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*Research Article*

# PI3K Inhibitors of Novel Hydrazide Analogues Linked 2-Pyridinyl Quinazolone Scaffold as Anticancer Agents

**Ibrahim F. Zeid,[1] Neama A. Mohamed,[2] Nagy M. Khalifa ,[2,3] Emad M. Kassem,[2] Eman S. Nossier,[4] Ahmed A. Salman,[1] Khaled Mahmoud,[5] and Mohamed A. Al-Omar[3]**

[1]*Department of Chemistry, Faculty of Science, Menoufiya University, Menoufiya, Egypt*
[2]*Department of Therapeutical Chemistry, National Research Centre, Dokki, Cairo 12622, Egypt*
[3]*Pharmaceutical Chemistry Department, Drug Exploration & Development Chair (DEDC), College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*
[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo 11754, Egypt*
[5]*Department of Pharmacognosy, National Research Centre, Dokki, Cairo 12622, Egypt*

Correspondence should be addressed to Nagy M. Khalifa; nagykhalifa@hotmail.com

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A series of novel 2-(pyridin-4-yl)quinazolin-4(3H)-ones bearing different heterocycle cores as potential PI3K inhibitors have been synthesized and evaluated via the MTT assay for their antiproliferative properties against selected HePG-2, MCF-7, and HCT116 cancer cell lines. Among them, compound **9** displayed significant activity against HePG-2 ($IC_{50}$ = 60.29 ± 1.06 $\mu$M) comparable to doxorubicin as a reference anticancer drug ($IC_{50}$ = 69.60 ± 1.50 $\mu$M). Kinase inhibitory assessment of target products against PI3K and docking studies revealed the promising binding affinities which match with the binding mode of the ligand, SW13 towards the active site of PI3K. Therefore, this work represents a promising matrix for developing novel potential anticancer candidates.

## 1. Introduction

Recently, cancer is considered as the second leading reason for dying. It is characterized by fast, uncontrolled, and pathological proliferation of abnormal cells. Almost 12 million deaths will be from cancer in 2030, according to statistics from the World Health Organization (WHO) [1, 2]. The phosphatidylinositol-3-kinase (PI3K) signaling pathway is a crucial signaling pathway, which plays a substantial role in the regulation of numerous cellular processes, such as cell growth, proliferation, differentiation, motility, and survival. Disturbance in regulation of this signal transduction way is related to the development of many cancers [3, 4]. Therefore, a great attention in the treatment of malignant tumors has been regarded to the compounds that inhibit the PI3K signal transduction pathway. In addition, quinazolinone derivatives constitute an important class of biologically active compounds. This heterocycle core has been associated with a broad spectrum of pharmacological properties as an anticancer agent [5]. Idelalisib derivatives (Figure 1) are known to contain quinazoline rings and are effective in the treatment of different kinds of cancer diseases via inhibition on PI3K. Furthermore, substituted quinazoline derivatives displayed various medicinal applications including anticholinesterase [6], anticonvulsant [7], anti-HIV [8], antidiabetic [9], anti-inflammatory [10], antihypertensive [11], antimalarial [12], antimicrobial [13], dihydrofolate reductase inhibition [14], antitubercular [15], antitumor [16], cellular phosphorylation inhibition [17], and kinase inhibitory activities [18]. Considering the much broader range of pharmacological effects, a vast number of synthetic approaches have been developed to prepare various quinazolinone compounds [19, 20]. Among them, quinazolin-4(3H)-one derivatives represent an attractive scaffold for

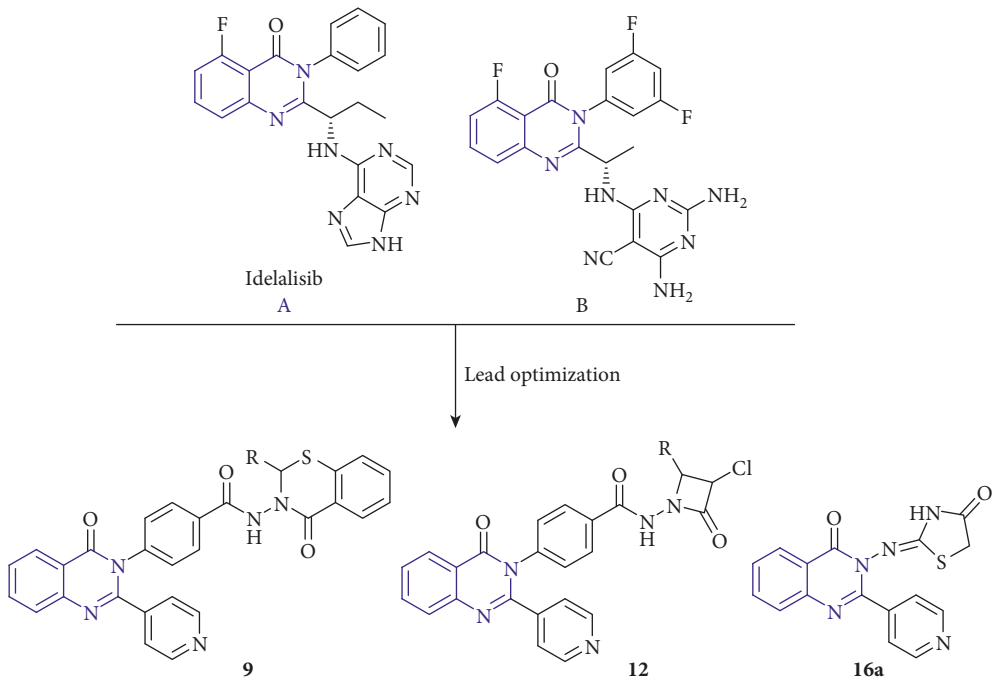



Figure 1: Structures of quinazoline-based PI3K inhibitors and synthesized quinazolines **9**, **12**, and **16a** as cytotoxic agents.

designing interesting anticancer drugs [21, 22], and they have attracted more interest because of their inhibitory activity for PI3K [23]. In our current study, we incorporate different substitutions at p-3 of a quinazolin-4(3H)-one scaffold to develop novel anticancer agents targeting PI3Ks.

## 2. Experimental

### 2.1. Chemistry

*2.1.1. Materials and Methods.* Electrothermal capillary apparatus was used for recording the melting points of all products and are uncorrected. Elemental analyses were found within ±0.4% of the theoretical values. Jasco FT/IR-6100 was used for measuring infrared spectra using potassium bromide pellets. Joel 270 MHz and Jeolsx 500 MHz spectrometers were used for recording NMR spectra downfield from TMS as a reference. Jeol JMS-AX 500 was used to determine mass spectra at 70 eV. TLC silica gel $F_{254}$ precoated sheets followed the reactions of all the new products.

*2.1.2. Ethyl 4-(4-Oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl) benzoate (**1**).* An equimolar mix (0.01 mol) of the starting compound 2-(pyridin-4-yl)-4H-benzo[*d*][1,3]oxazin-4-one and ethyl 4-aminobenzoate was fused in sand bath at 150°C for 45 min. The crude mass formed on cooling was collected and crystallized from ethanol twice to furnish reddish brown crystals in 80% yield.

*2.1.3. 4-(4-Oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)benzohydrazide (**2**).* In 30 mL of absolute ethyl alcohol, a mixture of benzoate derivative (0.01 mol) and hydrazine hydrate (0.04 mol) was heated under reflux for 4 h (followed by TLC). The product created on cooling collected and crystallized from AcOH. Yield 80%; mp 220–222°C; IR (KBr, $cm^{-1}$): 3438, 3315 ($NH_2$), 3236 (NH), and 1688 (CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.10–7.84 (m, 12H, ArH), 9.81(s, 2H, $NH_2$), and 10.65 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 116.78, 117.98, 123.86, 123.98, 126.65, 128.21, 128.29, 129.84, 131.51, 134.21, 139.92, 145.79, 149.86, 159.55, 160.70, and 165.98; MS (EI, 70 eV): *m*/*z* 357 ($M^+$, 10); anal. calcd. for $C_{20}H_{15}N_5O_2$ (357.37): C, 67.22; H, 4.23; and N, 19.60. Found: C, 67.17; H, 4.12; and N, 19.52.

*2.1.4. 1-(4-Oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)-3-substituted Thiourea (**3a, 3b**).* In dry ethyl alcohol (30 mL), an equimolar mix (0.01 mol) of the starting compound 2-(pyridin-4-yl)-4H-benzo[*d*][1,3]-oxazin-4-one and substituted thiosemicarbazide were heated for 2–4 h. After completion of the reaction, the residue obtained was filtered and crystallized from ethyl alcohol.

*(1) 1-(4-Oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)thiourea (**3a**).* Yield 92%; mp > 300°C; IR (KBr, $cm^{-1}$): 3380, 3280 ($NH_2$), 3140 (NH), 1693 (CO), and 1273 (C=S); $^1$H NMR (DMSO-$d_6$): $\delta$ 6.81–7.93 (m, 8H, ArH), 5.80 (s, 2H, $NH_2$), and 10.30 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 119.86, 121.14, 123.01, 127.38, 129.10, 134.02, 137.21, 146.79, 151.18, 161.01, 163.12, and 184.11; MS (EI, 70 eV): *m*/*z* 297 ($M^+$, 7); anal. calcd. for $C_{14}H_{11}N_5OS$ (297.34): C, 56.55; H, 3.73; and N, 23.55. Found: C, 56.23; H, 3.51; and N, 23.62.

*(2) 1-(4-Oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)thiourea (**3b**).* Yield 80%; mp 238–240°C; IR (KBr, $cm^{-1}$): 3190, 3165 (2NH), 1700 (CO), and 1278 (C=S); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.01–7.98 (m, 13H, ArH), 10.42, and 10.90 (2S, 2H, 2NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 119.97, 120.88, 122.16, 123.83, 125.74, 127.31,

128.43, 129.02, 132.90, 136.54, 137.13, 146.92, 149.24, 161.01, 163.42, and 182.20; MS (EI, 70 eV): $m/z$ 373 ($M^+$, 26); anal. calcd. for $C_{20}H_{15}N_5OS$ (373.43): C, 64.33; H, 4.05; and N, 18.75. Found: C, 64.21; H, 3.98; and N, 18.63.

*2.1.5. 1-(4-(4-Oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)benzoyl) pyrazolidine-3,5-dione (**4**).* Refluxing a mixture of compound **2** (0.002 mol), with diethylmalonate in (15 mL) of acetic acid for 8 h, (followed by TLC). The precipitated product formed on cooling was crystallized from ethyl alcohol. Yield 70%; mp 130–132°C; IR (KBr, $cm^{-1}$): 3334 (NH), 1682, and 1665 (3CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 3.20 (s, 2H, $-CH_2$), 7.31–7.88 (m, 12H, ArH), and 9.92 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 42.66, 117.70, 118.09, 119.35, 122.66, 123.83, 124.58, 127.75, 130.54, 130.75, 131.73, 134.53, 135.95, 140.47, 140.54, 150.40, 152.33, 153.07, 160.13, 160.59, 163.18, 172.04, and 174.28; MS (EI, 70 eV): $m/z$ 425 ($M^+$, 15); anal. calcd. for $C_{23}H_{15}N_5O_4$ (425.4): C, 64.94; H, 3.55; and N, 16.46. Found: C, 64.51; H, 3.34; and N, 16.55.

*2.1.6. N-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4-(4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)benzamide (**5**).* In 15 mL of acetic acid, an equimolar amount (0.002 mol) of starting **2** and maleic anhydride was heated for 8 h (followed by TLC). The product formed was collected and crystallized from isopropanol. Yield 63%; mp 248–250°C; IR (KBr, $cm^{-1}$): 3212 (NH), 1701, and 1688 (3CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.10–8.03 (m, 12H, ArH and maleimide-H) and 10.23 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 117.34, 117.70, 118.09, 118.90, 119.35, 122.66, 123.83, 124.58, 127.75, 130.75, 131.73, 131.83, 134.53, 134.57, 135.95, 140.16, 140.47, 140.54, 150.40, 153.07, 160.29, 160.59, 163.18, 172.04, and 174.28; MS (EI, 70 eV): $m/z$ 437 ($M^+$, 4); anal. calcd. for $C_{24}H_{15}N_5O_4$ (437.41): C, 65.90; H, 3.46; and N, 16.01. Found: C, 65.72; H, 3.28; and N, 15.89.

*2.1.7. 2-(Pyridin-4-yl)-3-(4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)quinazolin-4(3H)-one (**6**).* Equimolar amounts (0.001 mol) of hydrazide derivative **2** and potassium hydroxide with (1 mL) of carbon disulfide in ethyl alcohol (20 mL) was heated for 12 h. The excess solvent was evaporated, followed by neutralization with dil. HCl, and the solid separated was crystallized from isopropanol. Yield 82%; mp 165–167°C; IR (KBr, $cm^{-1}$): 3400 (NH), 1689 (CO), and 1260 (C=S); $^1$H NMR (DMSO-$d_6$): $\delta$ 6.90–7.85 (m, 12H, ArH), and 9.97 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 114.25, 115.22, 115.33, 116.26, 116.79, 118.00, 121.59, 124.12, 126.67, 128.28, 129.84, 131.55, 134.28, 139.99, 149.91, 151.74, 156.62, 159.25, 159.62, 163.41, and 189.84; MS (EI, 70 eV): $m/z$ 399 ($M^+$, 82); anal. calcd. for $C_{21}H_{13}N_5O_2S$ (399.43): C, 63.15; H, 3.28; and N, 17.53. Found: C, 62.96; H, 3.14; and N, 17.34.

*2.1.8. 5-Amino-1-(4-oxo-2-(pyridin-4-yl)-3,4-dihydroquinazoline-3-carbonyl)-1H-pyrazole-4-carbonitrile (**7**).* A solution of compound **2** (0.001 mol) in 20 mL ethyl alcohol and ethoxymethylene malononitrile (0.001 mol) was heated for 4 h (reaction progress followed by TLC). The residue obtained was crystallized from ethanol. Yield 95%; mp 170–172°C; IR (KBr, $cm^{-1}$): 3423, 3342 ($NH_2$), and 2197 (C≡N), 1684 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 4.61 (s, 2H, $NH_2$) and 7.61–8.35 (m, 9H, ArH + pyrazole-H); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 81.50, 114.65, 116.90, 118.00, 124.29, 125.58, 126.08, 126.76, 129.54, 129.71, 130.85, 131.59, 134.43, 140.96, 150.08, 154.10, 159.65, and 167.49; MS (EI, 70 eV): $m/z$ 433 ($M^+$, 4); anal. calcd. for $C_{24}H_{15}N_7O_2$ (433.42): C, 66.51; H, 3.49; and N, 22.62. Found: C, 66.37; H, 3.31; and N, 22.49.

*2.1.9. N′-(4-Hydroxy-3-methoxybenzylidene)-4-(4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)benzohydrazide (**8**).* In 25 mL of ethyl alcohol, a mixture of compound **2** (0.002 mol) and vanillin aldehyde (0.002 mol) were heated for 6 h, and the precipitate created was crystallized from ethanol. Yield 76%; mp 195–197°C; IR (KBr, $cm^{-1}$): 3426 (OH), 3216 (NH), 1701, and 1674 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 3.80 (s, 3H, $OCH_3$), 6.99–7.83 (m, 15H, ArH), 8.20 (s, 1H, CH=N), 10.11 (s, 1H, NH), and 11.74 (s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.58, 113.86, 113.94, 114.15, 116.75, 117.96, 124.20, 125.37, 125.84, 126.44, 127.98, 128.07, 128.50, 128.71, 128.81, 129.05, 129.64, 129.84, 131.62, 133.53, 134.38, 136.28, 141.20, 151.93, 155.92, 159.23, 159.45, and 166.70; MS (EI, 70 eV): $m/z$ 491 ($M^+$, 4); anal. calcd. for $C_{28}H_{21}N_5O_4$ (491.50): C, 68.42; H, 4.31; and N, 14.25. Found: C, 68.25; H, 4.16; and N, 14.09.

*2.1.10. N-(2-(3-Hydroxy-4-methoxyphenyl)-4-oxo-2H-benzo[e][1,3]thiazin-3(4H)-yl)-4-(4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)benzamide (**9**).* In benzene (10 mL), 0.001 mol of derivative **8** and 0.002 mol of thiosalicylic acid was heated for 8 h (followed by TLC). The residue formed was collected and crystallized from ethanol. Yield 70%; mp 145–147°C; IR (KBr, $cm^{-1}$): 3388 (OH), 3210 (NH), 1689, and 1674 (3CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 3.85 (s, 3H, $OCH_3$), 4.25 (s, 1H, thiazine-H), 6.93–8.06 (m, 19H, ArH), 9.15 (s, 1H, NH), and 11.40 (s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.63, 114.05, 114.40, 114.45, 114.50, 116.60, 117.38, 118.58, 121.72, 124.49, 125.33, 126.82, 128.53, 129.06, 129.17, 129.26, 130.42, 130.62, 130.90, 131.27, 131.59, 131.70, 131.81, 132.13, 132.19, 134.41, 134.56, 136.87, 140.70, 141.20, 151.38, 159.93, 161.51, 163.21, and 164.23; MS (EI, 70 eV): $m/z$ 627 ($M^+$, 7); anal. calcd. for $C_{38}H_{25}N_5O_5S$ (627.67): C, 66.97; H, 4.01; and N, 11.16. Found: C, 66.83; H, 3.91; and N, 10.96.

*2.1.11. N-(2-(3-Methoxy-4-hydroxyphenyl)-4-oxothiazolidin-3-yl)-4-(4-oxo-2-(pyridin-4-yl)-quinazolin-3-(4H)-yl)benzamide (**10**).* In benzene (10 mL), a mixture of benzylidene derivative **8** (0.001 mol) and thioglycolic acid (0.002 mol) were refluxed for 8 h (reaction progress followed by TLC). The residue obtained was collected and crystallized from isopropanol. Yield 60%; mp 253–255°C; IR (KBr, $cm^{-1}$): 3413 (OH), 3147 (NH), 1709, and 1675 (3CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 2.60 (s, 2H, $-CH_2$), 3.85 (s, 3H, $OCH_3$), 6.85–8.10 (m, 16H, ArH, and CH-thiazolidinone), 9.45 (s, 1H, NH), and 10.33 (s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 39.34, 55.59, 55.63, 104.60, 114.05, 114.50, 115.59, 116.60, 116.85, 117.06, 117.20, 118.28, 119.25, 122.97, 125.00, 126.60, 128.88,

130.06, 130.45, 131.84, 134.58, 147.52, 151.38, 156.70, 158.62, 159.93, 161.51, 163.21, and 164.23; MS (EI, 70 eV): $m/z$ 565 ($M^+$, 8). anal. calcd. for $C_{30}H_{23}N_5O_5S$ (565.6): C, 63.71; H, 4.10; and N, 12.38. Found: C, 63.54; H, 3.92; and N, 12.20.

*2.1.12. N-(2-(3-Methoxy-4-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-(4-oxo-2-(pyridin-4-yl)-quinazolin-3-(4H)-yl)benzamide (**11**).* In dioxane (5 mL), compound **8** (0.001 mol) and triethylamine (0.002 mol) were dissolved and cooled. To this cooled solution, acetyl chloride (0.001 mol) was added slowly at 0°C and stirring continued for 24 h and set aside for 48 h at room temperature. The formed solution was concentrated, and then the obtained product was poured onto ice water. The residue formed was washed with water and crystallized from ethyl alcohol. Yield 60%; mp > 300°C; IR (KBr, $cm^{-1}$): 3397 (OH), 3154 (NH), 1670, and 1661 (3CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 2.21–3.09 (dd, 2H, $-CH_2$), 3.82 (s, 3H, $OCH_3$), 4.36 (t, 1H, CH-azetidine), 6.93–8.06 (m, 15H, ArH), 9.66 (s, 1H, NH), and10.26 (s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 42.65, 55.65, 55.68, 114.38, 114.76, 117.70, 118.09, 119.35, 122.66, 123.83, 124.58, 127.08, 127.75, 130.51, 131.73, 134.53, 135.95, 140.16, 140.54, 150.40, 152.33, 153.07, 160.29, 163.18, 165.10, 172.04, and 184.98; MS (EI, 70 eV): $m/z$ 533 ($M^+$, 11); anal. calcd. for $C_{30}H_{23}N_5O_5$ (533.53): C, 67.53; H, 4.35; and N, 13.13. Found: C, 67.34; H, 4.19; and N, 12.95.

*2.1.13. N-(3-Chloro-2-(3-methoxy-4-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-(4-oxo-2-(pyridin-4-yl) quinazolin-3(4H)-yl)benzamide (**12**).* Chloroacetyl chloride (0.012 mol) in dioxane was added portionwise below 10°C to a well-stirred solution of compound **8** (0.001 mol) and triethylamine (0.003 mol). The mixture was stirred for 6 h, and then the excess of dioxane was evaporated in vacuum, and the precipitate formed was poured into ice water. The resulting product was collected and crystallized from ethyl alcohol. Yield 61%; mp 270–272°C; IR (KBr, $cm^{-1}$): 3399 (OH), 3214 (NH), 1670, and 1658 (3CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 2.44 (d, 1H, CH-azetidine), 3.38 (d, 1H, HC-Cl), 3.89 (s, 3H, $OCH_3$), 7.12–7.98 (m, 15H, ArH), 9.95 (s, 1H, NH), and 11.20 (s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.54, 55.71, 64.99, 114.40, 114.46, 115.02, 115.59, 116.85, 117.06, 117.20, 118.28, 119.25, 122.97, 125.00, 126.60, 128.88, 130.06, 130.45, 131.84, 134.58, 140.77, 147.52, 150.46, 156.70, 158.62, 159.29, 159.77, 160.28, 163.45, and 171.07; MS (EI, 70 eV): $m/z$ 569 ($M^+$ +2); anal. calcd. for $C_{30}H_{22}ClN_5O_5$ (567.98): C, 63.44; H, 3.90; and N, 12.33. Found: C, 63.26; H, 3.73; and N, 12.21.

*2.1.14. ((5-Acetyl-4-Substituted thiazol-2(3H)-ylidene) amino)-2-(pyridin-4-yl)quinazolin-4(3H)-ones (**13a, 13b**).* In dry ethanol (20 mL), a mixture of thiosemicarbazone derivatives **3a** and **3b** (0.002 mol), 3-chloro-2,4-pentadione (0.003 mol), and (0.003 mol) of sodium acetate were heated for 10–14 h (followed by TLC). The product obtained was crystallized from ethyl alcohol.

*(1) 3-((5-Acetyl-4-methylthiazol-2(3H)-ylidene)amino)-2-(pyridin-4-yl)quinazolin-4(3H)-one (**13a**).* Yield 66%; mp > 300°C; IR (KBr, $cm^{-1}$): 3204 (NH), 1715, and 1686 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 2.13 (s, 3H, $CH_3$-thiazole), 2.60 (s, 3H, $COCH_3$), 6.93–7.55 (m, 8H, ArH), and 9.95 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 13.32, 25.47, 101.45, 119.79, 120.99, 121.81, 126.78, 128.63, 133.28, 135.90, 143.17, 145.72, 149.38, 156.93, 159.75, 163.52, and 184.76; MS (EI, 70 eV): $m/z$ 377 ($M^+$, 45); anal. calcd. for $C_{19}H_{15}N_5O_2S$ (377.42): C, 60.46; H, 4.01; and N, 18.56. Found: C, 60.31; H, 3.89; and N, 18.38.

*(2) 3-((5-Acetyl-4-methyl-3-phenylthiazol-2(3H)-ylidene) amino)-2-(pyridin-4-yl)quinazolin-4(3H)-one (**13b**).* Yield 77%; mp 296–298°C; IR (KBr, $cm^{-1}$): 1718 and 1698 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 2.35 (s, 3H, $CH_3$-thiazole), 2.62 (s, 3H, $COCH_3$), and 7.01–7.92 (m, 13H, ArH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 13.09, 25.60, 114.28, 115.03, 115.13, 115.23, 116.13, 116.87, 118.07, 124.04, 126.72, 128.40, 128.89, 129.86, 131.57, 131.78, 133.35, 134.28, 139.99, 149.98, 151.99, 154.03, 156.30, 159.63, 163.46, and 184.10; MS (EI, 70 eV): $m/z$ 453 ($M^+$, 30); anal. calcd. for $C_{25}H_{19}N_5O_2S$ (453.52): C, 66.21; H, 4.22; and N, 15.44. Found: C, 66.09; H, 4.12; and N, 15.26.

*2.1.15. 5-Methyl-2-((4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl) imino)-3-substituted thiazolidin-4-ones (**14a, 14b**).* In dry ethanol (20 mL), equimolar mixture (0.002 mol) of derivatives **3a** and **3b**, ethyl 2-bromopropanoate, and anhydrous sodium acetate were refluxed for 6–10 h (followed by TLC). The resulting solid was washed with water and crystallized from ethyl alcohol.

*(1) 5-Methyl-2-((4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)imino)thiazolidin-4-one (**14a**).* Yield 62%; mp > 300°C; IR (KBr, $cm^{-1}$): 3140 (NH), 1702, and 1685 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.60 (d, 3H, $CH_3$), 4.91 (q, 1H, thiazolidinone), 7.12–7.93 (m, 8H, ArH), and 10.41 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 20.64, 43.12, 114.81, 117.43, 117.60, 118.66, 124.45, 127.05, 129.31, 130.13, 131.83, 134.57, 134.87, 140.53, 152.88, 160.24, and 171.70; MS (EI, 70 eV): $m/z$ 351 ($M^+$, 9); anal. calcd. for $C_{17}H_{13}N_5O_2S$ (351.38): C, 58.11; H, 3.73; and N, 19.93. Found: C, 57.90; H, 3.56; and N, 19.84.

*(2) 5-Methyl-2-((4-oxo-2-(pyridin-4-yl)quinazolin-3(4H)-yl)imino)-3-phenylthiazolidin-4-one (**14b**).* Yield 70%; mp 283–285°C; IR (KBr, $cm^{-1}$): 1715 and 1699 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.52 (d, 3H, $CH_3$), 4.90 (q, 1H, thiazolidinone), and 7.01–7.96 (m, 13H, ArH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.60, 55.15, 115.13, 116.13, 116.87, 118.07, 124.04, 128.85, 129.86, 131.79, 133.35, 134.28, 139.99, 149.98, 151.99, 154.03, 156.30, 159.63, and 172.90; MS (EI, 70 eV): $m/z$ 427 ($M^+$, 10); anal. calcd. for $C_{23}H_{17}N_5O_2S$ (427.48): C, 64.62; H, 4.01; and N, 16.38. Found: C, 64.51; H, 4.13; and N, 16.29.

*2.1.16. 3-((4-Substituted thiazol-2(3H)-ylidene)amino)-2-(pyridin-4-yl)quinazolin-4(3H)-ones (**15a, 15b**).* In (20 mL) absolute ethanol, 0.005 mol of derivatives **3a** and **3b**, (0.005 mol) of phenacyl bromide, and 0.02 mol of anhydrous sodium acetate were refluxed for 4–6 h (followed by TLC).

The excess concentrated solvent was poured into ice, and the precipitate created was washed with water and crystallized from ethyl alcohol.

*(1) 3-((4-Phenylthiazol-2(3H)-ylidene)amino)-2-(pyridin-4-yl) quinazolin-4(3H)-one (**15a**).* Yield 87%; mp 288–290°C; IR (KBr, $cm^{-1}$): 3130 (NH) and 1698 (CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 6.52–7.43 (m, 14H, ArH and thiazole) and 11.18 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 117.43, 117.60, 118.00, 118.66, 121.36, 124.45, 127.05, 129.31, 130.13, 131.83, 134.57, 134.87, 140.53, 152.88, 160.24, and 166.70; MS (EI, 70 eV): $m/z$ 397 ($M^+$, 21); anal. calcd. for $C_{22}H_{15}N_5OS$ (397.45): C, 66.48; H, 3.80; and N, 17.62. Found: C, 66.29; H, 3.62; and N, 17.48.

*(2) 3-((3,4-Diphenylthiazol-2(3H)-ylidene)amino)-2-(pyridin-4-yl)quinazolin-4(3H)-one (**15b**).* Yield 70%; mp 297–299°C; IR (KBr, $cm^{-1}$): 1688 (CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 7.10–8.12 (m, 19H, ArH, and thiazole); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 104.56, 115.21, 119.24, 120.45, 121.09, 122.17, 125.87, 126.90, 127.62, 128.36, 128.74, 129.40, 132.29, 134.19, 137.05, 140.38, 145.21, 146.52, 150.26, 151.06, 161.01, and 162.31; MS (EI, 70 eV): $m/z$ 473 ($M^+$, 12); anal. calcd. for $C_{28}H_{19}N_5OS$ (473.55): C, 71.02; H, 4.04; and N, 14.79. Found: C, 70.98; H, 3.93; and N, 14.82.

*2.1.17. 3-(4-Oxo-3-substituted thiazolidin-2-ylideneamino)-2-(pyridin-4-yl)quinazolin-4(3H)-ones (**16a, 16b**).* In glacial acetic acid (20 mL), equimolar amounts (0.01 mol) of **3a** and **3b**, chloroacetic acid, and anhydrous sodium acetate were heated for 8–11 h (followed by TLC). The solid obtained on pouring onto ice water was crystallized from chloroform/per ether.

*(1) 3-(4-Oxothiazolidin-2-ylideneamino)-2-(pyridin-4-yl) quinazolin-4(3H)-one (**16a**).* Yield 70%; mp 252–254°C; IR (KBr, $cm^{-1}$): 3138 (NH), 1702, and 1685 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 4.07 (s, 2H, $CH_2$), 6.81–7.55 (m, 8H, ArH), and 10.21 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.61, 114.61, 118.67, 124.57, 125.31, 127.15, 129.43, 129.98, 143.27, 145.39, 150.13, 161.01, 163.35, and 172.15; MS (EI, 70 eV): $m/z$ 337 ($M^+$, 14); anal. calcd. for $C_{16}H_{11}N_5O_2S$ (337.36): C, 56.96; H, 3.29; and N, 20.76. Found: C, 56.85; H, 3.19; and N, 20.82.

*(2) 3-(4-Oxo-3-phenylthiazolidin-2-ylideneamino)-2-(pyridin-4-yl)quinazolin-4(3H)-one ( **16b**).* Yield 68%; mp 258–260°C; IR (KBr, $cm^{-1}$): 1701 and 1688 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 4.20 (s, 2H, $CH_2$) and 7.00–8.01 (m, 13H, ArH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 30.56, 120.17, 120.79, 121.36, 122.08, 123.95, 126.64, 128.72, 129.81, 133.12, 134.93, 136.44, 143.10, 146.20, 150.24, 161.01, 163.28, and 171.65; MS (EI, 70 eV): $m/z$ 413 ($M^+$, 11); anal. calcd. for $C_{22}H_{15}N_5O_2S$ (413.45): C, 63.91; H, 3.66; and N, 16.94. Found: C, 63.87; H, 3.69; and N, 17.01.

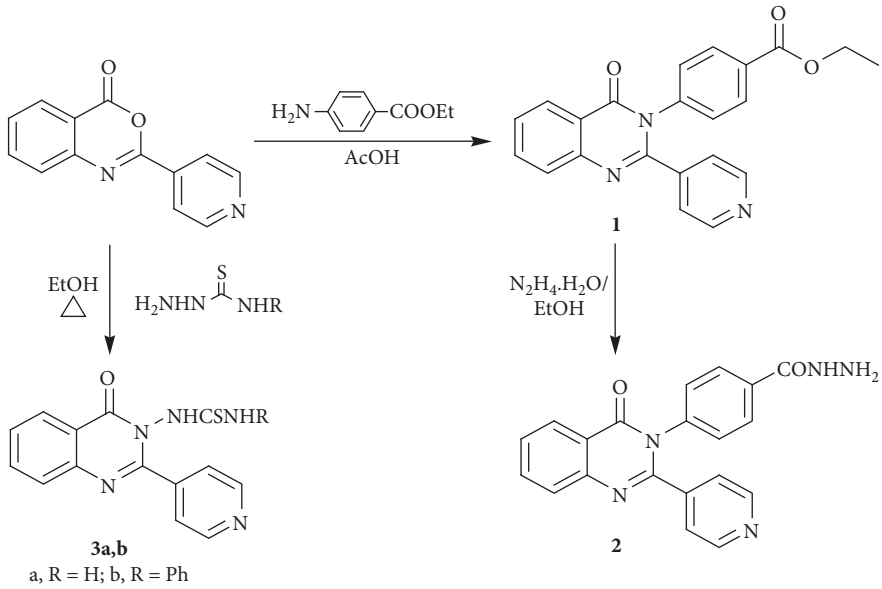
*2.1.18. 3-((Z)-5-(Substituted benzylidene)-4-oxothiazolidin-2-ylideneamino)-2-(pyridin-4-yl) quinazolin-4(3H)-ones (**17a–d**).* To a solution of glacial acetic acid (10 mL), a mix of 0.001 mol of derivatives **16a** and **16b**, 0.0015 mol of anhydrous sodium acetate, and 0.001 mol of aromatic aldehydes, namely, vanillin or 3,4,5-trimethoxybenzaldehyde were added and heated for 6–10 h (followed by TLC). The residue obtained on pouring onto ice was crystallized from ethyl alcohol.

*(1) 3-((Z)-5-(4-Hydroxy-3-methoxybenzylidene)-4-oxothiazolidin-2-ylideneamino)-2-(pyridin-4-yl)-quinazolin-4(3H)-one (**17a**).* Yield 70%; mp 235–237°C; IR (KBr, $cm^{-1}$): 3490 (br, OH), 3130 (NH), 1702, 1676 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 3.91 (s, 3H, $OCH_3$), 7.15–7.93 (m, 11H, ArH), 8.11 (s, 1H, olefinic CH=), 9.96 (s, 1H, NH), and 11.25 (s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.09, 113.26, 116.04, 117.30, 119.84, 121.14, 121.43, 122.19, 126.96, 128.69, 129.02, 132.79, 137.18, 143.28, 144.06, 145.67, 146.92, 149.37, 151.19, 161.01, 162.48, and 171.10; MS (EI, 70 eV): $m/z$ 471 ($M^+$, 18); anal. calcd. for $C_{24}H_{17}N_5O_4S$ (471.49): C, 61.14; H, 3.63; and N, 14.85. Found: C, 61.01; H, 3.69; and N, 14.30.

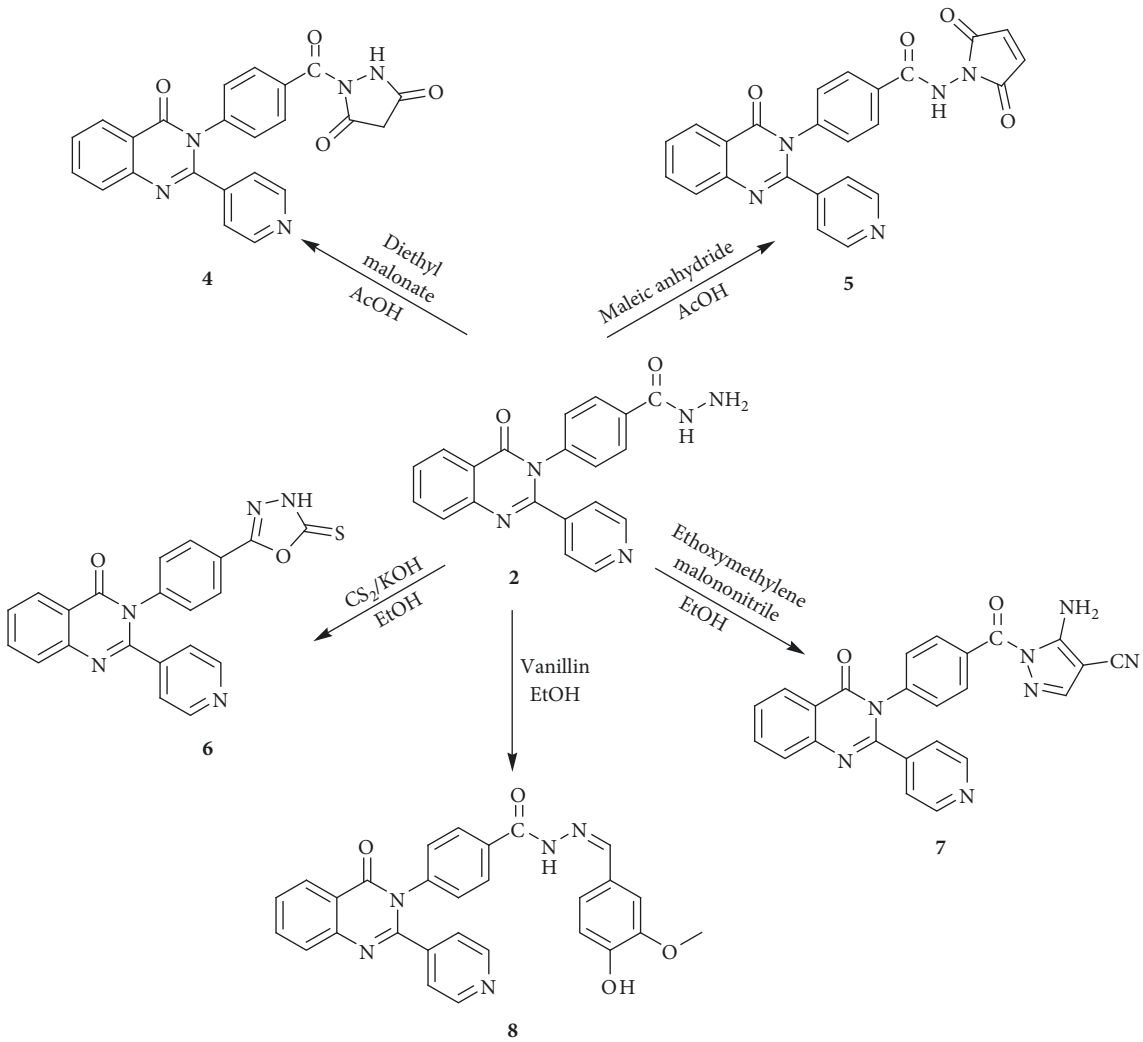
*(2) 3-((Z)-5-(3,4,5-Trimethoxybenzylidene)-4-oxothiazolidin-2-ylideneamino)-2-(pyridin-4-yl) quinazolin-4(3H)-one (**17b**).* Yield 68%; mp 240–242°C; IR (KBr, $cm^{-1}$): 3120 (NH), 1700, and 1685 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 3.87(s, 9H, $3OCH_3$), 7.09–7.98 (m, 10H, ArH), 8.12 (s, 1H, olefinic CH=), and 10.05 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.15, 55.66, 60.41, 114.29, 114.38, 115.07, 115.17, 116.18, 116.85, 118.06, 124.05, 124.32, 126.72, 128.38, 129.90, 131.58, 131.98, 134.28, 143.99, 149.97, 151.78, 154.03, 156.45, 159.62, 163.41, and 172.02; MS (EI, 70 eV): $m/z$ 515 ($M^+$, 11); anal. calcd. for $C_{26}H_{21}N_5O_5S$ (515.54): C, 60.57; H, 4.11; and N, 13.58. Found: C, 60.39; H, 3.96; and N, 13.42.

*(3) 3-((Z)-5-(3,4,5-Trimethoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylideneamino)-2-(pyridin-4-yl)quinazolin-4(3H)-one (**17c**).* Yield 72%; mp 245–247°C; IR (KBr, $cm^{-1}$): 1713 and 1699 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 3.90 (s, 9H, $3OCH_3$), 6.95–7.92 (m, 15H, ArH), and 8.20 (s, 1H, olefinic CH=); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.63, 113.75, 114.29, 114.93, 115.31, 116.87, 117.77, 118.51, 119.62, 121.87, 123.48, 124.68, 125.21, 127.94, 128.30, 129.20, 129.57, 129.82, 130.62, 130.93, 131.83, 134.57, 134.61, 140.27, 143.08, 150.68, 155.04, 156.67, 158.74, 159.88, 160.60, and 164.33; MS (EI, 70 eV): $m/z$ 591 ($M^+$, 13); anal. calcd. for $C_{32}H_{25}N_5O_5S$ (591.64): C, 64.96; H, 4.26; and N, 11.84. Found: C, 65.15; H, 4.31; and N, 11.93.

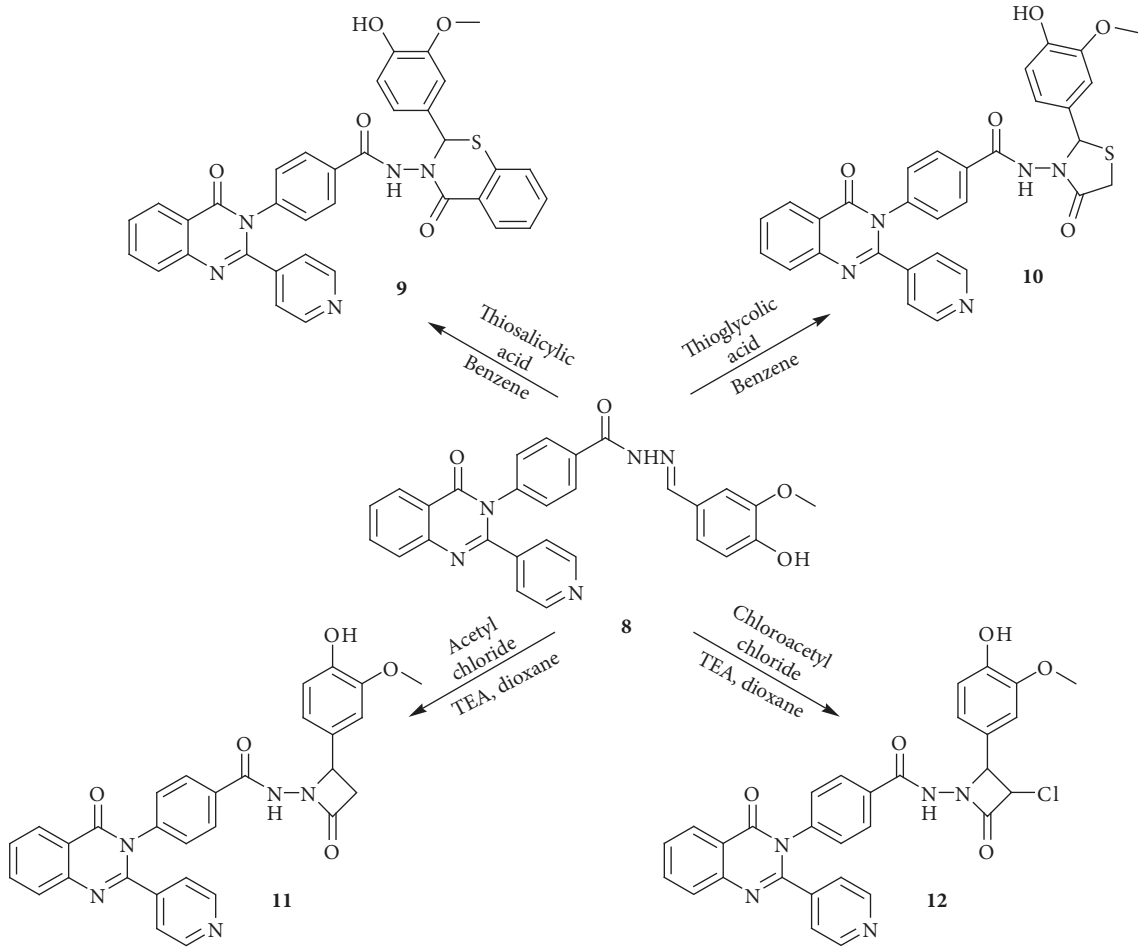
*(4) 3-((Z)-5-(4-Hydroxy-3-methoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylideneamino)-2-(pyridin-4-yl)quinazolin-4(3H)-one (**17d**).* Yield 71%; mp 255–257°C; IR (KBr, $cm^{-1}$): 3670–3481 (OH), 1711, and 1680 (2CO); $^1$H NMR (DMSO-$d_6$): $\delta$ 3.85 (s, 3H, $OCH_3$), 6.82–7.96 (m, 16H, ArH), 8.10 (s, 1H, olefinic CH=), and 11.33 (s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 56.14, 112.02, 115.86, 117.21, 119.80, 120.91, 121.38, 121.57, 122.06, 123.77, 127.11, 128.74, 129.02, 129.45, 131.60, 132.87, 137.25, 143.10, 144.12, 146.23, 147.05, 150.16, 151.22, 161.01, 163.15, and 169.46; MS (EI, 70 eV): $m/z$ 547 ($M^+$, 16); anal. calcd. for $C_{30}H_{21}N_5O_4S$ (547.58): C, 65.80; H, 3.87; and N, 12.79. Found: C, 65.69; H, 3.93; and N, 12.85.




Scheme 1: Synthesis of compounds **2** and **3**.




Scheme 2: Synthesis of compounds **4**–**8**.



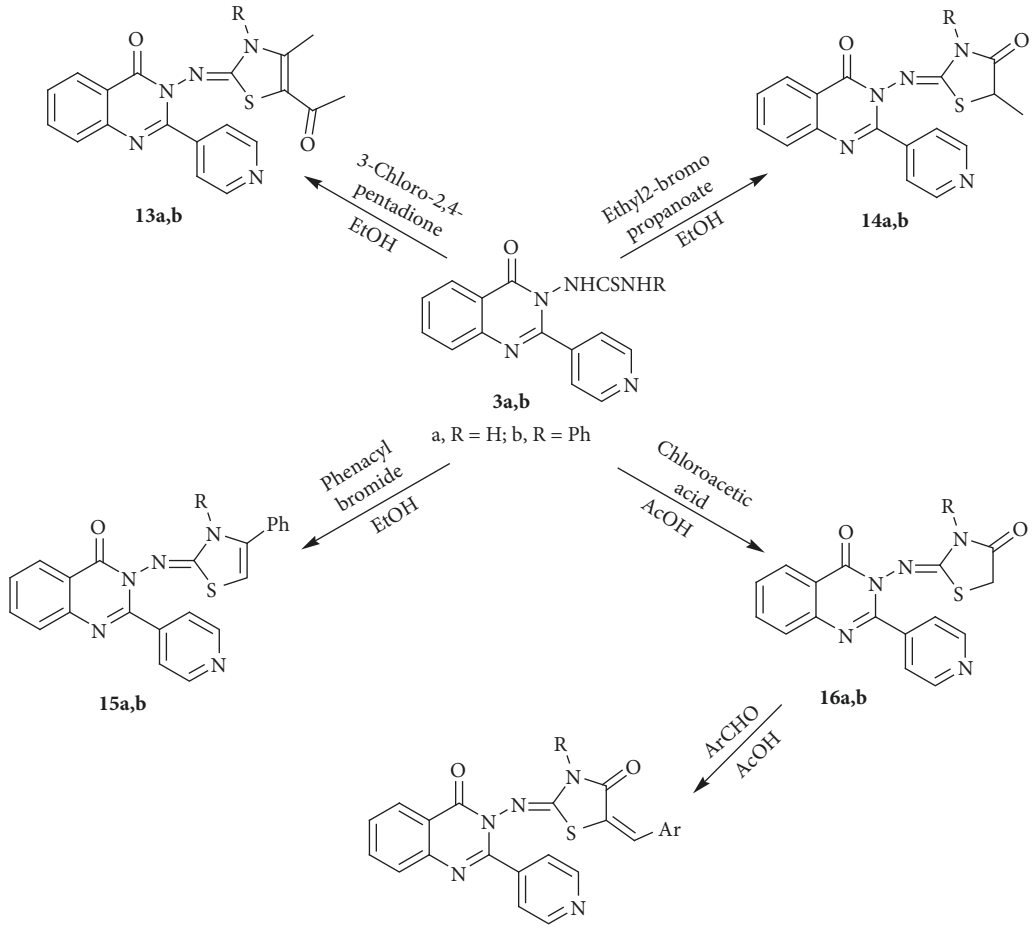

Scheme 3: Synthesis of compounds **9**–**12**.

## 3. Results and Discussion

*3.1. Chemistry.* Target products **2**–**17** were prepared based on the synthetic pathway summarized in Schemes 1–4. The key intermediates **2** and **3** were obtained via treatment of starting precursor 2-(pyridin-4-yl)-4H-benzo[*d*][1,3] oxazin-4-one [24] either with ethyl 4-aminobenzoate [25] followed by reaction with hydrazine hydrate and also with substituted thiosemicarbazides, respectively (Scheme 1). The structure of product **2** was proved by the existence of bands at 3438, 3315, 3236, and 1688 $cm^{-1}$ in its IR spectrum equivalent to amines and carbonyl functions, in addition to two singlet peaks at $\delta$ 9.81 and 10.65 ppm in the $^1$H NMR spectrum referred to $NH_2$ and NH protons. On the other hand, product **3b** exhibited bands at 3190, 3165, 1700, and 1278 $cm^{-1}$ in the IR spectrum and confirmed the existence of 2 NH, CO, and CS, respectively, besides two singlets at $\delta$ 10.42 and 10.90 ppm in the $^1$H NMR spectrum referred to 2 NH protons. Also, the appearance of the molecular ion peak at *m*/*z* 373 confirms the molecular formula ($C_{20}H_{15}N_5OS$) of compound **3b** in MS.

The key intermediate **2** can undergo cyclization with different reagents (diethyl malonate, maleic anhydride, carbon disulphide, ethoxymethylene malononitrile, or vanillin to form pyrazolidine-3,5-dione, 2,5-dioxo-2,5-dihydro-1H-pyrrole, 5-thioxo-1,3,4-oxadiazole, 5-aminopyrazole-4-carbonitrile, and Schiff base derivatives **4**–**8**, respectively (Scheme 2). Compound **4** as an example from this series displayed bands at 3334, 1682, and 1665 $cm^{-1}$ in its IR spectrum related to amines and carbonyl functions, respectively, besides one singlet peak at $\delta$ 3.20 ppm equivalent to cyclic methylene in pyrazolidinone ring residue in its $^1$H NMR spectrum. Also, the $^{13}$C NMR spectra indicating signals at $\delta$ 42.66, 163.18, and 172.04 ppm confirms the existence of the carbon in the methylene and carbonyl groups.

Additionally, treatment of product **8** with various reagents, namely, thiosalicylic acid, thioglycolic acid, acetyl chloride, or chloroacetyl chloride via different cyclocondensation reactions yielded the corresponding 4-oxobenzo[*e*][1,3]thiazine, 4-oxothiazolidine, and 4-oxoazetidine derivatives **9**–**12**, respectively (Scheme 3). The obtained products were proved with different spectral data. Compound **9** as an example from this group of compounds indicated in its IR spectrum bands for hydroxyl, amine, and carbonyl functions at 3388, 3210, 1689, and 1674 $cm^{-1}$, respectively. In addition, the hydroxyl and amine protons appeared in the $^1$H NMR spectrum as two singlets at $\delta$ 9.15 and 11.40 ppm. Furthermore, MS indicates the molecular formula ($C_{38}H_{25}N_5O_5S$) of product **9** by appearance of the molecular ion peak at *m*/*z* 627.




a, R = H; Ar = $C_6H_3$-3-($OCH_3$)-4-(OH)

b, R = H; Ar = $C_6H_2$-3,4,5-($OCH_3$)$_3$

c, R = Ph; Ar = $C_6H_2$-3,4,5-($OCH_3$)$_3$

d, R = Ph; Ar = $C_6H_3$-3-($OCH_3$)-4-(OH)

Scheme 4: Synthesis of compounds **13–17**.

Furthermore, reaction of compounds **3a** and **b** with 3-chloro-2,4-pentadione, ethyl 2-bromopropanoate, phenacyl bromide, or chloroacetic acid afforded the corresponding thiazole derivatives **13–16**. Compound **16a** as an example indicated the existence of bands corresponding to amine and carbonyl absorptions at 3138, 1702, and 1685 $cm^{-1}$ in the IR spectrum. In addition, $^1$H NMR spectrum characteristics of new singlet found at $\delta$ 4.07 ppm confirms methylene thiazole ring protons, besides another singlet was found at $\delta$ 10.21 ppm due to the amine proton. Also, the MS displayed the molecular ion peak at $m/z$ 337 confirming the molecular formula $C_{16}H_{11}N_5O_2S$ of product **16a**. Synthesis of derivatives **17a–d** was accomplished by reaction of **16a** and **16b** with vanillin or 3,4,5-trimethoxybenzaldehyde, respectively (Scheme 4).

The existence of bands at 3490, 3130, 1702, and 1676 $cm^{-1}$ in the IR spectrum of **17a** as an example confirmed the existence of -OH, -NH, and CO functionalities, respectively. Furthermore, $^1$H NMR spectrum clearly indicates the formation of olefinic CH= by existence of singlet at $\delta$ 8.11 ppm, besides two other singlets at $\delta$ 9.96 and 11.25 ppm confirmed the presence of hydroxyl and amine protons. Moreover, $^{13}$C NMR and MS spectrum revealed the carbons at their expected regions and the molecular formula of the target product.

### 3.2. Biology

*3.2.1. In Vitro Cytotoxic Screening against HePG-2, MCF-7, and HCT-116 Cell Lines.* The cytotoxic potencies of compounds **3a**, **3b**, **6**, **7**, **8**, **9**, **12**, **13b**, **14a**, **15a**, **15b**, **16a, 16b**, **17b**, and **17d** were preliminary investigated against human hepatocellular carcinoma (HePG-2), Caucasian breast adenocarcinoma (MCF-7), and colon carcinoma (HCT-116) cell lines at a concentration of 100 $\mu$M using the MTT assay [26–28] in comparison to marketed doxorubicin as a reference drug. Among the studied cell lines, HePG-2 was the most sensitive, while MCF-7 and HCT-116 had reduced

susceptibility to the screened derivatives (Table 1). Derivatives that exhibited cytotoxic activity more than 80% at a concentration of 100 $\mu$M were utilized to calculate their $IC_{50}$ values. The results in Table 2 revealed that, the analogues **9**, **12**, and **16a** demonstrated the best cytotoxic activity against HePG-2 cell line ($IC_{50}$ = 60.29 ± 1.06, 104.94 ± 2.46, and 126.40 ± 1.83 $\mu$M, respectively) in comparison with the reference drug doxorubicin ($IC_{50}$ = 69.60 ± 1.50 $\mu$M).

Regarding HePG-2 cell line, attachment of quinazolin-4(3H)-one scaffold to different moieties directly or through C=O group as a linker at position-3 decreased the cytotoxic activity drastically as in compounds **6**, **7**, and **8** (growth inhibition % = 5.3 ± 0.81, 4.3 ± 1.86, and 12.2 ± 2.27, respectively). Changing the linker with Ph-CONH increased the activity in a great manner as in compounds **9** and **12** (growth inhibition % = 100 and 90.1 ± 6.60, respectively). Quinazolin-4(3H)-one derivatives bearing thiazolidin-2-ylideneamino moieties at position-3 displayed remarkable increase in the activity specially with derivatives having R = H, e.g., **13a**, **15a**, **16a**, and **17b** (growth inhibition % = 41 ± 3.20, 20.1 ± 2.05, 98.2 ± 1.11, and 25.6 ± 4.51, respectively), than those with R = Ph, e.g., **13b**, **14b**, **15b** , and **17d** (growth inhibition % = 21 ± 2.08, 10 ± 2.25, 2.1 ± 2.15, and 22.1 ± 2.85, respectively).

*3.2.2. Kinase Inhibition Screening.* Regarding the cellular assay on HePG-2, MCF-7, and HCT-116, derivatives **9**, **12**, and **16a** displayed promising cytotoxic properties compared to doxorubicin as a reference. Therefore, they were submitting for inhibition estimation against PI3K at different concentrations using the ADP-Glo assay method [29]. Taking LY294002 as the reference compound ($IC_{50}$ = 57.30 ± 2.02 $\mu$M), the results revealed that compounds **9** and **16a** showed the highest inhibitory activities ($IC_{50}$ = 31.92 ± 3.26 and 74.48 ± 2.91 $\mu$M, respectively), which are very close to the standard. However, compound **12** showed weak inhibitory activity ($IC_{50}$ = 112.34 ± 11.14 $\mu$M) (Table 3).

*3.2.3. Molecular Modeling Study.* After *in vitro* evaluation, it was thought worthy to study the interaction of the promising compounds **9**, **12,** and **16a** with PI3K using MOE 2008.10 program [30]. The coordinates of the PI3K structure were obtained from the crystal structure of PI3K with its inhibitor (PDB ID: 2WXG) [31]. The root mean square difference (RMSD) between the top docking pose and original crystallographic geometry of cocrystallized ligand SW13 was 0.9 Å. The data of docking scores and interactive amino acid residues with the screened compounds are depicted in Table 4.

Molecular docking was performed to predict the binding forms and direction of the most active derivatives **9**, **12**, and **16a** at the active site of the ATP binding site of PI3K. The distinctive binding pattern of SW13 to PI3K active site would be discussed and compared to the tested compounds. SW13 engages in hydrogen bonding with Asp787 and Tyr813. Additionally, the phenolic OH group of SW13 serves as a hydrogen bond donor to the DFG Asp911 at the start of the activation loop. The amino group and *N*-3 of pyrazolopyrimidine moiety establish hydrogen bonds to the hinge residues Glu826 and Val828. The quinazolinone moiety of SW13 is sandwiched into the induced hydrophobic specificity pocket between Trp760 and Ile777 on the one side and two P-loop residues Met752 and Pro758 on the other side [30].

From the docking results, it was observed that the protonated nitrogen atom of amide groups in compounds **9** and **12** formed hydrogen bonds with the sidechain of Asp911 as H-donors (distance: 1.98, 1.30 Å, respectively) (Figures 2 and 3). In compound **9**, the arene cation interacts between the phenolic ring and Lys755. Oxygen of quinazolin-4(3H)-one moiety was linked to the sidechain of Lys779 via the H-bond acceptor (distance: 2.47 Å). Furthermore, the

Table 1: Cytotoxic activity of the newly synthesized compounds against human carcinoma cell lines at 100 $\mu$M.

| Compound[a] | Growth inhibition (mean ± SEM) (%) | | |
|---|---|---|---|
| | HePG-2 | MCF-7 | HCT-116 |
| 3a | 35.2 ± 2.52 | 52.5 ± 2.40 | 19.3 ± 2.23 |
| 3b | 33.5 ± 1.65 | 12.6 ± 1.15 | 0 |
| 6 | 5.3 ± 0.81 | 37.8 ± 1.13 | 0 |
| 7 | 4.3 ± 1.86 | 12.5 ± 2.45 | 14.4 ± 2.90 |
| 8 | 12.2 ± 2.27 | 9.6 ± 1.70 | 11.8 ± 3.58 |
| 9 | 100 ± 0.00 | 9.3 ± 1.45 | 20.4 ± 4.35 |
| 12 | 90.1 ± 6.60 | 39.1 ± 1.95 | 0 |
| 13a | 41 ± 3.20 | 8.4 ± 1.47 | 19.2 ± 2.00 |
| 13b | 21 ± 2.08 | 10.2 ± 2.35 | 0 |
| 14b | 10 ± 2.25 | 10.2 ± 1.56 | 11.2 ± 2.51 |
| 15a | 20.1 ± 2.05 | 6.2±1.10 | 17.1 ± 1.95 |
| 15b | 2.1 ± 2.15 | 2.3 ± 1.66 | 9.3 ± 0.90 |
| 16a | 98.2 ± 1.11 | 4.2 ± 1.00 | 37.3 ± 1.96 |
| 17b | 25.6 ± 4.51 | 67.3 ± 2.43 | 25.1 ± 4.99 |
| 17d | 22.1 ± 2.85 | 0 | 0 |
| Negative control[b] | 0 | 0 | 0 |
| Doxorubicin[a] | 100 ± 0.00 | 100 ± 0.00 | 100 ± 0.00 |

[a]Concentration of test compounds and positive control (doxorubicin) was 100 $\mu$M; [b]untreated cells in DMSO and its final concentration in the cells was less than 0.2%.

Table 2: $IC_{50}$ of highly cytotoxic active derivatives against human cancer cell lines.

| Compounds | $IC_{50}$ (mean ± SEM) ($\mu$M) HePG-2 |
|---|---|
| 9 | 60.29 ± 1.06 |
| 12 | 104.94 ± 2.46 |
| 16a | 126.40 ± 1.83 |
| Doxorubicin | 69.60 ± 1.50 |

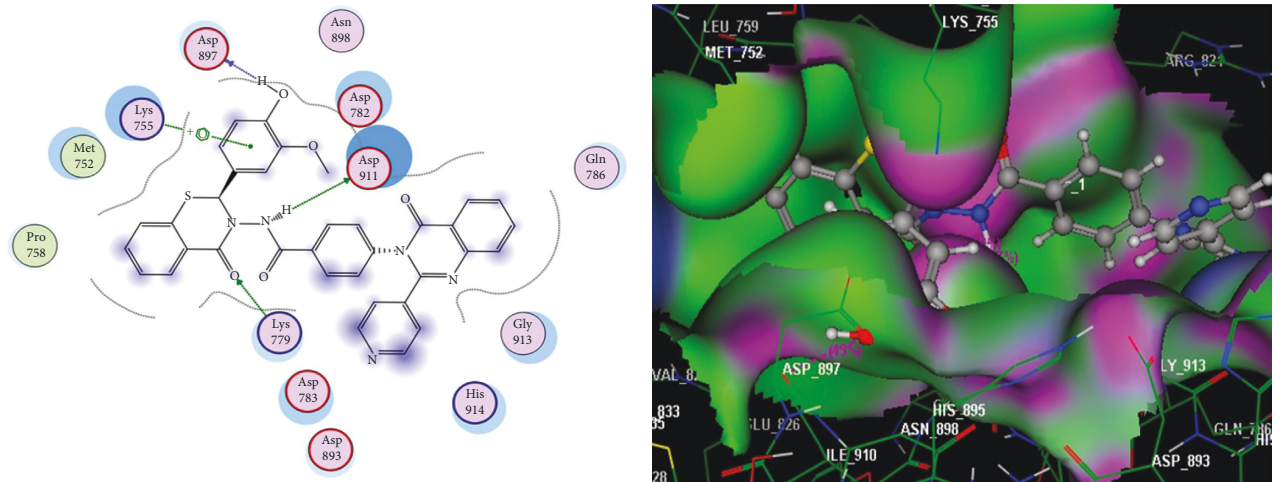
$IC_{50}$: compound concentration wanted to restrain the cell viability by 50%.

Table 3: Inhibitory activities of compounds **9**, **12**, and **16a** against PI3K.

| Compound | PI3K $IC_{50}$ (mean ± SEM) ($\mu$M) |
|---|---|
| 9 | 31.92 ± 3.26 |
| 12 | 112.34 ± 11.14 |
| 16a | 74.48 ± 2.91 |
| LY294002 | 57.30 ± 2.02 |

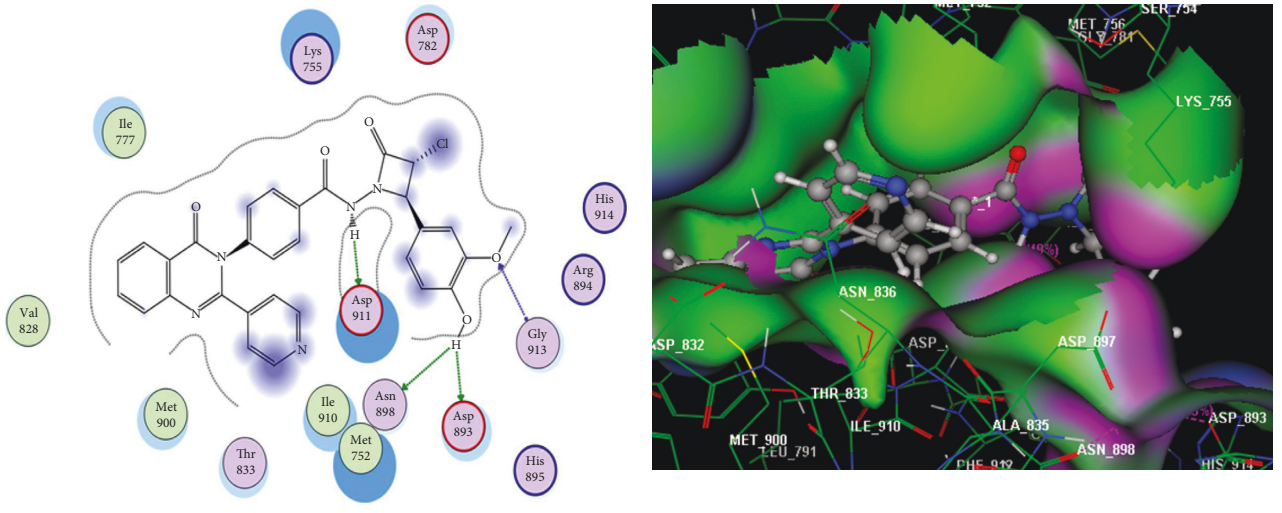
Table 4: Docking results of the synthesized compounds **9**, **12**, and **16a** with PI3K in comparison with the ligand SW13.

| Compd. no. | Docking score (kcal/mol) | Amino acid residues (bond length Å) | Atoms of compound | Type of bond |
|---|---|---|---|---|
| SW13 | −6.45 | Asp787 (1.46) | H(OH) | H-don |
| | | Tyr813 (2.55) | O(OH) | H-acc |
| | | Glu826 (1.95) | $H(NH_2)$ | H-don |
| | | Val828 (3.01) | *N*-3(Pyrazolo[3,4-d]pyrimidine) | H-acc |
| | | Asp911 (3.00) | O(OH) | H-acc |
| 9 | −6.22 | Lys755 | Methoxyphenol | Arene-cation |
| | | Lys779 (2.47) | O(Benzothiazone) | H-acc |
| | | Asp897 (1.75) | H(OH) | H-don |
| | | Asp911 (1.98) | H(CONH) | H-don |
| 12 | −6.93 | Asp893 (2.10) | H(OH) | H-don |
| | | Asn898 (3.27) | H(OH) | H-don |
| | | Asp911 (1.30) | H(CONH) | H-don |
| | | Gly913 (3.36) | $O(OCH_3)$ | H-acc |
| 16a | −6.26 | Lys779 (2.44) | O(Thiazolidinone) | H-acc |
| | | Asp911 (1.51) | H(Thiazolidine) | H-don |



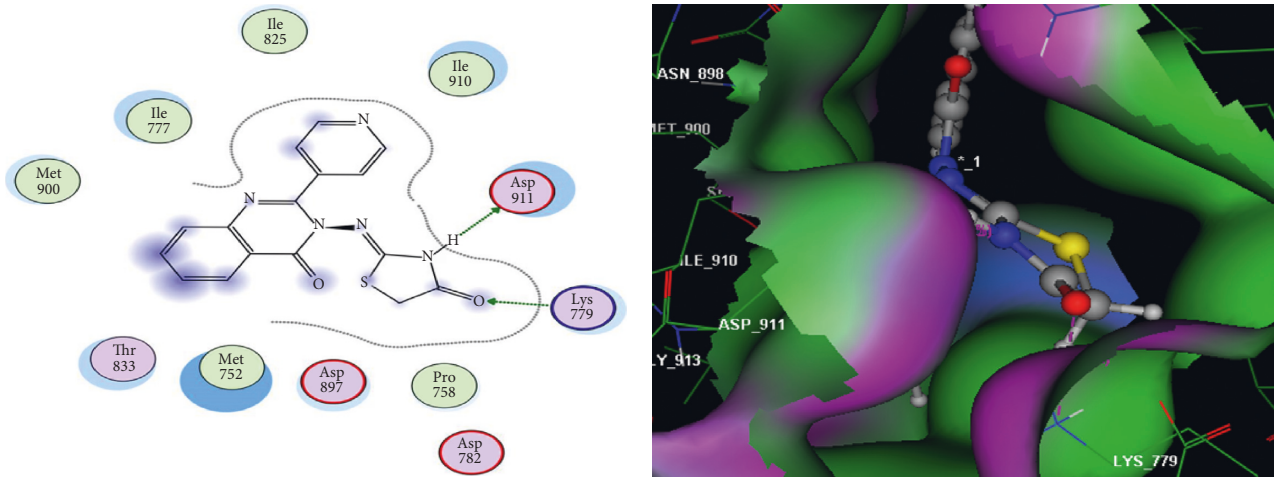

(a) (b)

Figure 2: The suggested binding form of derivative **9** docked in the active position of PI3K showing (a) 2D and (b) 3D ligand-receptor interactions, respectively (H bonds are clarified as arrows; C atoms are colored gray; N atoms are colored blue; O atoms are colored red).




(a) (b)

Figure 3: The suggested binding form of derivative **12** docked in the active position of PI3K showing (a) 2D and (b) 3D ligand-receptor interactions (H bonds are clarified as arrows; colored gray for C atoms, blue for N atoms, and red for O atoms).




(a) (b)

Figure 4: The suggested binding form of derivative **16a** docked in the active position of PI3K showing (a) 2D and (b) 3D ligand-receptor interactions, respectively (H bonds are clarified as arrows; C atoms are colored gray; N atoms are colored blue; O atoms are colored red).

phenolic OH formed the H-bond donor with the backbone of Asp897 (distance: 1.75 Å) (Figure 2).

In compound **12**, two H-bonds appeared as H-donors between hydrogens of the phenolic OH and the sidechains of Asp893 and Asn898 (distance: 2.10, 3.27 Å, respectively). Additionally, the backbone of Gly913 established the H-bond acceptor with the oxygen of the methoxy group (distance: 3.36 Å) (Figure 3).

It was noticed that quinazolin-4(3H)-one derivatives bearing thiazolidin-2-ylidenamino moieties at position-3 with free NH exhibited remarkable increase in the cytotoxic activity which may be due to formation of the H-bond donor with the sidechain of Asp911. Among this series, the excellent potency of **16a** may be due to unsubstitution on C-3 adjacent to the carbonyl group of thiazoilidinone moiety which facilitates formation of a characteristic H-bond acceptor between oxygen of the CO group and the sidechain of Lys779 (Figure 4).

## 4. Conclusions

In summary, we have designed and synthesized novel twenty-five-quinazolin-4(3H)-one-based derivatives incorporating different moieties and evaluated their cytotoxic activities against HePG-2, MCF-7, and HCT-116 cancer cell lines. Among them, compound **9** ($IC_{50}$ = 60.29 ± 1.06 $\mu$M) emerged as the most active member against HePG2, as it was equipotent with doxorubicin ($IC_{50}$ = 69.60 ± 1.50 $\mu$M). Also, compounds **12** and **16a** displayed excellent activity against HePG2 ($IC_{50}$ = 104.94 ± 2.46 and 126.40 ± 1.83 $\mu$M, respectively). Kinase inhibition assay against PI3K and docking studies were performed using the MOE 2008.10 program to justify the biological activities of the synthesized compounds. All active compounds could interact with a key amino acid **Asp911** with a characteristic hydrogen bond. Compounds **9** and **16a** as the most active compounds could interact by extra hydrogen bonds with **Lys779** signifying for their strongest PI3K inhibitory activities. So the applicability of quinazolin-based hybrids containing the benzo[*e*][1,3] thiazine function has the potential to be developed into anticancer active agents.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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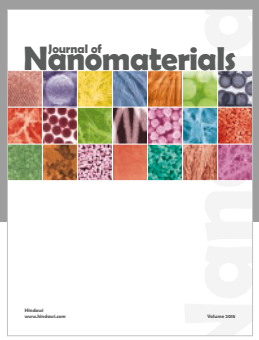

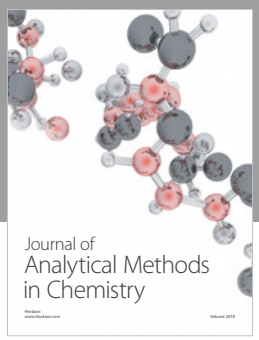

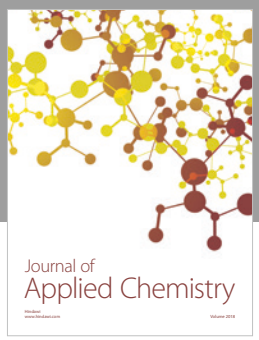

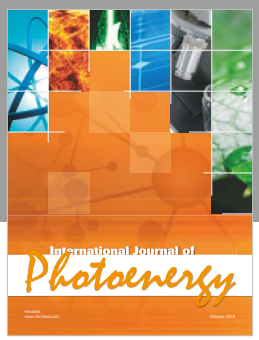

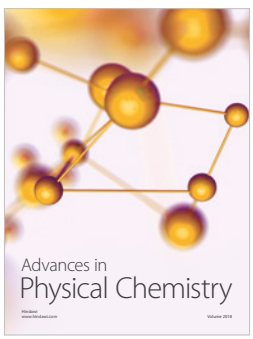

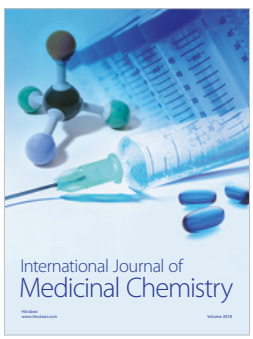

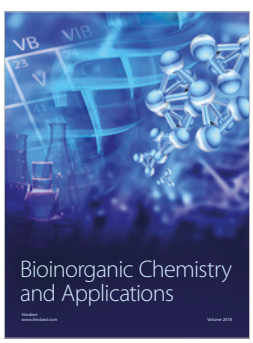

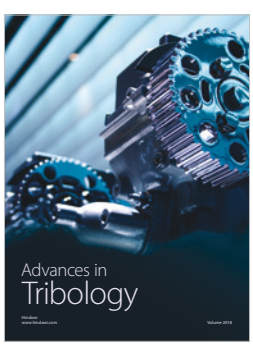

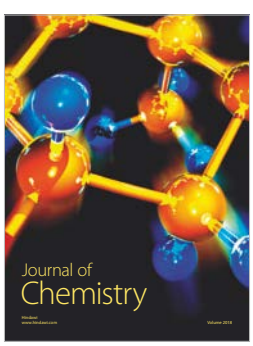

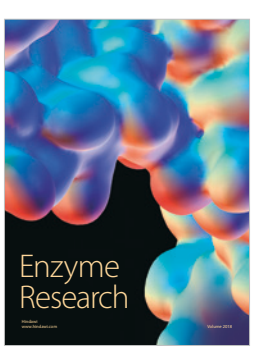

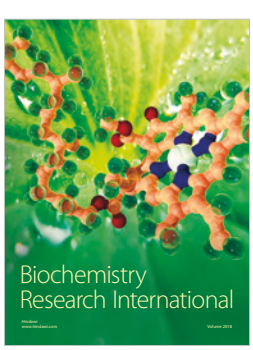


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