Semi-synthesis of Novel Thebaine Derivatives and their Anti-bacterial and Wound Healing Properties

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Abstract

In the present study, some new 1,2,3-triazole-tethered analogues of \( N \)-northebaine were designed and synthesized. The anti-bacterial properties of novel thebaine derivatives were studied on \( Staphylococcus aureus \) (\( S. aureus \)) and \( Escherichia coli \) (\( E. coli \)). Based on the results, compounds \( 5b, 5j \) and \( 5m \) showed the best activities against \( S. aureus \) (minimum inhibitory concentration (MIC) \( \sim 25 \) \( \mu M \)) compared to the parent compound (MIC = \( 321 \) \( \mu M \)). The most active anti-bacterial derivatives (\( i.e., 5b, 5j \) and \( 5m \)) and thebaine were considered as potent anti-bacterial wound healing agents. In this regard, fibroblast cell cytotoxicity and proliferation as well as anti-hemolytic activities of the mentioned compounds were studied. The cytotoxicity assay by using 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) on Human dermal fibroblast cell lines (HDF) revealed that products \( 5j \) and \( 5m \) didn't show inhibition of cell line growth after 24 hours and no further cytotoxic activity for a longer period (72 hours). Based on the investigation results on blood cell disruption for releasing of hemoglobin, compound \( 5j \) didn't exhibit any hemolysis activity in different doses.

Introduction

Thebaine (1, Fig. 1), as a natural alkaloid, is extracted from different species of \( Papaveraceae \) family [1]. This alkaloid has a structure similar to morphinane family, but it shows no significant biological properties like other morphine alkaloids [2].

Regardless of lack of biological properties of thebaine, it has a number of reactive sites such as 3, 4, 5, 6, 9, and 14, that have grabbed attention of scientists to synthesize new bioactive derivatives. Due to these chemically active sites, some new semi-synthetic bioactive compounds have been synthesized and introduced as drugs or prodrugs (\( e.g., \) buprenorphine, oxycodone and oxymorphone) [3–7].

Despite the attractive structure of thebaine for chemical modifications, several rearrangement pathways under acidic and nucleophilic conditions as well as cycloaddition reactions have limited the success of synthetic methodologies with formation of undesired byproducts [8–10].

Notwithstanding all attempts of researchers to introduce new methods for improving the yields of the targeted products, some major difficulties have not been circumvented yet. For example, Madyastha \( et al. \) implied that \( N \)-oxide of thebaine was not stable, and transformed to two major byproducts [11]. Therefore, several research groups have been engaged to find more efficient \( N \)-demethylation methods as an ongoing demand [6, 12, 13].

Previous studies on thebaine derivatives have introduced \( N \)-substituted congeners as potent bioactive compounds [12, 14]. Indeed, \( N \)-methyl can be replaced by other substituents such as \( N \)-allyl, \( N \)-cyclopropylmethyl and \( N \)-cyclobutylmethyl to improve its biological properties [12, 15]. Obviously, \( N \)-demethylation is a key step for delivering other \( N \)-substituted analogues.

Bacteria are one of the main causes of infectious diseases. The step by step progress of microorganism resistance to antibiotics, is the main reason to seek new compounds that are effective on pathogen [16]. A number of alkaloids in some \( Papaveraceae \) species revealed anti-microbial activity against some bacterial strains (including \( Listeria monocytogenes, Candida albicans, \) and \( S. aureus \)) [17].
Hybridization of some bioactive compounds with triazoles had a dramatic increase in their anti-bacterial properties [18–20]. A number of previous works have reported anti-bacterial activity of 1,2,3-triazole-tethered products on some different bacterial strains, like Enterococcus faecalis, S. aureus, Pseudomonas aeruginosa and E. coli [21–23].

Treatment of open and infected wounds is an important research area owing to the main functional and aesthetic role of skin [24]. In case of skin wounding, bacteria can damage and infect underlying tissues and make infectious diseases [25]. Also, wound healing and repairing process is regulated by various growth and releasing cytokine factors at the wound site [26]. Different studies on the anti-bacterial, antioxidant, anti-inflammatory and pro-collagen synthesis properties of natural products (such as, essential oils, alkaloids, phenolic compounds, tannins, flavonoids, and saponins) have been performed for wound healing [26, 27]. Moreover, contacting wound repairing agents with blood may cause hemolysis [28, 29].

In this paper, the synthesis of some novel thebaine derivatives containing triazole moiety is reported. Anti-bacterial activities of the synthesized compounds as well as the wound healing and anti-hemolytic properties of the most active products are reported.

Results And Discussion

Chemistry

Our strategy to synthesize the 1,2,3-triazole-tethered derivatives of thebaine is depicted in Scheme 1. As shown, final desired compounds were obtained from thebaine in three steps which were N-demethylation, N-propargylation and triazole formation.

N-Northebaine synthesis

In the first step, thebaine was treated with H₂O₂ to produce the N-oxide intermediate 2 by the oxidation reaction in methanol. Due to the lability of thebaine N-oxide, optimization of the reaction conditions including temperature, solvent and pH has a crucial impact on the yield of the product. The second step of N-demethylation was done by using iron, known as “Polonovski-type reaction” [30, 31]. This step was evaluated in the presence of different iron compounds such as iron powder, Fe(II) salts and stainless steel. According to our recent investigation, N-demethylation of thebaine was performed in the presence of stainless steel in good yield [6].

N-Propargylation of thebaine

To construct the 1,2,3-triazole ring on the nitrogen, as a pillar, a terminal alkyne group was needed. This goal was achieved by N-propargylation of the prepared secondary amine by the reaction of N-northebaine with propargyl bromide in the presence of a base. In previous researches, N-alkylation of thebaine was done by using potassium carbonate in ethanol as a polar solvent [14]. Therefore, at first, potassium carbonate was selected as a base and some polar solvents were studied under different conditions. Unfortunately, conducting the reaction of propargyl bromide with N-northebaine did not proceed well by using the reported methods [14]. So, optimization of the reaction conditions was studied by applying other bases and solvents at various
temperatures. The results are summarized in Table 1. It was observed that carrying out the reaction in long periods ended up with decomposition of \(N\)-northebaine (Table 1, entries 1–3). Also, sodium hydride (NaH) was found to be a superior base in conducting the reactions (Table 1, entries 8–10). As can be seen, the best yield was obtained by using THF as a solvent in the presence of NaH at 0° C to room temperature (Table 1, entry 10).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Condition</th>
<th>Results</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(K_2CO_3) (2eq)</td>
<td>Acetone</td>
<td>RT, 24 h</td>
<td>Decomposition</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>(K_2CO_3) (2eq)</td>
<td>Acetone</td>
<td>Reflux, 12 h</td>
<td>Decomposition</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>(K_2CO_3) (2eq)</td>
<td>Ethanol</td>
<td>Reflux, 12 h</td>
<td>Decomposition</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>(K_2CO_3) (2eq)</td>
<td>Methanol</td>
<td>RT, 24 h</td>
<td>No reaction</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>(K_2CO_3) (2eq)</td>
<td>Methanol</td>
<td>Reflux, 8 h</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>(K_2CO_3) (2eq)</td>
<td>Dichloromethane</td>
<td>RT, 8 h</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>(K_2CO_3) (2eq)</td>
<td>Methanol</td>
<td>35 °C, 4 h</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>NaH (3eq)</td>
<td>Dichloromethane (dry)</td>
<td>0–5 °C, 4 h</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>9</td>
<td>NaH (3eq)</td>
<td>Dichloromethane (dry)</td>
<td>0 °C to rt, 5 h</td>
<td>-</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>NaH (3eq)</td>
<td>Tetrahydrofuran (dry)</td>
<td>0 °C to rt, 5 h</td>
<td>-</td>
<td>80</td>
</tr>
</tbody>
</table>

### Synthesis of triazole ring, click chemistry

The concept of click chemistry was established by K. B. Sharpless in 2002 [32]. 1,3-Dipolar cycloaddition reaction of azides with an alkyne is the most used and studied click reaction. In the present study, various aryl, benzyl and aliphatic azides were chosen to build a diverse library and investigate the effects of different substituents on biological properties. So, \(N\)-propargylthebaine was treated with different azides to synthesize 1,2,3-triazole-tethered thebaine derivatives by 1,3-Huisgen cycloaddition. An overview of the new fourteen derivatives of thebaine, reaction conditions and yields of the products are summarized in Table 2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Reaction Conditions</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>NaH (3eq)</td>
<td>Dichloromethane (dry)</td>
<td>0–5 °C, 4 h</td>
<td>55</td>
</tr>
<tr>
<td>9</td>
<td>NaH (3eq)</td>
<td>Dichloromethane (dry)</td>
<td>0 °C to rt, 5 h</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>NaH (3eq)</td>
<td>Tetrahydrofuran (dry)</td>
<td>0 °C to rt, 5 h</td>
<td>80</td>
</tr>
</tbody>
</table>
Most of the target molecules were produced in high to excellent yields. The first group was synthesized based on the anchored 1,2,3-triazole rings with aryl derivatives (5a-5g). The substituents included halogens (F, Cl, Br) as well as ethyl, methoxy and nitro groups. The second category was delivered from the reaction of benzylic azides with substituents like Br, F and methyl, (5h-5k). In the third class, aliphatic azidoalcohols were used as the starting material to obtain three new thebaine analogues with triazole moiety (5l-5n).

### Biology

#### Anti-bacterial activity

The triazole-hybrid compounds synthesized by using anti-bacterial medicines and natural products in previous researches, showed variable inhibitory impacts on the growth of the tested Gram-positive and Gram-negative bacteria.
bacterial strains.[33, 34] Therefore, the anti-bacterial effects of the 1,2,3-triazole-tethered northebaine derivatives were investigated on *S. aureus* and *E. coli*.

Almost all of the novel thebaine analogues exhibited a better anti-bacterial effect on *S. aureus* than the parent compound. Among them, compounds 5b, 5j and 5m, with different substituents (4-methoxyphenyl, 4-flourobenzyl and benzyloxy hydroxypropyl, respectively) displayed a promising MIC value of 12.5 µg/mL (compared with that of thebaine, 100 µg/mL). Investigation of the new triazole-thebaine hybrids showed that the anchored triazole, regardless of the substituents, played a significant role on increasing the anti-bacterial properties of the base structure. The results showed that the synthesized derivatives did not prevent the *E. coli* growth better than the parent compound.

Table 3  MIC values of new thebaine derivatives on *S. aureus* and *E. coli*

<table>
<thead>
<tr>
<th>Product No.</th>
<th>MIC(µg/ml), (µM)</th>
<th>Product No.</th>
<th>MIC(µg/ml), (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td><em>E. coli</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>100, (321)</td>
<td>100, (321)</td>
<td>5g</td>
</tr>
<tr>
<td>3</td>
<td>50, (168)</td>
<td>100, (336)</td>
<td>5h</td>
</tr>
<tr>
<td>4</td>
<td>100, (298)</td>
<td>100, (298)</td>
<td>5i</td>
</tr>
<tr>
<td>5a</td>
<td>50, (110)</td>
<td>100, (220)</td>
<td>5j</td>
</tr>
<tr>
<td>5b</td>
<td>12.5, (26)</td>
<td>100, (103)</td>
<td>5k</td>
</tr>
<tr>
<td>5c</td>
<td>50, (100)</td>
<td>100, (200)</td>
<td>5l</td>
</tr>
<tr>
<td>5d</td>
<td>100, (212)</td>
<td>100, (212)</td>
<td>5m</td>
</tr>
<tr>
<td>5e</td>
<td>50, (102)</td>
<td>100, (204)</td>
<td>5n</td>
</tr>
<tr>
<td>5f</td>
<td>50, (94)</td>
<td>100, (188)</td>
<td></td>
</tr>
</tbody>
</table>

**Cytotoxic activity**

MTT assay of the most effective compounds and thebaine were done on Primary Dermal Fibroblast; Normal, Human, adult cell (HDFa). As shown in Fig. 2, the cell death was occurred in the presence of thebaine more than the other treatments. The effect of thebaine on cell mortality at 24 hours and 72 hours was greater than that of the control group. As can be evident, the cell growth inhibition in the first day of treatment with 5b, 5j and 5m was less than thebaine. As an outcome, compounds 5j and 5m showed inhibition effect on cell growth significantly less than the positive control. The impacts of compounds 5j and 5m on cell mortality increased within 72 hours. Therefore, unexpected cell growth in long periods is not being worried. The results showed that the cell viability rate was 53% after 72 hours by exposure to 5m. Since the compound 5m didn't inhibit growth of the tested cells, it was considered as the most proper analog.

These results were confirmed by optical microscopy to track the morphological changes of the characterized necrotic cells. Microscopic images of the fibroblast cells showed that the group exposed to thebaine (1) had a higher mortality rate after 72 hours than the other four studied groups. The optic microscopy of cell treated with compound 1 showed more nonadherent and suspended cells indicating death of the cells. In the contrary,
the synthesized derivatives, especially 5m, didn’t have any inhibitory for growth of cell line in 24 hours without unexpected cell growth in long time. Therefore, it can be concluded that tethering the 1,2,3-triazole ring to thebaine reduced its cytotoxicity on normal cells and improved its wound repairment ability.

**Anti-hemolytic assay**

To study of the erythrocyte lysis induced by compounds 5b, 5j, 5m and thebaine on open wound, the hemolytic assay was run. The hemolytic activity of the investigated compounds in the absence or presence of Fetal Bovine Serum (FBS) was negligible, and only compound 5b at the highest concentration exhibited 6% activity (Figs. 4 and 5). As can be seen, in concentration of 50 ppm, presence of 10% FBS enhanced the hemolytic activity of 5j. However, in the presence and absence of FBS, high treatment dose (100 ppm) of derivatives 5j and 5m didn’t reveal hemolytic activity, similar to thebaine. By comparison of two figs., 5m showed the lower hemolytic activity in different doses compared to other analyzed derivatives without being affected by existence or in-existence of FBS.

**Conclusion**

It has been shown that tethering triazole ring with biologically active compounds had a considerable impact on their activities [35]. In this research, fifteen hybrid derivatives of N-northebaine with 1,2,3-triazoles were designed and synthesized. The synthesized compounds showed a superior anti-bacterial activity on *S. aureus* than the parent compound, while did not have an inhibition effect on the growth of *E. Coli*.

The most active anti-bacterial derivatives (*i.e.*, 5b, 5j and 5m) and thebaine were investigated as the wound healing agents by HDF cell viability and hemolytic tests. In this regard, MTT assay was performed for HDF cell viability in the presence of synthesized compounds. As a result, the tested normal cell line was grown after treatment with compounds 5j and 5m better than positive control and thebaine. Based on the results of *in-vitro* tests, compounds 5j and 5m were nominated as the new possibly non-toxic effective anti-bacterial compounds with wound-healability.

**Material And Methods/experimental**

**Chemistry**

Thebaine was donated by Faran Shimi Pharmaceutical Company. Other solvents, chemicals and reagents such as propargyl bromide and NaH were provided from Sigma-Aldrich, Merck and Kimia Exir companies and were used without further purification. The reactions were monitored by thin layer chromatography (TLC) sheets pre-coated with silica gel F\textsubscript{254} from Merck. Purification steps of the synthesized compounds were carried out by preparative TLC plates coated with silica gel 60 PF\textsubscript{254} containing gypsum. Melting points were determined by a Branstead/Electrothermal 9200 apparatus. \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR spectra were recorded in CDCl\textsubscript{3} on a Bruker Avance spectrometer at 600 MHz and 125 MHz, respectively. The purity confirmation and analysis of the molecular weight of new compounds were done by using an analytical-scale Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA) consisting of a G1329B autosampler and a Bruker microTOF-Q mass spectrometer equipped with an electrospray ionization interface (Bruker Daltonik, Bremen,
Germany), respectively. Chromatographic separation and mass spectrometry were controlled using Hystar ver. 3.2 software, and analysis of chromatographic and mass spectrometric data were performed using Data Analysis ver. 4.0 software (Bruker Daltonik, Bremen, Germany). Different azides were prepared according to the previous procedures [23, 36, 37].

**Synthesis of N-Northebaine (3)**

Thebaine (2.0 g, 6.42 mmol) was mixed with methanol (20 mL), and hydrogen peroxide solution 30% (5.8 mL, 51.4 mmol) was added. The reaction mixture was stirred at room temperature for 8 hours. Solvent was removed by a rotary evaporator under reduced pressure. The resulting mixture was extracted by chloroform (3×100 mL) and 100 mL brine was used for washing the organic phase. The organic layer was dried by MgSO$_4$ and the solvent was evaporated by rotary evaporator. The afforded compound 2 (1.8 g) was dissolved in 2-propanol (25 mL) without any further purification. Stainless steel (0.9 g) was added to the reaction flask and the mixture was stirred at 50°C for 8 hours. After completion of this step approved by TLC, reaction mixture was filtered to pull out stainless steel and the filtrate was concentrated under reduced pressure. After addition of 50 mL water to the residue, it was extracted by chloroform (3×100 mL) and washed twice with 100 mL saturated solution of disodium ethylenediaminetetraacetate (EDTA). The organic phase was washed with NaOH solution (1 N, 2×50 mL). The pH of mother aqua phase was increased to 9 by NaOH (1 N, 50 mL) and extracted with chloroform (2×100 mL). All of the organic layers were combined and dried by anhydrous MgSO$_4$ and the solvent was evaporated by rotary evaporator. The afforded N-northebaine was purified by flash column chromatography by 15% of ethyl acetate in n-hexane as eluent. Finally, 1.0 g pure N-northebaine was obtained as a light-yellow powder, 60% yield.

**Synthesis of N-propargyl northebaine (4)**

Compound 3 (1.0 g, 3.37 mmol) was dissolved in dried THF (15 mL) and NaH, 60% dispersion in mineral oil, (0.4 g, 10.1 mmol) and propargyl bromide, 80% in toluene, (0.5 g, 3.70 mmol) were added. The mixture was stirred for 1 hour and the reaction was quenched by HCl (1N, 2 mL) and water (10 mL) which were added in order. The organic compounds were extracted with chloroform (3×100 mL) and the organic layers were combined and washed with HCl solution (1 N, 100 mL). The organic solution was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. The yield of this step after purification by column chromatography (n-hexane: ethyl acetate as the gradient eluent) was about 80% (yellow powder, 0.9 g).

**7,9-Dimethoxy-3-(prop-2-yn-1-yl)-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (4)**

Yellow powder; yield: 80%, C$_{21}$H$_{21}$NO$_3$, HRMS [M + 1]$^+$: calcd.= 336.1516, found = 336.1594, bp. 150–152 °C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ (ppm): 1.24–1.30 (m, 1H), 1.73 (d, $J$ = 12.5 Hz, 1H), 2.15–2.22 (m, 2H), 2.85–2.94 (m, 3H), 3.35 (d, $J$ = 18.1 Hz, 1H), 3.43 (d, $J$ = 2.4 Hz, 1H), 3.66 (s, 3H), 3.87 (s, 3H), 3.93 (d, $J$ = 7.1 Hz, 1H), 5.05 (d, $J$ = 6.4 Hz, 1H), 5.30 (s, 1H), 5.61 (d, $J$ = 6.4 Hz, 1H), 6.62 (d, $J$ = 8.2 Hz, 1H), 6.69 (d, $J$ = 8.2 Hz, 1H). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (ppm): 31.4, 36.1, 43.0, 44.1, 46.6, 54.9, 56.4, 53.8, 72.5, 80.7, 89.2, 95.8, 112.6, 113.2, 119.4, 127.7, 131.6, 133.6, 143.2, 144.9, 152.6.
General procedure for the synthesis of 1,2,3-triazole-tethered derivatives (5a-5n)

N-Propargyl northebaaine 4 (0.08g, 0.24 mmol) was dissolved in a mixture of DCM: MeOH: H2O (1:1:1, 1 mL). Then, azide (0.26 mmol), CuSO4·5H2O (0.0059 g, 0.024 mmol) and sodium ascorbate (0.0095 mg, 0.048 mmol) were added and the reaction was stirred for 5–40 minutes. Saturated solution of EDTA (3 mL) was poured to the reaction mixture and extracted with DCM (3×10 mL). The organic layers were mixed and concentrated under reduced pressure. Product was purified by preparative TLC using n-hexane and ethyl acetate (3:2) as the mobile phase.

7,9-Dimethoxy-3-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5a) Orange powder; yield: 90%, C27H26N4O3, HRMS [M + 1]^+: calcd.= 455.1999, found = 455.2063, bp. 165–167 °C decomposition point, 1H NMR (600 MHz, CDCl3) δ (ppm): 1.75 (m, 1H), 2.29–2.31 (m, 1H), 2.79–2.82 (m, 2H), 3.03–3.06 (m, 1H), 3.42 (d, J = 18.1 Hz, 1H), 3.63 (s, 3H), 3.79 (d, J = 6.9 Hz, 1H), 3.88 (s, 3H), 4.02 (s, 2H), 5.07 (d, J = 6.4 Hz, 1H), 5.33 (s, 1H), 5.58 (d, J = 6.4 Hz, 1H), 6.65 (d, J = 8.1 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.55 (t, J = 7.9 Hz, 2H), 7.77 (d, J = 7.9 Hz, 2H), 8.03 (s, 1H), 13C NMR (125 MHz, CDCl3) δ (ppm): 31.6, 36.4, 44.3, 46.5, 49.4, 54.9, 56.5, 58.8, 89.2, 95.9, 112.4, 113.0, 119.4, 120.4 (2CH), 120.9, 127.6, 128.6, 129.7 (2CH), 133.4, 137.2, 142.9, 142.9, 144.8, 146.5, 152.8.

7,9-Dimethoxy-3-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5b) Orange powder; yield: 95%, C28H28N4O4, HRMS [M + 1]^+: calcd.= 485.2105, found = 485.2150, bp. 133–135 °C decomposition point, 1H NMR (600 MHz, CDCl3) δ (ppm): 1.74–1.77 (m, 1H), 2.22–2.26 (m, 1H), 2.83–2.89 (m, 2H), 3.02–3.05 (m, 1H), 3.41–3.43 (d, J = 17.8 Hz, 1H), 3.63 (s, 3H), 3.79 (d, J = 6.7 Hz, 1H), 3.88 (s, 3H), 3.89 (s, 3H), 4.02 (s, 2H), 5.07 (d, J = 6.4 Hz, 1H), 5.33 (s, 1H), 5.58 (d, J = 6.4 Hz, 1H), 6.67 (d, J = 8.1 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 7.04 (d, J = 8.9 Hz, 2H), 7.67 (d, J = 8.9 Hz, 2H), 7.94 (s, 1H), 13C NMR (125 MHz, CDCl3) δ (ppm): 31.9, 36.4, 36.5, 44.2, 46.5, 49.2, 55.1, 56.1, 56.4, 58.8, 89.4, 96.2, 112.4, 113.2, 115.2 (2CH), 119.6, 121.0, 122.0 (2CH), 122.1, 130.8, 133.5, 143.1, 145.1, 152.8, 155.1, 159.7.

7,9-Dimethoxy-3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5c) Orange powder; yield: 60%, C27H25N5O5, HRMS [M + 1]^+: calcd.= 500.1850, found = 500.1915, bp. 224–226 °C decomposition point, 1H NMR (600 MHz, CDCl3) δ (ppm): 1.75–1.79 (m, 1H), 2.29 (td, J = 4.7, 12.7 Hz, 1H), 2.82 (dd, J = 4.7, 13.2 Hz, 1H), 2.87 (dd, J = 7.0 Hz, 18.0 Hz, 1H), 3.07 (td, J = 3.4, 12.9 Hz, 1H), 3.42 (d, J = 18.0 Hz, 1H), 3.63 (s, 3H), 3.78 (d, J = 7.0 Hz, 1H), 3.88 (s, 3H), 4.03 (s, 2H), 5.07 (d, J = 6.4 Hz, 1H), 5.33 (s, 1H), 5.58 (d, J = 6.4 Hz, 1H), 6.65 (d, J = 8.2 Hz, 1H), 6.70 (d, J = 8.2 Hz, 1H), 8.02 (d, J = 9.0 Hz, 2H), 8.15 (s, 1H), 8.44 (d, J = 9.0 Hz, 2H), 13C NMR (125 MHz, CDCl3) δ (ppm): 31.9, 36.4, 44.5, 46.5, 49.2, 49.6, 55.0, 56.8, 59.1, 89.4, 95.8, 112.5, 113.2, 119.6, 120.3 (2CH), 125.7 (2CH), 127.4, 131.4, 133.5, 141.7, 143.1, 145.1, 147.2, 147.8, 153.3.

3-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-7,9-dimethoxy-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5d) Orange powder; yield: 90%, C27H25FN4O3, HRMS [M + 1]^+: calcd.= 473.1905, found = 473.1980, bp. 194–195 °C decomposition point, 1H NMR (600 MHz, CDCl3) δ (ppm): 1.76
3-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-7,9-dimethoxy-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5e) Orange powder; yield: 95%, C$_{27}$H$_{25}$ClN$_{4}$O$_{3}$, HRMS [M + 1]$^+$: calcd. = 489.1609, found = 489.1671, bp. 140–141 °C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ (ppm): 1.78 (d, $J$ = 12.7 Hz, 1H), 2.32–2.36 (m, 1H), 2.82–2.86 (m, 2H), 3.07–3.10 (m, 1H), 3.44 (d, $J$ = 17.6 Hz, 1H), 3.64 (s, 3H), 3.87 (s, 4H), 4.08–4.11 (m, 2H), 5.07 (d, $J$ = 6.4 Hz, 1H), 5.34 (s, 1H), 5.65 (d, $J$ = 6.1 Hz, 1H), 6.65 (d, $J$ = 8.2 Hz, 1H), 6.70 (d, $J$ = 8.2 Hz, 1H), 7.51 (d, $J$ = 8.7 Hz, 2H), 7.73 (d, $J$ = 8.7 Hz, 2H), 8.12 (s, 1H), $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (ppm): 31.5, 31.9, 35.6, 36.6, 43.8, 46.2, 48.4, 55.1, 56.4, 58.8, 88.9, 95.8, 113.2, 113.7, 119.5, 121.5 (2CH), 126.9, 130.0 (2CH), 133.2, 134.5, 135.5, 142.9, 144.7, 153.1, 162.6.

3-((1-(4-Bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-7,9-dimethoxy-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5f) Orange powder; yield: 96%, C$_{27}$H$_{25}$BrN$_{4}$O$_{3}$, HRMS [M + 1]$^+$: calcd. = 533.1104, found = 535.1153, bp. 180–181 °C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ (ppm): 1.75 (d, $J$ = 12.7 Hz, 1H), 2.28 (td, $J$ = 4.9, 12.7 Hz, 1H), 2.80–2.86 (m, 2H), 3.04 (td, $J$ = 3.4, 12.9 Hz, 1H), 3.40 (d, $J$ = 17.9 Hz, 1H), 3.63 (s, 3H), 3.77 (d, $J$ = 7.0 Hz, 1H), 3.88 (s, 3H), 4.00 (s, 2H), 5.065 (d, $J$ = 6.4 Hz, 1H), 5.33 (s, 1H), 5.58 (d, $J$ = 6.4 Hz, 1H), 6.65 (d, $J$ = 8.2 Hz, 1H), 6.69 (d, $J$ = 8.2 Hz, 1H), 7.68–7.75 (m, 4H), 8.01 (s, 1H), $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (ppm): 31.6, 36.4, 44.3, 46.5, 49.3, 54.9, 56.4, 58.9, 89.1, 95.9, 112.4, 113.0, 119.3, 120.7, 121.7 (2CH), 122.3, 127.5, 131.8, 132.9 (2CH), 133.5, 136.1, 142.9, 144.7, 146.9, 152.7.

3-((1-(4-Ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-7,9-dimethoxy-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5g) Orange powder; yield: 92%, C$_{29}$H$_{30}$N$_{4}$O$_{3}$, HRMS [M + 1]$^+$: calcd. = 483.2312, found = 483.2374, bp. 156–157 °C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ (ppm): 1.31 (t, $J$ = 7.8 Hz, 3H), 1.76 (d, $J$ = 12.5 Hz, 1H), 2.30 (td, $J$ = 4.6, 12.6 Hz, 1H), 2.74 (q, $J$ = 7.8 Hz, 2H), 2.81–2.88 (m, 2H), 3.05 (td, $J$ = 3.2, 12.5 Hz, 1H), 3.42 (d, $J$ = 18.0 Hz, 1H), 3.63 (s, 3H), 3.80 (d, $J$ = 6.9 Hz, 1H), 3.88 (s, 3H), 4.03 (s, 2H), 5.07 (d, $J$ = 6.4 Hz, 1H), 5.33 (s, 1H), 5.59 (d, $J$ = 6.4 Hz, 1H), 6.65 (d, $J$ = 8.2 Hz, 1H), 6.70 (d, $J$ = 8.2 Hz, 1H), 7.36 (d, $J$ = 8.3 Hz, 2H), 7.67 (d, $J$ = 8.3 Hz, 2H), 8.00 (s, 1H), $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (ppm): 14.2, 15.4, 22.6, 28.5, 31.6, 36.4, 44.2, 46.4, 46.5, 49.2, 55.1, 58.7, 89.1, 95.9, 112.5, 113.0, 119.4, 120.4 (2CH), 120.9, 127.5, 129.1 (2CH), 133.4, 135.0, 142.9, 144.7, 145.1, 152.7.

3-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-7,9-dimethoxy-2,3,4,7a-tetrahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline (5h) Orange powder; yield: 90%, C$_{28}$H$_{26}$FN$_{4}$O$_{3}$, HRMS [M + 1]$^+$: calcd. = 469.2156, found = 469.2252, bp. 136–138 °C, $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ (ppm): 1.72 (d, $J$ = 12.8 Hz, 1H), 2.24 (td, $J$ = 4.7, 12.8 Hz, 1H), 2.73–2.83 (m, 2H), 2.96–2.98 (m, 1H), 3.37 (d, $J$ = 18.0 Hz, 1H), 3.62 (s, 3H), 3.72 (d, $J$ = 6.6 Hz, 1H), 3.87 (s, 3H), 3.89–3.93 (m, 2H), 5.04 (d, $J$ = 6.3 Hz, 1H), 5.30–5.32 (m, 2H), 5.54 (s, 2H), 6.63 (d, $J$ = 8.2 Hz, 1H), 6.69 (d, $J$ = 8.2 Hz, 1H), 7.24–7.33 (m, 2H), 7.35–7.44 (m, 3H), 7.50 (s, 1H), $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$
7,9-Dimethoxy-3-((1-(4-methylbenzyl)-1 H -1,2,3-triazol-4-yl)methyl)-2,3,4,7a-tetrahydro-1 H -4,12-methanobenzofuro[3,2-e]isoquinoline (5i) Orange powder; yield: 88%, C$_{29}$H$_{30}$N$_4$O$_3$, HRMS [M + 1]: calcd. = 483.2383, found = 483.2383, bp. 162–164 °C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$) δ (ppm): 1.73 (d, $J$ = 11.9 Hz, 1H), 2.24–2.26 (m, 1H), 2.37 (s, 3H), 2.74–2.76 (m, 1H), 2.82–2.84 (m, 1H), 2.98–3.20 (m, 1H), 3.41 (d, $J$ = 17.8 Hz, 1H), 3.62 (s, 3H), 3.71–3.78 (m, 1H), 3.89 (s, 3H), 3.85–2.94 (m, 1H), 3.93–3.97 (m, 1H), 5.07 (d, $J$ = 6.0 Hz, 1H), 5.31 (s, 1H), 5.49 (s, 2H), 5.57 (d, $J$ = 6.0 Hz, 1H), 6.63 (d, $J$ = 8.7 Hz, 1H), 6.69 (d, $J$ = 8.7 Hz, 1H), 7.18–7.21 (m, 4H), 7.59 (s, 1H), $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm): 21.1, 22.7, 29.9, 31.7, 43.8, 46.3, 48.9, 54.0, 55.0, 56.4, 59.1, 63.8, 65.3, 89.4, 95.8, 113.5, 119.6, 127.1, 128.8 (2CH), 129.4, 129.7 (2CH), 129.8, 131.9, 138.9, 143.1, 145.1, 153.3.

3-((1-(4-Fluorobenzyl)-1 H -1,2,3-triazol-4-yl) methyl)-7,9-dimethoxy-2,3,4,7a-tetrahydro-1 H -4,12-methanobenzofuro[3,2-e]isoquinoline (5j) Orange powder; yield: 95%, C$_{28}$H$_{27}$FN$_4$O$_3$, HRMS [M + 1]: calcd. = 487.2062, found = 487.2167, pb. 147–148 °C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$) δ (ppm): 1.76 (d, $J$ = 11.2 Hz, 1H), 2.23 (td, $J$ = 4.8, 12.6, 1H, CH$_2$), 2.72 (dd, $J$ = 4.4, 13.2 Hz, 1H), 2.79 (dd, $J$ = 7.1, 18.0 Hz, 1H), 2.96 (td, $J$ = 3.1, 12.8 Hz, 1H), 3.35 (d, $J$ = 18.0 Hz, 1H), 3.62 (s, 3H), 3.69 (d, $J$ = 7.1 Hz, 1H), 3.87 (s, 3H), 3.89 (s, 2H), 5.03 (d, $J$ = 6.4 Hz, 1H, CH), 5.30 (s, 1H), 5.49–5.62 (m, 3H), 6.62 (d, $J$ = 8.2 Hz, 1H), 6.68 (d, $J$ = 8.2 Hz, 1H), 7.09 (t, $J$ = 8.5 Hz, 2H), 7.30 (m, 2H), 7.48 (s, 1H), $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm): 31.4, 36.5, 44.2, 46.5, 49.2, 53.4, 54.9, 56.4, 58.8, 89.1, 95.9, 112.2, 112.9, 116.0–116.2 (2CH-C-CF), 119.3, 122.5, 127.6, 129.9–130.0 (2CH-C-CF), 130.5, 131.9, 133.4, 142.8, 144.7, 146.3, 152.7, 162.1–163.7.

3-((1-(4-Bromobenzyl)-1 H -1,2,3-triazol-4-yl)methyl)-7,9-dimethoxy-2,3,4,7a-tetrahydro-1 H -4,12-methanobenzofuro[3,2-e]isoquinoline (5k) Orange powder; yield: 98%, C$_{28}$H$_{27}$BrN$_4$O$_3$, HRMS [M + 1]: calcd. = 547.1261, found = 547.1313, bp. 183–185 °C, $^1$H NMR (600 MHz, CDCl$_3$) δ (ppm): 1.70–1.73 (m, 1H), 2.22 (td, $J$ = 5.1, 12.7 Hz, 1H), 2.72 (dd, $J$ = 4.7, 13.0 Hz, 1H), 2.79 (dd, $J$ = 7.2, 18.0 Hz, 1H), 2.97 (td, $J$ = 3.4, 13.0 Hz, 1H), 3.36 (d, $J$ = 18.0 Hz, 1H), 3.62 (s, 3H), 3.69 (d, $J$ = 7.0 Hz, 1H), 3.87 (s, 3H), 3.89 (s, 1H), 5.05 (d, $J$ = 6.4 Hz, 1H), 5.30 (s, 1H), 5.49 (s, 2H), 5.52 (d, $J$ = 6.4 Hz, 1H, CH), 6.62 (d, $J$ = 8.2 Hz, 1H), 6.68 (d, $J$ = 8.2 Hz, 1H), 7.18 (d, $J$ = 8.4 Hz, 2H), 7.47 (s, 1H), 7.52 (d, $J$ = 8.4 Hz, 2H), $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm): 31.4, 36.5, 44.2, 46.5, 49.3, 53.5, 55.0, 56.4, 58.8, 89.1, 95.9, 112.1, 113.0, 119.3, 122.6, 122.9, 127.6, 129.7, 132.1, 132.3, 133.4, 133.7, 142.8, 144.7, 146.5, 152.7.

2-(4-(7,9-Dimethoxy-1,2,3-triazol-1-yl)-1-phenylethan-1-ol) (5l, Mixture of two diastereomers 70:30) Orange powder; yield: 65%, C$_{29}$H$_{30}$N$_4$O$_4$, HRMS [M + 1]: calcd. = 499.2262, found = 499.2348, bp. 142–144 °C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$, Mixture of two diastereomers) δ (ppm): 1.75 (d, $J$ = 12.7 Hz, 1H, major diastereomer), 1.80 (d, $J$ = 12.7 Hz, 1H, minor diastereomer), 2.26–2.29 (m, 2H, two diastereomers), 2.64 (s, 2H, two diastereomers), 2.81–2.86 (m, 4H, two diastereomers), 3.04–3.08 (m, 2H, two diastereomers), 3.39 (d, 2H, two diastereomers), 3.64 (s, 6H, two diastereomers), 3.82–3.89 (m, 10H, two diastereomers), 3.99–4.02 (m, 2H, two diastereomers), 4.18–4.25 (m, 4H, two diastereomers), 4.54–4.58 (m, 2H, two diastereomers), 5.05–5.08 (m,
2H, two diastereomers), 5.32 (s, 1H, major diastereomer), 5.33 (s, 1H, minor diastereomer), 5.67–5.70 (m, 2H, two diastereomers), 6.62–6.66 (m, 2H, two diastereomers), 6.69–6.71 (m, 2H, two diastereomers), 7.15–7.18 (m, 3H, two diastereomers), 7.37–7.44 (m, 8H, minor and two diastereomers), 7.61 (s, 1H, major diastereomer),

$^{13}$C NMR (125 MHz, CDCl$_3$, mixture of two diastereomers) $\delta$ (ppm): 14.2, 21.8, 22.7, 29.7, 31.6, 32.0, 34.2, 35.0, 40.3, 40.8, 43.3, 43.5, 44.9, 45.6, 46.1, 48.1, 55.2, 56.4, 58.8, 59.0, 60.8, 65.0, 67.2, 88.6, 88.8, 95.7, 95.7, 113.2, 113.4, 114.3, 115.2, 119.6, 119.7, 124.5, 125.9, 125.9, 126.1, 126.6, 127.1 (2CH), 127.2, 128.7, 128.9, 129.1 (2CH), 129.3, 129.5, 132.4, 133.0, 136.0, 143.1, 143.2, 143.4, 144.7, 153.2, 153.4.

1-(4-(7,9-Dimethoxy-1,2,4,7a-tetrahydro-3H-4,12-methanobenzofuro[3,2-e]isoquinolin-3-yl)methyl)-1H-1,2,3-triazol-1-yl)-3-phenoxypropan-2-ol (5m, Mixture of two diastereomers 60:40) Orange powder; yield: 58%, C$_{30}$H$_{32}$N$_4$O$_5$, HRMS [M + 1]$^+$: calcd. = 529.2367, found = 529.2456, b.p. 171–172°C decomposition point, $^1$H NMR (600 MHz, CDCl$_3$, mixture of two diastereomers) $\delta$ (ppm): 1.74 (d, $J = 12.8$ Hz, 2H, two diastereomers), 2.26–2.30 (m, 2H, two diastereomers), 2.80–2.84 (m, 4H, two diastereomers), 3.01–3.05 (m, 1H, minor), 3.37–3.40 (m, 2H, two diastereomers), 3.41–3.46 (m, 2H, two diastereomers), 3.48–3.53 (m, 4H, two diastereomers), 3.63 (s, 6H, two diastereomers), 3.75–3.78 (m, 1H, major), 3.85–3.89 (m, 8H, two diastereomers), 3.96 (s, 2H, two diastereomers), 4.03–4.08 (m, 4H, two diastereomers), 4.23 (s, 2H, two diastereomers), 4.42–4.44 (m, 2H, two diastereomers), 4.56–4.58 (m, 2H, two diastereomers), 5.04–5.07 (m, 2H, two diastereomers), 5.21–5.26 (m, 2H, two diastereomers), 5.29–5.33 (m, 4H, two diastereomers), 5.55–5.60 (m, 2H, two diastereomers), 5.89–5.92 (m, 4H, two diastereomers), 6.63 (d, $J = 8.1$ Hz, 2H, two diastereomers), 6.69 (d, $J = 8.1$ Hz, 2H, two diastereomers), 6.99 (t, $J = 7.6$ Hz, 2H, two diastereomers), 7.31 (t, $J = 7.6$ Hz, 4H, two diastereomers), 7.94 (s, 2H, two diastereomers), $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (ppm): 31.8, 36.1, 44.0, 46.5, 48.8, 53.0, 55.0, 56.5, 58.8, 69.3, 70.9, 72.4, 89.1, 95.9, 112.9, 113.1, 117.9, 119.4, 124.5, 127.5, 127.5, 133.4, 134.1, 142.9, 143.2, 144.8, 153.0.

Biology

The anti-bacterial tests were performed by using Muller Hinton Agar (MHA) and Hinton Broth Mueller as solid and liquid cultures and 96 sterile microplates. Sterile microplates (96 wells plates) were purchased from SPL.
Life Sciences, South Korea. Two bacteria strains (S. aureus, ATCC: 1431 and E. coli, PTCC: 1339) were utilized to study the activities of the new thebaine derivatives.

Triton X100, FBS and peripheral blood smear (PBS) were supplied from Gibco, United States and Merck Companies. Released hemoglobin absorbance was recorded by using Biotek PowerWave XS2 ELISA.

The HDF cell lines were purchased from Pasteur Institute (Iran). Cells are adherent and have fibroblastic appearance. MTT reagent and other using solvents and materials were bought from Sigma Aldrich Company. The absorbance was measured at 570 nm by using a microplate reader (Epoch, BIO-TEK, USA). Inverted microscope (Olympus, Japan) was utilized to captured the optical microscopic image of cells.

**Anti-bacterial test**

In this work, the MHA dilution method was used to calculate the MIC amount of the synthesized derivatives. So, a batch of the materials was prepared from concentrations 0.04–0.1 µL/mL (with final volume of 100 µL). A suspension of fresh bacterial culture (18–20 hours) was prepared in normal saline and the turbidity was adjusted with a half McFarland tube. Based on MHA, dilution of suspension was adjusted to 1: 100 and the volume of each well was increased to 100 µL. The bacteria colony forming units were assayed at 0.5 × 10⁵ CFU/mL. With the increase of the bacterial suspension, the final concentration of the investigated substance was halved in each well.

After at least 20 hours of incubation at 37°C, the wells were examined for turbidity and the lowest growth inhibition concentration was determined and recorded in µL/mL. For the samples that caused turbidity after dissolving in the culture medium, resazurin reagent was added to recognize wells with growth from the wells without growth. The stock solution of the reagent was prepared with a final concentration of 4 mg/mL in distilled and sterilized water. The afforded solution (5 µL) was added to well treated with thebaine, its derivatives and positive control; and the plate was placed on the shaker. MIC was determined and recorded as soon as the color of positive control well was changed (purple to magenta or pink). The experiment was performed with testing of replicates and cefixime as a standard antibiotic.[38]

**Hemolytic test**

In this assay, the hemolytic potential of the new thebaine analogues was measured according to their ability to lyse and release hemoglobin of red blood cells (RBC). The assay was performed as described by Al Badri et al. with a little modifications [39]. After blood sampling, whole blood samples were collected in EDTA coated tubes and incubated for 10 min at 37°C. Then, in order to eliminate plasma and lysed red blood cells, whole blood samples were centrifuged at 1500 xg for 10 min and washed with PBS repeatedly until the supernatant became clear. The supernatant was removed carefully and a 2% suspension of red blood cells in PBS was prepared. Subsequently, 100 µL of 2% RBC suspension was dispensed into the wells of 96 well plate. A serial dilution (100, 50, 25 and 12.5 ppm) of compounds 5b, 5j, 5m and thebaine (1) were prepared in PBS and were added to the microplate wells in a total volume of 200 µL. The positive and negative control wells were prepared by adding 100 µL of 1% Triton X100 solution (i.e., 100% RBC lysis control) and 100 µL of PBS, respectively. The test plate was incubated at 37°C for 2 hours and then centrifuged at 1500 xg for 10 min. After centrifugation, 100 µL of the supernatant was transferred to a new plate and released hemoglobin
absorbance was recorded at 540 nm. Moreover, in order to evaluate the inhibitory effect of FBS on hemolytic activity of the compounds, a 2% suspension of RBC supplemented with 10% FBS was prepared and tested similarly. All samples were tested in triplicates and data were analyzed in Microsoft Excel. Compounds hemolytic activity was calculated according to the following formula and reported in percent compared to 1% Triton X100 controls:

\[
\text{% Hemolytic Activity} = \frac{(\text{sample OD}_{540nm} - \text{neg. cont. OD}_{540nm})}{(\text{Pos. cont. OD}_{540nm} - \text{Neg. Cont. OD}_{540nm})} \times 100
\]

Cellular Cytotoxic activity

Cell viability was determined by the colorimetric MTT assay in three independent experiments. Briefly, the fresh prepared HDF culture was used to seed in 96-well plates (5×10³ cells/well). After 24 hours incubation at 37°C, HDF cells were treated with the different compounds for 24 hours and 72 hours. Cell viability was determined by incubating 20 µL of MTT solution (5 mg/mL) in total volume of 100 µL in each well. After 4 hours incubation, the media was removed by decanting and 100 µL DMSO was added to each well and incubated again for 15 min. The absorbance of each well for every compound was observed at 570 nm by using an ELISA reader. Also, cells were photographed using an inverted microscope.

Abbreviations

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<tr>
<th>Entry</th>
<th>abbreviation</th>
<th>completely</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>Escherichia coli</td>
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<tr>
<td>2</td>
<td>S. aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>3</td>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>4</td>
<td>MTT</td>
<td>(3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>5</td>
<td>HFD</td>
<td>Human dermal fibroblast cell lines</td>
</tr>
<tr>
<td>6</td>
<td>HDFa</td>
<td>Primary Dermal Fibroblast; Normal, Human, adult cell</td>
</tr>
<tr>
<td>7</td>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>8</td>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>9</td>
<td>EDTA</td>
<td>disodium ethylenediaminetetraacetate</td>
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<tr>
<td>10</td>
<td>MHA</td>
<td>Müller Hinton Agar</td>
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<tr>
<td>11</td>
<td>PBS</td>
<td>peripheral blood smear</td>
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<tr>
<td>12</td>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>13</td>
<td>RBC</td>
<td>red blood cells</td>
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Declarations

Acknowledgement

We appreciate fruitful scientific discussion with Professor Lennart Bunch, University of Copenhagen, Denmark.

References


Scheme 1

Scheme 1 is available in the Supplementary Files section.

Figures

Figure 1

Thebaine (1)
Figure 2

Cell viability (MTT assay) was measured after treatment with compounds thebaine (1), 5b, 5j, and 5m. HDF were grown for 24 hours and 72 hours in the presence of thebaine (1), 5b, 5j, and 5m (25 μM). After 24 h and 72 h the cell numbers were measured with a MTT assay. Results expressed as % of control are the mean (±STDEV) of three independent experiments versus appropriate cell line control (PC) (without any treatment).
Figure 3

Photos were taken under phase contrast microscopy from control cell, cell with thebaine (1) and other synthetic derivatives (5b, 5j and 5m). Dark cell debris (red arrowheads) is hypoxic and dead cells that are suspended in the medium.
Figure 4

Hemolytic activity of four doses of the compounds (1, 5b, 5j and 5m) in the absence of FBS
Figure 5

Hemolytic activity of four doses of the compounds (1, 5b, 5j and 5m) in the presence of 10% FBS

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.pdf
- SupplementaryData.docx
- scheme1.jpg