Pembuatan dan uji khasiat antibakteri *p*-Anisil etil fumarat dan etil *n*-fenil fumaramat

Preparation and antibacterial activity of *p*-Anisyl ethyl fumarate and ethyl *n*-phenyl fumaramate

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Abstrak

Dalam rangka mendapatkan turunan antibiotik C-9154 yang cukup aktif, di dalam penelitian ini telah dilakukan sintesis dan uji aktifitas antibakteri terhadap *p*-anisil etil fumarat dan etil *N*-fenil fumaramat. Kedua molekul target tersebut dipilih dengan pertimbangan bahwa *p*-anisil etil fumarat akan merupakan turunan antibiotik C-9154 yang memiliki substituen metoksi pada posisi para, sedangkan etil *N*-fenil fumaramat akan menjadi contoh turunan antibiotik C-9154 tanpa substituen yang berada dalam bentuk amido-ester.

Pembuatan *p*-anisil etil fumarat dilakukan dari *p*-anisaldehida melalui reduksi menggunakan $NaBH_4$, kondensasi *p*-anisil alkohol dengan maleat anhidrid, dan esterifikasi asam *p*-anisil maleat hasil dengan etanol menggunakan asam benzena sulfonat sebagai katalis. Keseluruhan tahapan reaksi tersebut memberikan rendemen hasil yang memuaskan (55-81 %). Dalam hal etil *N*-fenil fumaramat, senyawa ini diperoleh dengan rendemen 84 % melalui kondensasi antara anilin dengan maleat anhidrid, diteruskan dengan esterifikasi asam *N*-fenil maleamat hasil dengan etanol menggunakan katalis asam sulfat pekat.

Hasil uji khasiat antibakteri menunjukkan bahwa harga konsentrasi hambatan minimum (MIC) dari *p*-anisil etil fumarat dan etil *N*-fenil fumaramat terhadap *Staphyllococcus aureus* masing-masing adalah 15 dan 25 μg/mL. Serupa dengan itu, harga MIC dari kedua senyawa tersebut terhadap *Eschericia coli* juga sebesar 15 dan 25 μg/mL. Data uji aktivitas antibakteri tersebut menunjukkan bahwa kedua senyawa turunan antibiotik C-9154 yang diperoleh adalah cukup aktif dan memiliki aktivitas antibakteri yang sebanding dengan harga aktivitas antibakteri dari antibiotik C-9154 sendiri yang memberikan harga MIC 6,25 – 37,5 μg/mL terhadap beberapa spesies *Staphyllococcus aureus* dan 25 - 50 μg/mL terhadap sejumlah spesies *Eschericia coli.*

Kata kunci: sintesis, anti bakteri, fumarat, dan fumaramat.

Abstract

In order to generate potent C-9154 antibiotic derivatives, it has been conducted the synthesis and antibacterial activity evaluation of *p*-anisyl ethyl fumarate and ethyl *N*-phenyl fumaramate. These target molecules were chosen as the former would be a *para*-methoxy substituted C-9154 derivative, whereas the latter would be an example of unsubstituted C-9154 derivative bearing an amido-ester fumaric side chain.

p-Anisyl ethyl fumarate was synthesized from *p*-anisaldehyde by reduction with $NaBH_4$, condensation of *p*-anisyl alcohol with maleic anhydride, and esterification of the resulting *p*-anisyl maleic acid with ethanol

in the presence of benzenesulfonic acid as the catalyst. These reactions gave satisfactorily yields (55-81 %) in all steps involved. In the case of ethyl *N*-phenyl fumaramate, this molecule was synthesized in 84 % yield through condensation of aniline with maleic anhydride, followed by esterification with ethanol in the presence of concentrated sulfuric acid.

The antibacterial activity test showed that the minimum inhibition concentration (MIC) of *p*-anisyl ethyl fumarate and ethyl *N*-phenyl fumaramate towards *Staphyllococcus aureus* were 15 and 25 μg/mL. Interestingly, the MIC values of these two compounds towards *Eschericia coli* were also 15 and 25 μg/mL respectively. Thus, the data showed that the two C-9154 derivatives obtained are sufficiently potent and possess antibacterial activities which are comparable to the antibacterial activity of C-9154 itself towards *Staphyllococcus aureus* (12.5 – 37.5 μg/mL) and *Eschericia coli* (25 – 50 μg/mL).

Key words: synthesis, antibacterial activity, fumarate and fumaramate.

Introduction

The derivatives of maleic acid (cis) and fumaric acid (trans) are two groups among the known compounds which have been used in the field of pharmacy, medicine, and agriculture since a long time ago. Specifically, the derivatives of these two isomeric compounds have been utilized for the preparation of antibiotics, antifungis, antihistamins and plant growth regulators (Stecher, 1968). Just to mention some examples are the ester form of melanilic acid which has been patented as an antifungi (Ligett, 1968), A-19009 antibiotic discovered by Molloy *et al.* (1972), fumaryl DL-alanin antibiotic, and maleic hydrazides which are known to be able to inhibit the growth of several horticulture plants (Birkingshaw *et al.* , 1942). In addition, combination of maleic acid and chlorpheniramine known as chlorpheniramine maleate is commonly found in some cough formulaes such as Benadryl, Formula 44 and Alerin.

Beside the active compounds as stated above, fumaric fragment also becomes part of the structure of C-9154 antibiotic. C-9154 (1) is a class of antibiotic isolated from *Streptomyces ishigakiensis* (Hasegawa *et. al.*, 1975) or *Streptomyces kurssanovii* (Maruyama *et al.* , 1975) through a fermentation process. The compound showed a broad spectral activity against Gram-positive and Gram-negative bacteria such as *Escherichia coli* and *Staphylococcus aureus* with minimum inhibition concentration (MIC) varied from 10-100 □g/mL and LD_{50} in mice of 75 mg/kg. The chemical structure of C-9154 antibiotic itself basically consists of two fragments i.e. phenylacetic acid and fumaramide (Hasegawa et.al, 1975; Suhara *et al.* , 1975).

A new strategy for the synthesis of C-9154 antibiotic derivatives from vanillin has been developed recently by our research group (Jumina et.al, 2000 and 2001). Within this strategy, vanillin was converted to 4-ethoxy-3-methoxybenzylaniline in three stages. This benzylaniline was then reacted with maleic anhydride to be followed by esterification of the related acid with absolute ethanol in the presence of H_2SO_4. This method is very efficient and satisfactory yields (70-95%) were obtained in each step. The biological activity of both C-9154 derivatives obtained (2 and 3) have already been investigated in which the MIC of 2 towards *Staphyllococcus aureus* and *Escherichia coli* were 2500-3000 μg/mL, whereas that of 3 towards the same bacteria were 500-1000 μg/mL. Thus, conversion of the carboxylic acid group in 2 to an ester functionality in 3 has led to an increase of anti microbe activity to approximately four times.

The above method has also been extended on the case of 4-ethoxy-3-methoxybenzyl alcohol simply prepared via reduction of ethyl vanillin with $NaBH_4$. This type of benzyl alcohol derivatives was reacted with maleic anhydride to be followed by treatment with absolute ethanol in the presence of H_2SO_4. Again, excellent yields (78-93 %) were achieved in all steps. The acid form 4 of the derivative obtained still gave a weak anti microbe effect (MIC 2000-2500 μg/mL) towards *Staphyllococcus aureus* and *Escherichia coli*. However, a significant anti microbe activity towards those two microbes (MIC 400-700

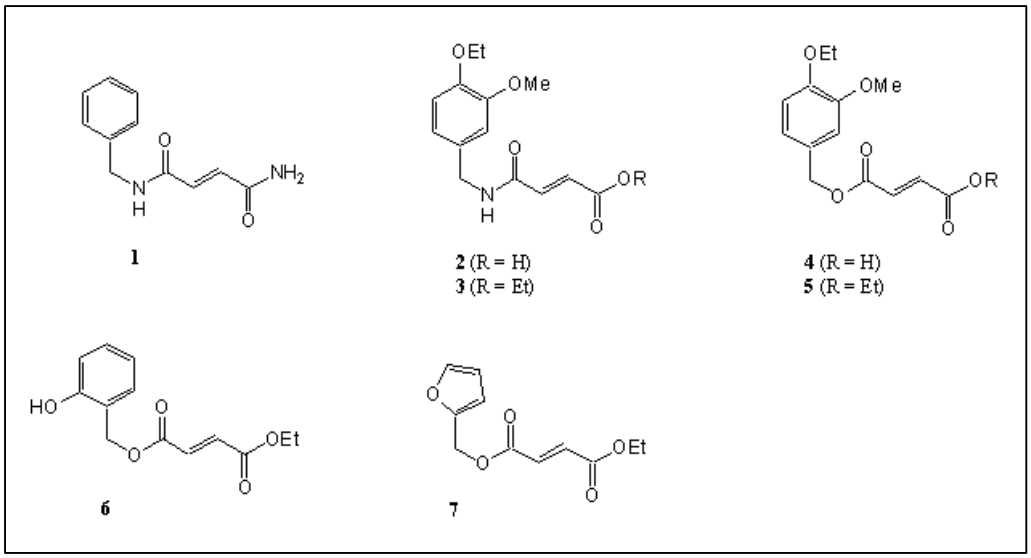

Figure 1. Structure of C-9154 antibiotic and its derivatives

μg/mL) were observed on the case of the ester derivative 5.

More recently, C-9154 antibiotic derivatives have also been synthesized from methyl salicylate and furfural (Jumina *et al.* , 2002). The preparation of C-9154 antibiotic derivative from methyl salicylate was conducted through reduction of methyl salicylate with lithium aluminium hydride, condensation of salicyl alcohol with maleic anhydride, and esterification of the resulting salicyl maleic acid with ethanol in the presence of benzenesulfonic acid to afford ethyl salicyl fumarate 6. In the case of furfural, the synthetic route performed were reduction of furfural with sodium borohydride, condensation of furfuryl alcohol with maleic anhydride, and esterification of the resulting furfuryl maleic acid with ethanol in the presence of benzenesulfonic acid to give ethyl furfuryl fumarate 7. This furfuryl fumarate ester 7 showed significantly strong activities towards *Staphyllococcus aureus* and *Escherichia coli* with MIC of 100 μg/mL towards both bacteria. Ethyl salicyl fumarate 6 also demonstrated a sufficiently strong activity towards *Staphyllococcus aureus* with MIC of 100 μg/mL, but this compound only exhibited a weak activity towards *Escherichia coli* with MIC of 4000 μg/mL.

In order to obtain a more potent C-9154 antibiotic derivatives, it was of interest to synthesize *p*-anisyl ethyl fumarate 8 and ethyl *N*-phenyl fumaramate 9. These target molecules were chosen as all would be less bulky than compound 2, 3, 4, 5, and 6 previously obtained. In addition, the aromatic moiety of target compound 9 would be exactly the same as that of C-9154 antibiotic. Thus, on the basis of molecular size and similarity of aromatic ring between the target molecules and C-9154 antibiotic, it was hoped that the target compound 8 and 9 would be more potent than compound 2, 3, 4, 5, 6, and 7 previously synthesized.

Methodology

Chemicals. All chemicals used in this research were reagent grade from E.Merck. Apparatus. The equipment used in this experiment involved JEOL MY60 proton NMR spectrometer, Shimadzu FTIR 8201 PC spectrophotometer, and Shimadzu QP 5000 Gas Chromatograph-Mass Spectrometer.

Reduction of p-anisaldehyde

Sodium borohydride (0.35 g; 9.2 mmol) was added into a solution of p-anisaldehyde (0.5 g; 3.67 mmol) in absolute ethanol (4 ml). The mixture was stirred and heated at reflux for 3 hours, then the solvent was removed using a Buchii evaporator. The residue was diluted with water (15 ml), and extracted with dichloromethane (3x20 ml). The combined organic layers were washed with water (2x70 ml), dried over anhydrous sodium sulfate and evaporated to give a brown oil. This oil was passed through a suction silica-chromatographic column eluted with

dichloromethane/petroleum ether (7:3) to yield *p*-anicyl alcohol (0.30 g; 55%) as a light yellow oil. This product was identified by means of IR and GC-MS spectrometers.

Condensation of p-anisyl alcohol with maleic anhydride

A solution of *p*-anisyl alcohol (1.5 g, 10.87 mmol) in benzene (5 mL) was added dropwise into a solution of maleic anhydride (1.28 g, 13.0 mmol) in benzene (10 mL). The mixture was stirred at 90°C for 2.5 h, then allowed to cool, and diluted with ethyl acetate (50 mL). The resulted solution was washed with water (3x70 mL), and the organic phase was dried over anhydrous sodium sulfate and evaporated to leave a white sticky solid. Recrystallization of this solid from ethanol afforded p-anisyl maleic acid as a white solid (2.01 g, 78 %). This p-anisyl maleic acid was characterized by means of IR, proton NMR, and GC-MS spectrometers.

Synthesis of p-anisyl ethyl fumarate (derivative of C-9154)

A mixture of *p*-anisyl maleic acid (1.0 g, 4.23 mmol), absolute ethanol (10 mL), and benzene sulfonic acid (ca. 0.3 g) was stirred and heated at reflux for 3 h. The resulted mixture was allowed to cool, then the solvent was removed by means of rotary evaporator. The residue was diluted with water (40 mL), then extracted with dichloromethane (3x40 mL). The combined organic layers were washed with water (2x70 mL), dried over anhydrous sodium sulfate and evaporated to afford the desired ester which was found as a colorless oil. This oil was passed through a suction silica-chromatographic column eluted with dichloromethane/petroleum ether (4:1) to afford p-anisyl ethyl fumarate as a colorless oil (0.90 g, 81 %). This product was characterized by means of IR and proton NMR spectrometers.

Condensation of aniline with maleic anhydride

Into a solution of maleic anhydride (5.0 g, 51.0 mmol) in benzene (20 mL) was added in portions aniline (4.0 g, 43.0 mmol). The resulted emulsion was then heated at reflux for 2 h. The mixture was allowed to cool down, then diluted with ethanol (120 mL) and acidified using 5 % HCl solution. The resulted suspension was filtered, and the solid obtained was dried and recrystallized from dichloromethane/petroleum ether (1:1) to give *N*-phenylmaleamic acid (6.2 g, 76 %) as a fine white solid.

Synthesis of ethyl N-phenyl fumaramate (derivative of C-9154*)*

A mixture of N-phenyl maleamic acid (1.5 g, 7.32 mmol), absolute ethanol (15 mL), and concentrated sulfuric acid (3 drops) was stirred and heated at reflux for 3 h. The resulted mixture was allowed to cool, then the solvent was removed by means of rotary evaporator. The residue was diluted with water (40 mL), then extracted with dichloromethane (3x40 mL). The combined organic layers were washed with water (2x70 mL), dried over anhydrous sodium sulfate and evaporated to leave a yellow oil. This oil was passed through a suction silica-chromatographic column eluted with dichloromethane/petroleum ether (4:1) to yield the desired ethyl ester (1.45 g, 84 %) as a light yellow oil. This product was characterized by means of IR and proton NMR spectrometers.

Determination of anti bacterial activity of the derivatives synthesized

A series of solutions of each C-9154 derivative were prepared and placed in tubes containing *Staphyllococcus aureus* and *Escherichia coli*. The samples were incubated at 37°C, and the inhibition effect of each sample was observed.

The C-9154 antibiotic derivatives obtained from the experiment were placed on petri dish containing Gram positive and negative bacteria. The samples were incubated at 37°C using agar media and the inhibition effect of each sample was observed.

Result And Discussions

Synthetic approaches to the targeted C-9154 antibiotic derivatives

This experiment was designed in order to synthesize 2 types of C-9154 antibiotic derivatives i.e. *p*-anisyl ethyl fumarate and *N*-phenyl fumaramate. Whereas the first example would be a fumarate diester, the second target molecule will be a fumarate amido-ester. In the case of *p*-anisyl ethyl fumarate, the whole reactions performed consisted of reduction of *p*-anisaldehyde (8) with sodium borohydride, condensation of *p*-anisyl alcohol (9) with maleic anhydride, and esterification of *p*-anisyl maleic acid (10) with ethanol (Figure 2).

The reduction of *p*-anisaldehyde with sodium borohydride was conducted in absolute ethanol at reflux for 3 hours to give the desired *p*-anisyl alcohol in 72 % yield which was found as light yellow oil. The reality that the reaction

required a relatively long time (3 hours) under a hard condition (78°C) could be correlated by the presence of the electron donating methoxy group which would decrease the reactivity of *p*-anisaldehyde carbonyl group towards nuchleophiles including hydride ions.

The IR spectrum of the resulted *p*-anisyl alcohol clearly shows the existence of a strong broad band at 3383 cm^{-1} originating from the OH stretching frequency. In addition, the disappearance of C=O absorption, which arises at 1681 cm^{-1} in the IR spectrum of the starting material, gave more evidence for the success of the reduction. Other absorption bands arising at 3100-3000 cm^{-1} (CH stretching), 1600-1500 cm^{-1} (C=C aromatic stretching), 1249 cm^{-1} (CH bending), and 1009 cm^{-1} (C-O stretching) also support the proposed structure.

Further structural assignment of the product conducted using GC-MS gave a chromatogram which indicated that the product essentially consists of a single component (t_R= 11.46 minutes, 91.5%). The mass spectrum of this major product gave a molecular ion peak at m/z = 138 which fits with the molecular weight of *p*-anisyl alcohol. Furthermore, fragments arising at m/z = 121 and m/z = 109 in the mass spectrum are respectively originated from the loss of OH (M-17) and CO (M-28) groups.

The resulted *p*-anisyl alcohol was then reacted with maleic anhydride in benzene at 70-80°C for 2.5 hours to afford *p*-methoxybenzyl maleic acid in 86 % yield. Identification of the product was conducted by means of IR and mass spectrometers. The IR spectrum showed stretching frequencies of OH group (3200-3700 cm^{-1}, medium), C=O group (1780 and 1732 cm^{-1}), and C=C group (1612 and 1516 cm^{-1}). The CH bending frequency of the alkyl groups appears at 1412-1463 cm^{-1} (weak), while the C-O stretching frequencies emerge as strong bands at 1175 and 1248 cm^{-1}. Identification using GC-MS indicated that the product consisted of 4 main components in which the expected anisyl maleic acid (t_R = 25.2 minutes) became the major component (60 %). The mass spectrum of this acid did not show molecular ion peak at the expected value (m/z 236), but it revealed a molecular ion peak at m/z 240. However, the fragmentation pattern of the spectrum exhibiting peaks at m/z 91, 121 (base peak), and 137 gave sufficient evidence for the formation of the desired *p*-anisyl maleic acid.

The esterification of *p*-methoxybenzyl maleic acid was first performed with absolute ethanol in the presence of concentrated sulfuric acid, which has been becoming the most common catalyst for esterification reaction. The reaction mixture was heated at reflux for 4 hours to be followed by evaporation of excess solvent and regular extraction to yield a light brown oil. The results of identification using GC-MS indicated that this product consisted of two components having molecular ion peak at m/z 172 and 166. Based on this data and on the fragmentation pattern given in the mass spectra, it was concluded that these two components were diethyl maleat (43 %) and ethyl *p*-methoxybenzyl ether (44 %). The mechanism of the reaction apparently involved normal esterification of COOH side of *p*-methoxybenzylmaleic acid to be followed by CH_3CH_2OH attack of the benzylic carbon to yield *p*-methoxybenzyl ethyl ether and ethyl maleat monoacid. This last compound then underwent esterification further to form diethyl maleat.

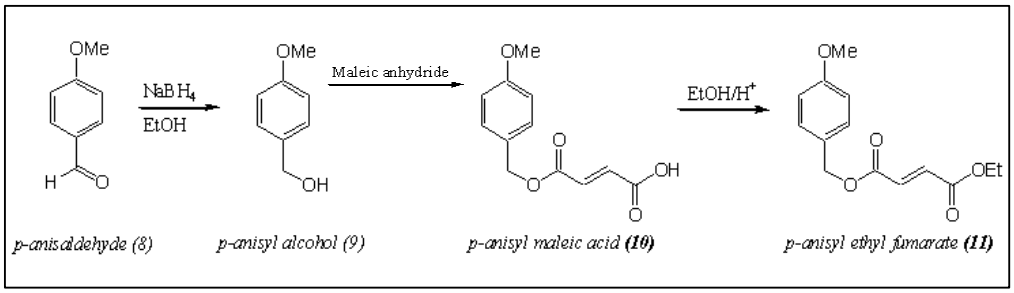

Figure 2 Synthetic scheme of *p*-anisyl ethyl fumarate

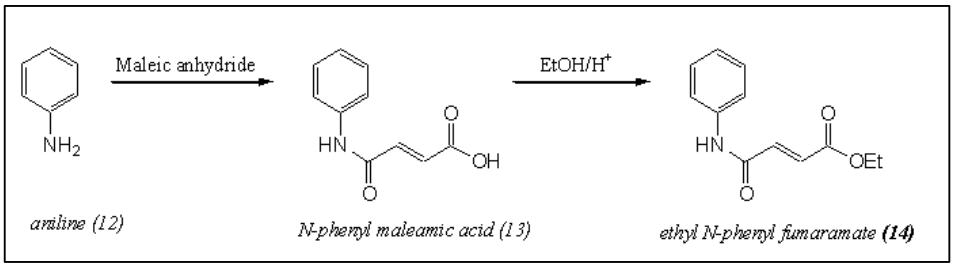

Figure 3 Synthetic scheme of ethyl N-phenyl fumaramate

The esterification of *p*-anisylmaleic acid with ethanol was then conducted using benzenesulfonic acid as the catalyst, which is weaker than sulfuric acid. The reaction was carried out at reflux for 3 hours to afford the desired ethyl *p*-anisylfumarate in 81 % yield which is presumably formed via isomerization of ethyl *p*-anisylmaleate. This product was identified by means of proton NMR spectrum which gives two doublets at 6.90 and 7.25 ppm (5 H) of the phenyl protons and two singlets at 6.15 and 6.25 ppm (2 H) of the fumaric CH=CH protons. The existence of CH_2 and OCH_3 protons are respectively indicated by singlets at 4.40 ppm (2 H) and 3.8 ppm (3 H). Likewise, the presence of CH_2CH_3 groups are indicated by quartet at 3.50 ppm (2 H) and triplet at 1.20 ppm (3 H). Therefore, it is clear that the result of the esterification is the desired *p*-anisyl ethyl fumarate.

The preparation of C-9154 antibiotic derivatives so far has been conducted towards *p*-anisyl alcohol. In these cases, the resulted C-9154 derivatives possess benzyl moiety existing as an ester. It was also of interest to prepare C-9154 derivatives from aniline in order to obtain C-9154 molecules having aromatic ring directly attached to the amide group. In this case the strategy consists of 2 reaction steps i.e. condensation of aniline (12) with maleic anhydride and esterification of the resulted *N*-phenylmaleamic acid (13) (Figure 3).

The condensation of aniline with maleic anhydride was conducted in diethyl ether at room temperature for 2 hour according to the method as described by Vogel (1968) to give 92 % yield of the desired *N*-phenylmaleamic acid. The resulted *N*-phenylmaleamic acid was simply isolated through filtration. This molecule was then treated with absolute ethanol at reflux for 3 hours in the presence of concentrated sulfuric acid to afford the desired ethyl *N*-phenyl fumaramate in 76 % yield. Interestingly, when this esterification was performed using benzenesulfonic acid as the catalyst, the outcome was only starting material. Thus, it should be clear here that N-phenylmaleamic acid is less reactive compared to *p*-anisylmaleic acid employed in the preparation of *p*-anisyl ethyl fumaramate.

The IR spectrum of the above ethyl *N*-phenylmaleamate revealed NH absorption band at 3307 cm^{-1}, C=O stretching frequency at 1716 cm^{-1}, and C=C stretching frequencies at 1548-1598 cm^{-1}. The proton NMR spectrum showed 5 signals indicating the presence of 5 types of protons. Broad singlet at 10.8 ppm (1 H) is originated from NH proton, whereas multiplet at 7.2-8.0 ppm (5 H) is corresponding to the aromatic protons. The appearance of a pair singlets at 6.3 and 6.4 ppm (2 H) clearly indicated the presence of CH=CH maleic protons. Similarly, the presence of a quartet at 4.5 ppm (2 H) and a triplet at 1.3 ppm (3 H) are strong evidence for the existence of CH_2CH_3 group.

The chromatogram obtained from GC-MS showed that the product consisted of two components. On the basis of mass spectrum, the major component having retention time of 18.867 minutes gave molecular ion peak at m/z 219 which is consistent with the molecular weight of the expected *N*-phenyl ethyl fuma-ramate. Thus, on the basis of proton NMR and GC-MS data, it was strongly indicated that the outcome of the above reaction was *N*-phenyl ethyl fumaramate.

Tabel I MIC values of C-9154 antibiotic derivatives

Compound	MIC SA (μg/mL)	MIC EC (μg/mL)
p-Anisyl ethyl fumarate 11	15	15
Ethyl *N*-phenyl fumaramate 14	25	25

Antibacterial evaluation

The experiments carried out within this research so far have led to the generation of two examples of C-9154 antibiotic derivatives i.e. *p*-anisyl ethyl fumarate 11 and ethyl *N*-phenyl fumaramate 14. Antibacterial activity test towards these compounds was conducted using *Staphyllococcus aureus (SA)* dan *Escherichia coli (EC)* as the representative of Gram positive and Gram negative bacteria. The experiment was carried out using agar media and ethyl acetate as the solvent. The results of measurement of minimum inhibition concentration (MIC) of the above compounds towards the growth of *Staphyllococcus aureus (SA)* and *Escherichia coli (EC)* are presented in Table I.

It can be seen from Table I that the antibacterial activity of *p*-anisyl ethyl fumarate 11 towards both *Staphyllococcus aureus* and *Escherichia coli* are 15 μg/mL. Nevertheless, slightly weaker antibacterial activities were found in the case of ethyl N-phenyl fumaramate 14 giving MIC values of 25 μg/mL towards both *Staphyllococcus aureus* and *Escherichia coli*. Even though a direct comparison based on the structure of *p*-anisyl ethyl fumarate 11 and ethyl N-phenyl fumaramate 14 is a bit difficult to be justified, the above data perhaps shows that the antibacterial activity of fumarate antibiotic derivatives are stronger when the molecules do present as diesters (as the case of *p*-anisyl ethyl fumarate 11) rather than when the molecules present as amido-esters (as the case of ethyl *N*-phenyl fumaramate 14). In addition, the data perhaps also shows that the presence of additional lipophilic groups such as OCH_3 and $–CH_2-$ in the structure of fumarate antibiotics could also slightly enhance their antibacterial activities. Furthermore, the data also shows that the activity of *p*-anisyl ethyl fumarate 11 and ethyl *N*-phenyl fumaramate 14 towards the Gram positive and Gram negative bacteria are the same. Therefore, the data proved that *p*-anisyl ethyl fumarate 11 and ethyl *N*-phenyl fumaramate 14 are both sufficiently effective to inhibit the growth of the Gram positive and Gram negative bacteria.

The activity of C-9154 antibiotic itself (Maruyama *et al.* , 1975; Hasegawa *et al.* , 1975) towards several types of *Staphyllococcus aureus* species (FDA 16, MS 3937, MS 4018, and MS 9261) are ranging from 6.25 - 37.5 μg/mL. Slightly different, the activity of C-9154 antibiotic towards several types of *Escherichia coli* species (NIHJ, K12, K12 ML1630, and JR66/W677) lies in the range of 25 - 50 μg/mL. Therefore, it is clear that the activities given by the synthesized *p*-anisyl ethyl fumarate 11 (15 μg/mL) and ethyl *N*-phenyl fumaramate 14 (25 μg/mL) towards *Staphyllococcus aureus* and *Escherichia coli* are comparable or similar to those given by C-9154 antibiotic. Thus, this finding should be significantly valuable as the structures and the method of preparations of *p*-anisyl ethyl fumarate 11 and ethyl *N*-phenyl fumaramate 14 are much simpler than those of C-9154 antibiotic.

Conclusions

p-Anisyl ethyl fumarate 11 could be prepared in a good yield from *p*-anisaldehyde through reduction with sodium borohydride, condensation with maleic anhydride, and esterification with ethanol in the presence of benzenesulfonic acid as the catalyst. Likewise, ethyl *N*-phenyl fumaramate 14 could also be synthesized in a satisfactorily yield through condensation of aniline with maleic anhydride followed by esterification of the resulting *N*-phenyl maleic acid with ethanol in the presence of concentrated sulfuric acid as the catalyst.

p-Anisyl ethyl fumarate 11 is sufficiently active to inhibit the growth of *Staphyllococcus aureus* and *Eschericia coli* with MIC value of 15 μg/mL for both bacteria. Slightly weaker activities towards the same bacteria were seen in the case of ethyl *N*-phenyl fumaramate 14

which give MIC value of 25 μg/mL. The antibacterial activities given by *p*-anisyl ethyl fumarate 11 and ethyl *N*-phenyl fumaramate 14 towards *Staphyllococcus aureus* and *Eschericia coli* are already comparable to those of C-9154 antibiotic towards the same bacteria.

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