drugs (De et al., 2011). Another factor is the presence of hydroxyl groups (phenoxy) as active sides by donating radical H atoms to neutralize cancer-causing free radical species and form more stable phenoxy radicals (Nakamura et al., 2014). Therefore, this compound is best used as a precursor to synthesize anticancer compounds.

In recent years, one of the derivatives of hydroxycinnamic acid, especially caffeic acid, has been used as a precursor in the synthesis of anticancer compounds and other bioactivity, especially their esters derivatives. One of the most common esters derived from caffeic acid is chlorogenic acid. This compound has the potential to be an antioxidant, anti-inflammatory, inhibitor of apoptotic cell, and anticancer (Lee and Zhu, 2006; Morishita and Ohnishi, 2001; Liang and Kitts, 2016; Kim et al., 2019; Clifford et al., 2017). In addition, analog compounds that show interesting bioactivity are CAPE (Caffeic Acid Phenethyl Ester) or phenethyl caffeate esters which have bioactivity as antituberculosis, antioxidants, anti-inflammatory, and anticancer (Guzman, 2014; Wu et al., 2011; Zhang et al., 2014; Kuo et al., 2015; Chen et al., 2001). Although both of these compounds exhibit strong anticancer activity, the ester compound usually is less important to be a drug because this compound easily hydrolyzed before reaching treatment targets in the body (Son and Lewis, 2002). One way to overcome this problem is by converting ester compounds into an amide compounds for use as anticancer drugs (Firdaus et al., 2018).

One method used to convert acid groups into amide groups is through an indirect conversion method with four stages of synthesis, namely the acetylation, chlorination, amidation and deacetylation stages (Firdaus et al., 2018). This method has successfully synthesized amide derivatives from hydroxycinnamic acid such as phenethyl trans-3-(4-hydroxy-3-methoxyphenyl) acrylate, trans-3-(4-hydroxy-3-methoxyphenyl)-N-phenethyl acrylamide, N-(o-tolyl) caffe amide, and N-(o-tolyl) p-coumaramide which have bioactivity to murine leukemia P-388 cells with IC_{50} values of 10.79 µg/mL, 29.14 µg/mL, 0.91 µg/mL and 16.97 µg/mL, respectively (Firdaus et al., 2019). This method has been used in this research to obtain compound 1 and compound 3. This compound is a new amide derived from hydroxycinnamic acid because using amine piperidine which has the potential to be used as an anticancer by testing the activity of both compounds by the Brine Shrimp Lethality Test method because this method shows a correlation with other in vitro cytotoxic test methods (Carballo et al., 2002).

Experimental

Material and Methods

The materials used in this study were 3-(3,4-dihydroxyphenyl)acrylic acid, TLC plate, acetic anhydride, toluene p.a., distilled water, chloroform p.a., ethyl acetate p.a., n-hexane p.a., acetone p.a., Whatman 42 filter paper, thionyl chloride pa, piperidine pa, dichloromethane pa, pyridine pa, triethylamine pa, and materials used in BSLT testing.

The Equipment used in this study were three-neck round bottom flasks, condensers, thermometers, magnetic stirrers, melting point gauges, UV lamps, rotary evaporators, FTIR spectrophotometers, and glassware commonly used in laboratories.

Procedures

Acetylation (Synthesis of Compound 1b)

Precursor, 3-(3,4-dihydroxyphenyl) acrylic acid as much as 1.00 g (6.00 mmol) was put into the round bottom flask, then added 1.80 mL pyridine and 1.60 mL (0.017 mmol) acetic anhydride. The reaction mixture is stirred using a magnetic stirrer for 4 hours at room temperature. Next, the reaction mixture was added ± 50 mL cold distilled water while stirring. The formed white precipitate is filtered, washed with distilled water, and dried in a desiccator to obtain a dry white solid. The solid is recrystallized from hexane-ethyl acetate solvent and filtered to obtain white amorphous solid. Furthermore, its purity was tested through TLC analysis with three kinds of eluent systems and then testing its melting point. The pure compounds obtained were analyzed by FTIR spectrometer. The reaction mechanism for the formation of compound 1b can be seen in Figure 1.

Chlorination

Product of acetylation step, 3-(3,4-diacetoxyphenyl) acrylic acid compound was put into a three-neck round bottom flask and dissolved using 20 mL toluene and 1 mL (7,2 mmol) of thionyl chloride was added. The reaction mixture is refluxed for 4 hours. After reflux, the reaction mixture is cooled to room temperature and evaporated, then proceed immediately to the next reaction step and the reaction mechanism can be seen in Figure 2.

Amidation (Synthesis of Compound 3c)

The product of the chlorination step was added to mixture 0,8 mL (4,18 mmol) piperidine, 1 mL pyridine (0,72 mmol), and 1 mL (2,46 mmol) triethylamine in 20 mL dichloromethane (CH_2Cl_2). The mixture is stirred for 1

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hour. Next, it was washed with 3% HCl (3x20 mL) then with saturated NH_4Cl (3x20 mL), dried using anhydrous Na_2SO_4, and the solvent is evaporated to obtain a yellowish solid. After recrystallization from an acetone-hexane solvent, purity of crystalline is tested through TLC analysis with 3 kinds of eluent systems and then measuring its melting point. The pure compounds obtained were analyzed by the FTIR spectrometer and the mechanism reaction in Figure 3.

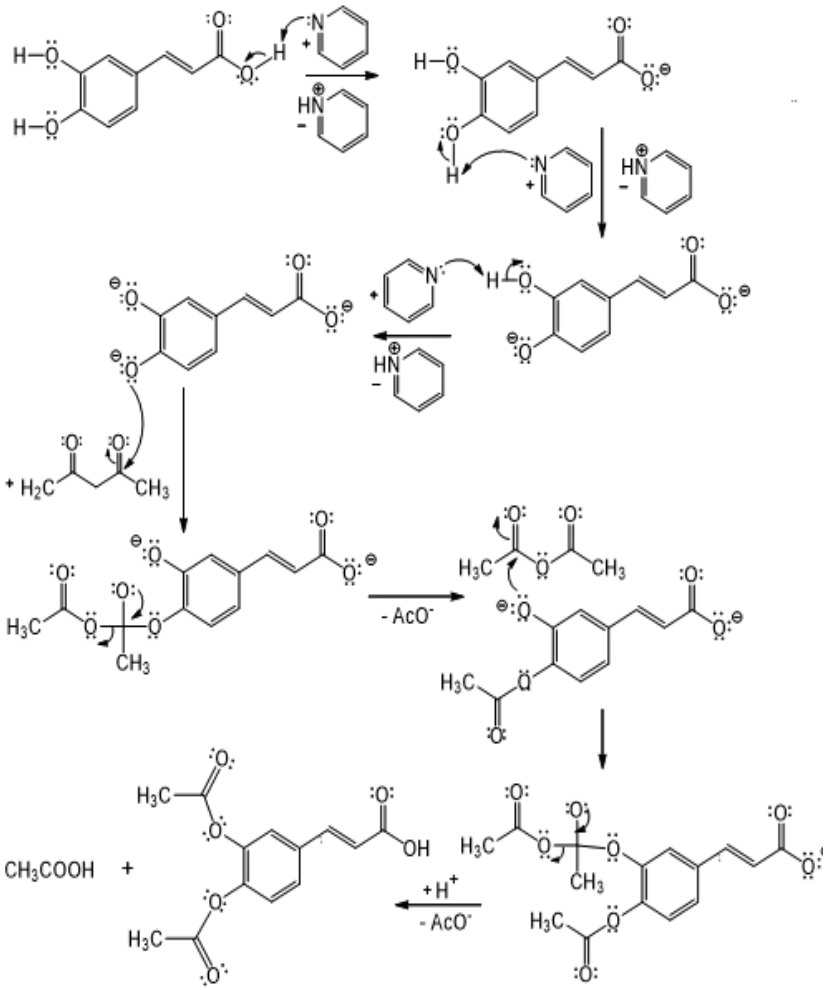

Figure 1. Reaction Mechanism of compound 1b.

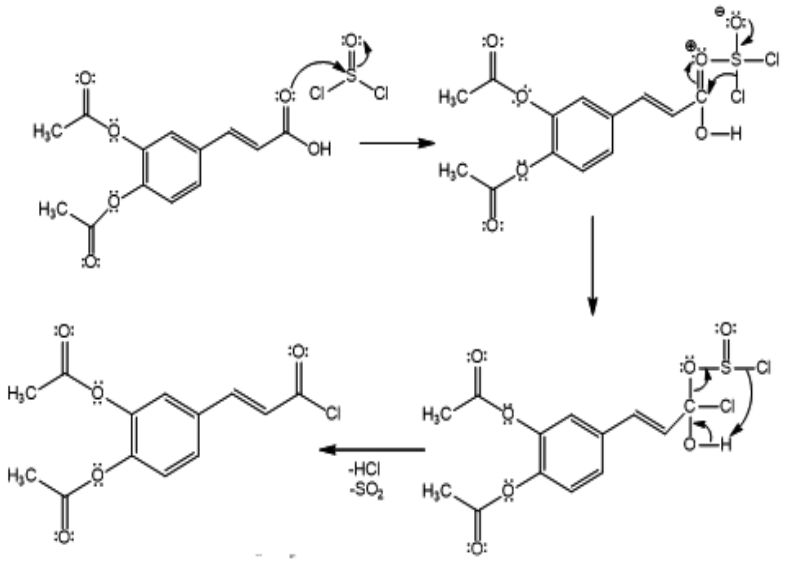

Figure 2. Chlorination reaction mechanism.

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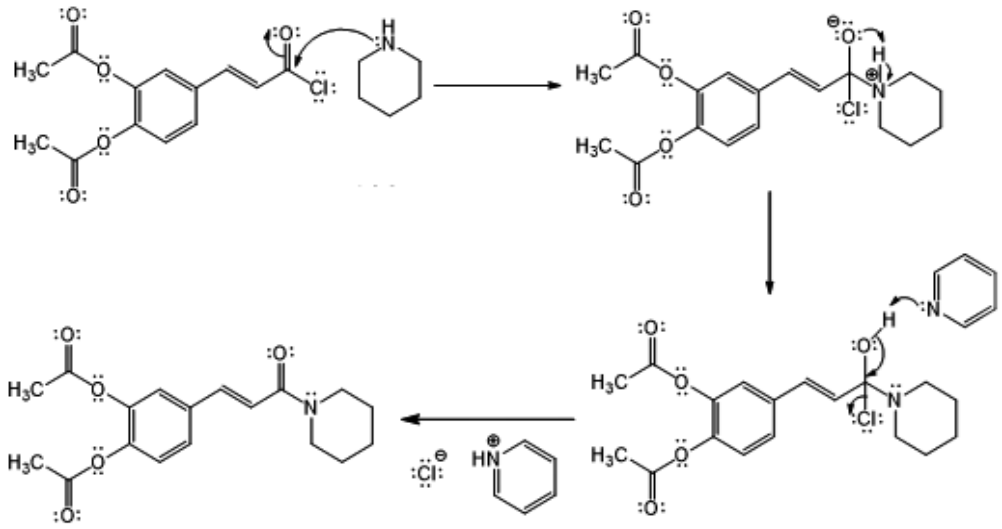

Figure 3. The mechanism of the amidation reaction of compound 3c.

Toxicity Test

The precursor (a), Compound 1b and Compound 3c were then tested for their activity against *A. salina* shrimp larvae by the BSLT method.

Preparation of A. Salina Leach Larvae. Shrimp eggs *A. Salina* Leach, 15 mg put in a container filled with seawater and aerated under a 40-60 watt incandescent lamp. The lights are turned on for 48 hours until the eggs of *A. salina* hatch into larvae.

Sample Preparation. Preparation of the sample solution is done by making an initial concentration of 2000 µg/ mL as mother liquor by dissolving 20 mg of sample dissolved with 200 µL DMSO, then diluted with 9800 µL of seawater until the total volume becomes 10.000 µL. Dilution and measurement were carried out in triplo in a vial tube with a series of concentrations of 1000, 100, 10, 1, and 0.1 µg/mL.

Toxicity Test. 10 Larvae of A. Salina L. that had hatched were put into vial tubes at each concentration and incubated for 24 hours. After 24 hours, the number of A. Salina Leach dead and alive on the vial tube was calculated to obtain the LC_{50} value.

Result and Discussion

Synthesis of Compound 1b

Synthesis of compound 1b aims to reduce the polarity of 3-(3,4-dihydroxyphenyl)acrylic acid compounds through the conversion of the hydroxyl phenolic group to acetoxy. This is done because nonpolar compounds more easily pass cell membranes composed of lipids (Shargel et al., 2012), so that it is expected to increase its activity as an anticancer. The reaction product is a white amorphous solid with a melting point of 181-183 °C and a yield of 85.68%.

The purity test was carried out using TLC analysis with three different eluent systems (a) acetone/ *n*-hexane 6:4, (b) acetone/ DCM 2:8, (c) ethyl acetate/ *n*-hexane 7:3. The success of each this step in the synthesis reaction has also been analyzed using an FTIR spectrometer. In the FTIR spectrum of compound 1b shown in Figure 4, there is no apparent absorption band of the phenolic OH group, which usually appears at wave number 3435.22 cm^{-1}, but a new sharp and strong absorption band appears at wave number 1764.87 cm^{-1}. The new absorption band indicates the presence of a carbonyl group (C=O) ester in the compound. The absorption band is also supported by the absorption band at wavenumbers 1213.23 cm^{-1} and 1114.86 cm^{-1} from C-O. The absorption bands at 2835.36–2981.95 cm^{-1} and 1431.18 and 1373.32 cm^{-1} originated from the CH_3 group (Firdaus et al., 2019). All absorption bands correspond to the structure of compound 1b shown in Figure 6.

Synthesis of Compound 3c

Before the amidation reaction is being carried out, the reactivity of the carbonyl group is carried out through the chlorination reaction. The reaction lasted for 4 hours with a reflux temperature of 80 °C. Then immediately proceed with the in-situ reaction in the middle so that the reaction is free from water vapor that can react with thionyl chloride which will again produce compound 1b (Firdaus et al., 2018).

Product of compound 3c is a white crystal with a melting point of 139-141°C and a yield of 39.45%. Crystal purity was tested through TLC analysis with three different eluent systems (a) ethyl acetate/chloroform 1:9, (b) ethyl

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acetate/n-hexane 6:4, (c) acetone/*n*-hexane 5:5. FTIR spectrum Compound 3c in Figure 5 does not show the OH carboxylate absorption band which usually widens in the area of 2500-3000 cm^{-1}. The sharp and strong absorption band at wavenumber 1647.21 cm^{-1} originates from the carbonyl group (C=O amide), 1764.87 cm^{-1} (C=O ester), 3032.10 cm^{-1} (C-H unsat.), 1602.85 dan 1508.41 cm^{-1} (C=C Ar), 2943.37 dan 2858.51 cm^{-1} (C-H sat.), 1369.46 dan 1444.68 cm^{-1} (methyl) (Firdaus et al., 2019). This explanation is in accordance with the structure of compound 3c in Figure 6.

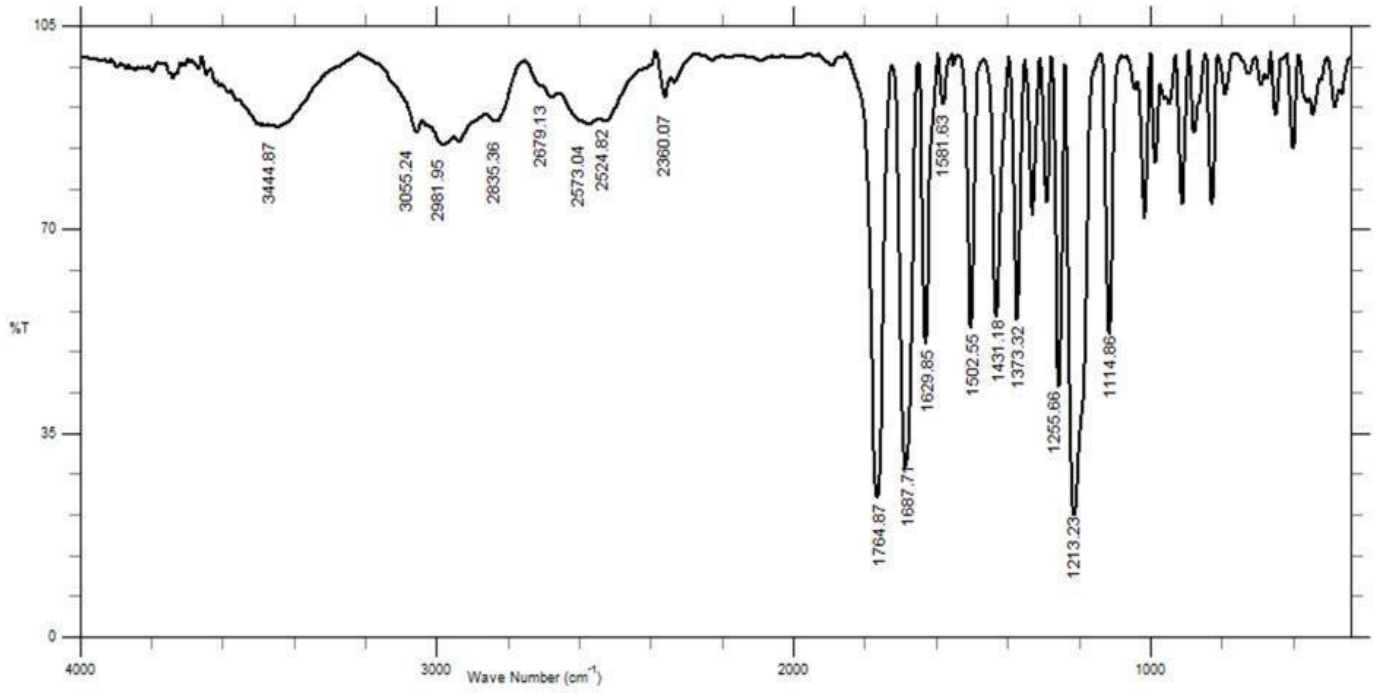

Figure 4. FTIR Spectrum of compound 1b.

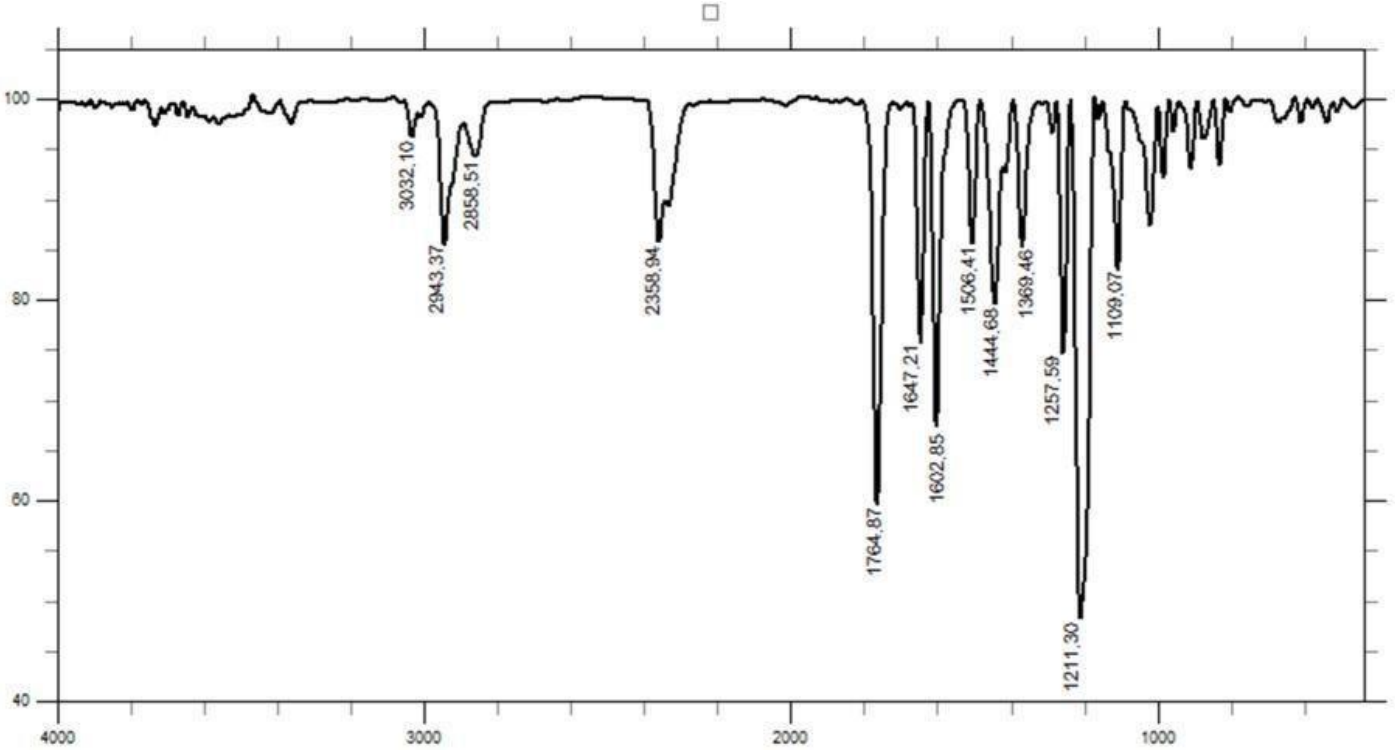

Figure 5. FTIR spectrum of compound 3c.

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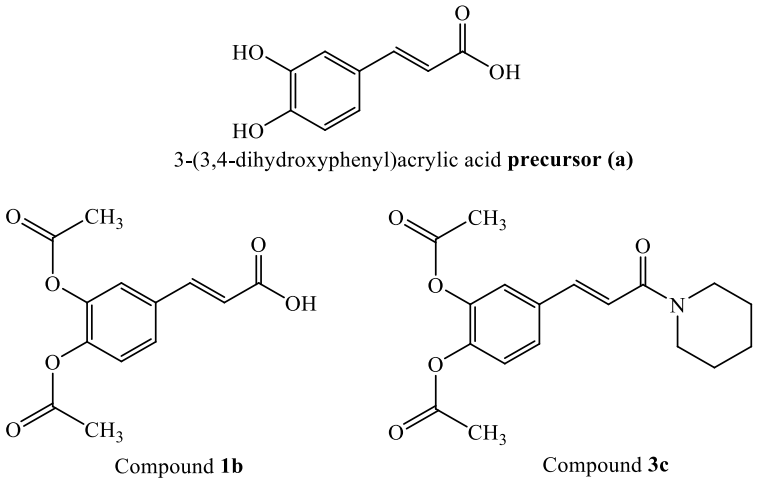

Figure 6. Structure of precursor (a), compound 1b and compound 3c.

Toxicity Test

The toxicity test with the BSLT method is the initial screening of active compounds. Toxicity test results for compound 1b, compound 3c, and precursor (a) with the BSLT method can be seen in Table 1. This test is carried out with three replications at each concentration of 1000, 100, 10, 1, and 0,1 µg/mL. Table 1 shows that variations in the concentration of compounds affect activity. The lower the concentration of the compound the smaller the effect of death on *A. salina*.

Table 1. Toxicity results of compound 1b, compound 3c, and precorsor (a) testing BSLT methods

Sample	Consentration (µg/mL)	Log consentration	% Mortality	Probit	LC_{50}
Compound 1b	1000	3	87	6,13	8,919
	100	2	60	5,25	
	10	1	50	5,00	
	1	0	33	4,56	
	0,1	-1	20	4,10	
Compound 3c	1000	3	67	5,44	84,511
	100	2	47	4,92	
	10	1	40	4,75	
	1	0	23	4,26	
	0,1	-1	13	3,87	
Precursor (a)	1000	3	87	6,13	46,504
	100	2	53	5,08	
	10	1	27	4,39	
	1	0	13	3,87	
	0,1	-1	7	3,52	

A compound is considered toxic if it has an LC_{50} value <100 µg/mL (Meyer et al., 1982). analysis results from the toxicity testing of each compound 1b, compound 3c, and Precursor (a) obtained LC_{50} value = 8,919 µg/mL, 84,511 µg/mL, and 46,504 µg/mL, respectively. These results also show that all compounds are toxic, and the toxicity of compound 1b is much stronger compared to 3-(3,4-dihydroxyphenyl)acrylic acid as a precursor. This corresponds to the decrease in polarity of the precursor compound after undergoing acetylation.

Conclusion

The compounds 1b and compound 3c can be synthesized through the acetylation, chlorination, and amidation reactions. Both of these compounds are toxic to *A. Salina* with LC_{50} values of 8.919 µg/mL and 84.511 µg/mL, and compound 1b is far more active than its Precursor (a) compound.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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