Synthesis and Biological Activity of Thiazolyl-Acetic Acid Derivatives as Possible Antimicrobial Agents

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5a-h, a series of (5-substituted-2-methyl-1,3-thiazole-4-yl) acetic acids as heterocyclic acetic acid derivatives, was designed and synthesized from ethyl acetoacetate. The synthesized compounds were screened for their antimicrobial activities against bacterial and fungal strains, and their characteristics were investigated by assays under various temperature and pH conditions. Cytotoxicity was evaluated with the use of sheep erythrocytes and human neonate dermal fibroblasts. Similarly, agents such as lauric acid 6 and parabens 7a-b, which are used as preservative agents for commercial cosmetics and detergents, were assayed for comparison. Although the structure of 5a is simple, comprising a thiazole attached with an octyl group and acetic acid moiety, the compound showed stronger and broader antibacterial and antifungal activities among the 5 series against the tested microbes other than gram-negative bacteria. Interestingly, 5a overcame the weak antifungal activity of parabens 7a-b. Also, the cytotoxicity of 5a was less than that of parabens 7a-b, especially to human dermal fibroblasts. These results suggest that thiazolyl-acetic acid 5a is a potentially effective biocide, and that it could be used as a preservative agent in commercially sold cosmetics and detergents, facilitated by the hydrophilic and charge properties of its carboxylic acid moiety.

Key words: Thiazolyl-acetic acid derivatives/Antibacterial/Antifungal/Cytotoxicity/Surfactant.

INTRODUCTION

Biocides such as parabens (esters of 4-hydroxy benzoic acid), glycerol esters of fatty acids and sorbic acid are added to cosmetics, detergents, pharmaceuticals, and food products to prevent biodeterioration caused by bacteria, yeasts and fungi (Soni et al., 2001; Steinberg, 2006). The extensive use of parabens, which are used in most bodycare cosmetics and detergents, including hair and face soaps, deodorants, creams, and lotions, is based on their broad-spectrum antimicrobial activity. For example, the minimum inhibitory concentrations (MICs) of propyl paraben against Staphylococcus aureus, Klebsiella pneumoniae and Saccharomyces cerevisiae are 500, 500 and 125 mg L⁻¹, respectively (Thomas and Donald, 1984). They are also cheap, and are active over a wide pH range (Rosen and Berke, 1973). However, ethyl and propyl parabens showed little growth inhibition of fungi at 1000 mg L⁻¹, as shown in our results in Table 2, indicating that these molecules are not effective against fungi. At the same time, parabens are known to be generically more effective against yeasts and fungi than bacteria. It is reported that pairs of different parabens provide efficient antifungal action (Neves et al., 2009).

This study focuses on the development of new biocides with simple structures that may confer antimicrobial activity similar to the way the structure of parabens does in cosmetics and detergents. Thiazoles and their derivatives, which are being studied by many chemists worldwide, have attracted continuing interest in their antibacterial (Carter et al., 1999), antimicrobial (Badorcí et al., 1997; Líras,
One thiazole derivative, 2-(4-thiazolyl)benzimidazole (TBZ), is commonly used as an antifungal agent and as a food preservative.

Our biocide design was based on simple thiazole derivatives, which incorporate a thiazole skeleton corresponding to that of sulfuroil, 5-(2-hydroxyethyl)-4-methylthiazole and are used as safe food additives, with an added hydrophobic group and acetic acid moiety. The added hydrophobic group and acetic acid moiety are designed to confer surfactant activity that leads to higher antimicrobial activity. Because many commercially available cosmetics and detergents include several anionic and nonionic surfactants, our derivatives are suitable as antimicrobial additives due to the hydrophilic and charge properties of carboxylic acid or its salts. Alkyl chains (octyl, decyl, dodecyl, tetradecyl or hexadecyl), which are widely responsible for surface activity, or bulky benzyl groups (benzyl, 4-fluorobenzyl or 4-chlorobenzyl), which are used in the structure of tebuconazole, fluconazole and chlorhexidine as beneficial biocides and an azole-type antifungal agent (Che et al., 2009), are substituted to the thiazole body as hydrophobic groups. The primary purpose of this investigation was to assess the antimicrobial activity against bacteria and fungi of simple structural thiazole derivatives synthesized from ethyl acetoacetate 1, with substitutes of hydrophobic and acetic acid moieties. We report herein not only on their broad-spectrum antibacterial activity but also the effect of temperature and pH on their bactericidal activity. In addition, the thiazolyl-acetic acids synthesized for use as possible antimicrobial agents were found to possess low cytotoxicity against sheep erythrocytes and human dermal fibroblasts. Comparison with the biological properties of parabens suggested the potency of thiazolyl-acetic acids as preservatives in cosmetics and detergents.

**MATERIALS AND METHODS**

**Chemistry**

All chemicals for the synthesis of compounds were reagent grade commercially available materials and were used without further purification. Ethanol was freshly distilled over magnesium turnings to give absolute alcohol, and chloroform was similarly distilled after being pre-dried with calcium chloride to obtain highly purified chloroform. All the reactions were monitored by thin-layer chromatography (TLC) using precoated Merck silica gel 60 F$_{254}$ plates (thickness 0.25 mm). NMR spectra were recorded on an NMR spectrometer (JEM-EX 400, JOEL, Japan) using tetramethyilsilane as an internal standard. The purity of compounds was checked by TLC, and by the melting point which was determined on a micro-melting apparatus (Fisher Scientific, USA). Mass spectra were recorded on an EI-MS system (QP1000, Shimadzu, Japan). Elemental analyses were performed with a Micro Corder JM10 instrument (J-Science Lab Co., Ltd., Japan) and were consistent with theoretical values within ±0.3%. IR spectra were acquired on a Bio-Rad FTS 3000MXT spectrometer. Optical rotations were taken on a DIP-360 digital polarimeter (Jasco, Japan). The crude compounds were purified through silica gel (mesh 70-230) by using flash column chromatography and a Hitachi L-2000 HPLC system equipped with a COSMOSIL column (MS-II Waters, 20 × 250 mm). Except for the hydrolysis reaction by lithium hydroxide (LiOH), all reactions were conducted in a stream of nitrogen. Lauric acid 6 and a commercially available antibacterial agent, parabens 7a-b, were purchased from Kanto Chemical Co., Inc., and Midori Chemical Co., Inc., Japan, respectively (Fig. 1).

**Synthesis**

As shown in Scheme 1, eight thiazolyl-acetic acid derivatives, composed of 1,3-thiazole-4-yl acetic acid, TAA-n and -benzyl series 5a-h were synthesized by hydrolyzing ethyl acetate portions such as ethyl (5-substituted-2-methyl-1,3-thiazole-4-yl) acetates 4a-h possessing various substituent groups at the 5-position of 1,3-thiazole ring. 4-Substituted-3-oxobutyric acid ethyl esters 2a-h (Hamze et al., 2005) and their brominations 3a-h (Svendsen and Boll, 1973) were prepared according to the method of previous reports. 1,3-Thiazole acetates 4a-h were then obtained by the reaction of brominated compounds 3a-h with thioacetamide by a previously reported synthetic method (Rudolph et al., 2007).

**Preparation of 4-substituted-3-oxobutyric acid ethyl ester (2a-h)**

$$6: \text{C}_1\text{H}_{23}^{\text{COOH}}$$

$$7a: R = \text{C}_2\text{H}_5$$

$$7b: R = \text{C}_3\text{H}_7$$

**FIG. 1.** Chemical structures of two agent series, lauric acid 6, and ethyl and propyl parabens 7a-b, used for comparison.
Scheme 1. Synthesis of (5-substituted-1,3-thiazole-4-yl) acetic acids 5a-h.

To a stirred solution of ethyl acetoacetate (3.00 g, 23.1 mmol) in anhydrous tetrahydrofuran (THF, 60 mL) at 4°C was added sodium hydride (NaH, 0.83 g, 34.7 mmol, 1.5 equiv.). After stirring the resultant solution for 10 min to 1 h, 1.6 mol L⁻¹ n-butyl lithium in n-hexane (n-BuLi, 21.6 mL, 34.7 mmol, 1.5 equiv.) was added dropwise. Stirring was done for 10 min, and then 0.8 mol L⁻¹ (0.8 equiv.) n-octyl bromide, n-decyl bromide, n-dodecyl bromide, n-tetradecyl bromide or n-hexadecyl bromide in anhydrous THF (23.3 mL) was added dropwise at room temperature, and the suspension was stirred for 1 h. In the case of 2f-h, the equivalent of NaH and n-BuLi was 2.2, and the reaction with unsubstituted or 4-substituted, benzyl bromide (benzyl bromide, 4-fluorobenzyl bromide or 4-chlorobenzyl bromide) was carried out at 4°C for 3.5 h. Most of the organic solvent in reactions, after being quenched with saturated ammonium chloride solution, was evaporated. The ethyl acetate layer extracted from the residue was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient elution of n-hexane/ethyl acetate (110:1-50:1) yielded the intended compounds (2a-f and 3h). The crude compound of 3g was purified by the HPLC method using a gradient elution of methanol/H₂O (1:1-7:3) as the mobile phase.

Preparation of ethyl (5-substituted-2-methyl-1,3-thiazole-4-yl) acetate (4a-h)

Compounds 3a-h (6.64 mmol) and thioacetamide (8.30 mmol, 1.25 equiv.) were dissolved in ethanol (120 mL). The mixture was stirred at 70°C for 3 h, and the reaction was quenched with saturated sodium bicarbonate solution (10 mL). After removal of the organic solvent under reduced pressure, more water was added, then extraction was performed with ethyl acetate. The organic phase was dried with sodium sulfate and removed under reduced pressure. The purified thiazole compounds (4a-h) were obtained by silica gel column chromatography using a gradient elution of n-hexane/ethyl acetate (40:1-1:5).

Preparation of (5-substituted-2-methyl-1,3-thiazole-4-yl) acetic acid (5a-h)

To a solution of compounds 4a-h (3.4 mmol) in 50 mL of THF/H₂O (5:1) was added lithium hydroxide monohydrate (4.8 mmol, 1.4 equiv.), and the mixture was stirred at room temperature for 16 h. The mixture was acidified with 1 mol L⁻¹ HCl at 4°C to give crystals, which were purified by washing with a mixed solvent of n-hexane and ethyl acetate.

In vitro assay for antimicrobial activity

The tube standard dilution method was used to determine antimicrobial activities: MBC against exponential-phase cells in a sterilized water system, and MIC against stationary-phase cells in a nutrient broth system (Okazaki et al., 1997; Shirai et al., 2005). Antimicrobial tests were performed using bacteria.
and fungi which had been stored in our laboratory. The microorganisms used were Bacillus cereus NITE Biological Resource Center (NBRC) 3001, B. subtilis American Type Culture Collection (ATCC) 6633, Kocuria rhizophila NBRC 12708, Staph. aureus NBRC 12732, Staph. aureus COL1 (MRSA), Escherichia coli NBRC 12713, Kl. pneumoniae ATCC 4352, Proteus mirabilis NBRC 3849, Pseudomonas aeruginosa ATCC 27583, Serratia marcescens ATCC 13880, Sac. cerevisiae NBRC 1136, Candida albicans NBRC 1385, Aspergillus brasiliensis NBRC 105650, Penicillum pinophilum NBRC 6345, Rhizopus stolonifer NBRC 4781, and Trichophyton mentagrophytes NBRC 32409.

Bacteria and fungi (conidia) were prepared according to previous reports (Okazaki et al., 1997; Shirai et al., 2005). The stationary-phase cell suspension was diluted to 2 × 10⁶ cells mL⁻¹ with nutrient broth (Difco Laboratories, Detroit, MI, USA). For MBC, exponentially growing S. aureus NBRC 12732 was prepared by 2 h incubation in nutrient broth under shaking, then washed and diluted to 2 × 10⁶ cells mL⁻¹ with sterilized ion-exchanged water. The number of conidia was determined with a hemocytometer (depth 0.1 mm, 1/400 qmm, Thoma) and adjusted to 2 × 10⁶ cells mL⁻¹ with Sabouraud broth [polypepton 1%, (w/v), glucose 4% (w/v)].

Except where noted, the solutions of TAA-n and -benzyl compounds, and of comparative agents tested, preliminarily dissolved in 80% dimethyl sulfoxide (DMSO) at a high concentration, were prepared by 1.25- or 2-fold stepwise dilution. All experiments were performed at least in triplicate, and the error bars indicate the standard deviations from the obtained mean values.

**In vitro assay for cytotoxic activity**

Hemolytic toxicity was determined on sheep red blood cells (Nippon Biotest Labo.) and by the serial 1.25-fold dilution method (Shirai et al., 2005). The concentration of TAA-n and -benzyl compounds which induced a 50% release of hemoglobin from erythrocytes was defined as Hₐₐ, determined from the plot of the percentage of hemolysis against concentrations of TAA-s. Likewise, a 50% inhibitory concentration against human neonate dermal fibroblasts was defined as ICₐₐ. The ICₐₐ value was determined by measuring cell viability using MTT assay on the basis of the reduction reaction by intracellular dehydrogenase. For all toxic experiments, the solutions of TAA-n and -benzyl compounds, and of comparative agents tested were preliminarily dissolved in 80% DMSO at a high concentration. Also, all experiments were performed at least in triplicate, and the error bars indicate the standard deviations from the obtained mean values.

Cytotoxicity was assessed by using the human neonate dermal fibroblasts NB1RGB (Riken Cell Bank, Japan). A minimum essential medium alpha medium (α-MEM) and Dulbecco’s modified Eagle’s medium (D-MEM), enriched with 10% fetal bovine serum and with 60 mg L⁻¹ of kanamycin sulfate as an antibiotic, were used for cell cultivation and to perform the tests. The cell viability was investigated using the MTT assay (Cell Counting Kit-8, Dojindo Molecular Technologies, Inc.). A cell suspension, which was pre-incubated with α-MEM and suspended with D-MEM, was diluted to 5 × 10⁵ cells mL⁻¹ with D-MEM, and distributed in 96-well culture plates (100 μL in each well). Then, the plates were incubated at 37°C in a humid atmosphere with 5% CO₂ until it was 2 or 3 days confluent. After disposing of 50 μL of the incubation supernatant in each well, 50 μL of the synthesized compound solutions which were prepared with the serial 1.5-fold dilution method was added to each well, then the plates were incubated at 37°C for 1 h. Then, 10 μL of the MTT solution was added to the wells, and the mixture was incubated at 37°C for 40 min. The supernatant was obtained by centrifuging the mixture (11800 g × 10 min, 4°C) and its absorbance was measured at 450 nm. The cell toxicity percentage was determined by comparing it with the absorbance for 0% viability (cells with medium including 10000 mg L⁻¹ sodium dodecyl sulfate). The control at 100% viability was that of cells with the medium including 0.1% DMSO.

**RESULTS**

**Synthesis of 1,3-thiazole derivatives**

In Scheme 1, the α-position of ethyl acetocetate 1 was alkylated by adding sodium hydride and n-butyl lithium, followed by 1-bromoalkane (n-octyl bromide, n-decyl bromide, n-dodecyl bromide, n-tetradeoxy bromide or n-hexadecyl bromide), giving yields of 29-48% (2a-e). This replacement reaction is a method to give thiazole series 4a-h which possesses several types of substituent groups at the 5-position. The α-position of the alkyl compounds 2a-e was subsequently bromo-substituted, giving yields of 89-98% (3a-3e). Five alkylated 1,3-thiazole acetates 4a-e were derived by heating the α-bromo-substituted compounds and thiacetamide in anhydrous ethyl alcohol, giving yields of 18-33%. The intended acetic acid derivatives 5a-e were obtained with yields of 69-93% by hydrolyzing compounds 4a-e with LiOH.
TABLE 1. MIC spectra of TAA-\(\eta\) and -benzyl series 5a-h, and of lauric acid 6 and parabens 7a-b as agents used for comparison against bacteria.

<table>
<thead>
<tr>
<th>TAA-(\eta) series</th>
<th>TAA-benzyl series</th>
<th>Comparative agents</th>
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<tbody>
<tr>
<td>5a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5c</td>
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<td>5f</td>
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<td>5g</td>
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<tr>
<td>5h</td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7b</td>
<td></td>
<td></td>
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</tbody>
</table>

\(\*\) All experiments were performed at least in triplicate, and the error bars indicate the standard deviations from the obtained mean values. \(\dagger\) TAA-series 5d-e were removed from this list because of no growth inhibition effect at 1000 mg L\(^{-1}\) against all bacteria. \(\ddagger\) Staphylococcus aureus NBRC 12732, \(\S\) Staphylococcus aureus COL1 (MRSA), \(\S\) Bacillus cereus NBRC 3001, \(\S\) Bacillus subtilis ATCC 6633, \(\S\) Kocuria rhizophila NBRC 12708, \(\S\) Escherichia coli NBRC 12713, \(\S\) Klebsiella pneumoniae ATCC 4352, \(\S\) Proteus mirabilis NBRC 3849, \(\S\) Pseudomonas aeruginosa ATCC 27583, \(\S\) Serratia marcescens ATCC 13880.

followed by neutralization with HCl solution. Likewise, the treatment of benzyl, 4-fluorobenzyl or 4-chlorobenzyl with ethyl acetoacetate yielded the precursors 4f-h, ethyl (5-benzyl-2-methyl-1,3-thiazole-4-yl) acetate 4f, ethyl [5-(4-fluorobenzyl)-2-methyl-1,3-thiazole-4-yl] acetate 4g, and ethyl (5-(4-fluorobenzyl)-2-methyl-1,3-thiazole-4-yl) acetate 4h, eventually leading to TAA-benzyl series 5f-h through hydrolysis reaction.

The synthesized compounds 2-5 containing thiazole derivatives were satisfactorily confirmed by IR, NMR, mass spectrometry and elemental analysis. For the final compound 5a, the IR spectrum showed absorptions at 2852 and 2920 cm\(^{-1}\) (CH\(_3\) corresponding to octyl group), and sharp bands 1722 cm\(^{-1}\) (C=O), 1564 cm\(^{-1}\) (C=N) and 1201 cm\(^{-1}\) (C=S). In the \(^1\)H-NMR spectrum 5a showed peaks at \(\delta\) 0.85 (CH\(_3\) corresponding to the termination of octyl group), 1.24-1.54 (polyethylene chain), 2.54 (CH\(_3\)) at the 2-position of 1,3-thiazole ring) and 3.56 (CH\(_3\)) in acetate acid residue), and a broad peak at 12.39 (COOH). Our results showed that the synthesized TAA-\(\eta\) and -benzyl series are the intended compounds. Eight thiazolyl-acetic acid derivatives were evaluated for their antimicrobial activity and biological toxicity, and compared with agents such as 6 and 7a-b.

**Antimicrobial activity**

As can be seen in Table 1, the results of the TAA-\(\eta\) series 5a-c possessing an alkyl chain showed effective growth inhibition of gram-positive bacteria; these compounds exhibited an MIC of below 170 mg L\(^{-1}\), except for 5d-e which had no effective bacteriostatic activity at 1000 mg L\(^{-1}\) (data not shown). In the case of TAA-benzyl series 5f-h, their activities, while broad against gram-positive bacteria, were inferior to those of 5a-c, except for the MIC of 5f against B. subtilis. Synthesized 5a-h exhibited little or no antibacterial effect against gram-negative bacteria. The agent used for comparison, lauric acid 6, demonstrated a similar antibacterial spectrum and bacteriostatic activity to 5a. Parabens 7a-b, which are commercially available biocides, were confirmed as possessing broader-spectrum antibacterial activity against both gram-positive and negative bacteria; however, their MICs were close to or exceeded 500 mg L\(^{-1}\), not lower than those of 5a-c.

Table 2 shows the MICs of the 5 series against fungi, except for 5d-e, which showed no antibacterial effect at 1000 mg L\(^{-1}\). Synthesized 5a was confirmed as working against a broader antifungal spectrum than other tested compounds against tested fungi.

**Antibacterial properties**

To investigate the antibacterial property of the TAA-\(\eta\) series, the minimum bactericidal concentrations (MBCs) of 5a-c were measured at various temperatures (10, 20, 30 and 40°C), and 6 and 7b were also examined in a similar way. As seen in Fig. 2 (A),
TABLE 2. MIC spectra of TAA-\(n\) and -benzyl series (5a-c and 5f-h), and of lauric acid 6 and parabens 7a-b as agents used for comparison against yeasts and fungi.

<table>
<thead>
<tr>
<th>MIC (mg L(^{-1}))</th>
<th>TAA-(n) series</th>
<th>TAA-benzyl series</th>
<th>Comparative agents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5a</td>
<td>5b</td>
<td>5c</td>
</tr>
<tr>
<td>Sacc. cerevisiae</td>
<td>63±0.0</td>
<td>16±0.0</td>
<td>1000±0.0</td>
</tr>
<tr>
<td>C. albicans</td>
<td>500±0.0</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>A. brasiliensis</td>
<td>500±0.0</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Pe. pinophilum</td>
<td>420±120</td>
<td>840±240</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Rh. stolonifer</td>
<td>670±240</td>
<td>1000±0.0</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Tr. mentagrophytes</td>
<td>42±18</td>
<td>12±5.5</td>
<td>42±15</td>
</tr>
</tbody>
</table>

* All experiments were performed at least in triplicate, and the error bars indicate the standard deviations from the obtained mean values.

* TAA-series 5d-e were removed from this test because of no growth inhibition effect at 1000 mg L\(^{-1}\) against all bacteria.


5a-c showed bactericidal activity at temperatures from 10 to 40°C at concentrations of 1000 mg L\(^{-1}\) or lower. Their MBCs fell with rising temperature in the following concentration ranges: 1000-125 mg L\(^{-1}\) for 5a, 500-63 mg L\(^{-1}\) for 5b, and 190-16 mg L\(^{-1}\) for 5c.

The MBCs of 5a-c and 6 were measured using 50 mmol L\(^{-1}\) Na-K phosphate buffer prepared at various pH values (6, 7 and 8). Figure 2 (B) shows that 5a-c have effective bactericidal activity from pH 5 to 8 and that the activity was higher in acidic solutions than in alkaline solutions. A similar effect of pH on bactericidal activity was observed in 6, with organic acids used as food preservatives showing similar effects (data not shown).

Cytotoxicity

As an index of the safety of antimicrobial agents, cytotoxicity was measured at likely concentrations. Table 3 summarizes the cytotoxic activity of 5a-c and 5f-h, and of 6 and 7a-b for comparison against sheep erythrocytes and human neonate dermal fibroblasts. Of the three thiazole series synthesized, 5a-c exhibited a higher cytotoxic activity against both cells with increasing alkyl chain length from 8 to 12. No cytotoxicity was exhibited by 5f-h when substituting a more bulky group in the thiazole ring on both cells. The hemolytic activity of compound 6 was comparable to that of 5a. Within the range of concentrations that could be dissolved in the tested buffer, parabens 7a-b showed no hemolytic activity. The IC\(_{50}\) of 7a-b was 340 and 290 mg L\(^{-1}\), respectively, and their values were lower than those of 5a-b, which exhibited no cytotoxic activity at 500 mg L\(^{-1}\).

![Fig. 2](image-url)  
**FIG. 2.** Effect of temperature (A) and pH (B) on the MBC of 5a (squares), 5b (triangles) and 5c (circles). Values are the mean and the error bar represents the standard deviation obtained from three independent experiments.

**DISCUSSION**

Eight 5-substituted-1,3-thiazolyl acetic acid derivatives synthesized newly, TAA-\(n\) and -benzyl series 5a-h, were evaluated for biological properties such as antimicrobial activities and cytotoxicity.

The MICs of the eight derivatives 5a-h were investigated against bacteria (10 strains) and fungi (6 strains) in comparison with those of lauric acid 6, consisting of only alkyl and carboxyl groups, which are also included in the structure of TAA-\(n\) series 5a-e; and those of the commercially available biocides ethyl and propyl parabens 7a-b, listed in Tables 1 and 2. From these results, 5a, even though its structure is a simple thiazole ring, alkyl chain and acetic moiety, indicated significantly stronger and broader-spectrum antibacterial and antifungal activities among the 5 series synthesized, and was more effective than agents 6 and 7a-b used for comparison against the tested microbes, but was less effective against gram-negative bacteria. Interestingly, 5a overcame the disadvantages of parabens 7a-b which have weak
TABLE 3. Cytotoxicities of TAA-η and -benzyl series (5a-c and 5f-h), and of 6 and 7 a-b as agents used for comparison against sheep red blood cells and human neonate dermal fibroblasts.

<table>
<thead>
<tr>
<th></th>
<th>TAA-η series</th>
<th>TAA-benzyl series</th>
<th>Comparative agents</th>
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<tbody>
<tr>
<td></td>
<td>5a</td>
<td>5b</td>
<td>5c</td>
</tr>
<tr>
<td>HC₅₀</td>
<td>121±23</td>
<td>61±0.94</td>
<td>15±0.36</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>190±24</td>
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* Cytotoxic activity of compounds were determined by the 50% hemolytic concentration (HC₅₀) in sheep erythrocytes, and by the 50% inhibitory concentration (IC₅₀) against human neonate dermal fibroblasts within concentrations at which agents can possibly be dissolved. Values are mean ± S.D. obtained from three independent experiments.

antifungal activity.

As the length of the alkyl chains increased from octyl to dodecyl, the bacteriostatic activity of TAA-η against gram-positive bacteria increased, but reduced with longer alkyl chains, tetradecyl and hexadecyl groups (Table 1). It appears that the elevation of activity and also the highest activity of 5c are due to the increase in molecular hydrophobicity, which is predominantly associated with alkyl chain length. The molecular hydrophobicity of simple surfactants possessing alkyl chains, such as quaternary ammonium salts, increases with lengthening of their alkyl chains (Maeda et al., 1998). The antibacterial potency of surfactants has been found to be related with physicochemical parameters such as CMC and molecular hydrophobicity (Devinsky et al., 1985; Maeda et al., 1998; Shirai et al., 2005). The TAA-η series are regarded as surfactants due to their hydrophobic long alkyl chains and/or hydrophilic carboxyl groups.

Generally, CMC is negatively correlated with molecular hydrophobicity. With decreasing CMC, the compounds show increasing antibacterial potency (Blois and Swarbrick, 1972) and binding ability to protein (Lien and Perrin, 1976), which enables the surfactants to adsorb onto the bacterial surface. Moreover, gram-positive bacteria have higher cell surface hydrophobicity than gram-negative bacteria (Maeda et al., 1998). Therefore, the alkyl chain of TAA-η potently interacted with the more hydrophobic cell surfaces of gram-positive bacteria, producing the elevation of activity seen from 5a to 5c, depending on alkyl chain length, and with 5c showing the highest activity. Unfortunately, the hydrophobic association between the long alkyl chains of 5d-e and medium components caused a decrease in the effective concentration, as in previously reported biocides (Okazaki et al., 1997). On the other hand, due to the major antibacterial effect of the hydrophobic interaction between the alkyl chain and bacterial cell membrane, the bacteriostatic activity of TAA-η series 5a-e is not evident in gram-negative bacteria, which have a more hydrophilic cell surface.

It was also expected that thiazole compounds 5g-h, which possess a halogen-substituted aromatic ring, would exhibit effective antibacterial and antifungal activities. Tebuconazole possesses a chlorophenyl group and has MIC values ranging from 13-110 mg L⁻¹ against the same gram-positive bacteria tested, and of 210 mg L⁻¹ or lower against the same yeasts and fungi tested (data not shown). Thiazole derivatives possessing halogen-substituted phenyl groups as described in previous papers (Holla et al., 2003; Narayana et al., 2004) showed effective antimicrobial activity at a concentration of 100 mg L⁻¹ or lower. However, 5f-h showed little effective bacteriostatic activity (Tables 1 and 2). For the thiazole derivatives synthesized, a stretching alkyl chain was considered to be important for effective activity. The antifungal activity of lauric acid 6 as a simple surfactant carrying undecyl and carboxyl groups, in which the thiazole moiety is free, confirmed the necessity of the thiazole ring for the carboxylic acid derivatives, as 5a showed strong and broad-spectrum antifungal activity.

We further investigated the antibacterial property of TAA-8, 10 and 12 (5a-c), which possessed effective bacteriostatic activity against the tested bacteria other than gram-negative bacteria (Table 1), at various temperatures and pH values. As seen in Fig. 2 (A), the MBCs of 5a-c fell with rising temperature until 40°C. This pattern corresponded to the fact that increased temperature enhances the bactericidal activity of ammonium salts (Holla et al., 2003; Kourai et al., 2006), which interact with the bacterial membrane to exhibit bactericidal action. Temperature is closely related to the fluidity of the bacterial cell membrane (Marr and Ingraham, 1962). The activity of 5a-c on the basis of hydrophobic interaction with the membrane must be influenced by the change in fluidity of the cell membrane at temperatures ranging from 10 to 40°C. The bactericidal activity of 6, which
possesses an alkyl chain, was found to increase as the temperature rose from 30 to 40°C, similar to the pattern seen in 5a-c (data not shown). In the case of 7b at temperatures of 10, 20 and 30°C, no bactericidal activity was exhibited at a concentration of 1000 mg L⁻¹ (data not shown), suggesting that 5a-c have high industrial applicability because of their effective bactericidal activity even at a low temperature. All assayed compounds indicated a higher activity in acidic solutions than in alkaline solutions (Fig. 2(B)). Under alkaline conditions, carboxylic acid changes to carboxylic ion. It appears that this change in electric charge generates an ionic repulsive interaction between the carboxylic ion in thiazole derivatives and the phosphatidyl group in the bacterial membrane, showing an attenuation of bactericidal action by these compounds on the cell surface.

Table 3 summarizes the cytotoxic activity of 5a-c and 5f-h, which exhibited antimicrobial activity as described in the above section. A relationship between the alkyl chain length of 5 series and the cytotoxic activity is the same as a relationship reported for other cationic biocides possessing a long alkyl chain (Dubíčková et al., 2000; Shirai et al., 2005; Kourai et al., 2006): the increasing alkyl chain length induces higher hemolytic activity. Although compound 6 has a longer alkyl chain than 5a, its hemolytic activity was comparable to that of 5a, implying that the thiazole ring in this series participates in hemolytic toxicity. Parabens 7a-b showed no hemolytic activity due to low hydrophobic interactions on the erythrocyte membrane since they might possess a bactericidal mechanism by inhibiting membrane transport and mitochondrial function rather than hydrophobic interaction. The low IC₅₀ of 7a-b indicates that even though these biocides are used as preservative agents in cosmetics and detergents in the marketplace, they have higher cytotoxicity than 5a-b.

Considering 5a’s biological characteristics such as stronger and broader antimicrobial activities (Tables 1 and 2), effective bactericidal activity at a low temperature (Fig. 2(A)), and low cytotoxicity (Table 3), 5a shows not only potential as an antimicrobial agent but also promise as a preservative for commercially sold products such as cosmetics and detergents, and also as a fungicide in products that come in contact with the skin.

CONCLUSION

This article reports for the first time the synthesis of 5a-h, which are simple structural thiazole derivatives with substitutions at the C₆ position with alkyl chains or bulky aromatic rings, starting from the commercially available ethyl acetatoacetate. Investigation of their activity revealed that the octyl group-substituted thiazole 5a was the most active compound, and was more broadly effective against bacteria and fungi than parabens 7a-b although unfortunately less effective against gram-negative bacteria. It is suggested that the bactericidal mechanism is the result of hydrophobic interaction between hydrophobic alkyl chains and the bacterial cell surface. Furthermore, the fact that the cytotoxicity of 5a was low, especially being less cytotoxic to human fibroblasts than parabens 7a-b, suggests that the compound could be used as a preservative agent in cosmetics and detergents, and as a fungicide, although further studies are required. The hydrophilic and charge properties of the carboxylic acid moiety also could facilitate its application as a preservative in commercially sold products, in which several anionic and nonionic surfactants are involved.

Appendices

Chemistry

4-Octyl-3-oxobutyric acid ethyl ester (2a)

Yellowish liquid; yield: 48%; IR (KBr, ν_max cm⁻¹): 2945 (CH₃ st as), 2918 (C-H st as), 2852 (C-H st sy), 1743 (C=O st), 1714 (C=O st), 1463 (CH₃ δ), 1367 (CH₃ δ sy), 1228 (C-O st as), 1028 (C-O st sy), 721 (CH₃ γ); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 0.88 (3H, t, J = 6.8 Hz, octyl group CH₂), 1.26-1.30 (15H, m, octyl group (CH₃)₉ and ethyl group CH₂), 1.55-1.63 (2H, m, octyl group CH₂), 2.53 (2H, t, J = 7.5 Hz, COCH₂), 3.44 (2H, s, COCH₂CO), 4.20 (2H, q, J = 7.0 Hz, COOCH₂); ¹³C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 13.14, 14.11, 22.7, 23.5, 29.0, 29.3, 29.4, 31.9, 43.1, 49.3, 61.3, 167.3, 203.1; MS (calcd/found) [M⁺]: 242.35/242; Anal. calcd. for C₉H₁₆O₂: C, 69.38; H, 10.81. Found: C, 69.40; H, 10.62.

4-Decyl-3-oxobutyric acid ethyl ester (2b)

Yellowish liquid; yield: 46%; IR (KBr, ν_max cm⁻¹): 2945 (CH₃ st as), 2916 (C-H st as), 2847 (C-H st sy), 1742 (C=O st), 1715 (C=O st), 1462 (CH₃ δ), 1367 (CH₃ δ sy), 1227 (C-O st as), 1025 (C-O st sy), 723 (CH₃ γ); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 0.86 (3H, t, J = 6.8 Hz, decyl group CH₂), 1.23-1.31 (19H, m, decyl group (CH₃)₉ and ethyl group CH₂), 1.54-1.62 (2H, m, decyl group CH₂), 2.51 (2H, t, J = 7.5 Hz, COCH₂), 3.41 (2H, s, COCH₂CO), 4.18 (2H, q, J = 7.0 Hz, COOCH₂); ¹³C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 13.14, 14.11, 22.7, 23.5, 29.0, 29.4, 29.5, 29.6, 29.7, 29.7, 31.9, 43.1, 49.3,
4-Dodecyl-3-oxobutyric acid ethyl ester (2c)

White crystal; yield: 43%; m.p.: 23-25°C; IR (KBrs, ν max cm⁻¹): 2945 (C-H st as), 2912 (C-H st as), 2848 (C-H st sy), 1739 (C=O st), 1710 (C=O st), 1467 (CH₂ δ), 1367 (CH₂ sy), 1230 (C-O st sy), 1029 (C-O st sy), 719 (CH₃ γ); ¹H-NMR (400 MHz, CDCl₃, Me,Si δ in ppm): 0.88 (3H, t, J = 6.8 Hz, dodecyl group CH₃), 1.25-1.33 (23H, m, dodecyl group (CH₃)₁₂ and ethyl group CH₂), 1.51-1.61 (2H, m, dodecyl group CH₂), 2.53 (2H, t, J = 7.5 Hz, COOH₂)₃, 3.43 (2H, s, COOH₂CO), 4.20 (2H, q, J = 7.0 Hz, COOCH₂CO); ¹³C-NMR (100 MHz, CDCl₃, Me,Si, δ in ppm): 14.1, 14.1, 22.7, 23.5, 29.1, 29.2, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7, 31.9, 43.1, 49.3, 61.4, 167.3, 203.1; MS (calcd(found) [M]+): 298/298; Anal. calcd. for C₂₈H₄₀O₂: C, 72.44; H, 11.48. Found: C, 72.50; H, 11.31.

4-Tetradecyl-3-oxobutyric acid ethyl ester (2d)

White crystal; yield: 35%; m.p.: 33-34°C; IR (KBrs, ν max cm⁻¹): 2943 (CH₃ st as), 2911 (CH₃ st as), 2845 (C-H st sy), 1740 (C=O st), 1712 (C=O st), 1463 (CH₂ δ), 1365 (CH₂ sy), 1233 (C-O st sy), 1027 (C-O st sy), 720 (CH₂ γ); ¹H-NMR (400 MHz, CDCl₃, Me,Si, δ in ppm): 0.88 (3H, t, J = 6.7 Hz, tetradecyl group CH₂), 1.25-1.30 (27H, m, tetradecyl group (CH₃)₁₂ and ethyl group CH₂), 1.53-1.60 (2H, m, tetradecyl group CH₂), 2.53 (2H, t, J = 7.4 Hz, COOH₂), 3.46 (2H, s, COOH₂CO), 4.20 (2H, q, J = 7.0 Hz, COOCH₂CO); ¹³C-NMR (100 MHz, CDCl₃, Me,Si, δ in ppm): 14.1, 14.1, 22.7, 23.5, 29.0, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7, 29.7, 32.0, 43.1, 49.3, 61.3, 167.3, 203.0; MS (calcd(found) [M]+): 326.51/326; Anal. calcd. for C₃₆H₅₂O₂: C, 73.57; H, 11.73. Found: C, 73.63; H, 11.97.

4-Hexadecyl-3-oxobutyric acid ethyl ester (2e)

White crystal; yield: 29%; m.p.: 43-44°C; IR (KBrs, ν max cm⁻¹): 2942 (CH₃ st as), 2911 (CH₃ st as), 2846 (C-H st sy), 1737 (C=O st), 1709 (C=O st), 1465 (CH₂ δ), 1364 (CH₂ sy), 1230 (C-O st sy), 1030 (C-O st sy), 722 (CH₂ γ); ¹H-NMR (400 MHz, CDCl₃, Me,Si, δ in ppm): 0.88 (3H, t, J = 6.7 Hz, hexadecyl group CH₂), 1.25-1.30 (31H, m, hexadecyl group (CH₃)₁₂ and ethyl group CH₂), 1.54-1.63 (2H, m, hexadecyl group CH₂), 2.53 (2H, t, J = 7.3 Hz, COOH₂), 3.42 (2H, s, COOH₂CO), 4.20 (2H, q, J = 7.2 Hz, COOCH₂CO); ¹³C-NMR (100 MHz, CDCl₃, Me,Si, δ in ppm): 14.1, 14.1, 22.7, 23.5, 29.0, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7, 29.7, 29.7, 29.7, 32.0, 43.1, 49.3, 61.3, 167.3, 203.0; MS (calcd(found) [M]+): 326.51/326; Anal. calcd. for C₃₆H₅₂O₂: C, 73.57; H, 11.73. Found: C, 73.63; H, 11.97.

4-(4-Fluorobenzy1)-3-oxobutyric acid ethyl ester (2g)

Yellow liquid; yield: 31%; IR (KBrs, ν max cm⁻¹): 3016 (ar-C-H st), 2976 (CH₃ st as), 2927 (C-H st as), 1743 (C=O st), 1716 (C=O st), 1510 (C-F st), 1220 (C-O st as), 1028 (C-O st sy), 821 (ar-C-H δ oop); ¹H-NMR (400 MHz, CDCl₃, Me,Si, δ in ppm): 1.26 (3H, t, J = 7.2 Hz, ethyl group-CH₂), 2.83-2.92 (4H, m, C(CH₃)₂CO), 3.41 (2H, s, COOH₂CO), 4.18 (2H, q, J = 7.2 Hz, COOH₂CO), 6.93-6.99 (2H, m, F-Bn meta-H), 7.11-7.16 (2H, m, F-Bn ortho-H); ¹³C-NMR (100 MHz, CDCl₃, Me,Si, δ in ppm): 14.1, 28.6, 44.5, 49.4, 61.5, 115.2, 115.4, 129.7, 129.8, 136.2, 160.2, 167.1, 201.8; MS (calcd(found) [M]+): 326.41/238; Anal. calcd. for C₁₇H₁₃NO₂: C, 65.53; H, 6.35. Found: C, 65.34; H, 6.11.

4-(4-Chlorobenzy1)-3-oxobutyric acid ethyl ester (2h)

Yellowish liquid; yield: 42%; IR (KBrs, ν max cm⁻¹): 3010 (ar-C-H st), 2980 (CH₃ st as), 2931 (C-H st as), 1743 (C=O st), 1714 (C=O st), 1367 (CH₃ δ sy), 1238 (C-O st as), 1091 (C-Cl st), 1014 (C-O st sy), 810 (ar-C-H δ oop); ¹H-NMR (400 MHz, CDCl₃, Me,Si, δ in ppm): 1.26 (3H, t, J = 7.1 Hz, ethyl group-CH₂), 2.83-2.92 (4H, m, C(CH₃)₂CO), 3.41 (2H, s, COOH₂CO), 4.18 (2H, q, J = 7.2 Hz, COOH₂CO), 7.11 (2H, d, J = 8.0 Hz, Cl-Bn ortho-H), 7.24 (2H, d, J = 8.3 Hz, Cl-Bn meta-H); ¹³C-NMR (100 MHz, CDCl₃, Me,Si, δ in ppm): 14.1, 28.7, 44.2, 49.4, 61.5, 128.6, 129.7, 129.7, 132.0, 139.0,
167.0, 201.6; MS (calcd/found) [M]+: 254.71/254 and 256; Anal. calcd. for C₃₃H₃₅ClO₃: C, 61.30; H, 5.94. Found: C, 61.46; H, 6.20.

(±)-4-Octyl-4-bromo-3-oxobutyric acid ethyl ester (3a)

Brownish oil; yield: 98%; [α]D25 0.00 (c 0.67 in CHCl₃); IR (KBr, ν max cm⁻¹): 2943 (CH₃ st as), 2926 (C-H st as), 2854 (C-H st sym), 1749 (C=O st), 1722 (C=O st), 1465 (CH₂ δ), 1367 (CH₃ δ sy), 1238 (C-O st as, CH₂ ω), 1028 (C-O st sy), 723 (CH₂ γ); 1H-NMR (400 MHz, CDCl₃, Me-Si, δ in ppm): 0.86 (3H, t, J = 6.8 Hz, octyl group CH₃), 1.25-1.30 (13H, m, octyl group (CH₃)₃ and ethyl group CH₂), 1.45 (2H, m, octyl group CH₂), 1.88-1.95 (1H, m, octyl group CHBrCH₂), 2.00-2.07 (1H, m, octyl group CHBrCH₂), 3.59 (1H, d, J = 15.9 Hz, COOCH₂), 3.81 (1H, d, J = 15.9 Hz, COOCH₂), 4.18 (2H, q, J = 7.2 Hz, COOCH₂), 4.43 (1H, dd, J = 6.3 and 8.0 Hz, CHBr); 13C-NMR (100 MHz, CDCl₃, Me-Si, δ in ppm): 13.9, 14.1, 22.6, 27.2, 29.0, 29.2, 29.3, 31.8, 33.0, 45.5, 53.3, 61.6, 167.0, 196.2; MS (calcd/found) [M+]: 321.15/322 and 322. Anal. calcd. for C₂₃H₃₄BrO₂: C, 52.34; H, 7.84. Found: C, 52.10; H, 7.97.

(±)-4-Decyl-4-bromo-3-oxobutyric acid ethyl ester (3b)

Brownish oil; yield: 94%; [α]D25 +0.75 (c 1.06 in CHCl₃); IR (KBr, ν max cm⁻¹): 2949 (CH₃ st as), 2924 (C-H st as), 2854 (C-H st sym), 1749 (C=O st), 1722 (C=O st), 1467 (CH₂ δ), 1367 (CH₃ δ sy), 1238 (C-O st as, CH₂ ω), 1029 (C-O st sy), 721 (CH₂ γ); 1H-NMR (400 MHz, CDCl₃, Me-Si, δ in ppm): 0.87 (3H, t, J = 6.8 Hz, decyl group CH₃), 1.25-1.41 (19H, m, decyl group (CH₃)₉ and ethyl group CH₂), 1.92-1.99 (1H, m, decyl group CHBrCH₂), 2.03-2.10 (1H, m, decyl group CHBrCH₂), 3.60 (1H, d, J = 15.9 Hz, COOCH₂), 3.82 (1H, d, J = 15.9 Hz, COOCH₂), 4.22 (2H, q, J = 7.1 Hz, COOCH₂), 4.44 (1H, dd, J = 6.3 and 8.0 Hz, CHBr); 13C-NMR (100 MHz, CDCl₃, Me-Si, δ in ppm): 14.1, 14.1, 22.7, 27.2, 27.2, 29.6, 29.3, 29.3, 29.5, 29.6, 31.9, 33.0, 45.5, 53.3, 61.6, 167.0, 196.2; MS (calcd/found) [M+]: 430.39/348 and 350; Anal. calcd. for C₃₉H₅₀BrO₂: C, 55.02; H, 8.37. Found: C, 55.16; H, 8.53.

(±)-4-Dodecyl-4-bromo-3-oxobutyric acid ethyl ester (3c)

Brownish oil; yield: 98%; [α]D25 +0.30 (c 0.67 in CHCl₃); IR (KBr, ν max cm⁻¹): 2947 (CH₃ st as), 2924 (C-H st as), 2854 (C-H st sym), 1749 (C=O st), 1718 (C=O st), 1465 (CH₂ δ), 1458 (CH₃ δ sy), 1367 (CH₂ δ sy), 1230 (C-O st as, CH₂ ω), 1026 (C-O st sy), 721 (CH₂ γ); 1H-NMR (400 MHz, CDCl₃, Me-Si, δ in ppm): 0.86 (3H, t, J = 7.0 Hz, dodecyl group CH₃), 1.24-1.47 (23H, m, dodecyl group (CH₃)₁₂ and ethyl group CH₂), 1.85-1.94 (1H, m, dodecyl group CHBrCH₂), 1.97-2.06 (1H, m, dodecyl group CHBrCH₂), 3.59 (1H, d, J = 16.0 Hz, COOCH₂), 4.18 (2H, q, J = 7.2 Hz, COOCH₂), 4.43 (1H, dd, J = 6.3 and 8.1 Hz, CHBr); 13C-NMR (100 MHz, CDCl₃, Me-Si, δ in ppm): 14.1, 14.1, 22.7, 27.2, 29.0, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7, 29.7, 29.7.
(±)-4-(4-Fluorobenzyl)-4-bromo-3-oxobutryric acid ethyl ester (3g)

Yellowish liquid; yield: 50%; [α]D +0.00 (c 0.30 in CHCl3); IR (KBr, ν max cm⁻¹): 3016 (ar-C-H st), 2978 (CH₃ st as), 2928 (C-H st as), 1780 (C=O st), 1720 (C=O st), 1507 (C=C st), 1369 (CH₃ δ sy), 1233 (C-O st as, CH₂), 1027 (C-O st sy), 820 (ar-C-H δ oop); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 1.23 (3H, t, J = 7.1 Hz, CH₃), 3.14 (1H, dd, J = 7.8 and 14.6 Hz, CHBrCH₂C), 3.47 (1H, dd, J = 6.8 and 14.6 Hz, CHBrCH₂C), 3.57 (1H, d, J = 15.9 Hz, COCH₂), 3.80 (1H, d, J = 15.9 Hz, COCH₂CO), 4.14 (2H, q, J = 7.1 Hz, COOCH₃), 4.68 (1H, dd, J = 6.8 and 7.6 Hz, CHBr), 6.98-7.02 (2H, m, F-Bn meta-H), 7.18-7.21 (2H, m, F-Bn ortho-H); ¹³C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 14.0, 38.1, 46.1, 52.0, 61.6, 115.2, 115.3, 130.5, 130.5, 136.5, 161.0, 166.6, 4, 195.5; MS (calcd/found) [M⁺]: 317.15/316 and 318. Anal. calcld. for C₁₅H₁₄NO₂S: C, 64.60; H, 9.15; N, 4.71. Found: C, 64.35; H, 8.98; N, 4.94.

Ethyl (5-octyl-2-methyl-1,3-thiazole-4-yl) acetate (4a)

Brown oily; yield: 33%; IR (KBr, ν max cm⁻¹): 2954 (CH₃ st as), 2926 (C-H st as), 2854 (C-H st sy), 1739 (C=O st), 1558 (C=N st), 1460 (CH₃ δ), 1458 (CH₃ δ as), 1367 (CH₃ δ sy), 1247 (C-O st as), 1180 (C=S st), 1031 (C-O st sy); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 0.80 (3H, t, J = 6.8 Hz, octyl group CH₃), 1.16-1.28 (13H, m, octyl group (CH₃)₂ and ethyl group CH₂), 1.48-1.55 (2H, m, octyl group CH₂), 2.55 (3H, s, CCH₃), 2.62 (2H, t, J = 7.6 Hz, CCH₃), 3.61 (2H, s, CH₃CO), 4.09 (2H, q, J = 7.2 Hz, COOCH₃); ¹³C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 14.1, 14.2, 19.1, 22.7, 26.4, 29.1, 29.2, 29.3, 31.8, 31.8, 35.0, 61.0, 135.3, 143.1, 162.3, 170.5; MS (calcd/found) [M⁺]: 297.46/297; Anal. calcld. for C₁₅H₂₀NO₂S: C, 64.60; H, 9.15; N, 4.71. Found: C, 64.35; H, 8.98; N, 4.94.

Ethyl (5-decyl-2-methyl-1,3-thiazole-4-yl) acetate (4b)

Brown oily; yield: 30%; IR (KBr, ν max cm⁻¹): 2947 (CH₃ st as), 2924 (C-H st as), 2852 (C-H st sy), 1739 (C=O st), 1558 (C=N st), 1458 (CH₃ δ), 1367 (CH₃ δ sy), 1247 (C-O st as), 1180 (C=S st), 1031 (C-O st sy); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 0.84 (3H, t, J = 7.0 Hz, decyl group CH₃), 1.17-1.37 (17H, m, decyl group (CH₃)₂ and ethyl group CH₂), 1.51-1.59 (2H, m, decyl group CH₂), 2.58 (3H, s, CCH₃), 2.65 (2H, t, J = 7.6 Hz, CCH₃), 3.63 (2H, s, CH₃CO), 4.12 (2H, q, J = 7.2 Hz, COOCH₃); ¹³C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 14.1, 14.2, 18.8, 22.7, 26.4, 29.1, 29.3, 29.3, 29.5, 29.5, 31.7, 31.9, 34.7, 61.0, 135.6, 142.6, 162.8, 170.3; MS (calcd/found) [M⁺]: 325.51/325; Anal. calcld. for C₁₇H₂₃NO₂S: C, 66.42; H, 9.60; N, 4.30. Found: C, 66.18; H, 9.32; N, 4.04.
Ethyl (5-dodecyl-2-methyl-1,3-thiazole-4-yl) acetate (4c)

Brown oily; yield: 33%; IR (KBr, ν max cm⁻¹): 2949 (CH₃ st as), 2922 (C-H st as), 2852 (C-H st sy), 1739 (C=O st), 1558 (C=N st), 1463 (CH₂ δ), 1456 (CH₂ δ as), 1367 (C=H δ), 1249 (C-O st as), 1180 (C=S st), 1033 (C-O st sy), 719 (CH₂ γ); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 0.81 (3H, t, J = 6.3 Hz, dodecyl group CH₃), 1.16-1.25 (21H, m, dodecyl group (CH₂)ₙ and ethyl group CH₂), 1.48-1.54 (2H, m, dodecyl group CH₂), 2.57 (3H, s, CH₃), 2.62 (2H, t, J = 7.6 Hz, CCH₂), 3.63 (2H, s, CH₂CO), 4.09 (2H, q, J = 7.2 Hz, COOCH₂); ᵃ¹C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 14.1, 14.2, 18.9, 22.7, 26.4, 29.1, 29.3, 29.3, 29.5, 29.6, 29.6, 31.7, 31.9, 34.7, 61.0, 135.6, 142.6, 162.7, 170.3; MS (calcd(found) [M⁺]: 409.67/409; Anal. calcd. for C₂₉H₄₃NO₃S: C, 70.36; H, 10.58; N, 3.42. Found: C, 70.12; H, 10.85; N, 3.50.

Ethyl (5-benzyl-2-methyl-1,3-thiazole-4-yl) acetate (4f)

Yellow low viscous oily; yield: 70%; IR (KBr, ν max cm⁻¹): 3072 (ar-C=H st), 3059 (ar-C-H st), 3028 (ar-C-H st), 2978 (CH₃ st as), 2920 (C-H st sy), 1737 (C=O st), 1546 (C=N st), 1454 (CH₂ δ), 1367 (CH₂ δ sy), 1247 (C-O st as), 1178 (C=S st), 1031 (C-O st sy), 704 (ar-C-H δ oop); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 1.25 (3H, t, J = 7.2 Hz, ethyl group-CH₂), 2.62 (3H, s, CH₃), 3.75 (2H, s, CH₂CO), 4.06 (2H, s, CCH₂), 4.15 (2H, q, J = 7.2 Hz, COOCH₂), 7.19-7.25 (3H, m, Bn ortho- and para-H), 7.28-7.32 (2H, m, Bn meta-H); ᵃ¹C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 14.2, 19.1, 32.3, 35.0, 61.0, 126.8, 128.3, 128.7, 128.7, 133.2, 134.9, 163.5, 170.3; MS (calcd(found) [M⁺]: 275.37/275; Anal. calcd. for C₂₀H₂₁NO₃S: C, 65.43; H, 6.22; N, 5.09. Found: C, 65.55; H, 6.45; N, 4.99.

Ethyl (5-[4-fluorobenzyl]-2-methyl-1,3-thiazole-4-yl) acetate (4g)

Yellow low viscous oily; yield: 87%; IR (KBr, ν max cm⁻¹): 3055 (ar-C=H st), 3035 (ar-C-H st), 2980 (CH₃ st as), 2924 (C-H st sy), 1548 (C=N st), 1508 (C-F st), 1369 (CH₂ δ sy), 1246 (C-O st as), 1176 (C=S st), 1033 (C-O st sy), 833 (ar-C-H δ oop); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 1.25 (3H, t, J = 7.1 Hz, ethyl group-CH₂), 2.62 (3H, s, CH₃), 3.73 (2H, s, CH₂CO), 4.03 (2H, s, CCH₂), 4.15 (2H, q, J = 7.1 Hz, COOCH₂), 6.95-7.01 (2H, m, F-Bn meta-H), 7.15-7.18 (2H, m, F-Bn ortho-H); ᵃ¹C-NMR (100 MHz, CDCl₃, MeSi, δ in ppm): 14.2, 19.1, 31.5, 35.0, 61.1, 115.3, 115.6, 129.8, 129.9, 133.6, 135.3, 144.1, 160.5, 163.6, 170.2; MS (calcd(found) [M⁺]: 293.36/293; Anal. calcd. for C₂₀H₁₉FNO₃S: C, 61.41; H, 5.50; N, 4.77. Found: C, 61.23; H, 5.78; N, 5.05.

Ethyl (5-[4-chlorobenzyl]-2-methyl-1,3-thiazole-4-yl) acetate (4h)

Yellow viscous oily; yield: 43%; IR (KBr, ν max cm⁻¹): 3032 (ar-C=H st), 2980 (CH₃ st as), 2924 (C-H st sy), 1732 (C=O st), 1556 (C=N st), 1435 (CH₂ δ), 1367 (CH₂ δ sy), 1249 (C-O st as), 1178 (C=S st), 1091 (C-Cl st), 1033 (C-O st sy), 837 (ar-C-H δ oop), 804 (ar-C-H δ oop); ¹H-NMR (400 MHz, CDCl₃, MeSi, δ in ppm): 1.25 (3H, t, J = 7.2 Hz, ethyl group-CH₂), 2.63 (3H, s, CH₃), 3.73 (2H, s, CH₂CO), 4.03 (2H, s,
CCH(C), 4.15 (2H, q, J = 7.2 Hz, COOCH2), 7.13-7.15 (2H, m, Cl-Bn ortho-H), 7.26-7.28 (2H, m, Cl-Bn meta-H); 13C-NMR (100 MHz, CDCl3, Me2Si, δ in ppm): 14.2, 18.9, 31.7, 34.8, 61.2, 128.8, 128.9, 129.7, 132.7, 133.3, 137.8, 143.7, 164.1, 170.1; MS (calcd/find) [M]+: 309.8/309 and 311; Anal. calcd. for C10H13ClNO2S: C, 58.15; H, 5.21; N, 4.52. Found: C, 58.39; H, 5.35; N, 4.43.

(5-Octyl-2-methyl-1,3-thiazole-4-yl) acetic acid (5a)
Yellowish crystal; yield: 77%; m.p.: 32-34°C; IR (KBr, ν max cm⁻¹): 3421 (COO-H st), 2953 (CH₃ st as), 2920 (C-H st as), 2852 (C-H st sy), 1722 (C=O st), 1564 (C=N st), 1465 (CH₂ δ), 1377 (CH₃ δ sy), 1346 (OC-Oh st), 1201 (C=S st), 705 (CH₃ γ); 1H-NMR (400 MHz, DMSO-d₆, Me2Si, δ in ppm): 0.85 (3H, t, J = 6.8 Hz, octyl group CH₃), 1.24 (10H, m, octyl group CH₂), 2.54 (3H, s, CCH₃), 2.67 (2H, t, J = 7.6 Hz, CCH₂), 3.56 (2H, s, C, CH₂), 12.39 (1H, bs, COOH); 13C-NMR (100 MHz, CDCl₃, Me2Si, δ in ppm): 11.1, 13.8, 24.4, 26.3, 28.2, 30.6, 31.0, 31.9, 34.1, 35.0, 142.2, 163.5, 172.4; MS (calcd/find) [M]+: 269.40/269; Anal. calcd. for C₁₁H₁₆ClNO₂S: C, 62.42; H, 8.61; N, 5.20. Found: C, 62.70; H, 8.85; N, 5.44.

(5-Decyl-2-methyl-1,3-thiazole-4-yl) acetic acid (5b)
Brownish crystal; yield: 82%; m.p.: 33-35°C; IR (KBr, ν max cm⁻¹): 3415 (COO-H st), 2940 (CH₃ st as), 2929 (C-H st as), 2853 (C-H st sy), 1728 (C=O st), 1564 (C=N st), 1465 (CH₂ δ), 1371 (CH₃ δ sy), 1334 (OC-Oh st), 1197 (C=S st), 723 (CH₃ γ); 1H-NMR (400 MHz, DMSO-d₆, Me2Si, δ in ppm): 0.85 (3H, t, J = 6.6 Hz, decyl group CH₃), 1.23 (14H, m, decyl group CH₂), 1.49-1.52 (2H, m, decyl group CH₂), 2.54 (3H, s, CCH₃), 2.67 (2H, t, J = 7.4 Hz, CCH₂), 3.56 (2H, s, C, CH₂), 12.31 (1H, bs, COOH); 13C-NMR (100 MHz, CDCl₃, Me2Si, δ in ppm): 11.4, 13.8, 24.0, 26.3, 28.2, 28.3, 30.6, 31.0, 31.9, 34.0, 35.4, 141.8, 164.0, 172.1; MS (calcd/find) [M]+: 279.46/279; Anal. calcd. for C₁₂H₂₀ClNO₂S: C, 64.60; H, 9.15; N, 4.71. Found: C, 64.37; H, 9.01; N, 4.45.

(5-Dodecyl-2-methyl-1,3-thiazole-4-yl) acetic acid (5c)
Yellowish crystal; yield: 85%; m.p.: 54-55°C; IR (KBr, ν max cm⁻¹): 3408 (COO-H st), 2937 (CH₂ st as), 2910 (C-H st as), 2846 (C-H st sy), 1722 (C=O st), 1564 (C=N st), 1465 (CH₂ δ), 1377 (CH₃ δ sy), 1344 (OC-Oh st), 1201 (C=S st), 723 (CH₃ γ); 1H-NMR (400 MHz, DMSO-d₆, Me2Si, δ in ppm): 0.85 (3H, t, J = 6.6 Hz, decyl group CH₃), 1.23 (18H, m, dodecyl group (CH₂)n), 1.48-1.52 (2H, m, dodecyl group CH₂), 2.54 (3H, s, CCH₃), 2.67 (2H, t, J = 7.4 Hz, CCH₂), 3.56 (2H, s, C, CH₂), 12.30 (1H, bs, COOH); 13C-NMR (100 MHz, CDCl₃, Me2Si, δ in ppm): 12.4, 15.1, 23.8, 26.4, 29.0, 29.6, 31.2, 31.8, 34.1, 135.0, 142.3, 163.5, 172.3; MS (calcd/find) [M]+: 381.62/381; Anal. calcd. for C₁₃H₂₄ClNO₂S: C, 69.24; H, 10.30; N, 3.67. Found: C, 69.51; H, 10.50; N, 3.42.

(5-Benzyl-2-methyl-1,3-thiazole-4-yl) acetic acid (5f)
Yellow crystal; yield: 34%; m.p.: 116-118°C; IR (KBr, ν max cm⁻¹): 3408 (COO-H st), 3070 (ar-C-H st), 3039 (ar-C-H st), 3024 (ar-C-H st), 2937 (CH₂ st as),
2904 (C-H str as), 1722 (C=O str), 1558 (C=N str), 1454 (CH, δ), 1379 (CH, δ sy), 1328 (OC-CH str), 1190 (C=S str), 754 (ar-C-H δ oop), 698 (ar-C-H δ oop); 1H-NMR (400 MHz, DMSO-d6, Me2Si, δ in ppm): 2.51 (3H, s, CH3), 3.69 (2H, s, CH2CO), 4.06 (2H, s, CH2C=O), 7.19-7.24 (3H, m, Br ortho and para-H), 7.27-7.31 (2H, m, Br meta-H), 12.3 (1H, bs, COOH); 13C-NMR (100 MHz, DMSO-d6, Me2Si, δ in ppm): 18.5, 31.3, 34.5, 126.4, 128.2, 128.8, 128.4, 133.4, 140.1, 144.5, 162.2, 171.5; MS (calcld/found) [M]+: 247.31/247; Anal. calcld. for C13H19NO3S: C, 63.13; H, 5.30; N, 5.66. Found: C, 62.88; H, 5.43; N, 5.36.

[5-(4-Fluorobenzyl)-2-methyl-1,3-thiazole-4-yl] acetic acid (5g)

Yellowish crystal; yield: 45%; m.p.: 124-126°C; IR (KBr, ν max cm⁻¹): 3410 (COO-H str), 3018 (ar-C-H str), 2933 (CH, str as), 2914 (C-H str as), 1714 (C=O str), 1558 (C-N str), 1506 (C-F str), 1435 (CH, δ), 1377 (CH, δ sy), 1203 (C=S str), 835 (ar-C-H δ oop), 673 (ar-C-H δ oop); 1H-NMR (400 MHz, DMSO-d6, Me2Si, δ in ppm): 2.52 (3H, s, CH3), 3.69 (2H, s, CH2CO), 4.06 (2H, s, CH2C=O), 7.09-7.13 (2H, m, F-Bn meta-H), 7.25-7.29 (2H, m, F-Bn ortho-H), 12.5 (1H, bs, COOH); 13C-NMR (100 MHz, DMSO-d6, Me2Si, δ in ppm): 18.6, 30.4, 34.5, 115.0, 115.2, 130.1, 130.1, 133.3, 136.0, 144.6, 159.6, 162.3, 171.5; MS (calcld/found) [M]+: 265.30/265; Anal. calcld. for C13H13FNO3S: C, 58.85; H, 4.56; N, 5.28. Found: C, 58.77; H, 4.58; N, 5.20.

[5-(4-Chlorobenzyl)-2-methyl-1,3-thiazole-4-yl] acetic acid (5h)

White crystal; yield: 74%; m.p.: 130-132°C; IR (KBr, ν max cm⁻¹): 3394 (COO-H str), 3049 (ar-C-H str), 3003 (ar-C-H str), 2937 (CH, str as), 2902 (C-H str as), 1722 (C=O str), 1556 (C-N str), 1433 (CH, δ), 1375 (CH, δ sy), 1207 (C=S str), 1087 (C-Cl str), 840 (ar-C-H δ oop), 810 (ar-C-H δ oop); 1H-NMR (400 MHz, DMSO-d6, Me2Si, δ in ppm): 2.52 (3H, s, CH3), 3.69 (2H, s, CH2CO), 4.07 (2H, s, CH2C=O), 7.25-7.27 (2H, m, Cl-Bn meta-H), 7.31-7.34 (2H, m, Cl-Bn ortho-H), 12.7 (1H, bs, COOH); 13C-NMR (100 MHz, DMSO-d6, Me2Si, δ in ppm): 18.5, 30.5, 34.5, 128.3, 128.3, 130.1, 131.0, 130.0, 144.7, 162.5, 171.5; MS (calcld/found) [M]+: 281.76/281 and 283; Anal. calcld. for C13H13ClNO3S: C, 55.42; H, 4.29; N, 4.97. Found: C, 55.54; H, 4.50; N, 4.60.

REFERENCES


