Exploring the Anticancer and Anti-Inflammatory Activities of Novel Diphenylthiazole-Amino Acid Conjugates

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ABSTRACT

Currently, there are sufficient evidences that there is a strong correlation between inflammation and cancer. In this regard, some NSAIDs such as celecoxib, were studied for the treatment and prevention of colon cancer. We herein have synthesized some novel diphenylthiazole-amino acids conjugates and evaluated their anticancer activity against three cancer cell lines; MCF-7, HT-29 and A549 using MTT assay. Furthermore, their anti-inflammatory activity was evaluated in vivo using carrageenan-induce paw edema assay. Compound 8a bearing methylglycine moiety exhibited the highest anticancer and anti-inflammatory activities with IC₅₀ between 0.6 µM and 4.0 µM and edema inhibition% between 80 and 84, respectively.

INTRODUCTION

Cancer is one of the most fatal diseases all over the world. It is characterized by unwanted, uncontrolled and purposeless cell growth that can spread to other essential organs in the body causing death (Rastogi et al., 2004). There is an increasing interest in development of new anticancer agents with higher efficacy and lower toxicity. Most anticancer therapeutic drugs can't differentiate between healthy cells and damaged cells. The main challenge in cancer treatment is to develop new anticancer drugs with high therapeutic index that can target the cancer cells (Valderrama et al., 2016). Cancer is correlated to inflammation. Acute infection and inflammation is one of the major cancer causes. Oxidants that protect our bodies from death by infection, can cause DNA damage and cancer (Yamashina et al., 1986; Shacter et al., 1988). Chronic hepatic inflammation caused by hepatitis B and C viruses, leads to hepatic cancer (Beasley, 1988; Yu et al., 1991; Tabor and Kobayashi, 1992). Most non-steroidal anti-inflammatory drugs (NSAIDs) act on COX-2 enzyme as a target. COX-2 over expression was observed in most malignant tumor as colon, breast and prostate (Suh et al., 2009; Limongelli et al., 2010; Ho et al., 2013). Peroxidative activity is one of the mechanisms that explain how COX-2 affects tumorigenesis as the reactive metabolites produced through synthesis of prostaglandin have a carcinogenic activity (Koki and Masferrer, 2002; Ghosh et al., 2010). Also, COX-2 inhibits apoptosis that is a process of programmed cell death, leading to an increase in tumor size (Ghosh et al., 2010). Another mechanism that explains COX-2 and cancer relationship is angiogenesis. Blood vessels generation is very necessary for cancer growth. COX-2 induces angiogenesis and so, promotes tumorigenesis (Ghosh et al., 2010). Moreover, COX-2 promotes tumor growth by aromatase transcription mechanism as COX-2-induced prostaglandins are vital for aromatase transcription, leading to an
increase in local estrogen levels, which in turn induces growth of estrogen-dependent breast cancer (Koki and Masferrer, 2002; Zhao et al., 1996; Purohit et al., 1999). Beside treatment of inflammation, COXs inhibitors were used clinically for treatment of other diseases such as atherosclerosis, neuroinflammation, endothelial dysfunction, Parkinson’s disease and preterm labor (Perrone et al., 2010; Biava et al., 2007; Tao et al., 2014).

Recently, diarylheterocycle compounds, such as Celecoxib (I), Etoricoxib (II) and Valdecoxib (III) were used as anticancer beside anti-inflammatory, Fig. 1 (Mozziconacci et al., 2005; El Miedany et al., 2006; Magda et al., 2011). Some diphenylthiazoles exhibited anticancer activity through targeting COX-2 enzyme such as compounds (IV) and (V), Fig. 1 (Carter et al., 1999; Abdelazeem et al., 2014). Also, amino acids play a vital role in human metabolism. Some of them were reported to enhance the physicochemical and biological characters of therapeutic agents (Nichifor and Schacht, 1994; Liu et al., 2002; Yin et al., 2008).

Based on the aforementioned data, some L-amino acid esters were attached to diphenylthiazole nucleus through a spacer that has a breaking point allowing releasing the drug at the target site.

**MATERIAL AND METHODS**

**CHEMISTRY**

(4,5-Diphenyl-thiazol-2-ylamino)-acetic acid hydrazide (6)

Hydrazine hydrate (0.5 mL, 0.015 mol) was added to a solution of 5 (1.01 g, 0.003mol) in absolute ethanol (20 mL). The reaction mixture was heated under reflux for 12 h, concentrated, cooled and diluted with water. The obtained precipitate was collected by filtration, washed with cold water, dried and purified by recrystallization from methanol. Off-white powder; m.p. 170-172 °C, yield 85%. IR (KBr, cm⁻¹): 3422 (NH₂); 3270 (NH); 3052, 3026 (CH aromatic); 2938 (CH aliphatic); 1630 (C=O); 1596 (C=N); 1526 (C=C). ¹H-NMR (DMSO-d₆): δ 4.12 (s, 2H, CH₂); 4.29 (s, 2H, NH₂ exchangeable with D₂O); 7.08-7.39 (m, 10H, aromatic H); 7.96 (s, 1H, NH₂ exchangeable with D₂O); 9.18 (s, 1H, NH exchangeable with D₂O). ¹³C-NMR (DMSO-d₆): δ 45.84; 119.82; 127.65; 127.85; 128.52; 128.99; 129.23; 129.41; 133.11; 135.83; 145.14; 166.19; 168.86. MS, m/z (%): 324 ((M)+, 15.49); 170 (100). Anal. Calcd. for C₁₇H₁₆N₄O₂S (324.40): C, 62.94; H, 4.97; N, 17.27. Found: C, 63.09; H, 5.04; N, 17.51.

General procedure for the preparation of compounds (8a-e)

To a cold solution (-5 °C) of hydrazide 6 (0.32 g, 1 mmol) in acetic acid (6 mL) and water (25 mL) was added a solution of NaN₃ (0.87 g, 1 mmol) in cold water (3 mL). The reaction mixture was stirred at -5 °C for 30 min. The yellow product formed was extracted with cold ethyl acetate (15 mL), washed with cold 3% NaHCO₃ and then with cold water. To this solution an amino acid ester hydrochloride (1 mmol) in ethyl acetate (10 mL) and few drops of triethylamine was added. The reaction mixture was kept at -5 °C overnight, then at 25 °C for another 48 h.

The solution was washed with 10% acetic acid, water, 5% NaHCO₃, and finally with water. Then the solution was evaporated to dryness and the residue was crystallized from ethanol.

[2-(4,5-Diphenyl-thiazol-2-ylamino)-acetylamino]-acetic acid methyl ester (8a)

Yellow powder; m.p. 205-207 °C, yield, 80 %. IR (KBr, cm⁻¹): 3334, 3315 (NHs); 3054(CH aromatic); 2952 (CH aliphatic); 1740, 1660 (C=Os); 1626 (C=N); 1491 (C=C). ¹H-NMR (CDCl₃-d₆): 6.375 (s, 3H, CH₃); 4.08 (s, 2H, CH₂CONH);
2-[2-(4,5-Diphenyl-thiazol-2-ylamo)-acetylamino]-propionic acid ethyl ester (8b)

Yellow powder; m.p. 191–193°C, yield 69 %. IR (KBr, cm⁻¹): 3331 (OH and NHs); 3064 (CH aromatic); 2930 (CH aliphatic); 1737, 1670 (C=O); 1450 (C=C).¹³C-NMR (CDCl₃): δ 128.32; 128.62; 128.92; 128.97; 129.05; 129.42; 131.13; 134.54; 146.14; 160.21; 163.37. MS, m/z (%): 381 (M⁺), 94; 265 (100). Anal. Calcd. For C₂₉H₂₃N₂O₅S (381.45): C, 62.97; H, 5.02; N, 11.02. Found: C, 63.15; H, 5.11; N, 11.23.

2-[2-(4,5-Diphenyl-thiazol-2-ylamo)-acetylamino]-3-hydroxy-propionic acid methyl ester (8c)

Yellow powder; m.p. 191–193°C, yield 69 %. IR (KBr, cm⁻¹): 3331 (OH and NHs); 3064 (CH aromatic); 2930 (CH aliphatic); 1737, 1670 (C=O); 1450 (C=C).¹³C-NMR (CDCl₃): δ 128.32; 128.62; 128.92; 128.97; 129.05; 129.24; 129.33; 129.70; 130.51; 134.18; 146.23; 155.11; 177.67; 178.41. MS, m/z (%): 501 (M⁺), 9.09; 107 (100). Anal. Calcd. For C₂₉H₂₃N₂O₅S (501.60): C, 67.05; H, 5.43; N, 8.38. Found: C, 67.31; H, 5.52; N, 8.52.

2-[2-(4,5-Diphenyl-thiazol-2-ylamo)-acetylamino]-3-(1H-indol-3-yl)-propionic acid ethyl ester (8e)

Yellow powder; m.p. 190–191°C, yield 72 %. IR (KBr, cm⁻¹): 3398 (NHs); 3056 (CH aromatic); 2946 (CH aliphatic); 1737, 1670 (C=O); 1442 (C=C).¹³C-NMR (CHCl₃-d₂): δ 28.19 (t, J = 7.2 Hz, 3H, CH₃), 2.07 (s, 3H, NH exchangeable with D₂O); 3.32 (d, J = 4 Hz, 2H, CH₂CH₃); 4.12 (m, 2H, CH₂CH₃), 4.82 (d, J = 6 Hz, 1H, upfield proton of CH₂NH); 4.88 (t, J = 4 Hz, 1H, CH); 5.02 (d, J = 16 Hz, 1H, downfield proton of CH₂NH); 5.63 (s, 1H, NH exchangeable with D₂O); 6.94–7.53 (m, 16H, 15 aromatic H and NH exchangeable with D₂O).¹³C-NMR (CDCl₃): δ 14.03; 26.70; 45.28; 53.29; 61.79; 111.14; 118.29; 122.05; 123.42; 125.53; 128.13; 128.36; 128.45; 128.60; 129.62; 129.31; 129.66; 129.78; 131.17; 134.33; 135.90; 143.64; 146.18; 163.46; 171.40. MS, m/z (%): 524 ((M⁺), 1.77; 130(100). Anal. Calcd. For C₃₉H₃₈N₂O₅S (524.63): C, 68.68; H, 5.38; N, 10.68. Found: C, 68.90; H, 5.35; N, 10.87.

PHARMACOLOGICAL SCREENING

Anticancer Activity

Cell Culture

Three human cancer cell lines MCF-7, HT-29 and A549 were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco’s modified Eagle’s medium/F12 medium (DMEM/F-12), DMEM or RPMI-1640 media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco) according to ATCC recommendation. All the cell lines were cultured at 37 °C in a humidified incubator containing 5% CO₂ atmosphere for 24 h before the cytotoxicity assessments.

Cell Viability Assay

All the tested diphenylthiazole derivatives were evaluated in vitro for their antitumor activity against three cancer cell lines; MCF-7, HT-29 and A549 using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay method (Gouda et al., 2014; Arafat et al., 2014). Tested samples were added to 6 wells with doxorubicin used as positive reference. Controls received DMSO at the same concentration as that in drug-treated cells. After 48 h, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well. Reduced MTT was solubilized in DMSO (200 µL/well) for determination of absorbance at 570 nm using a microplate reader, Table 1.

Anti-Inflammatory Assay

Wister adult albino rats of both sexes weighing between 120 and 150 g were uniformly hydrated by giving 3 ml water/rat orally to decrease variability to edema response. Animals were divided into 7 groups each of five animals. The control group was
given 10% DMSO aqueous solution (v/v). Indomethacin (100mg/kg) was used as a reference standard drug for comparison and compounds under examination (100 mg/kg) were administered orally in the form of 10% DMSO aqueous solutions 1 h before induction of inflammation. Induction of Paw edema was performed by S.C. injection of 50 µl of 1% carrageenan-sodium gel (Sigma-Aldrich, USA), into the sub-plantar region of the right hind paw. The dorso-ventral diameter (thickness) of the right and left hind paw of each rat was measured using a pair of dial thickness gauge callipers accurate to 0.001 cm 0.5, 1, 3 and 5 h after induction of inflammation. The left hind paw diameter was used as a control for the degree of inflammation in the right hind paw(Winter et al., 1962). The percentage of anti-inflammatory activity (% inhibition of inflammation) was calculated using the following equation: 
\[
\% \text{ inhibition} = \left( \frac{W_c - W_t}{W_c} \right) \times 100
\]

\( W_c \): is the mean increase in paw thickness in rats treated with the tested compounds.

\( W_t \): is the mean increase in paw thickness in the control group.

**RESULTS AND DISCUSSION**

**Chemistry**

The target compounds were prepared according to the general synthetic pathways shown in Schemes 1 and 2. Thionyl chloride was added drop-wise to benzoin in presence of pyridine to get the desyl chloride 2 followed by reflux with thiourea in absolute ethanol to afford the amine derivative 3 (Ren et al., 2008). 4,5-Diphenylthiazol-2-amine 3 was stirred with freshly prepared formic acetic anhydride to get the intermediate formamide derivative 4 which was stirred with ethyl chloroacetate in DMF in presence of sodium hydride to afford the ester derivative 5 following the reported procedure (Abdel-azeem et al., 2017).

The starting hydrazide derivative 6 was obtained through reacting the ester 5 with hydrazine hydrate in absolute ethanol under reflux, **Scheme 1**.

**Scheme 1.** Reagents and Reaction Conditions: (a) pyridine, SOCl₂, 1h; (b) thiourea, ethanol, reflux, 2 h; (c) formic acetic anhydride, ether, stir, rt, 24 h; (d) ethyl chloroacetate, NaH, DMF, rt, 24 h; (e) NH₂NH₂, ethanol, reflux 12 h; (f) NaNO₂, acetic acid, -5 °C, 30 min.

**Scheme 2.** Reagents and Reaction Conditions: (a) Appropriate amino acid ester, ethyl acetate, TEA, rt, 72 h.
Subsequently, the azide intermediate was obtained through the reaction of hydrazide 6 with sodium nitrite and glacial acetic acid in ice-bath which was directly coupled with various amino acid esters at room temperature to afford the final amino acid conjugates 8a-e. Scheme 2. All the final targeted compounds were confirmed by elemental analysis and various spectral data (IR, 1H-NMR, 13C-NMR and mass).

PHARMACOLOGICAL SCREENING

Anticancer Activity

The anti-cancer activity of the synthesized compounds was evaluated in vitro against three cancer cell lines; HT-29 (human colorectal adenocarcinoma), MCF-7 (human breast carcinoma) and A549 (human lung carcinoma) and doxorubicin was used as positive reference drug. IC₅₀ values in µM (the concentration that caused a 50% inhibition) were used to express the results, Table 1.

Most of the tested compounds had moderate activity. The ethylalanine substituted diphenyl thiazole 8b showed the least activity against the three cancer cell lines as its IC₅₀ values were between 28.85 and 47.90 µM. On the other hand, the methylglycine derivative 8a showed the highest activity against the tested cancer cell lines and its IC₅₀ values were between 0.6 and 4.0 µM indicating that substitution with small amino acid group gives much more better activity the bulky one. Cells were treated with the test compounds or vehicle for 48 h. Data were calculated in comparison to indomethacin. (N = 5).

Table 1: IC₅₀ values for the newly synthesized compounds on three cancerous cell lines; MCF-7, HT-29 and A549 cell lines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCF-7 (µM)</th>
<th>HT-29 (µM)</th>
<th>A549 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>4.0± 1.1</td>
<td>2.0± 1.3</td>
<td>0.6± 0.1</td>
</tr>
<tr>
<td>8b</td>
<td>43.65± 3.2</td>
<td>47.9± 2.7</td>
<td>28.85±2.8</td>
</tr>
<tr>
<td>8c</td>
<td>12.9± 2.5</td>
<td>13.8± 2.1</td>
<td>13.8± 3.1</td>
</tr>
<tr>
<td>8d</td>
<td>24.55± 1.9</td>
<td>13.5± 1.6</td>
<td>12.0± 1.4</td>
</tr>
<tr>
<td>8e</td>
<td>34.7± 3.2</td>
<td>47.9± 3.1</td>
<td>46.0± 2.6</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>1.0± 0.3</td>
<td>0.058± 0.05</td>
<td>0.27± 0.1</td>
</tr>
</tbody>
</table>

Cells were treated with the test compounds or vehicle for 48 h. Data were reported as mean ± S.D. (n = 6). Three human cancer cell lines were used; MCF-7 (human breast carcinoma), HT-29 (colorectal cancer cell line) and A549 (lung cancer cell line). Doxorubicin was used as a positive control.

ANTI-INFLAMMATORY ACTIVITY

All the newly synthesized compounds were evaluated in vivo for their anti-inflammatory activity and the carrageenan-induced rat paw edema method was used. Edema inhibition percentages (mean change in thickness of paw edema of rats pretreated with the tested compounds after 1, 3 and 5 h from injection with carrageenan) were used to express the results, Table 2.

Indomethacin was used as a positive standard. The results showed that the tested compounds have good anti-inflammatory activity. Interestingly, compound 8a that had the highest anticancer activities showed the highest in vivo anti-inflammatory activities. Also, a sharp decrease in activity was observed on substitution with bulky amino acid groups in comparison with the small methyl glycine one. So, results indicate that there is a correlation between the anticancer and anti-inflammatory activities that confirms our theory.

Table 2: Edema thickness, inhibition % and relative potency% of the tested compounds compared to indomethacin using “rat paw carrageenan edema”.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Edema thickness (mm) ± SEM</th>
<th>Edema inhibition % (µM)</th>
<th>Relative potency %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1h: 0.680±0.022</td>
<td>1.624±0.024</td>
<td>2.734±0.024</td>
</tr>
<tr>
<td></td>
<td>3h: 0.211±0.005</td>
<td>0.545±0.003</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>5h: ---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.59 ± 0.04</td>
<td>0.221 ± 0.103</td>
<td>0.545 ± 0.003</td>
</tr>
<tr>
<td>8a</td>
<td>(83)</td>
<td>(86)</td>
<td>(81)</td>
</tr>
<tr>
<td>8b</td>
<td>0.38 ± 0.016</td>
<td>0.82 ± 0.017</td>
<td>1.34 ± 0.015</td>
</tr>
<tr>
<td>8c</td>
<td>0.42 ± 0.016</td>
<td>0.97 ± 0.018</td>
<td>1.64 ± 0.009</td>
</tr>
<tr>
<td>8d</td>
<td>0.38 ± 0.013</td>
<td>0.79 ± 0.015</td>
<td>1.77 ± 0.011</td>
</tr>
<tr>
<td>8e</td>
<td>0.44 ± 0.017</td>
<td>0.64 ± 0.011</td>
<td>1.28 ± 0.007</td>
</tr>
</tbody>
</table>

All test compounds were given orally in a dose of 100 mg/kg. Treatments began 1 h before induction of inflammation by the injection of 1% carrageenan-sodium gel into the sub-planter region of the right hind paw. The mean size of the induced paw edema thickness of rats pretreated with the tested compounds was observed and measured at 0, 1, 3 and 5 h from the induction of inflammation. The percentage of inhibition in thickness of edema was calculated in comparison to indomethacin. (N = 5).

CONCLUSION

In summary, a novel series of diphenylthiazole-amino acids conjugates has been synthesized and evaluated their anticancer activity against a three cancer cell lines; MCF-7, HT-29 and A549 using MTT assay where doxorubicin was used as a positive standard. Meanwhile, the anti-inflammatory activity of the same series was evaluated in vivo using carrageenan-induced paw edema assay. It was found that compound 8a exhibited the highest anticancer and anti-inflammatory activities with IC₅₀ between 0.6 µM and 4.0 µM and edema inhibition% between 80 and 84, respectively. Totally, these new conjugates represent a promising anticancer and anti-inflammatory scaffold for further optimization and development.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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