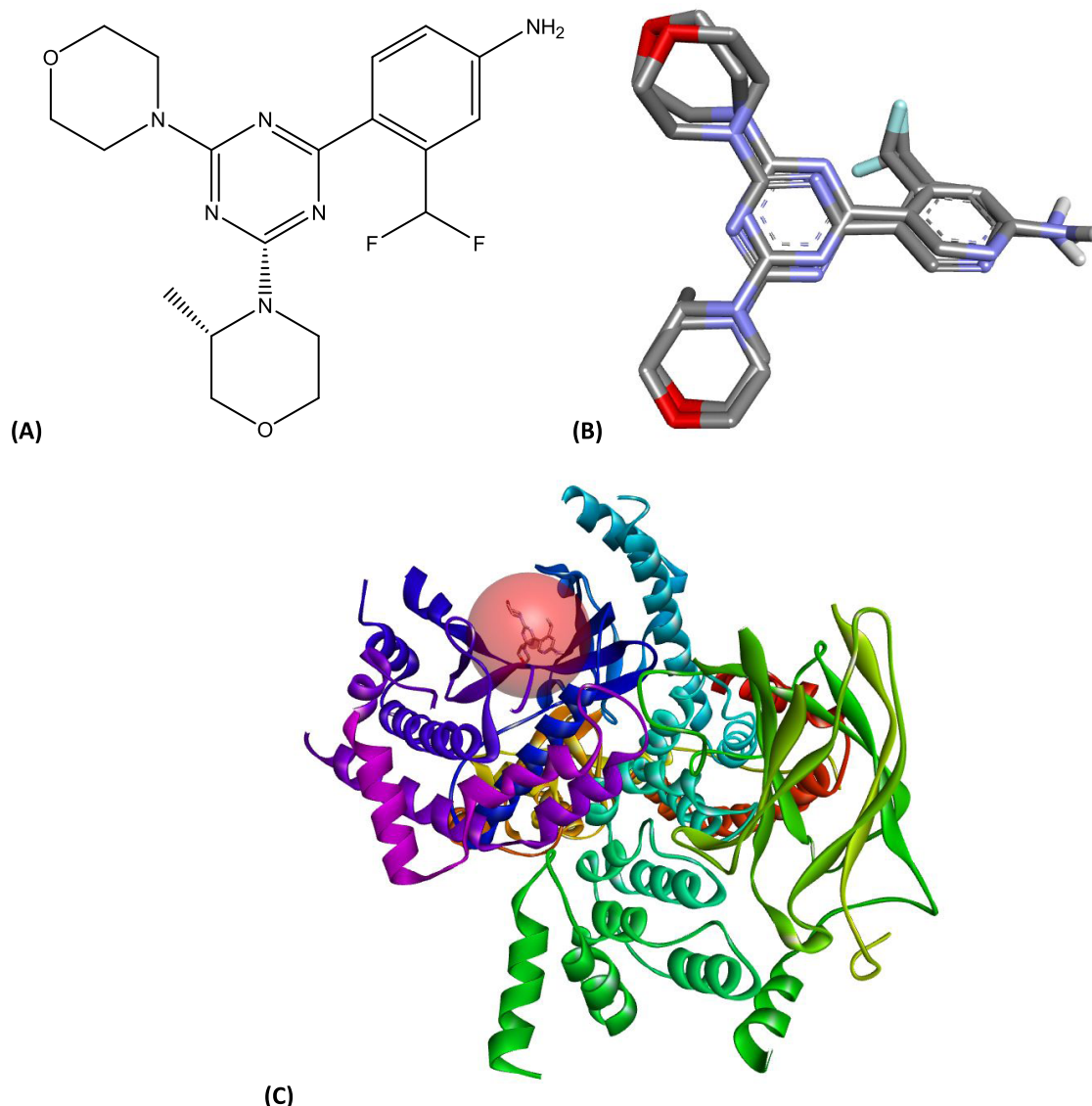
Figure 2. (A) Co-crystallized triazine ligand (PQR530), (B) co-crystallized pose and the docked pose of the co-crystallized ligand with RMSD = 0.67 Å, and (C) the binding site of the PI3Kα protein (PDB code: 6OAC, resolution: 3.15 Å).

(d, J = 8.1 Hz, 4H, H-C1″), 1.18 ppm (t, J = 7.0 Hz, 6H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 161.4 (C2, C4, C6), 157.8 (C7′), 142.1 (C1‴), 140.8 (C1′), 137.6 (C4′),128.3 (C3‴, C5‴), 126.4 (C3′, C5′), 121.7 (C4‴), 119.2 (C2‴, C6‴), 118.3 (C2′, C6′), 41.3 (2X C1″), 13.0 ppm (2X C2″), LC−MS/MS: calculated 490.98 found MH[+] = 490.25.

2.3.4.3. 1-(4-{[4-(Diethylamino)-6-[(4-fluorophenyl)-amino]-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)-guanidine (37). Compound (**37**) was prepared by reacting compound (**15**) (0.5 g, 1.25 mmol) with 4-fluoroaniline (0.7 g, 0.6 mL, 6.27 mmol) and recrystallized from ethanol to give blue solid powder (0.51 g, 87.2%): mp: 186−188 °C; FTIR (ATR): v = 1543, 1587, 1622, 3323 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ 10.17 (d, J = 61.5 Hz, 2H, NH-SO$_2$ and NH-triazine), 7.84 (d, J = 8.5 Hz, 2H, H-C3′/5′), 7.70 (d, J = 8.5 Hz, 4H, H-C2‴/6‴/2′/6′), 7.19 (t, J = 8.9 Hz, 2H, H-C3‴/5‴), 6.83 (s, 4H, guanidine), 3.66−3.58 (m, 4H, H-C1″), 1.18 ppm (t, J = 6.9 Hz, 6H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 159.8 (C2, C4, C6), 157.8 (C7′), 156.7 (C4‴), 141.4 (C1′), 137.9 (C4′), 134.7 (C1‴), 126.5 (C3′,C5′), 122.7

(C3‴,C5‴), 119.6 (C2′,C6′), 115.2 (C2‴,C6‴), 41.7 (2X C1″), 12.9 ppm (2X C2″), LC−MS/MS: calculated 474.52 found MH[+] = 474.26.

2.3.4.4. 1-(4-{[4-(Diethylamino)-6-[(4-nitrophenyl)amino]-1,3,5-triazin-2-yl]amino}benzene-sulfonyl)guanidine (38). Compound (**38**) was prepared by reacting compound (**15**) (0.5 g, 1.25 mmol) with melted 4-nitroaniline (0.87 g, 6.27 mmol) and recrystallized from ethanol to give a yellow solid powder (0.39 g, 62.3%): mp 245−248 °C (Decomposed); FTIR (ATR): v = 1556, 2986 cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ 10.20 (d, J = 72.6 Hz, 2H, NH-SO$_2$ and NH-triazine), 8.24−8.15 (m, 2H, H-C3‴/5‴), 8.06 (s, 2H, H-C2‴/6‴), 7.91 (d, J = 7.8 Hz, 2H, H-C3′/5′), 7.73 (d, J = 8.6 Hz, 2H, H-C2′/6′), 7.04 (s, 4H, guanidine), 3.64 (s, 4H, H-C1″), 1.27−1.14 ppm (m, 6H, H-C2″); ^{13}C NMR (75 MHz, DMSO) δ = 162.0 (C2, C4, C6), 157.3 (C7′), 146.3 (C4‴), 142.5 (C1‴), 141.1 (C1′), 136.4 (C4′), 126.7 (C3′,C5′), 124.7 (C3‴, C5‴), 119.3 (C2′,C6′), 119.0 (C2‴,C6‴), 41.5 (2X C1″), 13.0 ppm (2X C2″), LC−MS/MS: calculated 501.53 found MH[+] = 501.32.

Table 1. Synthetic Compounds and the Standard Inhibitors

Compound number	R₁	R₂	R₃
1	pyridin-2-yl-methyl	Cl	Cl
2	amidine	Cl	Cl
3	pyridin-2-yl-methyl	N-methyl-phenyl	Cl
4	pyridin-2-yl-methyl	N-benzyl	Cl
5	pyridin-2-yl-methyl	N-cyclohexyl	Cl
6	pyridin-2-yl-methyl	N-cyclopropyl	Cl
7	pyridin-2-yl-methyl	N-diethyl	Cl
8	pyridin-2-yl-methyl	N-(3-methylphenyl)	Cl
9	pyridin-2-yl-methyl	N-(3,4-dimethylphenyl)	Cl
10	pyridin-2-yl-methyl	morpholine	Cl
11	amidine	N-methyl-phenyl	Cl
12	amidine	N-benzyl	Cl
13	amidine	N-cyclohexyl	Cl
14	amidine	N-cyclopropyl	Cl
15	amidine	N-diethyl	Cl
16	amidine	N-(3-methylphenyl)	Cl
17	amidine	N-(3,4-dimethylphenyl)	Cl
18	amidine	morpholine	Cl
19	pyridin-2-yl-methyl	N-methyl-phenyl	N-diethyl
20	pyridin-2-yl-methyl	N-benzyl	N-diethyl
21	pyridin-2-yl-methyl	N-cyclohexyl	N-diethyl
22	pyridin-2-yl-methyl	N-cyclopropyl	N-diethyl
23	pyridin-2-yl-methyl	N-diethyl	N-diethyl
24	pyridin-2-yl-methyl	N-(3-methylphenyl)	N-diethyl
25	pyridin-2-yl-methyl	N-(3,4-dimethylphenyl)	N-diethyl
26	pyridin-2-yl-methyl	morpholine	N-diethyl
27	amidine	N-methyl-phenyl	N-diethyl
28	amidine	N-benzyl	N-diethyl
29	amidine	N-cyclohexyl	N-diethyl
30	amidine	N-cyclopropyl	N-diethyl
31	amidine	N-diethyl	N-diethyl
32	amidine	N-(3-methylphenyl)	N-diethyl
33	amidine	N-(3,4-dimethylphenyl)	N-diethyl
34	amidine	morpholine	N-diethyl
35	amidine	N-diethyl	N-(4-bromophenyl)
36	amidine	N-diethyl	N-(4-chlorophenyl)
37	amidine	N-diethyl	N-(4-fluorophenyl)
38	amidine	N-diethyl	N-(4-nitrophenyl)

Gedatolisib

Doxorubicin

2.3.5. In Vitro Biological Studies. 2.3.5.1. Cell Culture and Seeding. The MCF-7 and A549 cancer cell lines were provided by the University of Jordan (the American Type Culture Collection ATCC, Manassas, VA). The MCF-7 cancer cells

were cultured in RPMI 1640 media, and the A549 cancer cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc., Rockville, MD) supplemented with heat-inactivated fetal bovine serum (10%; Sigma-Aldrich, St. Louis, MO), streptomycin, penicillin (1%), and glutamine (2 mmol/L). The selected cancer cell lines were cultured at 37 °C in a humidified atmosphere with 5% CO_2 until the recovery reached 80%. The selected cancer cell lines, MCF-7 and A549, were seeded in 96-well plates at a density of 2000 cells/well and 10000 cells/well, respectively. The cells were incubated for 24 h before their treatment with the compounds to allow the cells to be attached to the wall of the wells. Then, the media were aspirated and replaced with fresh media (100 μL) containing the hits in different concentrations. The treated cells were incubated for 72 h at 37 °C in a humidified atmosphere with 5% CO_2.

2.3.5.2. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazo-lium-bromide (MTT) Assay. The MTT assay was carried out in the MCF-7 and A549 cell lines to determine the anticancer activity of compounds (**19−34**) and (**35−38**). Briefly, after 72 h of incubation, 20 μL of a 5 mg/mL solution of (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and incubated for further 4 h at 37 °C. At the end of the incubation, the medium was removed leaving the violet formazan crystals precipitated in the wells. The resulting formazan crystals were solubilized by adding 100 μL of DMSO to each well. The plates were then analyzed on a microplate reader (GLOMAX) to identify the absorbance of the samples at 450 and 540 nm. In three independent experiments, each compound was tested in triplicate for each cell line.[56] The IC_{50} of the most active compounds was calculated by nonlinear regression analysis using GraphPad Prism 9.3.1 software.

2.3.5.3. Anti-PI3Kα Enzymatic Activity. The biological activity of the final compounds (**19−34**) and (**35−38**) against PI3Kα enzyme was investigated *in vitro* using an Adapta universal kinase assay (ADP-fluorescent based immunoassay) by Invitrogen/Thermo Fisher Scientific.[55] The assay is divided into a kinase reaction phase and an ADP detection phase. In the first phase (kinase reaction phase), all components required for the kinase reaction are added to the well, and the reaction is allowed to incubate for 60 min. After the reaction, a detection solution consisting of a europium-labeled anti-ADP antibody, a Fluor 647 labeled ADP tracer, and ethylenediaminetetraacetic acid (EDTA) (to stop the kinase reaction) is added to the assay well. ADP formed by the kinase reaction (in the absence of an inhibitor) will displace the Alexa Fluor 647 labeled ADP tracer from the antibody, decreasing the TR-FRET signal. In the presence of an inhibitor, the amount of ADP formed by the kinase reaction is reduced, and the resulting intact antibody tracer interaction results in a high TR-FRET signal. ADP formation is determined by calculating the emission ratio from the assay well.

2.3.6. Molecular Modeling. 2.3.6.1. Computational Docking. The final compounds were docked into the active site of a PI3Kα protein. The three-dimensional (3D) coordinates of PI3Kα protein with known co-crystallized triazine inhibitor (PQR530) were retrieved from Protein Data Bank (PI3Kα, PDB code: 6OAC, resolution: 3.15 Å). First, the protein was prepared by adding hydrogen atoms using Biovia Discovery Studio software. The protein was cleaned, prepared, and repaired by adding missing atoms, correcting connectivity and names, and inserting missing loops. Second, the active site was

defined around the co-crystallized ligand (PQR530) using the (From Current Selection) option of the (Define and Edit Binding Site) tool in Biovia Discovery Studio 2021, as shown in Figure 2. The co-crystallized ligand (PQR530) was removed from the binding site for docking validation. The ligand was re-docked using the LibDock algorithm, and the root mean square deviation (RMSD) was calculated to validate and assess pose similarity between the ligand poses concerning the original pose of the ligand. The RMSD value for the pose with the highest LibDock scores in comparison to the co-crystallized pose was 0.67 (less than 2), which is acceptable. The final triazine compounds bearing sulfapyridine or sulfaguanidine moiety in their structures were docked into the binding site of the selected PI3Kα protein using the default LibDock algorithm.

2.3.6.2. Toxicity Prediction Using ED_{50} Daphnia Calculations. Toxicity prediction studies were performed using software suites implemented in Discovery Studio 4.5 from Biovia, Inc. (San Diego, California). Structures were drawn by ChemDraw Ultra 7.0 [Cambridge Soft Corp. (http://www.cambridgesoft.com)].

2.3.6.3. Data Set. The structures of 38 triazine derivatives (Table 1) were analyzed using the default parameters in Biovia 4.5 using the TOPKAT toxicity function after the addition of all parameters (a detailed PDF report has been attached as a Supporting Information).

3. RESULTS AND DISCUSSION

3.1. Chemistry. The final trisubstituted triazine derivatives, shown in Table 1, were synthesized via nucleophilic substitution of the chlorine atoms of cyanuric chloride with different amines as reported by Daoud and Taha.[30] The reactivity of cyanuric chloride toward nucleophilic substitution is reduced when it is substituted.[31] In general, the first chlorine atom of unsubstituted cyanuric chloride is substituted at a very low temperature (less than 5 °C). The second chlorine atom of mono-substituted triazine could be replaced at low temper-ature to room temperature depending on the reactivity of the nucleophile. The third chlorine atom of the disubstituted triazine is generally replaced at higher temperatures (more than 60 °C).[32]

In this study, compounds **1** and **2** were first synthesized by reacting sulfapyridine and sulfaguanidine with cyanuric chloride at low temperatures (0−5 °C), respectively. Sulfapyridine and sulfaguanidine were chosen to react with cyanuric chloride since the reactivity of sulfapyridine and sulfaguanidine as a nucleophile is less than the other selected amines because of the electron-withdrawing effect of the para SO_2 group. In the second step, the sulfonamide−triazine compounds, **1** and **2**, were then reacted with different amines (aniline, benzylamine, cyclohexyl amine, cyclopropylamine, diethylamine, *m*-toluidine, 3,4-dimethylaniline, and morpho-line) at 0−25 °C to give compounds **3−18** in acceptable yields. In the final substitution reaction, compounds **3−18** were then reacted with the most selected reactive amine, diethylamine, at reflux condition to give final compounds **19−34**. The final compounds **35−38** were synthesized by solvent-free/neat fusion method.[33] The reactivity of the selected substituted anilines as a nucleophile is highly reduced because of the electron-withdrawing effect of the halogens and nitro group of the anilines. Because of the weak nucleophilicity of the substituted anilines, the nucleophilic substitution of the mono- and disubstituted sulfonamide−triazine compounds

Table 2. Anticancer Activities of Compounds (19−34) and (35−38) against MCF-7 and A549 Cancer Cell Lines Using MTT Assay

| | | experimental % of inhibition/IC_{50} | | | | | | |
| | | MCF-7 | | | A549 | | | |
compound	100 μM	IC_{50} (μM)	R_2	hill slope	100 μM	IC_{50} (μM)	R_2	hill slope
19	36.9	>100			28.4	>100		
20	60.6	<100			31.3	>100		
21	41.5	>100			68	<100		
22	27.8	>100			59.7	<100		
23	55.5	<100			88.3	<100		
24	23.8	>100			66.3	<100		
25	15.5	>100			40.1	>100		
26	9.5	>100			39.6	>100		
27	98.1	17.5 ± 2.1	0.99	2.3	97.4	14.8 ± 0.5	0.98	2.1
28	99.5	21.1 ± 1.0	0.99	2.2	98.6	33.2 ± 0.7	0.99	3.5
29	99.9	18.4 ± 0.7	0.99	1.7	98.4	15.7 ± 0.9	0.99	2.0
30	28.3	>100			77.8	<100		
31	99.7	26.5 ± 2.5	0.98	2.1	97	27.4 ± 0.5	0.98	2.7
32	86.6	<100			99.5	28.5 ± 1.5	0.97	3.6
33	87.5	<100			97.8	27.5 ± 4.5	0.99	2.6
34	18.7	>100			7.9	>100		
35	74.5	21.1 ± 1.1	0.97	2.1	96.5	19.3 ± 3.5	0.99	2.6
36	35.7	>100			12.3	>100		
37	70	<100			99.8	23.4 ± 0.6	0.98	2.7
38	8.14	>100			95.1	22.4 ± 0.3	0.97	2.0
Ged[a]	66.4	13.1 ± 0.8	0.98	2.2	95.1	16.5 ± 2.2	0.96	2.7
Docx[b]	90	2.1 ± 0.2	0.97	2.0	93.6	16.6 ± 1.4	0.99	2.2

[a]Gedatolisib. [b]Doxorubicin.
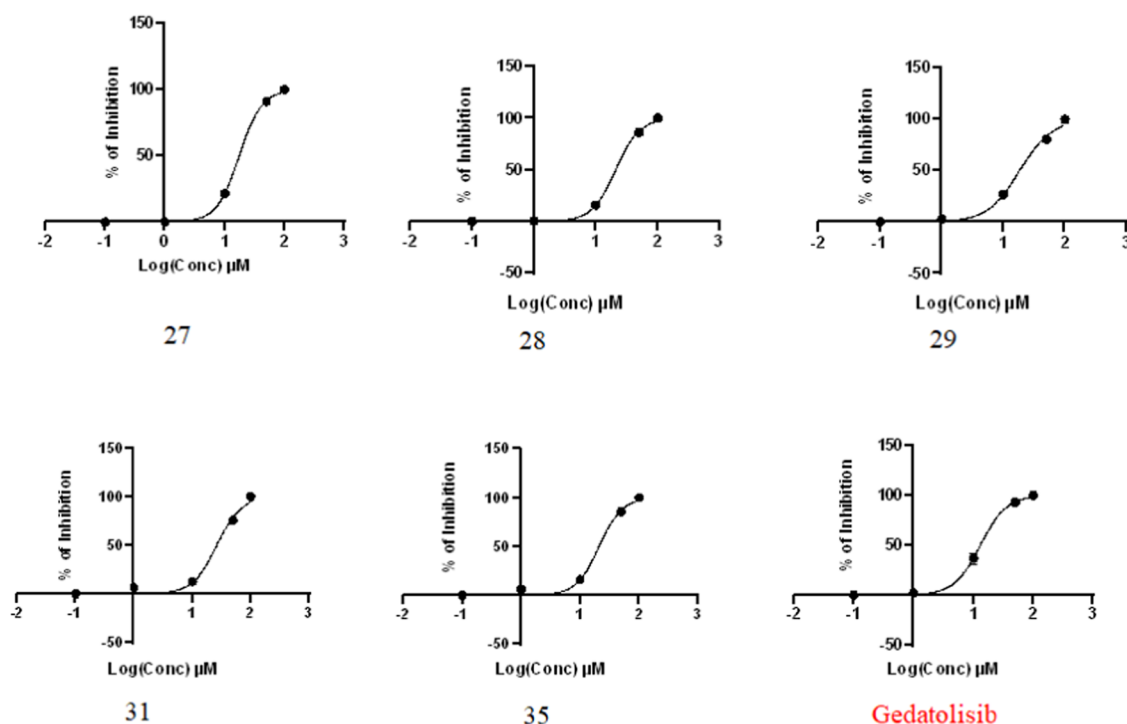
Figure 3. Percentage of inhibition vs log (concn) of the active compounds against MCF-7 cell line for the synthetic compounds **27−31**, **35**, and standard inhibitor (Gedatolisib).

with the halogenated anilines was unsuccessful using the conventional method at different temperatures from 25 to 110 °C using different high-boiling-point solvents. So, using the solvent-free/neat fusion method utilizing a higher-temperature reaction reaching 180 °C was successful to give compounds

35−38 in acceptable yields. Compounds **19−34** were recrystallized using the acetone/water antisolvent recrystallization method, whereas compounds **35−38** were recrystallized by the hot filtration method using ethanol as a solvent.
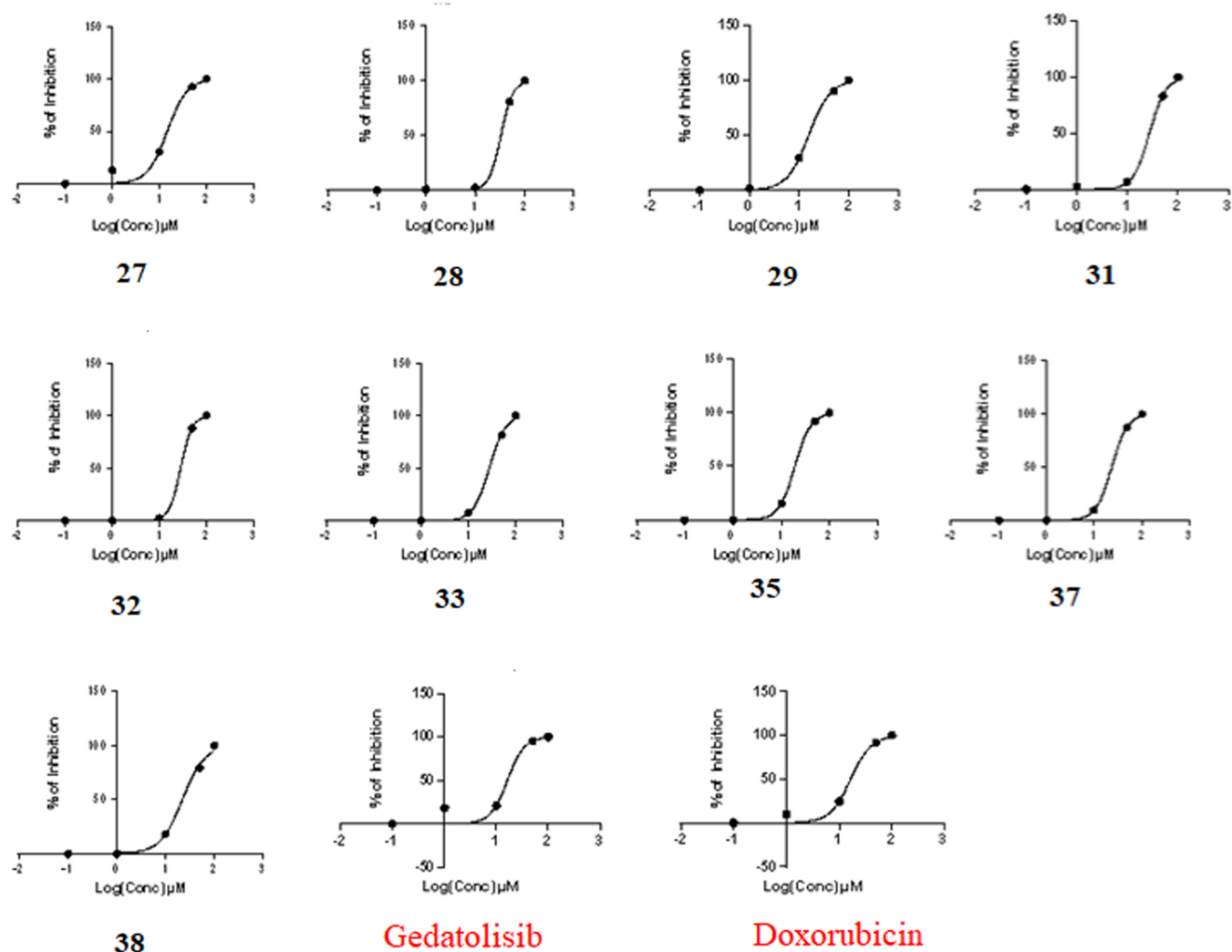
Figure 4. Percentage of inhibition versus log (conc) of the active compounds against A549 cell line for synthetic compounds **27−29**, **31−33**, **35**, **37**, **38**, and standard inhibitors Gedatolisib and Doxorubicin.

3.2. *In Vitro* Biological Studies.

The final compounds (**19−34**) and (**35−38**) were tested for their anticancer activity against MCF-7 and A549 cancer cell lines using an MTT assay.[56] Gedatolisib, a potent PI3Kα triazine inhibitor, and doxorubicin, one of the most effective and commonly used chemotherapeutic anticancer drugs, were used as positive standards for the MTT assay study. As shown in Table 2 and Figures 3 and 4, the most active compounds against MCF-7 and A549 cancer cell lines were compounds **27**, **28**, **29**, **31**, and **35**. It was noticed that most of the active compounds that displayed almost full inhibition against the selected cancer cell lines at 100 and 50 μM were bearing sulfaguanidine and diethylamine in their structures. The IC_{50} values of the most active aforementioned compounds were in a range between 14.8 and 33.2 μM, as shown in Table 2. The active compounds have a sulfaguanido group, diethyl amino group, and aryl amino group substituted with an electron withdrawal group, and further evaluation of QSAR analysis needs a larger number of compounds to derive an equation that correlates the physicochemical properties with the anticancer properties.

3.3. Computational Docking and PI3Kα Enzymatic Activity.

In computational docking, the ideal orientation and conformation for a small molecule to attach to a bigger receptor are anticipated to result in a stable complex molecule.[34] One of the most effective in silico strategies for predicting the interactions between chemicals and biological targets is molecular docking.[35] One program that leverages the properties of protein binding sites to direct docking is the LibDock algorithm.[36,37] The LibDock technique has four primary components: creating the ligand's conformations, identifying hot spots in terms of polar and apolar hot spots, matching the binding site image with the ligand, and lastly the optimization stage and scoring.[38] The effectiveness of docking to distinguish between active and inactive compounds can be significantly impacted by the protein used and the similarity between the co-crystallized ligand and the screened ligands.[39−42] With the aforementioned information in mind, the Protein Data Bank file (PDB code: 6OAC, resolution: 3.15 Å) was chosen for this. The document includes a triazine ligand and the PI3Kα protein co-crystallized (PQR530). By calculating the RMSD, the difference between the best-docked posture and the initial co-crystallized pose, it was possible to confirm the docking by choosing the best pose conformation based on the LibDock score (101.216). This resulted in a 0.67Å RMSD (less than 2Å). As illustrated in Figure 5, the co-crystallized ligand (PQR530) interacts with the PI3Kα enzyme's binding site amino acids through four hydrogen bonds: Lys 802 with the fluorine atom, Asp 810 with the NH2,
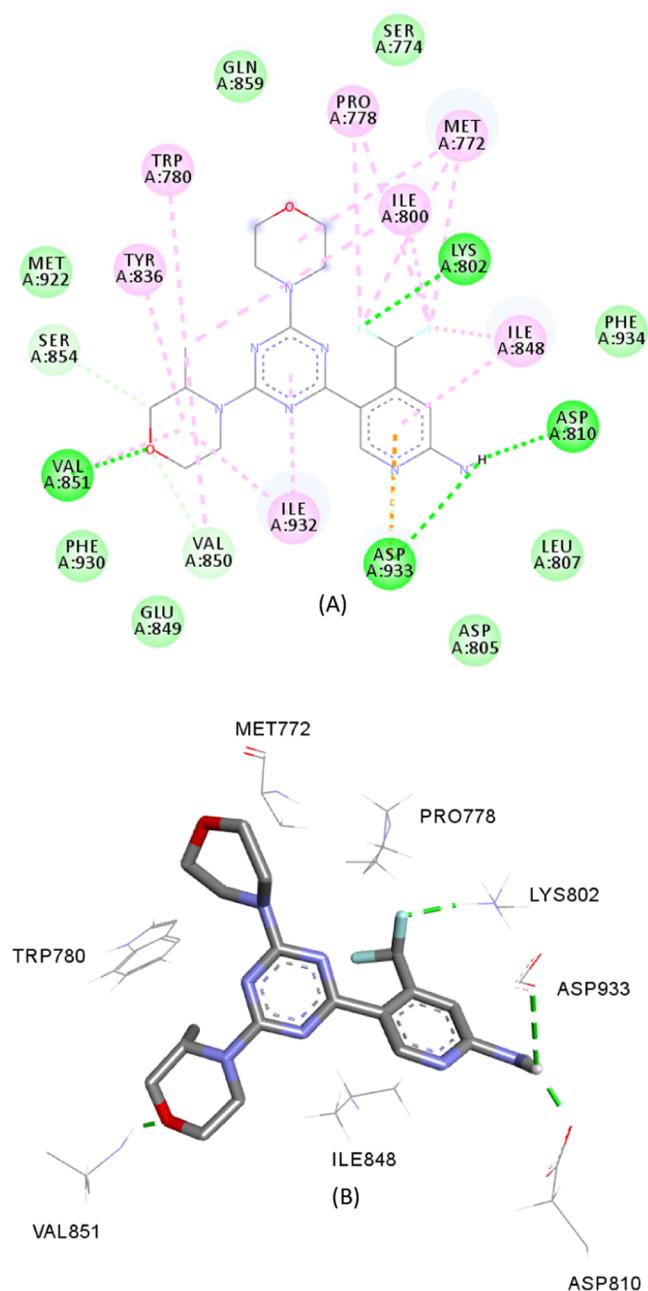
Figure 5. (A) Diagram of receptor−ligand interaction between the co-crystallized ligand (PQR530) and PI3Kα enzyme (PDB code: 6OAC). (B) A 3D diagram of receptor−ligand interaction between the co-crystallized ligand (PQR530) and PI3Kα enzyme (PDB code: 6OAC).

Asp 933 with the NH2, and Val 851 with the O-morpholine. These hydrogen bonds have an average length of 2.05 Å about Table 3, all of the final synthetic compounds had acceptable LibDock scores in comparison to the co-crystallized ligand, which has between one and five hydrogen bonds. This suggests that the selected compounds may bind to the PI3Kα enzyme.

Figure 6 illustrates how the most active substance (**27**; libDock score: 118.508) is predicted to interact with the PI3Kα protein's binding site via three hydrogen bonds: VAL 851 with N-triazine by a hydrogen bond of 2.03 Å, ASP 810 with NH−sulfaguanidine by a hydrogen bond of 2.57Å, and ASP 933 with SO2− sulfaguanidine by a hydrogen bond. We

Table 3. LibDock Score and Hydrogen Bonds of the Final Compounds into the Binding Site of the PI3Kα Enzyme

CPD	LibDock score	no. of H bonds	H bonds (CPD part-amino acid)	bond length (Å)
19	120.7	2	N-SER 854	2.82
			SO_2-VAL 851	1.92
20	137.6	1	NH-VAL 851	2.5
21	134.0	1	SO_2-VAL 851	2.22
22	133.1	3	SO_2-VAL 851	1.95
			NH-TYR 863	1.71
			NH-ASP 810	2.10
23	130.6	1	NH-VAL 851	2.58
24	131.7	1	NH-VAL 851	2.31
25	128.6	1	NH-VAL 851	1.90
26	127.3	1	N-SER 854	2.57
27	118.5	3	N-VAL 851	2.03
			NH-ASP 810	2.57
			SO_2-ASP 933	2.14
28	132.3	3	NH-VAL 851	2.35
			NH-VAL 851	2.35
			NH-GLU 849	2.92
29	127.1	3	NH-VAL 851	1.65
			SO_2-ASP 933	2.10
			NH-ASP 810	2.55
30	121.7	4	SO_2-VAL 851	1.96
			NH-ASP 810	2.48
			NH-SER 854	2.64
			NH-SER 854	2.28
31	117.0	3	NH-VAL 851	2.61
			SO_2-VAL 851	2.04
			N-ASP 933	2.51
32	121.5	4	NH-VAL 851	2.07
			N-VAL 851	1.95
			SO_2-ASP 933	2.18
			NH-ASP 810	2.61
33	115.2	2	NH-VAL 851	2.22
			NH-SER 854	3.06
34	121.5	1	SO_2-ASP 933	2.11
35	116.1	1	NH-ASP 805	1.91
36	111.9	5	NH-VAL 851	2.26
			NH-VAL 851	2.37
			NH-SER 854	2.84
			NH-ASN 853	2.40
			NH-GLU 849	2.89
37	115.4	3	SO_2-LYS 802	2.21
			NH-ASP 933	2.94
			NH-ASP 805	2.41
38	119.7	3	N-VAL 851	1.96
			SO_2-ASP 933	2.26
			NH-ASP 810	2.67
PQR530	101.2	4	O-VAL 851	1.76
			NH-ASP 933	2.92
			NH-ASP 810	1.90
			F-LYS 802	2.05

examined the synthetic compounds (**19−34**) and (**35−38**) for their anti-PI3Kα activity at 100 μM via Invitrogen Thermo Fisher Scientific Enzyme assay service.[55] Table 4 displays the inhibition percentage. The majority of the compounds displayed low to moderate levels of inhibition at 100 μM and were active. Since the site feature algorithm (LibDock) is primarily used to perform the rapid docking of combinatorial libraries of compounds to prioritize the selection of libraries
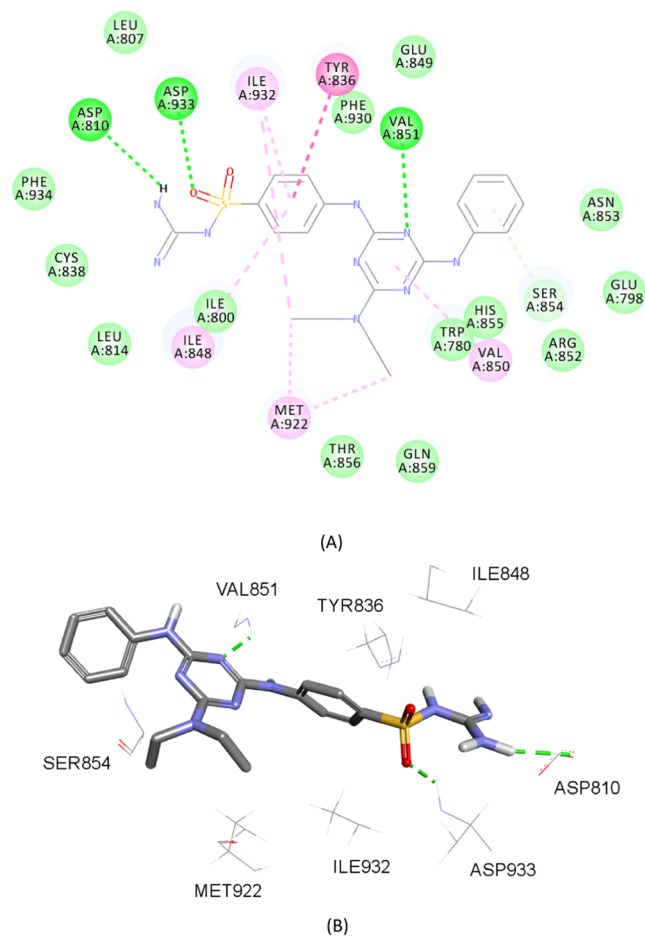
Figure 6. (A) Diagram of receptor−ligand interaction between compound (**27**) and PI3Kα enzyme (PDB code: 6OAC). (B) 3D diagram of receptor−ligand interaction between compound (**27**) PI3Kα enzyme (PDB code: 6OAC).

Table 4. Anti-PI3Kα Activity of Compounds (19−34) and (35−38)[a]

compound ID	% inhibition against PI3Kα enzyme ± SD[b]
19	28 ± 0.5
20	**46 ± 0.0**
21	23 ± 0.0
22	14 ± 1.0
23	37 ± 1.5
24	34 ± 4.0
25	18 ± 2.0
26	26 ± 2.0
27	24 ± 1.0
28	21 ± 2.5
29	29 ± 4.0
30	29 ± 3.0
31	20 ± 0.5
32	20 ± 0.5
33	24 ± 6.0
34	**68 ± 3.0**
35	21 ± 2.0
36	17 ± 5.0
37	25 ± 3.0
38	18 ± 3.0
Doxorubicin	95 ± 3.0%

[a]IC_{50} measured = 16nM. [b]% inhibition at 100 μM.

glucosidase inhibition,[53] sulfonamide derivatives may act as anticancer through tubulin polymerization inhibition,[54] and further chemical modification and exploration of various suggested mechanisms of anticancer activity are essential.

3.4. *Daphnia Magna* EC_{50}. The original citations for data in this TOPKAT model were obtained from the AQUIRE database. Each citation was then read to determine the reported values for *Daphnia magna* EC_{50}. The model was developed from 48 h assays. Results from assays on volatile chemicals that were performed in open beakers were not used. For those compounds for which there were multiple assay values, the median of the available values was used.

The acute aquatic toxicity model predicts the effective concentration of a substance that causes adverse effects on 50% (EC_{50}) of the test population *Daphnia magna* within a designated period.[57,58]

According to the results in Table 5, it is found that compound **34**, which is the most active compound in the MTT, is not carcinogenic with ED_{50} = 5.7 mg/L; however, low Daphnia toxicity indicates draggability and low toxicity of the synthetic triazine derivatives.

TOPKAT computes a probable value of toxicity for a submitted chemical structure from a quantitative structure−toxicity relationship (QSTR) equation. The equation is linear in the structure descriptors. The coefficients are optimized during the development of the equation.

The product of a structure descriptors value and its corresponding coefficient is the descriptors' contribution to the probable toxicity. Contributions from the products may be either positive or negative; a positive contribution will increase the probability of the chosen property, whereas a negative contribution will decrease it.

Toxicity values are computed by summing the individual contributions. For assessing toxicity values such as LD_{50} or LC_{50}, this sum is transformed into a weight/weight unit (mg/kg) or a weight/volume unit (mg/L).

rather than rank ordering the compounds themselves, it has many drawbacks. One of these is the lack of correlation between the docking results and the *in vitro* enzymatic activity. The poor accuracy of predictions of protein−ligand affinity is another major problem in computational chemistry.[43] However, there are significant differences in how well different protein systems fulfill scoring functions, and predictions frequently have poor correlations with experimental evidence.[44−47]

The final triazine-sulfaguanidine derivatives may exercise their anticancer effect via means other than the inhibition of PI3Kα enzyme, according to the moderate activity against PI3Kα enzyme. In light of numerous research investigations that introduced numerous sulfanilamide−triazine compounds as anticancer medicines, another mode of action is therefore postulated. These aforementioned sulfanilamide−triazine hybrid compounds were found to exert their anticancer activity by inhibiting the tumor-associated carbonic anhydrases IX and XII as reported in refs 48, 49. Since reported sulfonamide−triazine derivatives were shown to be active against FAK enzyme as reported in ref 50, it is also hypothesized that the synthetic chemicals in this work could exert their antiproliferative impact by acting on focal adhesion kinase enzyme (FAK). However, more research is required to identify a different potential mode of action.[51,52] Triazine derivatives may exert anticancer properties through α-

Table 5. Toxicity and Carcinogenic Predicted Properties of Triazine Derivatives

compound no.	daphnia EC_{50} mg/L[a]	carcinogenicity[b]	compound no.	daphnia EC_{50} mg/L[a]	carcinogenicity
1	2.9	carcinogen	20	1.04	carcinogen
2	4.3	noncarcinogen	21	0.62	carcinogen
3	0.74	carcinogen	22	5.18	carcinogen
4	1.1	carcinogen	23	1.62	carcinogen
5	0.43	carcinogen	24	0.98	carcinogen
6	3.5	carcinogen	25	0.91	carcinogen
7	5.07	carcinogen	26	3.87	carcinogen
8	0.97	carcinogen	27	1.24	noncarcinogen
9	0.94	carcinogen	28	1.70	noncarcinogen
10	4.31	carcinogen	29	1.31	noncarcinogen
11	0.84	noncarcinogen	30	10.7	noncarcinogen
12	1.82	noncarcinogen	31	2.39	noncarcinogen
13	0.89	noncarcinogen	32	1.37	noncarcinogen
14	7.27	noncarcinogen	33	1.36	noncarcinogen
15	7.45	noncarcinogen	34	5.72	noncarcinogen
16	1.34	noncarcinogen	35	0.60	noncarcinogen
17	1.39	noncarcinogen	36	1.11	noncarcinogen
18	6.34	noncarcinogen	37	1.57	noncarcinogen
19	1.08	carcinogen	38	0.85	noncarcinogen

[a]EC50; effective concentration that kills 50% of daphnia. [b]TOPKAT_Rat_Male_FDA_None_vs_Carcinogen_Prediction.

All of the quantitative structure−toxicity relationship (QSTR) models with two-group classifications, such as carcinogens/noncarcinogens, are derived via two-group linear discriminant analysis. The discriminant function defines a linear combination of the descriptor variables that best separates the cases into two groups, in this case, carcinogen or noncarcinogen. The discriminant score is the value of this function for the particular compound. The more positive the discriminant, the closer to 1 the computed probability of toxicity will be. The more negative the discriminant, the closer to 0 (nontoxic) the computed probability of toxicity will be.

The discriminant score is the sum of the contributions from each descriptor. In stand-alone TOPKAT, if you enter smiles, then pick a model, then look in the "descriptor contribution" window, this is the sum of the contributions for each descriptor that is shown in the report window. The value shown in the descriptor contribution window is the value for the descriptor for that compound times the model coefficient. The coefficients by themselves are not available.

Since positive descriptors contribute to increased toxicity and negative descriptors contribute to decreased toxicity, a positive discriminant score would indicate a toxic compound (carcinogen, mutagen, etc.), while a negative discriminant score would indicate a nontoxic compound (noncarcinogen, nonmutagen, etc.) However, rather than the discriminant score, the computed probability should be used to determine toxicity. If it is between 0 and 0.29, the compound is nontoxic; if it is between 0.3 and 0.69, the result is indeterminate; and if the score is between 0.7 and 1, the compound is toxic.[57,58]

4. CONCLUSIONS

A novel set of sulfapyridine/sulfaguanidine−triazine hybrid compounds were successfully synthesized by employing aromatic nucleophilic replacement of cyanuric chloride with various amines via either solvent-free/neat fusion procedure or reflux with DMF solvent. The synthetic compounds were evaluated for anti-PI3Kα inhibitory, in addition to measuring antiproliferative properties against the cancer cell lines MCF-7 and A549. The final compounds containing sulfaguanidine and diethylamine in their structures 27, 28, 29, 31, and 35 were the most successful compounds against the cancer cell lines MCF-7 and A549. Their IC_{50} ranged between 14.8 and 33.2 μM. The most active compounds against the PI3Kα enzyme was compound 20 and 34 with the percentage of inhibition at 100 μM 46.0 and 68%, respectively. This suggests that triazine-sulfaguanidine-diethylamine derivatives are good lead compounds that may exert their anticancer properties via additional mechanisms besides PI3Kα. Compound 34, which is the most active compound, shows promising properties with low toxicity ED_{50} = 5.7 mg/L, predicted to be noncarcinogenic, suggesting that compound 34 could be a good lead for future chemical modifications.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01273.

Instrumental analysis charts for the synthetic compounds 19−38; IR, ^1H NMR, ^{13}C NMR, and LC−MS/MS spectra (PDF)

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