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Synthesis and Anticancer Activity of New 2-Aryl-4-(4-Methoxybenzylidene)-5-Oxazolone Scaffolds

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Abstract: A new series of 4-(4-methoxybenzylidene)-2-substitutedphenyl-5(4*H*)-oxazolone derivatives has been synthesized through the application of Erlenmeyer condition (Ac_2O, AcONa, and 4-anisaldehyde) to the reaction products of the highly versatile p-aminohippuric acid with various electrophilic reagents such as aromatic aldehydes, phenyl isothiocyanate, diazotization-coupling reaction (malononitrile and 2-amino-4-phenylthiazole) and cyanoacetyl-pyrazole. IR, ^{1}H NMR, and mass spectroscopic techniques were utilized to confirm the structures of these oxazolone scaffolds. The synthesized oxazolone derivatives were evaluated against four human cancer cell lines (HepG2, HTC-116, PC-3, and MCF-7). Compound **3e** showed the best activity against hepatocellular carcinoma (IC_{50} 8.9±0.30 µg/mL) and colorectal carcinoma (IC_{50} 9.2±0.63 µg/mL) cell lines compared with the standard anticancer drug 5-fluorouracil.

Keywords: p-Aminohippuric acid; acetic anhydride; Erlenmeyer reaction; diazo coupling; malononitrile.

1. Introduction

Oxazole derivatives have been utilized as important precursors for the construction of many biologically active compounds as antimicrobial agents [1], photosensitive composition devices for proteins [2], and biosensors in the detection of ACh inhibitor [3]. Oxazole-based compounds could readily bind with a variety of enzymes and receptors in biological systems and show broad biological activities [4-13]. Especially, the 4-arylidene-5(4*H*)-oxazolones are important precursors for the construction of many organic molecules such as amino acids and peptides [14]. They display a broad domain of pharmacological activities [15-20], such as anti-inflammatory [21], anticancer [22], antagonistic and antiangiogenic [23,24] and tyrosinase inhibitor [25]. As a result of the synthesis of various substituted oxazolones is highly desirable, a number of synthetic protocols [26-29] have been reported for the preparation of azlactones. These include the utilization of acetic anhydride with sodium(lead) acetate, sulfur trioxide/dimethylformamide complex, polyphosphoric acid and perchloric acid, in addition to the methods that employ triphenylphosphonium chloride (Ph_3P/CCl_4), anhydrous zinc chloride or bismuth (III) acetate as catalysts [30-35]. In light of an urgent need to develop anticancer materials, recent research papers have been published to diminish the growth of tumor cells by cytotoxic agents [36,37]. The present research article reports on the preparation of some new

oxazolone scaffolds, their structures elucidation by IR, ^{1}H NMR & MS spectroscopic techniques, and evaluating their anticancer activity.

2. Materials and Methods

All melting points were measured on the Gallenkamp electric melting point apparatus. Infrared spectra were determined on Mattson 5000 FT-IR spectrometer (KBr discs). The 1H NMR spectra were recorded on a Varian XL 300 MHz apparatus. The Mass spectra were performed using a Shimadzu Qp-2010 mass spectrometer at 70 eV (EI mode). Elemental analyses (C, H, and N) were determined on Perkin-Elmer 2400 analyzer.

*2.1. Synthesis of (4-arylideneamino-benzoyl)-glycines **2a-e**.*

A mixture of 4-aminohippuric acid (0.97 g, 0.005 mol) and the appropriate aromatic aldehyde (0.005 mol) in 20 mL distilled ethyl alcohol was boiled under reflux for 4 hours. The precipitate that formed upon cooling was filtered to afford glycine derivatives **2a-e**.

*(4-Benzylideneamino-benzoyl)-glycine (**2a**).* White crystals in 63% yield; m.p. = 218-220 ºC; IR: 3318 (NH, OH), 1744 cm^{-1} (broad, C=O); ^{1}H NMR ($CDCl_3$): δ/ppm = 12.35 (s, 1H), 10.15 (s, 1H), 8.25 (s, 1H), 7.80-7.35 (m, 9H), 3.65 (d, *J* = 4.80 Hz, 2H); Analysis for $C_{16}H_{14}N_2O_3$ (282.30): Calcd. C, 68.08; H, 5.00; N, 9.92%. Found: C, 68.27; H, 5.05; N, 9.84%.

*[4-(4-Methylbenzylidene-amino)-benzoyl]-glycine (**2b**).* White crystals in 73% yield; m.p. = 196-198 ºC; IR: 3339 (NH, OH), 1734 (C=O), 1642 cm^{-1} (C=O); 1H NMR ($CDCl_3$): δ/ppm = 12.30 (s, 1H), 10.25 (s, 1H), 8.25 (s, 1H), 7.80-7.25 (m, 8H), 3.65 (d, *J* = 4.80 Hz, 2H), 2.35 (s, 3H); Analysis for $C_{17}H_{16}N_2O_3$ (296.33): Calcd. C, 68.91; H, 5.44; N, 9.45%. Found: C, 68.73; H, 5.48; N, 9.52%.

*[4-(4-Nitrobenzylidene-amino)-benzoyl]-glycine (**2c**).* Yellow powder in 62% yield; m.p. = 216-218ºC; IR: 3436 (NH, OH), 1730 (C=O), 1654 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 12.87 (s, 1H), 11.08 (s, 1H), 8.44 (s, 1H), 8.16-7.30 (m, 8H), 3.68 (d, *J* = 4.80 Hz, 2H); MS: m/z (%) = 327 (molecular ion peak, 23), 58 (base peak, 100); Analysis for $C_{16}H_{13}N_3O5$ (327.30): Calcd. C, 58.72; H, 4.00; N, 12.84%. Found: C, 58.54; H, 3.94; N, 12.67%.

*[4-(4-Chlorobenzylidene-amino)-benzoyl]-glycine (**2d**).* Light brown crystals in 55% yield; m.p. = 191-192 ºC; IR: 3294 (NH, OH), 1709 (C=O), 1644 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ/ppm = 12.35 (s, 1H), 10.15 (s, 1H), 8.45 (s, 1H,), 7.90-7.30 (m, 8H), 3.58 (d, *J* = 4.60 Hz, 2H); Analysis for $C_{16}H_{13}ClN_2O_3$ (316.74): Calcd. C, 60.67; H, 4.14; N, 8.84%. Found: C, 60.78; H, 4.08; N, 8.91%.

*[4-(4-Chloro-2-nitrobenzylidene-amino)-benzoyl]-glycine (**2e**).* Orange crystals in 78% yield; m.p. = 270-272 ºC; IR: 3262 (NH, OH), 1701 (C=O), 1653 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6): δ/ppm = 12.35 (s, 1H), 10.05 (s, 1H), 8.45 (s, 1H), 7.80-8.15 (m, 7H), 3.61 (d, *J* = 4.80 Hz, 2H); Analysis for: $C_{16}H_{12}ClN_3O_5$ (361.74): Calcd. C, 53.13; H, 3.34; N, 11.62%. Found: C, 53.05; H, 3.31; N, 11.70%.

*2.2. Synthesis of 2-(4-arylideneamino-phenyl)-4-(4-methoxy-benzylidene)-oxazol-5(4H)-one derivatives **3a-e**.*

A mixture of anhydrous sodium acetate (0.16 g, 0.002 mol), p-anisaldehyde (0.27 mL, 0.002 mol) and (4-(arylideneamino)-benzoyl)-glycines **2a-e** (0.002 mol) in acetic anhydride

(20 mL) was heated with stirring at 100 ºC for 4 hours. The reaction mixture was poured into ice-water, and the precipitate that formed was filtered and recrystallized from ethanol.

2-(4-Benzylideneamino-phenyl)-4-(4-methoxybenzylidene)-oxazol-5(4H)-one ***(3a)***. Yellow powder in 38% yield; m.p. = 248-250 ºC; IR: 1793 (C=O), 1652 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 8.31 (s, 1H), 8.22 (s, 1H), 7.75-7.24 (m, 13H), 3.78 (s, 3H); Analysis for $C_{24}H_{18}N_2O_3$ (382.42): Calcd. C, 75.38; H, 4.74; N, 7.33%. Found: C, 75.18; H, 4.68; N, 7.21%.

4-(4-Methoxybenzylidene)-2-[4-(4-methylbenzylideneamino)-phenyl]-oxazol-5(4H)-one ***(3b)***. Brown powder in 34% yield; m.p. = 214-215 ºC. IR: 1761 (C=O), 1646 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 8.35 (s, 1H), 8.10 (s, 1H), 7.83-7.20 (m, 12H), 3.78 (s, 3H), 2.35 (s, 3H); Analysis for $C_{25}H_{20}N_2O_3$ (396.45): Calcd. C, 75.74; H, 5.09; N, 7.07%. Found: C, 75.56; H, 5.15; N, 7.00%.

4-(4-Methoxybenzylidene)-2-[4-(4-nitrobenzylideneamino)phenyl]-oxazol-5(4H)-one ***(3c)***. Yellowish brown powder in 38% yield; m.p. = 263-264 ºC; IR: broad centered at 1713 (C=O), 1645 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 8.48 (s, 1H), 8.22 (s, 1H), 8.12-7.08 (m, 12H), 3.81 (s, 3H); MS: m/z (%) = 427 (molecular ion peak, 1.95), 84 (base peak, 100); Analysis for $C_{24}H_{17}N_3O_5$ (427.42): Calcd. C, 67.44; H, 4.01; N, 9.83%. Found: C, 67.59; H, 4.08; N, 9.77%.

2-[4-(4-Chlorobenzylideneamino)phenyl]-4-(4-methoxy-benzylidene)-oxazol-5(4H)-one ***(3d)***. Yellowish green powder in 30% yield; m.p. = 205-207 ºC; IR: 1790 (C=O), 1657 cm-1 (C=N); 1H NMR (DMSO-d_6): δ/ppm = 8.41 (s, 1H), 7.98 (s, 1H), 7.85-7.11 (m, 12H), 3.82 (s, 3H); MS: m/z (%) = 416 (molecular ion peak, 37), 162 (base peak, 100); Analysis for $C_{24}H_{17}ClN_2O_3$ (416.86): Calcd. C, 69.15; H, 4.11; N, 6.72%. Found: C, 69.32; H, 4.07; N, 6.64%.

2-[4-(4-Chloro-2-nitrobenzylideneamino)phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4H)-one ***(3e)***. Yellow powder in 46% yield; m.p. > 300 ºC; IR: 1796 (C=O), 1655 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 8.67 (s, 1H), 8.42-7.14 (m, 12H), 3.81 (s, 3H); MS: m/z (%) = 461 (molecular ion peak, 40), 120 (base peak, 100); Analysis for $C_{24}H_{16}ClN_3O_5$ (461.86): Calcd. C, 62.41; H, 3.49; N, 9.10%. Found: C, 62.31; H, 3.43; N, 9.19%.

*2.3. Synthesis of 4-(4-methoxybenzylidene)-2-(4-N'-phenyl-thioureido-phenyl)-oxazol-5(4H)-one (**5**).*

A mixture of anhydrous sodium acetate (0.16 g, 0.002 mol) and *p*-anisaldehyde (0.27 ml, 0.002 mol) and phenylthiourea derivative **4** (0.66 g, 0.002 mol) in acetic anhydride (20 mL) was heated with stirring at 100 ºC for 4 hours. After cooling, the reaction mixture was poured into ice-cold water. Then, the resulting solid was filtered and recrystallized by heating in ethanol to give **5**. Light brown crystals in 42% yield; m.p. = 169-171ºC; IR: broad centered at 3387 (NH), 1781 (C=O), 1652 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 13.25 (s, 1H), 11.75 (s, 1H), 7.95-7.24 (m, 14H), 3.79 (s, 3H); MS: m/z (%) = 429 (molecular ion peak, 28.35), 77 (base peak, 100). Analysis for $C_{24}H_{19}N_3O_3S$ (429.49): Calcd. C, 67.12; H, 4.46; N, 9.78%. Found: C, 67.32; H, 4.55; N, 9.65%.

*2.4. Synthesis of 4-[2-(dicyanomethylene)hydrazinyl]benzoyl-glycine (**7**).*

4-Aminohippuric acid (0.97 g, 0.005 mol) was firstly neutralized by stirring with 15 mL Na_2CO_3 solution (2.5%), and the solution that obtained was cooled to 0-5ºC. Sodium nitrite (0.35 g) was added to this neutralized solution with stirring. Then the solution was acidified by

conc. HCl (1.5 mL) and then water (1.5 mL H_2O) was added. The freshly prepared suspension of the diazonium chloride was added drop by drop with stirring to a cold solution of malononitrile (0.33 g, 0.005 mole) and 1.5 g sodium acetate in 30 mL EtOH. After stirring the reaction mixture for 3 hours at 0-5°C, the resulting precipitate was filtered and recrystallized from ethyl alcohol. Orange powder in 76% yield; m.p. = 145-146 ºC, lit. m.p. = 144-147 ºC [1]; IR: 3395, 3222 (NH, OH), 2226 (C≡N), 1753 (C=O), 1637 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ/ppm = 12.25 (s, 1H), 11.10 (s, 1H), 8.90 (s, 1H), 7.95 (d, *J* = 8.80 Hz, 2H), 7.70 (d, *J* = 8.80 Hz, 2H), 3.68 (d, *J* = 4.40 Hz, 2H); Analysis for $C_{12}H_9N_5O_3$ (271.24): Calcd. C, 53.14; H, 3.34; N, 25.82%. Found: C, 53.03; H, 3.29; N, 25.86%.

*2.5. Synthesis of 2-[4-(dicyanomethylene-hydrazinyl)-phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4H)-one (**8**).*

A mixture of 7 (0.54 g, 0.002 mol), with anhydrous sodium acetate (0.16 g, 0.002 mol) and p-anisaldehyde (0.27 mL, 0.002 mol) in acetic anhydride (15 mL) was heated with stirring at 100ºC for 4 hours. After cooling, the reaction mixture was poured on ice-cold water. Then, the solid that formed was picked up by filtration and purified by recrystallization from EtOH. Orange powder in 35% yield; m.p. = 250-252 ºC; IR: 3226 (NH), 2224 (C≡N), 1788 (C=O), 1688 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 10.58 (s, 1H), 7.96-7.13 (m, 9H), 3.86 (s, 3H); Analysis for $C_{20}H_{13}N_5O_3$ (371.36): Calcd. C, 64.69; H, 3.53; N, 18.86%. Found: C, 64.85; H, 3.49; N, 18.75%.

*2.6. Synthesis of 4-[(2-amino-4-phenylthiazol-5-yl)azo]benzoyl-glycine (**10**).*

4-Aminohippuric acid (0.97 g, 0.005 mol) was firstly neutralized by stirring with 15 mL Na_2CO_3 solution (2.5%), and the solution that obtained was cooled to 0-5ºC. Sodium nitrite (0.35 g) was added to this neutralized solution with stirring. Then the solution was acidified by conc. HCl (1.5 mL) and then water (1.5 mL H_2O) was added. The freshly prepared suspension of the diazonium chloride was added drop by drop with stirring to a cold solution of 2-amino-4-phenylthiazole (**9**) (0.88 g, 0.005 mole) and 1.5 g sodium acetate in 30 mL EtOH. After stirring of the reaction mixture for 3 hours at 0-5°C, the resulting precipitate was filtered purified by recrystallization from EtOH/DMF mixture (3:1). Dark red crystals in 80% yield; m.p. > 300 ºC; IR: 3402, 3337 (NH2, NH, OH), broad centered at 1627 cm-1 (C=O); 1H NMR ((DMSO-d_6)): δ/ppm = 12.15 (s, 1H), 9.84 (s, 1H), 8.05-7.30 (m, 11H), 3.66 (d, *J* = 4.40 Hz, 2H); MS: m/z (%) = 381 (molecular ion peak, 60), 176 (base peak, 100); Analysis for $C_{18}H_{15}N_5O_3S$ (381.41): Calcd. C, 56.68; H, 3.96; N, 18.36%. Found: C, 56.44; H, 3.91; N, 18.29%.

*2.7. Synthesis of 2-[4-(2-acetylamino-4-phenylthiazol-5-yl)azo-phenyl]-4-(4-methoxy-benzylidene)oxazol-5(4H)-one (**11**).*

A mixture of anhydrous sodium acetate (0.16 g, 0.002 mol), *p*-anisaldehyde (0.27 ml, 0.002 mol) and 2-amino-5-arylazothiazole derivative **10** (0.76 g, 0.002 mol) in acetic anhydride (20 mL) was heated with stirring at 100 ºC for 4 hours. The reaction mixture was poured into ice cold water. Then, the solid that obtained was picked up by filtration and purified by recrystallization from EtOH. Brown powder in 66% yield; m.p. = 279-281 ºC; IR: 3162 (NH), 1772 (C=O), 1684 (C=O), 1651 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 12.68 (s, 1H),

8.24-7.21 (m, 14H), 3.80 (s, 3H), 2.23 (s, 3H); Analysis for $C_{28}H_{21}N_5O_4S$ (523.57): Calcd. C, 64.23; H, 4.04; N, 13.38%. Found: C, 64.05; H, 4.11; N, 13.27%.

*2.8. Synthesis of 4-(2-cyanoacetamido)benzoyl-glycine (**13**).*

A suspension of 4-aminohippuric acid (0.97 g, 0.005 mol) and 3,5-dimethyl-1-cyanoacetyl pyrazole (0.82 g, 0.005 mol) was refluxed in 15 mL dioxane for 4 hours. On cooling, the solid that formed was separated by filtration, dried and purified by recrystallization from EtOH. White crystals in 57% yield; m.p. = 192-193 ºC, lit. m.p. = 191-193 ºC [1]. IR: 3406, 3319, 3193 (NH, OH), 2262 (C≡N), 1695 (C=O), 1641 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 10.51 (s, 1H), 8.73 (s, 1H), 8.25 (s, 1H), 7.87 (d, *J* = 8.80 Hz, 2H), 7.64 (d, *J* = 8.80 Hz, 2H), 3.93 (s, 2H), 3.65 (d, *J* = 4.60 Hz, 2H); MS: m/z (%) = 261 (molecular ion peak, 32), 187 (base peak, 100); Analysis for $C_{12}H_{11}N_3O_4$ (261.24): Calcd. C, 55.17; H, 4.24; N, 16.09%. Found: C, 54.98; H, 4.28; N, 16.22%.

*2.9. Synthesis of 2-[4-(2-cyanoacetamido)phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4H)-one (**14**).*

A suspension of cyanoacetamide derivative **13** (0.52 g , 0.002 mol), anhydrous sodium acetate (0.16 g, 0.002 mol) and *p*-anisaldehyde (0.27 mL, 0.002 mol) in acetic anhydride (20 mL) was heated at 100ºC with stirring for 4 hours. After cooling, the reaction mixture was poured on ice-cold water. Then, the solid that obtained was filtered and recrystallized from EtOH. Brown crystals in 30% yield; m.p. = 215-217 ºC; IR: 3353 (NH), 2213 (C≡N), 1748 (C=O), 1698 (C=O), 1641 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ/ppm = 9.45 (s, 1H), 7.80-6.90 (m, 9H), 3.90 (s, 2H), 3.80 (s, 3H); Analysis for $C_{20}H_{15}N_3O_4$ (361.36): Calcd. C, 66.48; H, 4.18; N, 11.63%. Found: C, 66.32; H, 4.25; N, 11.71%.

*2.10. Synthesis of 4-[2-cyano-3-(4-tolyl)-acrylamido]-benzoyl-glycine (**15**).*

A mixture of cyanoacetamide compound **13** (1.30 g, 0.005 mol) with *p*-tolualdehyde (0.60 ml, 0.005 mol) was refluxed in 20 mL of ethyl alcohol containing three drops of piperidine for 2 hours. The reaction mixture was left aside to cool, and the white crystals that formed was picked up by filtration and recrystallized from EtOH. White crystals in 66% yield; m.p. = 220-222 ºC; IR: 3321, 3212, 3157 (NH and OH), 2214 (C≡N), 1683 (C=O), 1649 cm^{-1} (C=O); ^{1}H NMR (hot DMSO-d_6): δ/ppm = 8.28 (s, 1H), 8.05-7.15 (four doublets, 8H), 3.66 (d, J = 4.80 Hz, 2H), 2.35 (s, 3H); Analysis for $C_{20}H_{17}N_3O_4$ (363.37): Calcd. C, 66.11; H, 4.72; N, 11.56%. Found: C, 66.24; H, 4.70; N, 11.50%.

*2.11. Synthesis of 2-[4-(2-cyano-3-(4-tolyl)acrylamido)phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4H)-one (**16**).*

A mixture of anhydrous sodium acetate (0.16 g, 0.002 mol), cyanoacrylamide compound **15** (0.76 g, 0.002 mol) and *p*-anisaldehyde (0.27 ml, 0.002 mol) in 15 mL acetic anhydride was heated with stirring at 100 ºC for 4 hours. After cooling, the reaction mixture was poured on ice-cold water. Then, the solid that formed was filtered off and purified by recrystallization from EtOH. Yellow powder in 35% yield; m.p. = 280-282 ºC; IR: 3352 (NH), 2213 (C≡N), 1741 (C=O), 1692 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 10.55 (s, 1H), 8.23 (s, 1H), 8.04-7.15 (m, 13H), 3.87 (s, 3H), 2.31 (s, 3H); Analysis for $C_{28}H_{21}N_3O_4$ (463.49): Calcd. C, 72.56; H, 4.57; N, 9.07%. Found: C, 72.39; H, 4.73; N, 9.25%.

*2.12. Synthesis of 4-[2-cyano-2-(4-tolylhydrazono)-acetamido]-benzoyl-glycine (**17**).*

To a cold suspension of *p*-toluidine (0.54 g, 0.005 mol) in 1.5 mL conc. HCl, a solution of $NaNO_2$ (0.35 g in 5 mL water) was added dropwise with continuous stirring at 0-5°C. The prepared diazonium chloride solution was then added dropwise to a cold and stirred solution of cyanoacetamide compound **13** (1.30 g, 0.005 mol) and sodium acetate (1.5 g) in 30 mL ethyl alcohol. After stirring of the reaction mixture for 3 hours at 0-5°C, the precipitate was filtered off and purified by heating in EtOH. Brown crystals in 74% yield; m.p. = 165-167 ºC; IR: 3395, 3230 (NH and OH), 2215 (C≡N), 1683 (C=O), 1646 cm^{-1} (C=O); 1H NMR (DMSO-d_6): δ/ppm = 12.25 (s, 1H), 11.10 (s, 1H), 9.65 (s, 1H), 8.90 (s, 1H), 7.75-7.30 (m, 8H), 3.71 (d, *J* = 4.80 Hz, 2H), 2.35 (s, 3H); MS: m/z (%) = 379 (molecular ion peak, 18.30), 65 (base peak, 100); Analysis for $C_{19}H_{17}N_5O_4$ (379.38): Calcd. C, 60.15; H, 4.52; N, 18.46%. Found: C, 60.29; H, 4.48; N, 18.36%.

*2.13. Synthesis of 2-[4-(2-cyano-(4-tolylhydrazono)-acetamido)-phenyl]-4-(4-methoxy-benzylidene)- oxazol-5(4H)-one (**18**).*

A mixture of anhydrous sodium acetate (0.16 g, 0.002 mol) and *p*-anisaldehyde (0.27 ml, 0.002 mol), cyanoacetamide compound **17** (0.75 g, 0.002 mol) in 15 mL acetic anhydride was heated at 100ºC for 4 hours. After cooling, the reaction mixture was poured on ice-cold water. Then, the solid that produced was picked up by filtration and purified by recrystallization from hot EtOH. Dark red crystals in 45% yield; m.p. = 231-233 ºC; IR: 3393 (NH), 2214 (C≡N), 1785 (C=O), 1681 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 11.45 (s, 1H), 10.20 (s, 1H), 7.92-6.90 (m, 13H), 3.82 (s, 3H), 2.32 (s, 3H); MS: m/z (%) = 479 (molecular ion peak, 12.30), 43 (base peak, 100); Analysis for $C_{27}H_{21}N_5O_4$ (479.50): Calcd. C, 67.63; H, 4.41; N, 14.61%. Found: C, 67.47; H, 4.49; N, 14.46%.

2.14. Anticancer screening.

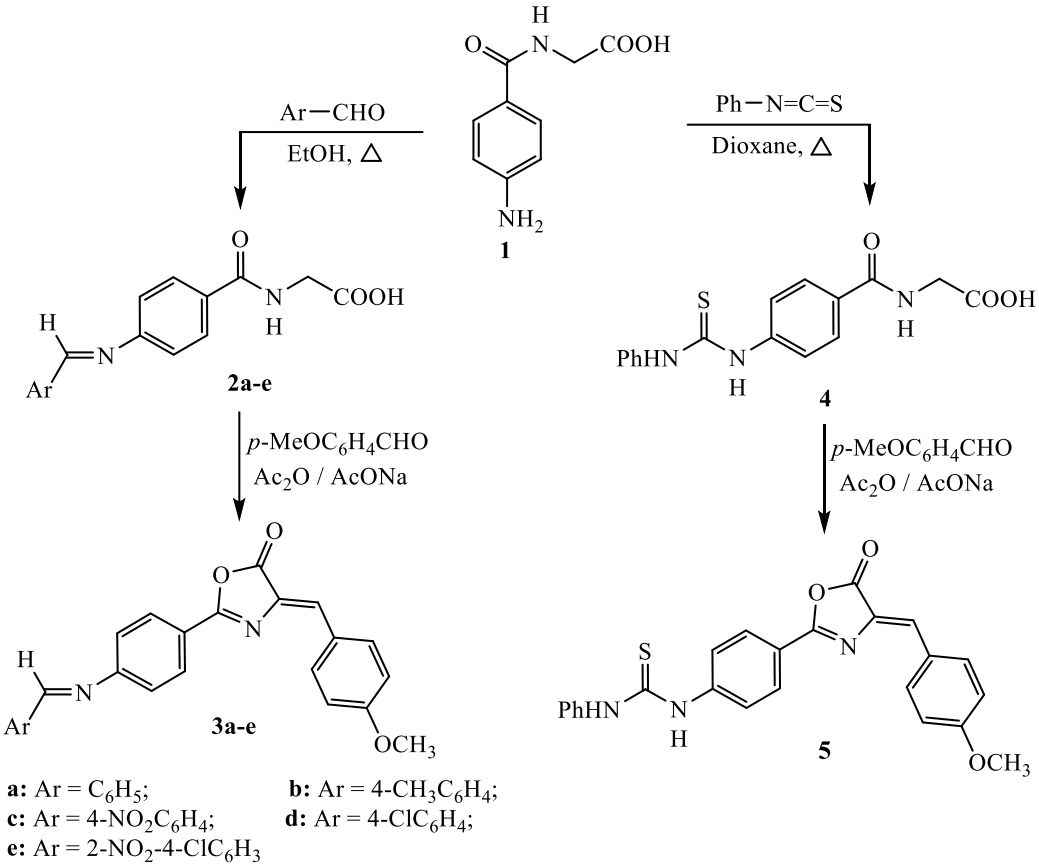
For the estimation of the cytotoxicity effects of the investigated oxazolone scaffolds, four human cancer cell lines were used (namely, hepatocellular carcinoma HepG-2, colorectal carcinoma HCT-116, human prostate cancer PC-3 and mammary gland breast cancer MCF-7). These cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Cytotoxicity determinations are based on the transformation of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in practical cells. The method of this MTT test was performed as previously described in detail [38-40].

3. Results and Discussion

3.1. Chemistry.

Condensation of *p*-aminohippuric acid **(1)** with different substituted aromatic aldehydes yielded the corresponding 4-(arylideneamino-benzoyl)-glycine derivatives **2a-e** (Scheme 1), which were analyzed and secured by their compatible IR, ^{1}H NMR and elemental analysis. The IR spectrum of **2a** (as an example) revealed the characteristic absorptions of (NH & OH) and carbonyl groups at 3318 and 1744 cm^{-1}, respectively. The ^{1}H NMR spectrum of **2b** exhibited three singlet signals for the protons of OH, NH and CH=N function at 12.30, 10.25 and 8.25 ppm, respectively, multiplet for the aromatic protons (7.80-7.25 ppm), doublet for two protons

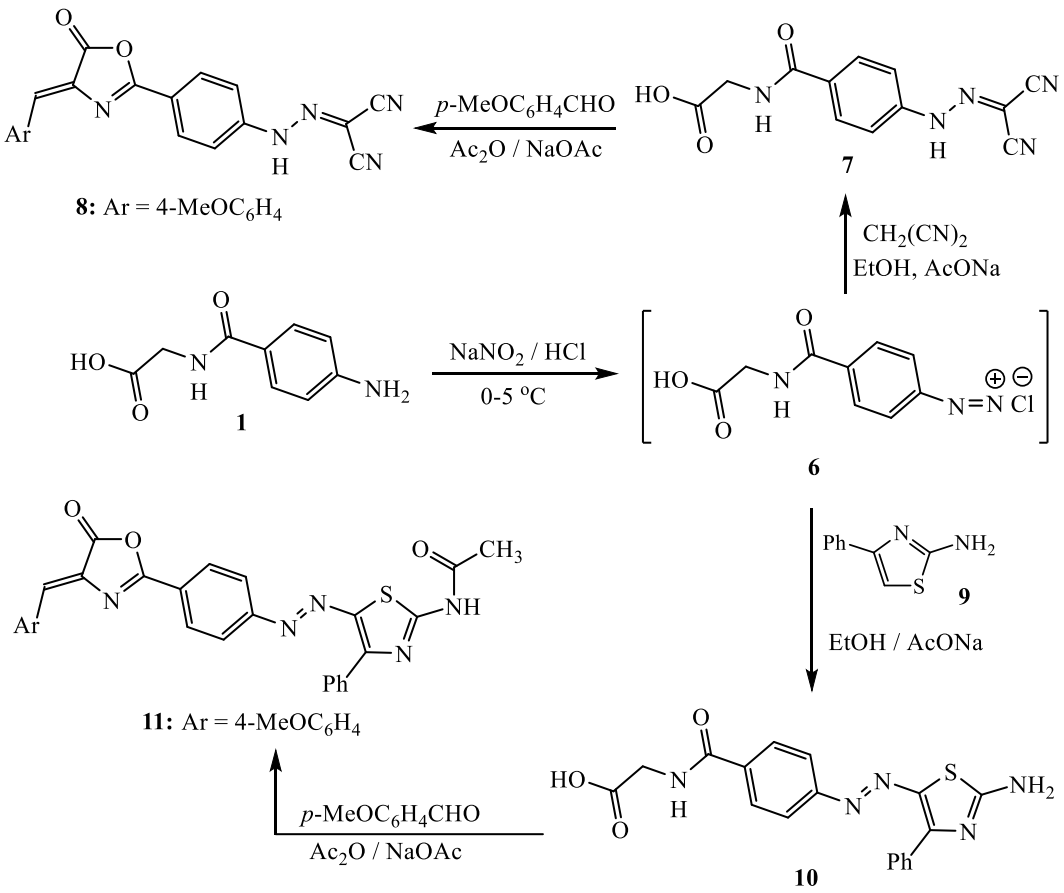
at 3.65 ppm (-CH_2-) and singlet for three protons at 2.35 ppm (-CH_3). Then, the formation of 2-(4-arylideneamino)-phenyl)-4-(4-methoxybenzylidene)-oxazol-5(4*H*)-ones **3a-e** were achieved by classical Erlenmeyer reaction [41] that involve heating of compounds **2a-e** with *p*-anisaldehyde at 100°C in acetic anhydride and sodium acetate. The IR spectrum of **3a** revealed the characteristic absorption band of the carbonyl group of the oxazolone ring at 1793 cm^{-1}. The reaction of *p*-aminohippuric acid **(1)** with phenyl isothiocyanate has been previously reported in the literature [42] to afford 4-(3-phenylthioureido)-benzoyl-glycine **(4)**, which was utilized in the synthesis of 4-(4-methoxybenzylidene)-2-(4-*N'*-phenylthioureido-phenyl)-oxazol-5(4*H*)-one **(5)** by classical Erlenmeyer reaction. The reaction involves heating of **4** with *p*-anisaldehyde at 100°C in acetic anhydride and sodium acetate. The spectral data (IR and ^1H NMR) has been utilized to establish the proposed structure of **5**. In the infrared spectrum of **5**, the NH and carbonyl functional groups were absorbed in 3387 and 1781 cm^{-1}, respectively. ^1H NMR spectrum exhibited two singlet signals for the protons of NH functions at 13.25 and 11.75 ppm, multiplet for the aromatic and olefinic protons (7.95-7.24 ppm) and singlet for three protons at 3.79 ppm (OCH_3).



Scheme 1. Synthesis of 2-(4-substitutedamino-phenyl)-4-(4-methoxybenzylidene)-oxazol-5(4*H*)-ones **3** and **5.**

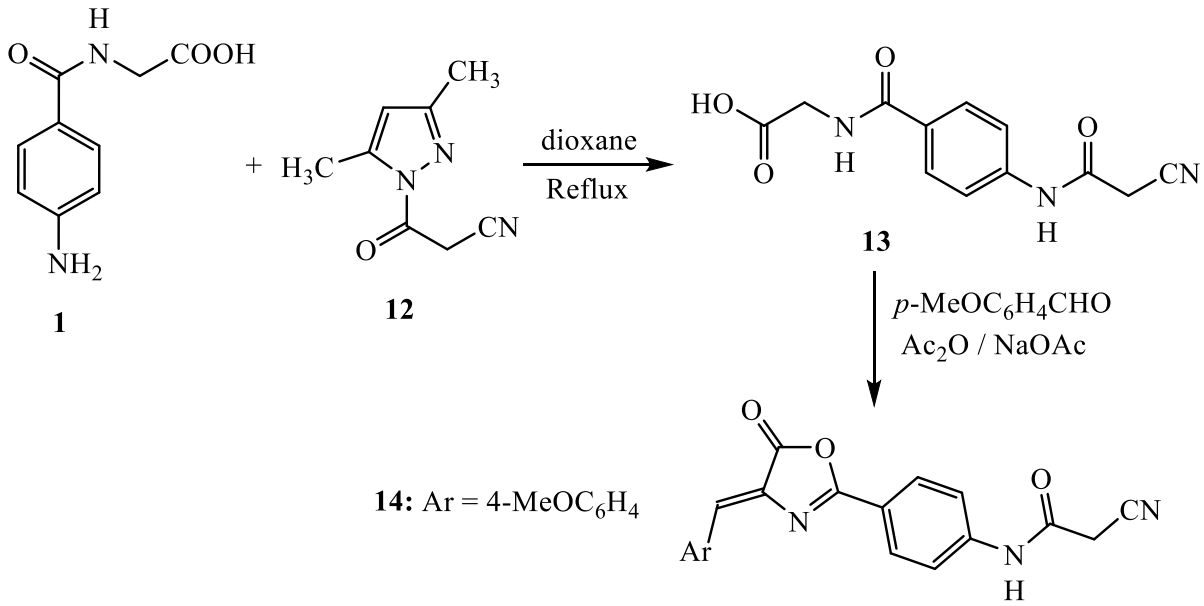
In addition, 4-[2-(dicyanomethylene)hydrazinyl]benzoyl-glycine **(7)** has been synthesized in 76% yield by diazotization of *p*-aminohippuric acid at 0-5°C with sodium nitrite and hydrochloride acid followed by diazo coupling with malononitrile as active methylene component in ethyl alcohol buffered with sodium acetate (Scheme 2). The characteristic NH and OH functions (IR spectrum of **7**) absorbed at 3395 and 3222 cm^{-1}. In comparison, absorptions at 2226 and 1753 cm^{-1} indicated the presence of nitrile and carbonyl groups, respectively. ^1H NMR spectrum showed singlet for the carboxylic proton at 12.25 ppm, two singlet signals for the protons of NH functions at 11.10 and 8.90 ppm, two doublet signals for

the aromatic protons at 7.95 and 7.70 ppm and doublet for the methylene protons at 3.68 ppm. Synthesis of our target 2-[4-(dicyanomethylene-hydrazinyl)-phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4*H*)-one **(8)** has been achieved by the application of classical Erlenmeyer method, involving condensation of benzoyl glycine derivative **7** with *p*-anisaldehyde in acetic anhydride and sodium acetate by heating at 100 °C. The IR spectrum of **8** indicated absorptions at 3226 cm^{-1} for the NH function, 2224 cm^{-1} for the nitrile group, and 1788 cm^{-1} for the carbonyl group (oxazolone ring). ^1H NMR spectrum displayed singlet for hydrazone proton at 10.58 ppm, multiplet for the aromatic and olefinic protons at 7.96-7.13 ppm, and singlet for the methoxy protons at 3.86 ppm. In addition, 4-[(2-amino-4-phenylthiazol-5-yl)azo]benzoyl-glycine **(10)** was synthesized in good yield (%80) by diazotization of **1** with sodium nitrite at 0-5°C and hydrochloric acid followed by diazo coupling with 2-amino-4-phenylthiazole **(9)** in ethanol buffered by sodium acetate solution. The IR spectrum of **10** exhibited broad absorption bands for the NH_2, NH, and OH groups centered at 3402 and 3337 cm^{-1} and broad absorption at 1627 cm^{-1} indicating the carbonyl function. The formation of 2-[4-(2-acetylamino-4-phenylthiazol-5-yl)azo-phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4*H*)-one **(11)** was achieved by condensation of 5-arylazothiazole derivative **10** with *p*-anisaldehyde in acetic anhydride and sodium acetate at 100°C. The proposed structure of **11** was elucidated by spectral analyses (IR and ^1H NMR). The infrared spectrum of **11** exhibited the absorption of NH group at 3162 cm^{-1} and absorptions due to two carbonyl functions at 1772 (oxazolone ring) and 1684 cm^{-1} (amide). ^1H NMR spectrum displayed singlet for the proton of NH at 12.68 ppm, multiplet for the aromatic and olefinic protons in the region 8.24-7.21 ppm, and two singlet signals for the protons of methoxy and methyl groups at 3.80 and 2.23 ppm.



Scheme 2. Synthesis of 4-(4-methoxybenzylidene)-2-[4-substituted-azophenyl]-oxazol-5(4*H*)-ones **8** and **11.**

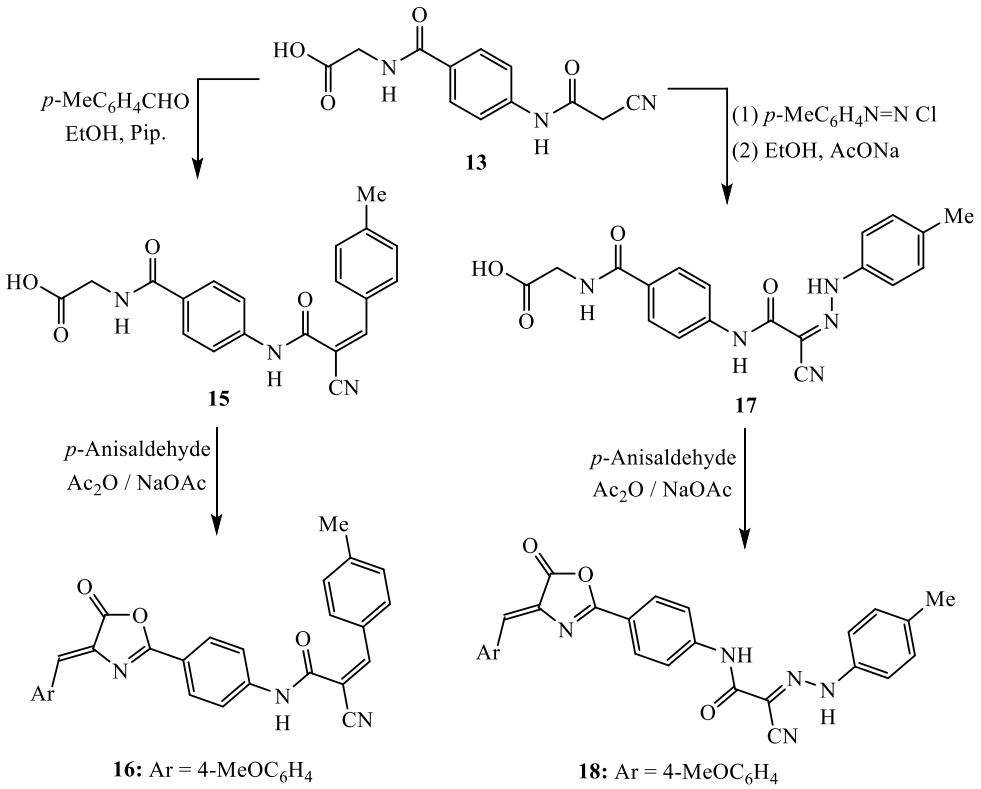
The formation of cyanoacetamide scaffolds has been achieved by different methods. The literature provided several methods to prepare many *N*-aryl or *N*-heteroaryl-cyanoacetamides [43,44]. Cyanoacetyl pyrazole, which was synthesized by Ried *et al.* [45], is a very handily and successfully utilized for the preparation of many *N*-alkyl and *N*-aryl cyanoacetamide compounds. Thus, cyanoacetylation of *p*-aminohippuric acid was achieved by heating with 3,5-dimethyl-1-cyanoacetyl pyrazole in dioxane to furnish the corresponding *p*-(*N*-cyanoacetylamino)-hippuric acid **(13)** (Scheme 3). Heterocyclization of cyanoacetamide derivative **13** into our target oxazolone was achieved by condensation with *p*-anisaldehyde in acetic anhydride and sodium acetate at 100°C to furnish 2-[4-(2-cyanoacetamido)phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4*H*)-one **(14)**. The IR spectrum of **14** revealed absorption bands of NH and nitrile functions at 3353 and 2213 cm^{-1}, in addition to absorption bands of two carbonyl groups (C=O) at 1748 and 1698 cm^{-1}. ^1H NMR spectrum exhibited singlet for the proton of NH function at 9.45 ppm, multiplet for the aromatic and olefinic protons in the region 7.80-6.90 ppm, and two singlet signals for the protons of $-CH_2-$ and $-OCH_3$ at 3.90 and 3.80 ppm, respectively.



Scheme 3. Synthesis of 2-[4-(2-cyanoacetamido)phenyl]-4-(4-methoxybenzylidene)-oxazol-5(4*H*)-one **(14).**

In addition, 4-[2-cyano-3-(4-tolyl)-acrylamido]benzoyl-glycine **(15)** has been formed by condensation of compound **13** with *p*-tolualdehyde by heating under reflux in ethanol containing drops of piperidine (Scheme 4). IR and ^1H NMR spectral analyses have been employed to elucidate the proposed structure of **15**. In the IR spectrum, NH and OH groups absorbed at 3321, 3212, and 3157 cm^{-1}, nitrile group absorbed at 2214 cm^{-1} while absorptions of the two carbonyl groups (C=O) were recognized at 1683 and 1649 cm^{-1}. ^1H NMR spectrum showed singlet for the olefinic proton (C=CH) at 8.28 ppm, four doublet signals for the aromatic protons (8.05-7.15 ppm), doublet for two protons at 3.66 ppm ($-CH_2-$) and singlet for three protons at 2.35 ppm ($-CH_3$). After that, 2-[4-(2-cyano-3-(4-tolyl)acrylamido)phenyl]- 4-(4-methoxybenzylidene)-oxazol-5(4*H*)-one **(16)** was prepared by classical Erlenmeyer method, involving heating of the unsaturated nitrile scaffold **15** with *p*-anisaldehyde in acetic anhydride and sodium acetate at 100 °C. The ^1H NMR spectrum of oxazolone **16** showed singlet for the proton of NH function at 10.55 ppm, singlet for one olefinic proton at 8.23 ppm, multiplet for the aromatic and olefinic protons in the region 8.04-7.15 ppm and two singlet

signals for the protons of methoxy and methyl groups at 3.87 and 2.31 ppm, respectively. The reactivity of the methylene group of cyanoacetamide derivative **13** has been examined towards electrophilic diazo coupling reaction. Thus, diazo coupling reaction of cyanoacetamide derivative **13** with *p*-tolyl diazonium chloride at 0-5°C in ethanol buffered with sodium acetate solution furnished 4-[2-cyano-2-(4-tolylhydrazono)-acetamido]-benzoyl-glycine **(17)**. The ^{1}H NMR spectrum showed singlet for the carboxylic proton at 12.25 ppm, three singlet signals for the protons of NH functions at 11.10, 9.65 and 8.90 ppm, multiplet for the aromatic protons (7.75-7.30 ppm), doublet for the methylene protons at 3.71 ppm and singlet for three protons of the methyl group at 2.35 ppm. Finally, the synthesis of 2-[4-(2-cyano-(4-tolylhydrazono)-acetamido)-phenyl]- 4-(4-methoxy-benzylidene)-oxazol-5(4*H*)-one **(18)** was achieved by heating of **17** with *p*-anisaldehyde in acetic anhydride and sodium acetate at 100°C. The IR spectrum of **18** displayed the absorptions of NH function at 3393 cm^{-1} and cyano function at 2214 cm^{-1} in addition to two absorptions 1785 and 1681 cm^{-1} for the carbonyl groups. In the ^{1}H NMR spectrum, the protons of NH groups resonated as two singlet signals at 11.45 and 10.20 ppm. The multiplet in the region 7.92-6.90 ppm indicated the aromatic and olefinic protons. In comparison, the protons of -OCH_3 and -CH_3 groups resonated as two singlet signals at 3.82 and 2.32 ppm.



Scheme 4. Synthesis of 2-[4-(substituted-2-cyanoacetamido)-phenyl]-4-(4-methoxybenzylidene)oxazol-5(4*H*)-ones **16** and **18.**

3.2. In vitro anticancer activity.

The pharmacological activities of oxazolone compound **3a-e**, **5**, **8**, **11**, **14**, **16** and **18** were performed against four types of human cancer cell lines: HepG2 (hepatocellular carcinoma), HTC-116 (colorectal carcinoma), PC-3 (prostate cancer) and MCF-7 (mammary gland breast cancer). As seen in Table 1, the majority of the synthesized oxazolone scaffolds reveal moderate to strong cytotoxic effects toward the four tested human cancer cell lines. The

oxazolone compound **3e** exhibited the highest cytotoxic effect against two of the tested cell lines HepG2 (IC_{50} 8.9±0.30 µg/mL) and HTC-116 (IC_{50} 9.2±0.63 µg/mL), their IC_{50} values were near to the standard anticancer drug 5-fluorouracil (5-FU). These promising results may be attributed to the presence of electron-withdrawing groups (chlorine and nitro) at the phenyl moiety. Besides, it showed strong cytotoxic effects among the other two cancer cell lines PC-3 (IC_{50} 13.1 ± 1.06 µg/mL) and MCF-7 (IC_{50} 12.1 ± 1.06 µg/mL). Furthermore, against all tested cancer cell lines, the oxazolone compound **18** was found to show strong cytotoxic effects. In contrast, the oxazolone compounds **3d, 5,** and **14** exhibited moderate cytotoxic effects.

Table 1. Cytotoxicity data of the prepared oxazolone scaffolds against human tumor cells.

Compound No.	*In vitro* Cytotoxicity IC50 (µg/mL)			
	HepG2	HCT-116	PC-3	MCF-7
5-FU	7.91 ± 0.28	5.20 ± 0.14	8.30 ± 0.25	5.51 ± 0.21
3a	70.5 ± 4.83	61.8 ± 4.85	47.5 ± 3.69	59.0 ± 4.30
3b	81.5 ± 5.60	86.2 ± 5.07	92.5 ± 6.11	89.6 ± 5.38
3c	47.7 ± 4.67	51.9 ± 4.39	67.7 ± 4.96	62.4 ± 4.31
3d	16.4 ± 1.63	20.0 ± 1.67	27.3 ± 2.32	30.8 ± 2.90
3e	8.9 ± 0.30	9.2 ± 0.63	13.1 ± 1.06	12.1 ± 1.06
5	25.8 ± 1.35	19.2 ± 1.32	29.9 ± 1.98	33.5 ± 2.87
8	73.0 ± 5.31	80.9 ± 5.67	82.3 ± 4.69	87.4 ± 5.21
11	62.3 ± 3.96	55.4 ± 4.31	71.3 ± 4.37	85.4 ± 4.87
14	22.6 ± 2.08	32.8 ± 2.42	33.2 ± 2.63	31.3 ± 2.26
16	89.5 ± 5.62	93.1 ± 6.37	96.4 ± 5.23	92.9 ± 5.65
18	14.7 ± 1.48	13.4 ± 1.26	15.9 ± 1.55	18.9 ± 2.00

4. Conclusions

New series of oxazolone scaffolds **3a-e**, **5**, **8**, **11**, **14**, **16,** and **18** were synthesized via application of Erlenmeyer reaction to p-substituted amino-hippuric acid derivatives. Most of the synthesized compounds exhibited moderate to strong cytotoxic effects toward the tested human cancer cell lines (HepG2, HTC-116, PC-3, and MCF-7). The best activity was obtained with compound **3e** against hepatocellular carcinoma (IC_{50} 8.9±0.30 µg/mL) and colorectal carcinoma (IC_{50} 9.2±0.63 µg/mL) cell lines compared with the standard anticancer drug 5-fluorouracil, long or complex.

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Conflicts of Interest

The authors declare no conflict of interest.

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