1,3-Dipolar Cycloaddition: Free Catalytic Synthesis and Esophageal Cancer Activity of New 1,2,3-Triazole-Oxydianiline-Maleimide Hybrids

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Abstract

A new series of 1,2,3-triazole-oxydianiline-maleimide hybrids 12-15 was synthesized by using 1,3-dipolar cycloaddition reaction of N-Arylmaleimides 6-9 with 4,4'-oxybis(azidobenzene) 11 under an efficient and free catalytic reaction. All the newly synthesized hybrids were characterized by their 1H NMR, F-TIR, Mass spectral data and melting points. The cytotoxic activities (in vitro) of selected hybrids against esophageal cancer of human cell line (SKG) were evaluated by MTT assay. Among them, hybrid 13 exhibited a potent inhibition activity with the IC50 value of 1.61±0.01 μM against esophageal cancer cell (SKG). Cellular mechanism investigations in esophageal carcinoma cells (SKG) elucidated that hybrid 13 inhibited cell growths in vitro and arrested cell cycle at an environmental phase. These results revealed that hybrid 13 holds a promising anticancer agent with the enhancement of further clinical applications in drug discovery field.

Keywords: Esophageal cancer; 1,3-dipolar cycloaddition; free catalytic synthesis; 1,2,3-triazole hybrids.

1. Introduction

Cancer is an uninhibited growth of cells, considered as one of the threatening diseases which causes fatal dangers to human lives in the worldwide [1-3]. Esophageal cancer is being ranked as the sixth deadly cancer type in the worldwide [4]. Esophageal cancer is a malignant tumor which occurs in the inner layers of the mucosa (lining) of the esophagus cell [5]. The esophageal cancer cells possess the ability to spread out into other organs of the human body [6]. The esophageal cancer's progression is a multistep process which starts with an accumulation of genetic and epigenetic alterations which leads to the activation of oncogenes [7]. By looking at the reported incidence of esophageal cancer it can be concluded that esophageal cancer is the most common cancer type among men than women. Although the real numbers of incidences of esophageal cancer remains moderate approximately 15,500 cases in year, it is increasingly growing cancer in the United States [8]. There are two types of esophageal cancer, including adenocarcinoma and squamous cell carcinoma. Squamous cell carcinoma is often combined with several factors involving achalasia, advanced age, alcohol abuse, tobacco use and high-starch diets [9, 10]. Esophageal cancer's treatment normally includes healing strategies such as surgery, radiotherapy and chemotherapy. Chemotherapy is an effective strategy for suppressing cancer growth and eradication of tumors [11, 12]. The developed resistance of cancer cells toward the chemotherapeutic agents and association with side effects are a major obstacle toward an efficient cure of cancer [13]. Therefore, the development of novel anti-esophageal cancer agents with merits like a significant cytotoxicity, better efficacy and reduction of side effects is urgently needed [14, 15]. The creation of anti-esophageal cancer compounds is the top target to overcome these obstacles and leads to effective clinical therapies [16].

The hybridization of pharmacological molecules is a powerful strategy to drug discovery and applied to target various diseases. The hybridization of two or more drug pharmacophores into a single molecule supply a prospect of best drugs for the cure of a number of diseases, particularly for cancer [17, 18]. The synthesis of effective anticancer hybrids has recently emerged as an efficient protocol for...
esophageal squamous cell carcinoma [19, 20]. Moreover, the synthesis of hybrid organometallics has been systematically investigated as it can offer potent drugs, for example, “Hybrid anticancer drugs” reported by Saha et al [21].

On the other hand, 1,2,3-triazole ring has attracted a paramount attention of the medicinal and organic chemists due to this moiety which offers a broad spectrum of biological activities [22]. The 1,2,3-triazole derivatives are well known as privileged pharmacophores because of their efficient biological activities [23]. Literatures have reported several methodologies for the synthesis of 1,2,3-triazole derivatives, but thermal 1,3-dipolar cycloaddition (Huisgen reaction) of terminal alkynes with azides is the best traditional methodology for the synthesis of these derivatives [24].

Hybridization of known pharmacophores with 1,2,3-triazole ring to enhance their physiochemical properties and anticancer activity has become a challenging scope in drug discovery [25]. Triazole moiety which conjugates with indole (compound A) showed a significant role to inhibit growth of cancer cells and induce apoptosis [26]. Compound B, a 1, 2, 3-triazol-dithiocarbamate-urea hybrid exhibited a potency with IC50 values of 1.86 and 1.62 μM against cell lines MCF-7 and MGC-803, respectively [27]. In addition, 1,2,3-triazol-picolinamidine hybrid (compound C) was found to be potent and better activity against breast cancer cell Line MCF-7 (IC50 = 0.046 ± 0.022 μM) and lymphoma cell Line U937 (IC50 = 0.58 ± 0.42 μM) [28].

Several molecular hybrids were efficiently synthesized using a 1,3-dipolar cycloaddition reaction to afford versatile 1,2,3-triazole hybrid systems [29, 30]. The study of new hybrid system in that 1,2,3-triazole, oxydianiline and maleimide are combined considered an unexplored field of organic chemistry. These findings have encouraged us to study the potential anticancer effect of 1,2,3-triazole, oxydianiline and maleimide hybrids. Thus, as a part of our interest with 1,3-dipolar cycloaddition reaction and in order to examine new 1,2,3-triazole-oxydianiline-maleimide hybrids with a good antiesophageal cancer activity, herein we report the design, synthesis and anti-esophageal cancer of human cell line (SKG) evaluation of a new series of 1,2,3-triazole-oxydianiline-maleimide-hybrids 12-15.

2. Materials and Methods

All chemicals and solvents were ordered from Sigma-Aldrich. All chemicals were at least of ACS grade, and solvents were obtained in analytical grade. Trypsin/EDTA was purchased from Capricorn (USA), RPMI 1640 and Fetal bovine serum from Gibco (USA), MTM stain from Sigma (USA), Cell culture plates from Thermo Fisher Scientific (USA), CO2 incubator from Cypress Diagnostics (Belgium). Analytical properties of TLC were performed by using plates precoated with a silica gel 60 UV 254 (Merck). The dot spots of all synthesized compounds were visualized by UV light at 254 nm. The final purification of the synthesized compounds was performed with purity >95%. Melting points were obtained from a Gallenkamp melting point apparatus in capillary tubes. 1H NMR spectra were recorded on a Bruker inova AV-400 spectrometer (Iran) at room temperature in DMSO-d6 as solvent with a signal peak of 1H spectra at δ 2.50 ppm. Chemical shifts were given in ppm (δ scale) and the coupling constant values (J) are given in Hz. The splitting pattern was abbreviated as follows: (s, singlet), (d, doublet), (t for triplet) and (m, multiplet). Fourier transform infrared (FTIR) spectra were run on Shimadzu FTIR-8300 spectrophotometer (Iraq) using KBr (1%) discs and the absorbance was reordered between 4000-600 cm⁻¹. Accurate mass was performed by using a Micro Mass LCT operating in Electrospray mode (ES-MS) (Iran).

2.1. Synthesis of N-arylmaleimides (6-9) [31]

To a solution of maleic anhydride 5 (20 mmol) in diethyl ether (25 mL), an equivalent of various anilines 1-4 (20 mmol) dissolved in diethyl ether (5 mL) was added. The suspension mixture was subjected to stirring at room temperature for an hour and the resulting mixture was monitored by TLC until the starting materials were consumed. The resulting N-substituted maleic acids were filtered, dried, and
delivered to a flask (50 mL) containing an anhydrous solution of sodium acetate (8 mmol) in acetic anhydride (6.7 mL). The mixture was additionally stirred for 30 min over a steam bath, cooled to room temperature and transferred into an iced water (100 mL). The resulting precipitate was filtered and washed thoroughly with cold water (30 mL). The pure N-arylmaleimides 6-9 were obtained through recrystallization from EtOH: H2O (1:1) as follows: N-(4-Methylphenyl)maleimide 6 as yellow solid: Yield 84%, m.p 158-160 °C. 4-Maleimidobenzoic acid 7 as colorless solid: Yield 85%, m.p 232-234 °C. N-(2-Methoxyphenyl) maleimide 8 as brown solid: Yield 70%, m.p 158-160 °C and N-(2-Methylphenyl)maleimide 9 as yellow solid: Yield 85%, m.p 147-148 °C.

2.2. Synthesis of 4,4′-oxybis(azido benzene) (11)[32]

To a solution of 4,4’-oxydianiline 10 (2.0 mmol), conc. HCl (0.7 mL) and water (10 mL), sodium nitrite (2.0 mmol) dissolved in water (5 mL) was added drop wise at 0 °C. The mixture was stirred at 0 °C for 15 min and water (10 mL) was added. To above mixture, sodium azide (2.0 mmol) dissolved in water (5 mL) was gradually added and the resulting mixture was stirred at 0 °C till a beige solid would precipitate. The collected precipitate was filtered, dried and re-crystallized from chloroform: hexane (1: 1) to give 4,4′-oxybis(azidobenzene) 11 as a beige solid: Yield 90%, m.p 311-312 °C.

2.3. General method for the synthesis of 1,2,3-triazole-oxydianiline-maleimide hybrids (12-15) [33]

To a solution of appropriate N-arylmaleimides 6-9 (2.0 mmol) in EtOH: H2O (1:1, 10 mL), 4,4′-oxybis(azido benzene) 11 (1.0 mmol) was slowly added. The reaction mixture was refluxed for an appropriate time as shown in Table 1. The resulting mixture was checked by TLC until the starting materials were consumed. The resulting reaction was cooled to precipitate crude products. The crude products were separated, washed with cooled ethanol, re-crystallized from THF: hexane (1:1) and dried under vacuum desiccators to afford the pure hybrids 12-15 in yield 55-75%. The purification of synthesized hybrids 12-15 was performed with purity >95%. The structures of these hybrids were confirmed by their 1H NMR, FTIR and Mass spectral data (see supplementary materials).

1,1′-(Oxybis(4,1-phenylene))bis(5-(p-toly)-3a,6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6 (1H, 5H)-dione) (12)

Hybrid 12 was obtained as a white solid, yield 65%. m.p 154-155 °C. FTIR spectrum (KBr, cm⁻¹):

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2982 (C-H), 1724 (C=O), 1502 (C=C, Ar), 1381 (N=N). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 7.13-7.64 (m, 16H, CH of phenyl ring), 5.98 (d, 2H, J = 10.1 Hz, 2CH), 5.30 (d, 2H, J = 10 Hz, 2CH). 1.82 (s, 6H, 2CH3). Mass (EI⁺) (M⁺) calculated for C34H23N6O8: 626.2026 found: 625.2016.

4,4′-(Oxybis(4,1-phenylene))bis(4,6-dioxo-3a,4,6a-tetrahydropyrrolo[3,4-d][1,2,3]triazole-1,5 (1H)-dily)benzoic acid (13)

Hybrid 13 was obtained as a white solid, yield 75%. mp 173-175 °C. FTIR spectrum (KBr, cm⁻¹): 3053 (C=H, Ar), 1726 (C=O), 1500 (C=C, Ar), 1377 (N=N). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 7.12-7.65 (m, 16H, CH of phenyl ring), 5.96 (d, 2H, J = 10 Hz, 2CH), 5.28 (d, 2H, J = 10 Hz, 2CH). Mass (EI⁺) (M⁺) calculated for C34H23N6O8: 686.1509 found: 685.1500.

1,1′-(Oxybis(4,1-phenylene))bis(5-(2-methoxy phenyl)-3a,6a-dihydropyrrolo[3,4-d]) [1,2,3]triazole-4,6(1H,5H)-dione) (14)

Hybrid 14 was obtained as a white solid, yield 55%. mp 148-150 °C. FTIR spectrum (KBr, cm⁻¹): 3061 (C=H, Ar), 2976 (C-H) 1728 (C=O), 1500 (C=C, Ar), 1377 (N=N). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 7.11-7.64 (m, 16H, CH of phenyl ring), 5.94 (d, 2H, J = 9.2 Hz, 2CH), 5.26 (d, 2H, J = 9.2 Hz, 2CH), 2.34 (s, 6H, 2OCH3). Mass (EI⁺) (M⁺) calculated for C34H23N6O8: 658.1924 found: 657.1918.

1,1′-(Oxybis(4,1-phenylene))bis(5-(o-toly)-3a, 6a-dihydropyrrolo[3,4-d][1,2,3]triazole-4,6 (1H, 5H) -dione) (15)

Hybrid 15 was obtained as a white solid, yield 60%. mp 160-162 °C. FTIR spectrum (KBr, cm⁻¹): 3060 (C=H, Ar), 2970 (C-H) 1728 (C=O), 1500 (C=C, Ar), 1386 (N=N). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 7.12-8.06 (m, 16H, CH of phenyl ring), 5.99 (d, 2H, J = 6.2 Hz, 2CH), 5.30 (d, 2H, J = 6.1 Hz, 2CH), 2.08 (s, 6H, 2OCH3). Mass (EI⁺) (M⁺) calculated for C34H23N6O8: 626.2026 found: 625.2019.

2.4. Cell culture

Human SKG cancer cell line was obtained from the IRAQ Biotech Cell Bank Unit in Basrah, maintained in in plate (10 cm) containing RPMI-1640 and supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were conducted through Trypsin-EDTA, reseeded at 50% confluence twice a week and incubated with a humid atmosphere (CO₂, 5%) at 37 °C.

2.5. Cytotoxicity assays and dose response

To determine the cytotoxic effect, the MTT assay of cell viability was carried out on 96-well plates for 48 h. SKG cells were seeded at 1 × 10⁴ cells/well. After 24 h, a confluent monolayer was performed
and cells were treated with the screened compounds with final concentration (1000 µg/mL). After treatment for 72 h, the cell viability was measured by removing the medium, adding 28 µL of solution (2 mg/mL, MTT) and incubating at 37 °C for 2 h. After the MTT solution was removed, the remaining crystals in the wells were solubilized by an addition of DMSO (100 µL) and followed by shaking at 37 °C for 15 min. The absorbance of cell viability was determined on a micro-plate reader (Thermo Fisher Scientific) at 620 nm (tested wavelength) and all experiments were repeated in triplicate.

The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated by using the following Equation 1:

\[
Proliferation \ Rate \ (PR) = \frac{B}{A} \times 100\%
\]

Where, \(A\) = an optical density of untreated wells.

\(B\) = an optical density of treated wells.

2.6. Statics analysis

Results and data were expressed as mean ± SEM, GraphPad Prism 8.1 and the values of *\(p\) < 0.05 were taken as a statistical significant.

3. Results and Discussion

3.1. Chemistry

\(N\)-Arylmaleimides 6-9 were synthesized from various anilines 1-4 and malic anhydride 5 by stirring the mixture in diethyl ether at temperature room for an hour. Then, the resulting mixtures were treated with sodium acetate and acetic anhydride under refluxing for 30 min over a steam path. The formation of \(N\)-arylmaleimides 6-9 was indicated by TLC (petroleum ether/ethyl acetate, 6:4) as an eluent for the crude reaction mixture (Scheme 1).

Next, the 4,4’-oxydianiline 10 treated with con. HCl and NaNO\(_2\) at 0-5 °C offered the solution of diazonium salt which was immediately used for the next step without purification. Thus, an addition of an aqueous solution of NaN\(_3\) to diazonium salt solution led to produce 4,4’-oxybis(azido benzene) 11 (Scheme 2).

The final step includes synthesis of new 1,2,3-triazole-oxydianiline-maleimide hybrids 12-15 through a 1,3-dipolar cycloaddition reaction. Free catalytic reaction was applied for the synthesis of new 1,2,3-triazole-oxydianiline-maleimide hybrids 12-15. Initially, the model reaction was carried out by refluxing \(N\)-arylmaleimides 6 and 4,4’-oxybis(azido benzene) 11 for an appropriate time as shown in the Table 1. As result, EtOH: H\(_2\)O (1:1) was chosen as the best appropriate solvent for the 1,3-dipolar cycloaddition synthesis of new 1,2,3-triazole-oxydianiline-maleimide hybrids 12-15 (Table 1).

Table 1. 1,3-Dipolar cycloaddition for the synthesis of new 1,2,3-triazole-oxydianiline-maleimide hybrids 12-15

<table>
<thead>
<tr>
<th>Com</th>
<th>R</th>
<th>Time (h)</th>
<th>M.p (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>4-CH(_3)</td>
<td>8</td>
<td>154-155</td>
<td>65</td>
</tr>
<tr>
<td>13</td>
<td>4-CO(_2)H</td>
<td>19</td>
<td>173-175</td>
<td>75</td>
</tr>
<tr>
<td>14</td>
<td>2-OCH(_3)</td>
<td>3</td>
<td>148-150</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>2-CH(_3)</td>
<td>2</td>
<td>160-162</td>
<td>60</td>
</tr>
</tbody>
</table>
The success of synthesis of new 1,2,3-triazole-oxydianiline-maleimide hybrids 12-15 was clearly evidenced by their \(^1\)H NMR analysis through the appearance of two distinct doublets at \(\delta\) 5.24–6.01 ppm for fused 1,2,3-triazole-maleimide protons (CH groups) at the expected region. The \(^1\)H NMR spectrum confirmed that the absence of CH protons (CH=CH groups) of N-arylmaleimides 6-9 was a good evidence of the success of 1,3-dipolar cycloaddition reaction. The other aromatic protons were displayed in the expected regions. The formation of hybrids 12-15 was also confirmed by the presence of absorption bands at the expected region in FTIR spectra. FTIR spectrum clearly showed the absence of an azido benzene group at 2121 cm\(^{-1}\) and the appearance of strong absorption bands at 1377-1386 cm\(^{-1}\) attributed to the azo group (N=N) of 1,2,3-triazole moiety. Furthermore, mass spectra data of all 1,2,3-triazole-oxydianilinemaleimide hybrids 12-15 were in consistent with the expected structures. (See experimental section).

3.2. Cytotoxicity on esophageal cancer cells

The favorable properties of 1,2,3-triazole ring involve a hydrogen bonding capability, moderate dipole character and stability under \textit{in vivo} conditions [34]. 1,2,3-Triazole moiety can actively participate in dipole-dipole interactions, stable for oxidative and not be protonated by physiological pH due to its low basicity [35]. These properties are potentially responsible for its enhancement of biological activities. The action of new 1,2,3-triazole hybrids 12-15 against esophageal cancer cell line (SKG) was evaluated via a cytotoxic screening and estimation of IC\(_{50}\). When cell viability test (a cytotoxic assay) was used for new hybrids screening, it was observed that a significant role of these hybrids for reducing the living cells growth of human esophageal cancer cells (SKG). After 72 h of treatment with 1000 \(\mu\)g/mL for each hybrid, it was noted that hybrid 13 showed the best action against cancer cell line (SKG). GraphPad Prism 8.1 was used to calculate the IC\(_{50}\) values for new hybrids 12-15. In particular, hybrid 13 displayed a significant cytotoxic activity with IC\(_{50}\) value of 1.61±0.01 \(\mu\)M, meanwhile, hybrids 12, 14 and 15 exhibited low cytotoxic activity against human esophageal cancer cells (SKG). It is clear from Figs. 2 and 3, the triazole residue hybrid with N-arylmaleimides 7 compound enhance the effect of cytotoxicity of hybrid 13 on human esophageal cancer cell line (SKG), while the nature of the combination of the triazole moiety with N-arylmaleimides 6, 8 and 9 (hybrids 12, 14 and 15) does not influence the relevant cytotoxicity. This can be described to its disparity in either binding properties of protein or bioavailability. The IC\(_{50}\) value of hybrid 13 confirms that hybrid 13 is being considered as a promising anti-esophageal cancer agent.

\[
\text{IC}_{50} = 1.61 \, \mu\text{M}
\]

Fig. 2. GraphPad Prism 8.1 for estimation of IC\(_{50}\) value of hybrid 13.

\[
\text{Absorbance}\% \\
0 \quad 1 \quad 2 \quad 3 \\
0 \quad 50 \quad 100 \quad 150
\]

Concentration of hybrid 13

![Absorbance % vs Concentration of hybrid 13](image)

Fig. 3. Rate of inhibition for four concentrations of hybrid 13.

4. Conclusions

A new series of 1,2,3-triazole-oxydianiline-maleimide hybrids was synthesized through a simple, efficient and free catalytic 1,3-dipolar cycloaddition reaction. As the discovery of new anticancer agents is the top target for medicinal chemistry, the new 1,2,3-triazole-oxydianiline-maleimide hybrids have been evaluated \textit{in vitro} against esophageal cancer. The results exhibited that one hybrid molecule showed a significant activity as a novel potential anti-esophageal cancer agent. Particularly, hybrid 13 showed an excellent growth inhibition against esophageal cancer cell (SKG) with IC\(_{50}\) value of 1.61±0.01 \(\mu\)M and less toxic to normal cells \textit{in vitro} cytotoxicity. It is expected to be a promising anti-esophageal cancer agent. In addition, a further investigation to evaluate its mechanistic study and action is in progress.
Conflicts of interest

There are no conflicts to declare.

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