Research Article

Syntheses and In Vitro Biological Activity of Some Derivatives of C-9154 Antibiotic

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In our continued attempts at designing new antibiotics based on the structure of the C-9154 antibiotic, to simultaneously improve activity and lower toxicity, an analogue to the C-9154 antibiotic and six derivatives of this analogue were synthesized. The approach was to significantly reduce the polarity of the synthesized analogue in the derivatives to achieve increased permeability across cell membranes by conversion of the highly polar carboxylic group to an ester functional group. The compounds were synthesized using a two-step reaction which involved an additional reaction between benzyl amine and maleic anhydride and then conversion of the terminal carboxylic acid functional group to an ester functional group using a thionyl chloride mediated esterification reaction. The compounds were fully characterized using Infrared, GC-MS, and 1D and 2D NMR experiments. The in vitro biological activity of the compounds showed that the derivatives were more active than the analogues as was anticipated with minimum inhibitory concentration in the range 0.625–5 μg/mL. The analogue had minimum inhibitory concentration in the range 2.5–10 μg/mL. These values are significantly better than that obtained for the original C-9154 antibiotic which had activity in the range 10–>100 μg/mL.

1. Introduction

In 1889, Vuillemin, a French bacteriologist, suggested using the word “antiobiosis”, meaning “against life,” to describe the group of drugs that had action against microorganisms [1]. Selman Waksman, an American microbiologist and the discoverer of streptomycin, later changed this term to antibiotic in 1942 [2]. The term “antibiotic” as coined by Selman Waksman is used to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution (low concentration). This definition excluded substances that kill bacteria but are not produced by microorganisms such as gastric juices and hydrogen peroxide [3].

Antibiotics today, with advances in medicinal chemistry, are semisynthetic modifications of various natural compounds [4]. These include, for example, the Beta-lactam antibiotics, which include the penicillins, the cephalosporins, and the carbapenems. Some antibiotic compounds are still isolated from living organisms like the aminoglycosides, whereas other antibiotics like the sulfonamides, the quinolones, and the oxazolidinones are produced solely by chemical synthesis [4]. This implies that synthesis of antibiotic compounds plays an important and vital role in the fight against disease-causing organisms.

In light of emerging resistance to antibiotic drugs, it has become imperative to synthesize new drugs with improved activity to combat various illnesses that have developed
strain NR-7GGI. This Streptomyces was isolated from the whole agar culture of totics, a new antibiotic with a broad antibacterial spectrum provides a way to assist man in this great battle.

During studies on screening for antibiotics that showed activity against bacteria resistant to various known antibiotics, a new antibiotic with a broad antibacterial spectrum was isolated from the whole agar culture of Streptomyces strain NR-7GGI. This Streptomyces species was called Streptomyces kurssanovii and the isolated antibiotic referred to as fumaramidmycin [5].

Another researcher working independently and slightly earlier than the previous researcher also found that a new species of Streptomyces, Streptomyces ishigakiensis produced a novel antibiotic which was named C-9154 [6].

The two new antibiotics were found from structural studies to be the same compound [6, 7]. This new antibiotic was found to inhibit the growths of various microorganisms at concentrations between 10–100 μg/mL [6]. It was also shown to be active against certain strains that were resistant to ampicillin, cephalosporin, chloramphenicol, gentamicin, kanamycin, macrolides, neomycin, sulfonamides, streptomycin, and tetracyclines at concentrations between 1.25–2.5 g/kg [5]. Its intraperitoneal LD50 value in mice was found to be between 75–100 mg/kg while its oral LD50 was found to be 1.25–2.5 g/kg [5].

The structure of C-9154 (Figure 1) was determined using elemental analysis procedures, IR and UV measurements, and NMR and GC-MS experiments [6, 7].

Analysis of the structure of C-9154 antibiotic showed that it was made up of two fragments, namely, phenylacetic acid and fumaramide [6, 7].

Analogues of the C-9154 antibiotic have been previously synthesized [7–10]. These analogues were shown to have antibacterial activity against Staphylococcus aureus and Escherichia coli ranging from 15 μg/mL [10] to 4000 μg/mL [9].

2. Materials and Method

Infrared spectra were determined using a PerkinElmer Spectrum 100 series Universal ATR. 1D and 2D NMR experiments were carried out using a Bruker AV400 MHz NMR. The gc-ms spectra were taken using an Agilent Technologies 6890 series GC coupled with an Agilent 5973 Mass Selective detector. All chemicals and reagents unless otherwise stated were obtained from Merck Chemicals, Germany.

2.1. Synthesis of C-9154 Analogue. The analogue was synthesized using the following procedure. N-benzyl fumaramic acid was prepared according to reaction scheme 1 (Figure 2).

Benzyamine (1.0 g, 9.3 mmol) in toluene (5 mL) was transferred to a round bottom flask containing maleic anhydride (1.1 g, 11.2 mmol) in toluene (5 mL). The mixture was refluxed with stirring for 3 hrs. The reaction was allowed to cool to room temperature and filtered using a Buchner funnel. The residue was washed using ethyl acetate and dried to afford a shiny white solid labeled IA/12/1 (1.76 g, 91.7%). TLC was used to determine that the reaction had gone to completion. IR, 1D and 2D NMR, and GC-MS were used to identify the compound as the desired N-benzyl fumaramic acid. Its melting point was determined to be 125–127°C.

2.2. Synthesis of Derivatives of C-9154 Analogue. This analogue was then converted to its ester derivatives using methanol, ethanol, n-propanol, isopropanol, n-butanol, and 2-butanol, respectively, using a thionyl chloride (SOCl2) mediated esterification process, according to reaction scheme 2 (Figure 3).

Six portions of IA/12/1 (0.5 g, 2.4 mmol) were individually transferred to six round-bottomed flasks in ice baths. Thionyl chloride (2 mL) was added in drops with constant stirring. The excess thionyl chloride was removed using a rotary evaporator. Methanol (10 mL), ethanol (10 mL), n-propanol (10 mL), isopropanol (10 mL), n-butanol (10 mL), or 2-butanol (10 mL) were added to each flask and the mixtures refluxed. At the end of the reactions as determined by TLC, saturated sodium carbonate (Na2CO3) solution was added to each flask until the solutions just turned alkaline as indicated by litmus paper. Water (20 mL) was added to each flask and the mixtures were individually transferred to different separatory funnels. The mixtures in the different separatory funnels were extracted using dichloromethane (2 × 25 mL). The combined dichloromethane fractions were then individually dried using anhydrous sodium sulphate (Na2SO4) and concentrated to give clear oils. These were chromatographed on silica gel columns and eluted using ethyl acetate : hexane (3:7), to give the desired esters which crystallized on standing. All the esters were obtained as crystalline solids (Table 1) except the methyl and butyl esters.
The media were prepared according to the manufacturer’s instructions, sterilized at 121°C, and used for the bacteria and fungi, respectively. England) and Sabouraud agar, (Oxoid, England) were the media used for the bacteria and fungi, respectively. Mueller Hinton, (Oxoid, England) was the initial concentration used to check the antimicrobial activities of the compounds. The sterilized media were seeded with a standard inoculum (0.1 mL) of the test microorganisms. This was spread evenly over the surface of the plate by using a sterile swab. The plates were dried at 37°C for 30 minutes. Using a standard cork-borer of 6 mm in diameter, a well was cut at the centre of each seeded plate. 0.1 mL of the compounds was then introduced into the well. The plates were then incubated at 37°C for 24 hrs for the bacteria and 30°C for 48 hrs for the fungi, after which the plates were observed for zones of inhibition of growth. The zones were measured using a pair of dividers and a ruler and the result recorded in millimeters.

The activity of the compounds was compared against two standard drugs; Sparfloxacin (antibacterial) and Fluconazole (antifungal).

2.5. Minimum Inhibitory Concentration. The minimum inhibitory concentrations (Table 3) of the compounds were carried out using broth dilution method. Mueller Hinton, (Oxoid, England) and Sabouraud dextrose broth, (Oxoid, England) were prepared and 10 mL was dispensed into test tubes and the broths were sterilized at 121°C for 15 minutes, the broths were allowed to cool.

McFarland’s turbidity scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared and the test microorganisms were inoculated and incubated at 37°C for 6 hrs. Dilution of the test microorganisms was done continuously in the normal saline until the turbidity matched that of the McFarland’s scale by visual comparison. At that point the test microbe was at a concentration of about 1.5×10⁸ CFU/mL. Twofold serial dilutions of the compounds in the broth were made to obtain the different concentrations of the compounds in the broth. Having obtained the different concentrations, 0.1 mL of the standard inoculum of the test microorganisms in the normal saline was then inoculated into the different concentrations, and then incubated at 37°C for 24 hrs for the bacteria and 30°C for 48 hrs for the fungi, after which each test tube was observed for turbidity (growth). The MIC was the test tube with the lowest concentration of the compounds which showed no turbidity.

2.6. Minimum Bactericidal/Fungicidal Concentration. MBC (Table 3) was carried out to check whether test microorganisms were killed or only their growths were inhibited. Mueller Hinton, (Oxoid, England) and Sabouraud dextrose agar, (Oxoid, England) were prepared, sterilized, and poured into sterile Petri dishes. These were allowed to cool and solidify. The content of the MIC in the serial dilution was then subcultured onto the prepared media. These were then incubated at 37°C for 24 hrs for the bacteria and 30°C for 48 hrs for the fungi after which each plate was observed for colony growth. The MBC/MFC was the plate with lowest concentration of the compounds without colony growth.

3. Results and Discussion

A total of seven compounds were synthesized and fully characterized using 1D and 2D NMR experiments, infrared
Table 1: Synthesized C-9154 analogue and its derivatives.

| Sample code | Type                  | Yield (mg) | Melting point (°C) | Physical State               |
|-------------|-----------------------|------------|--------------------|------------------------------|
| IA/12/1     | C-9154 analogue       | 91.7%      | 125–127            | Shiny white crystalline solid |
| IA/27/1/B   | Methyl ester          | 140,       | 125,               | Colourless oil               |
|             | (0.64 mmol)           |            |                    |                              |
| IA/28/1/B   | Ethyl ester           | 105,       | 79                 | Light yellow crystalline solid|
|             | (0.45 mmol)           |            |                    |                              |
| IA/29/1/B   | n-propyl ester        | 170,       | 40                 | White crystalline solid      |
|             | (0.69 mmol)           |            |                    |                              |
| IA/30/1/B   | Isopropyl ester       | 185,       | 67                 | White crystalline solid      |
|             | (0.75 mmol)           |            |                    |                              |
| IA/31/1/B   | n-butyl ester         | 100,       | not determined     | Colourless oil               |
|             | (0.38 mmol)           |            |                    |                              |
| IA/32/1/B   | 2-butyl ester         | 125,       | not determined     | Colourless oil               |
|             | (0.48 mmol)           |            |                    |                              |

*Synthesized for the first time. Confirmed from available data and on Scifinder.

Table 2: Zones of inhibition (mm) of the analogue and derivatives.

|                 | IA/12/1 (10 μg/mL) | IA/27/1/B (5 μg/mL) | IA/28/1/B (5 μg/mL) | IA/29/1/B (5 μg/mL) | IA/30/1/B (5 μg/mL) | IA/31/1/B (5 μg/mL) | IA/32/1/B (5 μg/mL) | DMSO (20 μg/mL) | Sparfloxacin (20 μg/mL) | Fluconazole (50 μg/mL) |
|----------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|----------------|------------------------|----------------------|
| MRSA           | 27                 | 32                  | 33                  | 30                  | 30                  | 31                  | 31                  | 0              | 22                     | 0                    |
| S. aureus      | 26                 | 30                  | 29                  | 32                  | 32                  | 30                  | 30                  | 0              | 24                     | 0                    |
| S. pyogenes    | 21                 | 31                  | 32                  | 27                  | 30                  | 30                  | 30                  | 0              | 24                     | 0                    |
| B. subtilis    | 33                 | 37                  | 38                  | 34                  | 34                  | 31                  | 29                  | 0              | 30                     | 0                    |
| C. ulcerans    | 24                 | 0                   | 30                  | 32                  | 30                  | 0                   | 0                   | 0              | 0                      | 0                    |
| E. coli        | 27                 | 27                  | 29                  | 26                  | 24                  | 26                  | 24                  | 0              | 27                     | 0                    |
| P. mirabilis   | 27                 | 24                  | 27                  | 25                  | 27                  | 24                  | 25                  | 0              | 22                     | 0                    |
| P. aeruginosa  | 0                  | 0                   | 0                   | 0                   | 0                   | 0                   | 0                   | 20             | 0                      | 0                    |
| S. typhii      | 29                 | 27                  | 26                  | 24                  | 27                  | 24                  | 27                  | 0              | 21                     | 0                    |
| S. dysenteriae | 31                 | 29                  | 26                  | 22                  | 27                  | 24                  | 25                  | 0              | 27                     | 0                    |
| K. pneumoniae  | 30                 | 27                  | 25                  | 26                  | 24                  | 26                  | 25                  | 0              | 25                     | 0                    |
| C. albicans    | 26                 | 22                  | 23                  | 20                  | 20                  | 22                  | 21                  | 0              | 0                      | 24                   |
| A. niger       | 21                 | 0                   | 0                   | 0                   | 0                   | 0                   | 0                   | 0              | 0                      | 0                    |
| T. rubrum      | 0                  | 0                   | 0                   | 0                   | 0                   | 0                   | 0                   | 20             | 0                      | 0                    |

spectrophotometry, and gas chromatography-mass spectrometry.

The analogue was synthesized by reaction between benzylamine and maleic anhydride to get the desired fumaramic acid. This analogue was then converted to its methyl, ethyl, n-propyl, isopropyl, n-butyl, and 2-butyl esters using a modified method of the Fischer-Speier esterification.

The results are presented below.

IA/12/1 (N-benzylamino fumaramic acid). Shiny white needle-like solid; melting point, 125–127°C.

**13C-NMR (400 MHz, CDCl3)**: 165.7 (C11), 165.2 (C8), 137.9 (C1), 132.4 (C10), 131.5 (C9), 128.4 (C2 and C6), 127.6 (C3 and C5), 127.2 (C4), 42.6 (C7).

**1H-NMR (400 MHz, CDCl3)**: δ 3.65 (3H, s, H-12), 4.40 (2H, d, J = 5.88 Hz, H-7), 6.27 (1H, d, J = 12.41 Hz, H-10), 6.45 (1H, d, J = 12.41 Hz, H-9), 7.26–7.37 (5H, H-2, H-3, H-4, H-5 and H-6), 9.42 (1H, s, 11-OH), 14.57 (1H, s, 7-NH). EI-MS: m/z 187 [{[m – H]+, 100%}. IR<sub>v</sub>max (neat) cm<sup>−1</sup>: 3247.81 (N–H), 3064.54 (O–H), 2947.47 (C–H), 1696.92, 1629.53 (C=O).

IA/27/1/B (Methyl N-benzylamino fumaramate). Colourless oil.

**13C-NMR (400 MHz, CDCl3)**: 166.6 (C8), 164.4 (C11), 137.9 (C1), 135.9 (C9), 126.0 (C10), 128.5 (C2 and C6), 127.8 (C3 and C5), 127.3 (C4), 52.2 (C12), 43.4 (C7).

**1H-NMR (400 MHz, CDCl3)**: δ 3.65 (3H, s, H-12), 4.40 (2H, d, J = 5.80 Hz, H-7), 6.02 (1H, d, J = 12.53 Hz, H-10), 6.23 (1H, d, J = 12.53 Hz, H-9), 7.19–7.29 (5H, H-2, H-3, H-4, H-5 and H-6), 8.31 (1H, s, 7-NH). EI-MS: m/z 219 {[M]+, 25%}. IR<sub>v</sub>max (neat) cm<sup>−1</sup>: 3284.69 (N–H), 2950.59 (C=H), ~1700.00, 1634.63 (C=O).

IA/28/1/B (Ethyl N-benzylamino fumaramate). Light yellow crystalline solid; melting point, 79°C.
Table 3: Minimum Inhibitory Concentration (above) and Minimum Bactericidal/Fungicidal Concentration (below) of the analogue and
derivatives (μg/mL).

|             | IA/12/1 | IA/27/1/B | IA/28/1/B | IA/29/1/B | IA/30/1/B | IA/31/1/B | IA/32/1/B | DMSO | Sparfloxacin | Fluconazole |
|-------------|---------|-----------|-----------|-----------|-----------|-----------|-----------|------|--------------|-------------|
| MRSA        | 2.5     | 0.625     | 0.625     | 0.625     | 0.625     | 0.625     | 0.625     | ND   | 10           | ND          |
|             | 5       | 2.5       | 2.5       | 5         | 2.5       | 2.5       | 5         | ND   | ND           | ND          |
| S. aureus   | 2.5     | 0.625     | 1.25      | 0.625     | 0.625     | 0.625     | 0.625     | ND   | 10           | ND          |
|             | 5       | 5         | 5         | 2.5       | 5         | 2.5       | 5         | ND   | ND           | ND          |
| S. pyogenes | 2.5     | 0.625     | 1.25      | ND        | 1.25      | 0.625     | 0.625     | ND   | 10           | ND          |
|             | 10      | 2.5       | 2.5       | ND        | 5         | 5         | 2.5       | ND   | ND           | ND          |
| B. subtilis | 1.25    | 0.625     | 0.625     | ND        | 0.625     | 0.625     | 1.25      | ND   | 10           | ND          |
|             | 5       | 1.25      | 1.25      | 2.5       | 2.5       | 2.5       | 5         | ND   | 5            | ND          |
| C. ulcerans | 2.5     | ND        | 0.625     | 0.625     | 0.625     | ND        | ND        | ND   | ND           | ND          |
|             | 10      | ND        | 2.5       | ND        | 5         | ND        | ND        | ND   | ND           | ND          |
| E. coli     | 2.5     | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | ND   | 10           | ND          |
|             | 5       | 5         | 5         | 5         | 5         | 5         | 5         | ND   | ND           | ND          |
| P. mirabilis | 2.5   | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | ND   | 10           | ND          |
|             | 5       | 5         | 5         | 5         | 5         | 5         | 5         | ND   | ND           | ND          |
| P. aeruginosa | ND   | ND        | ND        | ND        | ND        | ND        | ND        | ND   | ND           | ND          |
| S. typhi    | 1.25    | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | ND   | 10           | ND          |
|             | 5       | 5         | 5         | 5         | 5         | 5         | 5         | ND   | ND           | ND          |
| S. dysenteriae | 1.25 | 1.25     | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | ND   | 5            | ND          |
|             | 5       | 5         | 5         | 5         | 5         | 5         | 5         | ND   | ND           | ND          |
| K. pneumoniae | 1.25 | 1.25     | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | ND   | 5            | ND          |
|             | 5       | 5         | 5         | 5         | 5         | 5         | 5         | ND   | ND           | ND          |
| C. albicans | 2.5     | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | 1.25      | ND   | ND           | 25          |
|             | 5       | 5         | 5         | 5         | 5         | 5         | 5         | ND   | ND           | ND          |
| A. nigre    | 2.5     | ND        | ND        | ND        | ND        | ND        | ND        | ND   | ND           | 25          |
|             | 10      | ND        | ND        | ND        | ND        | ND        | ND        | ND   | ND           | ND          |
| T. rubrum   | ND      | ND        | ND        | ND        | ND        | ND        | ND        | ND   | ND           | 25          |

Upper values are MIC and lower values are MBC or MFC as the case may be. ND: Not Determined.

IA/29/1/B (n-propyl N-benzylamino furamate). White crystalline solid; melting point, 40°C.

13C-NMR (400 MHz, CDCl3). 166.3 (C8), 164.0 (C11), 137.9 (C1), 138.1 (C9), 125.6 (C10), 128.6 (C2 and C6), 127.8 (C3 and C5), 127.4 (C4), 67.2 (C7), 43.7 (C12), 21.8 (C13), 10.3 (C14).

H-NMR (400 MHz, CDCl3) δ 8.75 (1H, d, J = 12.77 Hz, H-10), 6.23 (1H, d, J = 12.93 Hz, H-9), 7.18–7.27 (5H, H-2, H-3, H-4, H-5 and H-6), 8.46 (1H, s, 7-NH). EI-MS: m/z 106 [{m + H}]+, 100%. IRmax (neat) cm⁻¹: 3320.01 (N-H), 2989.28 (C-H), 1706.65, 1664.23 (C=O).

IA/30/1/B (Isopropyl N-benzylamino furamate). White crystalline solid; melting point, 67°C.

13C-NMR (400 MHz, CDCl3). 165.8 (C8), 164.0 (C11), 137.9 (C1), 138.3 (C9), 126.0 (C10), 128.6 (C2 and C6), 127.8 (C3 and C5), 127.4 (C4), 69.5 (C12), 43.7 (C7), 21.7 (C13 and C14).

1H-NMR (400 MHz, CDCl3) δ 1.27 (6H, d, J = 6.52 Hz, H-3 and H-5 and H-6), 8.75 (1H, s, 7-NH). EI-MS: m/z 106 [{m + H}]+, 100%. IRmax (neat) cm⁻¹: 3293.92 (N-H), 2931.87 (C-H), ~1700.00, 1650.74 (C=O).

IA/31/1/B (n-butyl N-benzylamino furamate). Colourless oil.

13C-NMR (400 MHz, CDCl3). 166.3 (C8), 164.3 (C11), 138.0 (C1), 136.2 (C9), 126.2 (C10), 128.5 (C2 and C6), 127.7 (C3 and C5), 127.3 (C4), 65.2 (C12), 43.4 (C7), 30.4 (C13), 19.0 (C14), 13.6 (C15).

1H-NMR (400 MHz, CDCl3) δ 0.80 (3H, t, J = 7.34 Hz, H-14), 1.60 (2H, m, H-13), 4.03 (2H, t, J = 7.34 Hz, H-12), 4.43 (2H, d, J = 5.68 Hz, H-7), 6.05 (1H, d, J = 13.05 Hz, H-10), 6.25 (1H, d, J = 13.01 Hz, H-9), 7.16–7.27 (5H, H-2, H-3, H-4, H-5 and H-6), 8.54 (1H, s, 7-NH). EI-MS: m/z 106 [{m + H}]+, 100%. IRmax (neat) cm⁻¹: 3275.38 (N-H), 2967.66 (C-H), 1721.33, 1651.91 (C=O).
Some in vivo work is being carried out to establish the activity of the synthesized compounds as anticancer, anti-HIV, antimalarial, antitrypanosomiasis, antituberculosis, and so forth.

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References

[1] S. M. Aronson, “The naming of antibiotics,” Medicine and Health, vol. 80, no. 6, p. 180, 1997.
[2] M. Radetsky, “The discovery of penicillin,” The Pediatric Infectious Disease Journal, vol. 15, no. 9, pp. 811–818, 1996.
[3] S. A. Waksman, “What Is an antibiotic or an antibiotic substance?” Mycologia, vol. 39, no. 5, pp. 565–569, 1947.
[4] F. Von Nussbaum, M. Brands, B. Hinzen, S. Weigand, and D. Häbich, “Antibacterial natural products in medicinal chemistry—exodus or revival?” Angewandte Chemie, vol. 45, no. 31, pp. 5072–5129, 2006.
[5] H. B. Maruyama, Y. Suhara, J. Suzuki Watanabe et al., “A new antibiotic, fumaramidimycin. I. Production, biological properties and characterization of producer strain,” Journal of Antibiotics, vol. 28, no. 9, pp. 636–647, 1975.
[6] T. Hasegawa, M. Asai, and K. Haibara, “A new antibiotic, C-9154,” Journal of Antibiotics, vol. 28, no. 9, pp. 713–716, 1975.
[7] Y. Suhara, H. B. Maruyama, and Y. Kotoh, “A new antibiotic, fumaramidimycin. II. Isolation, structure and syntheses,” Journal of Antibiotics, vol. 28, no. 9, pp. 648–655, 1975.
[8] J. Jumina, D. Swastika, and A. K. Zulkarnain, “Sintesis dan Uji Aktivitas Biologis Turunan Antibiotik C-9154 Dari Vanillin,” Majalah Farmasi Indonesia, vol. 12, no. 3, pp. 24–35, 2001.
[9] J. Jumina, I. Tahir, and A. K. Zulkarnain, “Synthesis and antimicrobe activity evaluation of ethyl salicyl fumarate and ethyl furfuryl fumarate,” Majalah Farmasi Indonesia, vol. 13, no. 4, pp. 207–214, 2002.
[10] J. Jumina, A. K. Zulkarnain, and P. Mulyono, “Preparation and antibacterial activity of p-Anisyl ethyl fumarate and ethyl N-phenyl fumaramate,” Majalah Farmasi Indonesia, vol. 16, no. 2, pp. 116–123, 2005.
[11] F. L. Strand, “The plasma membrane as a regulatory organelle,” in Physiology: A Regulatory Systems Approach, pp. 49–67, MacMillan, New York, NY, USA, 2nd edition, 1983.
[12] R. Eckert, D. Randell, and G. Augustine, “Permeability and transport,” in Animal Physiology, pp. 65–99, W. H. Freeman, New York, NY, USA, 3rd edition, 1988.
[13] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, “The Plasma membrane,” in Molecular Biology of the Cell, pp. 276–337, Garland Publishing, New York, NY, USA, 2nd edition, 1989.