New pyrimidine-benzoxazole/benzimidazole hybrids: Synthesis, antioxidant, cytotoxic activity, in vitro cyclooxygenase and phospholipase A2-V inhibition

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ARTICLE INFO
Keywords:
Benzoxazole
Benzimidazole
Cytotoxicity
COXs
Antioxidant

ABSTRACT
To enhance the cytotoxicity of benzimidazole and/or benzoxazole core, the benzimidazole/benzoxazole azopyrimidine were synthesized through diazo-coupling of 3-aminophenylbenzimidazole (6a) or 3-aminophenylbenzoxazole (6b) with diethyl malonate. The new azo-molanates 6a&b mixed with urea in sodium ethoxide to afford the benzimidazolo/benzoxazolopyrimidine 7a&b. The structure elucidation of new synthesized targets was proved using spectroscopic techniques NMR, IR and elemental analysis. The cytoxicity screening had been carried out against five cancer cell lines: prostate cancer (PC-3), lung cancer (A-549), breast cancer (MCF-7), pancreas cancer (PaCa-2) and colon cancer (HT-29). Furthermore, the antioxidant activity, phospholipase A2-V and cyclooxygenases inhibitory activities of the target compounds 7a&b were evaluated and the new compounds showed potent activity (cytotoxicity IC\textsubscript{50} range from 4.3 to 9.2 µM, antioxidant activity from 40% to 80%, COXs or LOX inhibitory activity from 1.92 µM to 8.21 µM). The docking of 7a&b was made to confirm the mechanism of action.

1. Introduction
Cancer disease is the most dangerous disease coming after cardiovascular disease, and in 2018, it produced death of about 9.6 million deaths [1]. Although there is a great advance in treating cancer through targeting therapy, there is a need for process of anticancer drug discovery and development. So, efforts have been made for synthesis of potential anticancer drugs. As a result from that millions of variants organic or natural compounds have been synthesized and shown cytotoxic activity against different types of cancer cell [2]. Moreover, COX enzymes and phospholipase A2 had been reported to be highly found in solid tumors such as bladder and breast cancer [3–5].

Benzoxazole and benzimidazole scaffolds constitute many bioactive compounds possessing significant pharmacological activities for example antihistaminic [6], and antimicrobial [7,8], anti-inflammatory [9–11], antioxidant [12] and anticancer [7,13,14].

In 2017, the benzimidazol-2-yl-phenyl-hydrazono-pyrazol-3-one (1) (Fig. 1) had been prepared and was found to exhibit anticancer activity against A549 and MCF-7 and cell lines with IC\textsubscript{50} = 8.46 and 6.42 µM, respectively [15]. In addition, benzimidazole derivative 2 (Fig. 1) was recorded to demonstrate cancer inhibitory effect against both HepG-2 and HCT-116 with IC\textsubscript{50} = 2.10 and 1.25 µM [16]. Also, hybridizing benzoxazole ring with piperazinemoiety produced compound 3 (Fig. 1) which showed anticancer activity towards MCF-7 cell lines with IC\textsubscript{50} = 12 nM [17]. On the other hand, pyrimidine ring drawn much attention due to its biological importance as anti-inflammatory [18,19], antioxidant [20,21] and anticancer [22,23]. For example, the pyrimidin-9-carbonitrile had been prepared by Fathalla et al. [24a] and was evaluated for its anticancer activity towards liver cancer cell line (HEPG-2). The result of evaluation detected that compound 4 (Fig. 1) had better anticancer activity (IC\textsubscript{50} = 3.74 µg/ml) than the standard drug 5-fluorouracil (IC\textsubscript{50} = 5 µg/ml).

COX-2 inhibitors could use it to prompt apoptosis is TRAIL receptor (cytokine that is normally secreted by all of our cells and could induce apoptosis in tumor cells). Another target for COX-2 inhibitors is PI3 Kinase; PI3 Kinase is a provocateur for Bad protein, the protein that has a major role in cancer development. COX-2 inhibitors stop the activity of Bcl-XL and induce the activity of Bcl-2 and by this, they restrain
apoptosis. Bcl-xL is a promoter for cell proliferation. COX-2 inhibitors activate procaspase-8 and convert it in to its active form (Caspase 8). This is a major step in the process of cell apoptosis [24b–24d].

Based on these studies and our research for preparing bioactive candidates with anticancer activity [25–27], we decided to synthesize novel benzimidazole and/or benzoxazole derivatives mixed with pyrimidine ring hoping that the new hybrids might exhibit anticancer activity with less side effects.

2. Results and discussion

2.1. Chemistry

The steps of compounds 6a&b and 7a&b preparations are displayed in Scheme 1. First, compounds 5a&b were prepared as the reported procedures [4]. The new compounds were prepared following reported methods for diazocoupling of diazonium salts with active methylene in presence of sodium acetate [27]. The structure of azo-diethylmalonate 6a and 6b was elucidated from NMR spectra which showed aliphatic peaks at δ (m) 1.22 to 1.56 and (m) 4.20–4.46 indicating diethyl groups of ester. The azo-pyrimidinone derivatives 7a and 7b prepared as result of reaction of azo-compound 6a and 6b with urea or/and thiourea in basic solution of sodium ethoxide. The absence of aliphatic NMR peaks and presence of new NH peaks of azo-diethylmalonate 6a and 6b prove the cyclization and formation of new pyrimidinones 7a and 7b. The target compound structure was recognized by elemental and spectroscopic analyses.

2.2. Pharmacological screening

2.2.1. Screening of antioxidant activity using DPPH

Free radical scavenging effect of novel benzoxazole 7b demonstrated better scavenging activity against the DPPH radical than benzimidazole derivative 7a at all the used concentrations.

2.2.2. In vitro sPLA2-V and COXs inhibitory activity assay

The potency of the newly prepared target compounds 7a and 7b as cyclooxygenase (COX) and phospholipase A2-V (sPLA2-V) inhibitors was determined as the concentration causing 50% inhibition (IC50) for COX and sPLA2-V enzymes using an enzyme immunoassay (EIA) kit. The results showed that the target compounds (7a and 7b) potent inhibitory activity against COX-1 (IC50 = 2.76, 1.92µM), and moderate activity towards COX-2 (IC50 = 7.47, 8.21µM), but both compounds have moderate inhibitory against secretory Phospholipase A2-V (sPLA2-V) (Table 1).

2.2.3. Cytotoxic activity

The cytotoxic activity of the newly target compounds was recorded using MTT assay against breast carcinoma (MCF-7), lung cancer (A549), human prostate cancer (PC-3), human pancreatic cancer (PaCa-2) and colorectal adenocarcinoma (HT-29) cell lines. From the obtained data, both the target compounds 7a and 7b showed moderate activity against all the tested cell lines (Table 2) with IC50 range = 4.3–8.8µM. In addition, the benzimidazole derivative 7a showed higher cytotoxic activity than the benzoxazole derivative 7b against all the cell lines except non-small cell lung cancer (A549). Moreover, the target 7a demonstrated comparable anticancer activity against colorectal adenocarcinoma (HT-29) cell lines (IC50 = 5.9 µM) to that showed by the standard drug doxorubicin (IC50 = 5.36 µM) (see Table 2).

2.3. Molecular docking study

The target compounds 7a&b were exposed to molecular modeling study to explore modes of binding interactions with COX-2 enzyme by the use of MOE.2010 software (Molecular Operating Environment). The 3D crystal structure of COX-2 enzyme combined with the cocristallized ligand (PDB code: 1CX2) was used for this study. Bromocelecoxib, S-58 binded with COX-2 isofrom forming two hydrogen bonding interactions with His90 and ArgS13 with binding affinity = −11.93 kcal/mol. Docking of compound 7a within COX-2 binding site displayed that 7a was good fitted with the receptor with three hydrogen bonding interactions with energy score = −11.50 kcal/mol (Table 3, Fig. 3).
Scheme 1. Reagent and reaction conditions; (a) PPA, reflux for 5 h, neutralization with Na2CO3, (b), HCl, sodium nitrite, DEM, sodium acetate, stirring for 2 h, (c) Sodium ethoxide, urea, ethanol, reflux for 4 h, water, HCl, cooling for 2 h.

Fig. 2. DPPH radical scavenging effect of the target compounds 7a, 7b and Torlox at different concentrations (10, 50 and 100μM). SD = 0.23.
Cytotoxicity activity of the target compounds 7a and 7b.

<table>
<thead>
<tr>
<th>Compound</th>
<th>sPLA2 IC50 (µM)</th>
<th>COX-1 IC50 (µM)</th>
<th>COX-2 IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>7.51 ± 1.84</td>
<td>2.76 ± 0.5</td>
<td>7.47 ± 1.3</td>
</tr>
<tr>
<td>7b</td>
<td>5.72 ± 2.15</td>
<td>1.92 ± 0.9</td>
<td>8.21 ± 1.6</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.69 ± 0.01</td>
<td>ND*</td>
<td>3.82 ± 0.08</td>
</tr>
<tr>
<td>Indomethacin*</td>
<td>ND*</td>
<td>0.29 ± 0.07</td>
<td>3.82 ± 0.08</td>
</tr>
</tbody>
</table>

ND* not done.

Moreover, docking of compound 7b within COX-2 enzyme recorded that this compound showed docking score (S) = −11.50 kcal/mol. Furthermore, this compound 7b showed two hydrogen bonding interactions; (i) C=O with Arg513 (2.51 Å), (ii) C=O with Gln192 (2.79 Å) (Table 3, Fig. 4).

3. Material and methods

3.1. Chemistry

Thomas-Hoover apparatus was used for determination of melting points (uncorrected). The chemical structure of the prepared compounds was proved by NMR (using a Bruker Avance III 100 MHz for 13C and 400 MHz for 1H, Bruker AG, Switzerland). IR (by Nicolet 550 Series II Magna FT-IR), Mass (by Hewlett Packard 5988) spectroscopy and elemental analysis (by Perkin Elmer 2400 analyzer, Perkin-Elmer, Norwalk, CT, USA). Compounds 5a & b were synthesized according to the literature procedure [4].

3.1.1. General methods for synthesis of 6a & b

To a cooled diazonium solution prepared from stirring compound 5a or 5b (0.01 mol) in 10%, 10 mL hydrochloric acid and solution of sodium nitrite (0.01 mol, 5 mL water), a mixture of (0.01 mol) diethyl malonate and (0.01 mol) sodium acetate in (50%, 20 mL) aqueous ethanol was added and stirred for 4 h in ice bath. The product was filtered and crystallized from (95%) ethanol.

3.1.2. 2-[[3-(1H-Benzimidazol-2-yl)-phenyl]-hydrazono]-malonic acid diethyl ester (6a)

Yellowish white crystals in 90% yield; mp 145–147°C; IR: 3445.6 (NH), 3000.3 (CH, Aromatic), 2900.4 (CH, Aliphatic), 1720.7, 1680.4 (C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 1.18–1.43 (m, 6H, CH₂CH₃), 4.21–4.49 (m, 4H, CH₂CH₃), 7.25–7.33 (m, 3H, aminophenyl H-2,4,6), 7.41–7.58 (m, 1H, phenyl H-3), 6.97–7.88 (m, 2H, benzoxazole H-5, 6), 8.28–8.36 (m, 2H, benzoxazole H-4,7), 11.98 (s, 1H, NH–N=)=; 13C NMR (DMSO-d₆) δ 142.93, 144.33, 150.29, 151.10; Anal. Calcd for C₁₇H₁₁N₃O₄: C, 58.62; H, 3.17; N, 20.05. Found: C, 58.70; H, 3.10; N, 20.10.

3.1.4. General procedure for synthesis of compound 7a & b

A mixture of 6a & b (0.01 mol), urea (0.01 mol) in sodium ethoxide solution (0.03 mol sodium, 20 mL absolute ethanol) was heated under reflux for 4 h. (20 mL) Hot water was added to the mixture, and then sufficient quantity of hydrochloric acid was added till the mixture became acidic and then kept in the refrigerator for 5 h. The products 7a & b were filtered and dried then crystallized from ethanol.

3.1.5. 5-[[3-(1H-Benzimidazol-2-yl)-phenyl]-hydrazono]-pyrimidinone-2,4,6-trione (7a)

Dark reddish crystals in 90% yield. mp 300°C; IR: 3460, 3300, 3250, 3169 (4NH), 3043 (CH, Aromatic), 1720, 1687, 1681 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 7.20–7.37 (m, 2H, aminophenyl H-6), 7.5 (s, 1H, aminophenyl H-6), 7.65–7.67 (m, 1H, aminophenyl H-3), 7.75–7.76 (m, 2H, benzimidazole H-5, 6), 8.41–8.48 (m, 2H, benzimidazole H-4,7), 11.39 (s, 1H, NH–N=)=; 13C NMR (DMSO-d₆) δ 111.75, 117.46, 119.00, 119.24, 122.22, 123.05, 127.86, 128.29, 135.51, 142.93, 144.33, 150.29, 151.10; Anal. Calcd for C₁₇H₁₂N₆O₃: C, 58.62; H, 3.47; N, 24.13. Found: C, 58.60; H, 3.50; N, 24.10.

3.1.6. 5-[[3-(1H-Benzoxazol-2-yl)-phenyl]-hydrazono]-pyrimidinone-2,4,6-trione (7b), pale

Reddish crystals in 80% yield. mp 300°C; IR: 3450, 3400, 3280 (3NH), 3040 (CH Aromatic), 1730, 1690, 1675 (3C=O) cm⁻¹; 1H NMR (DMSO-d₆) δ 7.28–7.44 (m, 2H, aminophenyl H-2,4), 7.74–7.92 (m, 1H, aminophenyl H-3), 8.01–8.18 (m, 2H, benzimidazole H-4,7), 12 (s, 1H, NH–N=)=; 13C NMR (DMSO-d₆) δ 143.92, 144.35, 151.48, 162.12, 162.80; Anal. Calcd for C₂₀H₁₉N₃O₄: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.20; H, 5.20; N, 14.70.

3.1.3. 2-[[3-(1H-Benzoxazol-2-yl)-phenyl]-hydrazono]-malonic acid diethyl ester (6b)

Yellowish white crystals in 85% yield; mp 158–160°C; IR: 3200.5 (NH), 3000.3 (CH, Aromatic), 2900.4 (CH, Aliphatic), 1720.7, 1680.4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell viability %</th>
<th>Cytotoxicity activity IC50 ± SEM (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>7a</td>
<td>5.4 ± 1.2 9.2 ± 1.5 4.3 ± 0.8 4.5 ± 1.3 5.9 ± 1.4</td>
</tr>
<tr>
<td>7b</td>
<td>7b</td>
<td>7.2 ± 2.3 8.4 ± 1.2 7.9 ± 1.6 8.8 ± 1.2 8.8 ± 2.2</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>–</td>
<td>2.01 ± 0.87 1.17 ± 0.35 0.91 ± 0.12 1.57 ± 0.02 5.36 ± 1.98</td>
</tr>
</tbody>
</table>
3.2. Pharmacological screening

3.2.1. DPPH radical scavenging activity

Scavenging DPPH assay was determined using of 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reported [28]. 0.08mL solution of the new compounds was mixed with a 1.92mL 6×10−5M solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol and then absorbance reduction was calculated at λ=515nm over 3min against a blank sample (containing methanol only). The ability of the tested samples to quench DPPH free radicals was determined according to the equation: scavenging% = [(AC−AA)/AC] × 100 where: AC—absorbance of the control at 0min, AA—absorbance of the sample after 3min. The antioxidant activity was expressed as µmol of Trolox per 100g of fresh weight (FW) (TE—Trolox equivalent).

3.2.2. In vitro COX and sPLA2-V inhibition assay

The in vitro inhibition of ovine COX-1/COX-2 was calculated by enzyme immunoassay (EIA) kit as the reported procedure [29] and sPLA2-V inhibition was measured using Ellman’s method [30].

3.2.3. Cytotoxic activity

Cytotoxic activity of the newly prepared targets was measured by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay as reported [31].

3.3. Molecular docking study

The crystal structures of COX-2 isoform was obtained from protein data bank at research collaboration for structural Bioinformatics (RSCB) (PDB:ID 1CX2) [32]. Docking of the co-crystallized ligand had been performed to estimate amino acid binding and root mean standard deviation (rmsd) which is equal to 0.87 Å. 3D structure of the tested compounds was designed by Molecular Operating Environment (MOE, Version 2005.06, Montreal, Canada). The 3D structures were protonated, energy minimized and docked within COX-2 active site.

4. Conclusion

Novel hybrid structures of benzoxazole and/or benzimidazole with pyrimidine scaffold 7a and 7b had been designed and synthesized.
These new compounds had been evaluated for their antioxidant and anticancer activities. From the results, both candidates 7a and 7b exhibited good scavenger effect and the benzoxazole derivative 7b demonstrated better scavenging activity than benzimidazole derivative 7a. For their anticancer activity, both compounds 7a and 7b revealed moderate activity against breast carcinoma (MCF-7), non-small cell lung cancer (A549), human prostate cancer (PC-3), human pancreatic cancer (PâCa-2) and colorectal adenocarcinoma (HT-29) cell lines with IC50 range = 4.3–8.8 µM. Furthermore, the inhibitory activity of the cancer (PaCa-2) and colorectal adenocarcinoma (HT-29) cell lines with moderate activity against breast carcinoma (MCF-7), non-small cell

References


Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103218.

Acknowledgement

Authors would like to appreciate the support of Jouf University and the help of Mr. Saife M. Alshmarial, and Mr. Saeed A. Almohammadi for this work.

References


