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Synthesis of New Derivatives of Leaf-Eating Ant Toxic Gland Pheromone Compounds and Investigation of their Biological Properties

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ABSTRACT

Background: The anti-mycobacterial characteristics of 2-pyrazinoic acid esters have been discovered through recent research. Research has demonstrated that the pyrazine ring and the alkyl part of these compounds interact with the enzyme phytynetase, which in turn inhibits the interaction between NADPH and mycobacterial fatty acids synthase, the enzyme responsible for synthesizing the fatty acids required for the cell wall of mycobacteria.

Materials and Methods: Targeted pyrazinoic acid molecules have been synthesized, their structure verified by spectroscopic data, and their anti-mycobacterial activity against Mycobacterium TB H37Rv at a dose of 10 micrograms per milliliter assessed in order to test this theory. Significant anti-mycobacterial capabilities were shown by several compounds, including 3c, 3J, and 3M, which inhibited mycobacterial growth by 45.7%, 45.4%, and 51%, respectively.

Findings: The results showed that the compounds exhibited little toxicity and did not inhibit macrophage cell proliferation. Pyrazinamide had significant antibacterial and antifungal activity, despite its lack of fatal action against non-tubercular mycobacteria and both Gram-positive and Gram-negative bacteria. Compound 3B, for example, exhibited excellent antibacterial properties against Gram-positive bacteria, such as S. aureus, with a minimum inhibitory concentration (MIC) of 125 micrograms per milliliter. Conversely, the compounds showed equally potent antibacterial properties against Gram-negative bacteria. Interestingly, compared to Gram-positive bacteria, the ester produced from maltol exhibited greater antibacterial efficacy against Gram-negative bacteria. Compounds synthesized with biodegradable groups also exhibited time-dependent toxicity against K562 leukemia cancer cells in MTT measurements, with compounds 3e and 3J displaying IC50 values of 25 micromolars.

Conclusion: After synthesizing pyrazinoic acid monoterpene esters, spectral data was used to confirm their structures. When their biological characteristics were assessed, toxicity studies against K562 leukemia cells produced encouraging results. Through the use of Thio Ester-mediated activation of 2-pyrazinoic acid by 2, 2-dibenzothiazole disulfide and triphenylphosphine, 6-aminopenicillanic acid (6-APA) was coupled with pyrazinoic acid.

Keywords: Pyrazine, Pyrazinoic Acid, Pheromone Compounds, Anti-mycobacterial.

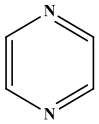
INTRODUCTION

Chemicals called pheromones are released by animals that cause a collective reaction in other members of the same or different species. Pheromones are chemical substances that work similarly to hormones but outside of the secretary body, affecting the recipient's behavior. There are many different kinds of pheromones, including sex, alarm, and food-trail pheromones. They affect pheromone receptors in the body and in behavior (Karlson et al., 1995). Pheromones are employed across the biological spectrum, from simple unicellular prokaryotes to complex multicellular eukaryotes. They are found in the Plantae, insect, and mammal kingdoms (Kleerebezem et al., 2001). For example, plants employ pheromones to deceive insects for pollination, using mosquito sex pheromones to lure them and aid in pollination. Orchid flowers, in particular, use alkylpyrazines to sexually deceive specific insects into aiding them in pollen transport (Bohman et al., 2014).

An important part of leaf-eating insect pheromone structure is played by alkylpyrazines. These pyrazines are frequently discovered in the venom of Attini, a type of insect that feeds on leaves. These insects mark their tracks with alkylpyrazines, which act as trail pheromones and help worker ants and other species find food sources (David Morgan, 2009). With populations of up to five million ants per colony, these leaf-eating ants are among the most significant animal communities in nature and cause significant agricultural damage across the globe (Beckers et al., 1989). To ensure effective communication within such a large community, a robust chemical communication system is imperative.

The chemical composition of the specific message vector should have an optimal shelf life to avoid congestion resulting from the prolonged presence of the chemical. Therefore, the pheromone message's chemical composition should possess an ideal lifespan. For example, in the case of path pheromones, the pheromone's lifespan and that of the food source should be closely aligned to prevent directing insects to depleted food sources (Blum, 2009). This highlights the significance of the pyrazine ring's presence in the pheromone structure, characterized by nitrogen atoms, due to its unique structure, low bipolar membrane volatility, and insect traceability (Dembitsky et al., 2014).

Two nitrogen atoms can be found in the significant class of 6-membered heterocyclic aromatic compounds known as pyrazine. It is a member of the pyrazine, pyridazine, and pyrimidine family of -1, 4-diazines (Zimhony et al., 2007). These heterocyclic compounds have characteristics and chemistry similar to aromatic compounds; nevertheless, pyrazine's chemistry differs greatly from that of benzene. Its resonance energy is also lower than that of pyridine and benzene (Casanova et al., 2010). This compound is stable and colorless, and it has a zero dipole moment (Scheme 1). The boiling point of pyrazine is 116 degrees Celsius, and its melting point is 54 degrees Celsius. Many lower homologues are liquid at room temperature and highly soluble in water, often miscible with water in any proportion. Pyrazine forms an azeotrope with water (boiling point 95.5 degrees Celsius, 60% pyrazine and 40% water).



Scheme 1. Structure of Pyrazine

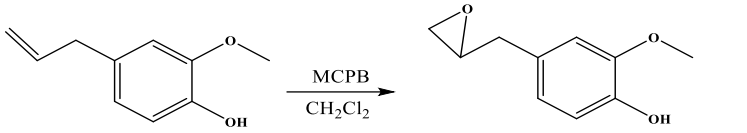
Pyrazine and its alkyl derivatives are useful and readily form complex compounds with copper (I), cobalt (II), nickel (II), and iron (II) as ligands (Joule et al., 2012). This structural unit is found in many natural products. They exist as fragrance and flavor components in food products. For example, the aroma and taste of green peas, coffee, pepper, and wine are formed by pyrazine compounds. Although they are present in very small amounts, they are highly aromatic and can be detected even at concentrations as low as 0.00001% (percent per million).

These compounds are naturally found in many vegetables, insects, terrestrial vertebrates, and marine organisms, and they are produced through primary or secondary metabolism by microorganisms. Pyrazines have also demonstrated interesting anti-cancer (Smith and March, 2002) and anti-tuberculosis properties (Rajini et al., 2011). The structures of pyrazinoic acids were confirmed but their biological characteristics and toxicity studies against K562 leukemia cells were not studied. Thus, this research was conducted to study the biological characteristics and toxicity of pyrazine. The significance of this discovery lies in the fact that Mycobacterium TB is one of the bacteria that produces the enzyme beta-lactamase, which has the ability to withstand medications that include the beta-lactam ring.

MATERIALS AND METHODS

Synthesis of Eugenol Oxide

Eugenol oxide, containing 82 milligrams, was produced when eugenol was oxidized with meta-chloroperoxybenzoic acid (MCPBA) in dichloromethane. First, in a 100 ml round-bottom flask, 5.0 mmol of eugenol was dissolved in 20 ml of dichloromethane while being agitated. Next, drop by drop, 2.17 mg (0.0750 mmol) of meta-chloroperoxybenzoic acid was added to the 180 ml of dichloromethane solution. The reaction proceeded at room temperature until no eugenol was detectable based on TLC paper analysis. The reaction mixture was then washed three times with a 10% sodium metabisulfite solution to eliminate the meta-chloroperoxybenzoic acid. Afterwards, it was consecutively washed with saturated solutions of sodium bicarbonate and sodium chloride. The organic phase, obtained after drying with magnesium sulfate, was evaporated, resulting in the separation of eugenol oxide as a white solid (Scheme 2).

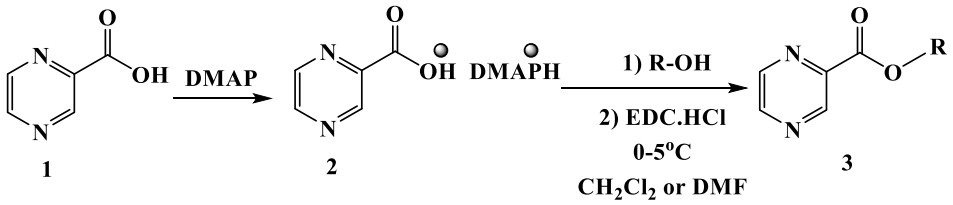


Scheme 2. Synthesis of Eugenol Oxide

Optimized Method for Synthesizing 2-Pyrazinoic Acid Esters (3a-3n)

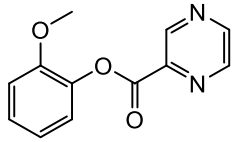
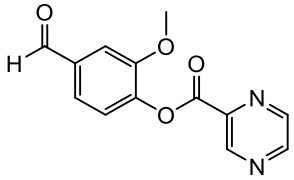
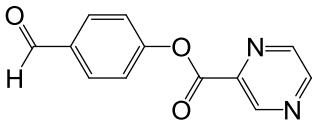
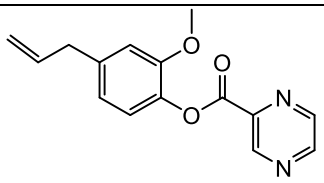
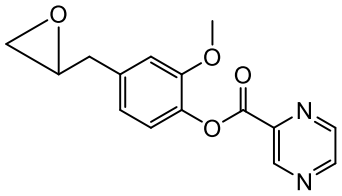
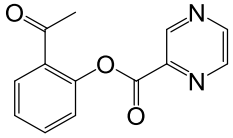
In a 50 mL round-bottom flask, pyrazinoic acid (124 mg, 1 mmol) was dissolved in dichloromethane or dimethylformamide (2 mL). Dimethylamino pyridine (37 mg, 0.3 mmol) was added, and after 10 minutes, the desired alcohol compound (0.7 mmol equivalent) was introduced into the mixture. The reaction mixture was then placed in an ice-water bath until the temperature reached zero degrees Celsius as shown in Table 1. Subsequently, ZnBr2 (22 mg, 0.1 mmol) was added, followed by the gradual addition of EDC.HCl (173 mg, 0.9 mmol) to the reaction mixture. After 30 minutes, the cold bath was removed, and the reaction mixture was stirred at room temperature for an additional 4 hours. The progress of the reaction was monitored by thin-layer chromatography (TLC) using an ethyl acetate/hexane mixture in a 1:3 ratio. Finally, the mixture was saturated

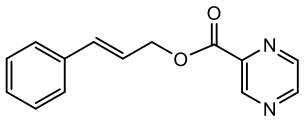
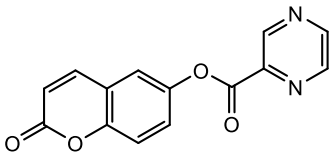
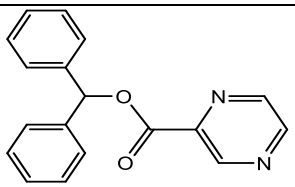
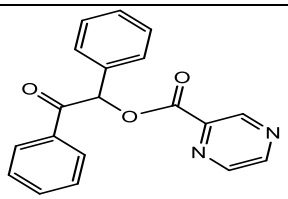
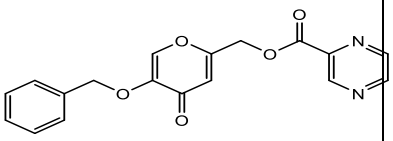
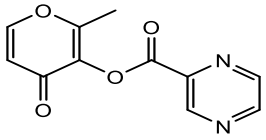
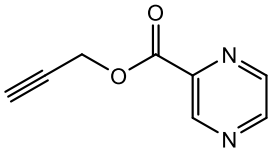
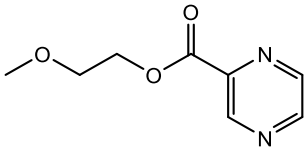
with a sodium bicarbonate solution and then washed with a 0.05 M HCl solution. The organic phase was dried using magnesium sulfate, and after evaporating the solvent under reduced pressure, the corresponding ester compounds were obtained. Some of the solid esters, such as 3f, 3c, 3b, 3a, 3i, were further purified using a crystallization method with the mentioned solvents, while two other (3g, 3e) compounds (3e) were purified on a chromatographic column (Wang et al., 2011). The products obtained are described in (Scheme 3).



Scheme 3. Discription of the product obtained by chromatographic column.

Table 1. Reaction of 2-Pyrazinoic Acid with Alcohol for Synthesis their Esters.

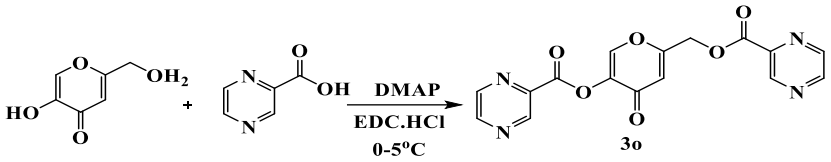
Compound Name	Structure	Appearance / Characteristics	Yield	Melting Point (°C)
Methoxyphenylpyrazine-2-carboxylate (3a)		Neo-crystallized	80%	68-65
4-formyl-2-methoxyphenylpyrazine-2-carboxylate (3b)		Noblated	75%	112-111
4-formylphenylpyrazine-2-carboxylate (3c)		Noblated	80%	135-134
4-allyl-2-methoxyphenylpyrazine-2-carboxylate (3d)		White solid material	90%	99
2-methoxy-5-(oxiran-2-Il methyl) phenylpyrazine-2-carboxylate (3e)		Purification with chromatographic column	65%	Low melting point brown-
2-acetylphenylpyrazine-2-carboxylate (3f)		Noblored	70%	63-62

2-cinnamylpyrazine-2-carboxylate (3g)		Purification with chromatographic column	75%	52-49
2-OXO-2--Hchromene-6-IL pyrazine-2-carboxylate (3h)		Solid sediment	87%	192-194
Benzhydrylpyrazine-2-carboxylate (3i)		Noblored	70%	90-89
2-OXO-1,2-diphenylethylpyrazine-2-carboxylate (3j)		White crystals	95%	108
5-(benzyloxy-4-OXO-4-Hpyran-2-Il) methylpyrazine-2-carboxylate (3k)		Crystalline solid	74%	135-133
2-Methyl-4-OXO-4-Hpyran-3-Il pyrazine-2-carboxylate (3l)		White crystals	80%	120-119
Prop-2-enylpyrazine-2-carboxylate (3m)		Solid white	85%	80-79
2-methoxyethylpyrazine-2-carboxylate (3n)		Colorless liquid	86%	-

Synthesis of (4-OXO-5-(pyrazine-2-carbonyloxy)-4-H-pyran-2yl) methylpyrazine-2-carboxylate (3o)

In a 20 mL round-bottom flask, 2-pyrazinoic acid (124 mg, 1 mmol) was dissolved in dichloromethane or dimethylformamide (2 mL), and dimethylaminopyridine (37 mg, 0.3 mmol) was added. After a 10-minute interval, Kojic Acid (0.4 mmol) was introduced, and the reaction mixture was cooled to zero degrees Celsius in an ice bath. Following this, ZnBr2 (22 mg, 0.1 mmol) was added, and EDC.HCl (173 mg, 0.9 mmol) was gradually introduced into the reaction mixture. After a 30-minute duration, the cooling apparatus was removed, and the reaction mixture was stirred at room temperature for 4 hours. The progress of the reaction was monitored using thin-layer chromatography (TLC) with an ethyl acetate/hexane mixture in a 2:1 ratio. The white solid precipitate formed during the reaction was washed with a small quantity of chloroform after filtration. This

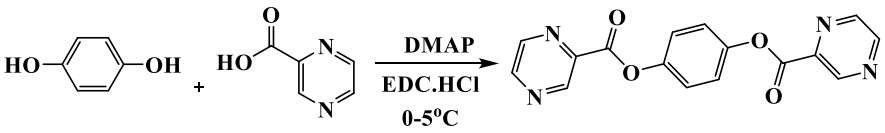
yielded diestercogic acid (2-4) as a white crystalline solid with a 72% yield and a melting point of 145-147 °C (Scheme 4).



Scheme 4. Synthesis (3o) from Kojic Acid.

Preparation of 2-Pyrazine Carboxylic Acid Ester from Hydroquinone (3p)

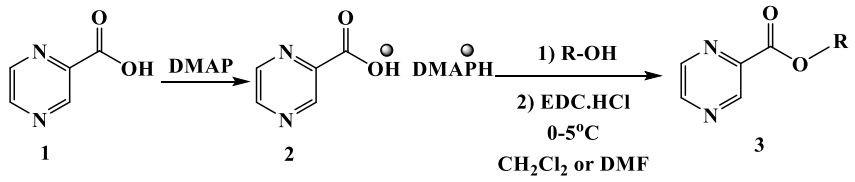
2-pyrazinoic acid (124 mg, 1 mmol) was added to either dimethylformamide (2 mL) or dichloromethane (2 mL) in a 20 mL round-bottom flask. Following the addition of dimethylaminopyridine (37 mg, 0.3 mmol), hydroquinone (44 mg, 0.4 mmol) was added after ten minutes. After that, the reaction mixture was submerged in an ice bath to reach a temperature of zero. Following this, ZnBr2 (22 mg, 0.1 mmol) and EDC.HCl (173 mg, 0.9 mmol) were gradually incorporated into the reaction mixture. After a 30-minute duration, the cooling source was removed, and the reaction mixture was stirred at room temperature for 4 hours. The reaction's progress was monitored using thin-layer chromatography (TLC) with an ethyl acetate/hexane mixture in a 2:1 ratio. The white solid precipitate formed during the reaction was washed with an adequate amount of water after filtration. Upon drying, Diester hydroquinone (Scheme 5) was obtained as white needle crystals with a 70% yield and a melting point of 222-225 °C.



Scheme 5. Synthesis (3p) from Hydroquinone

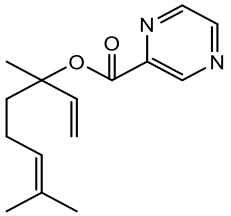
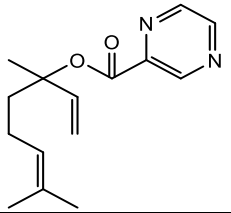
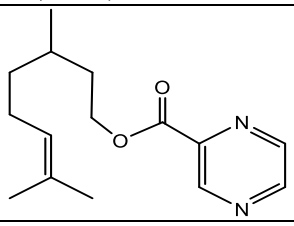
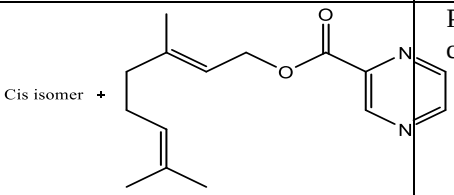
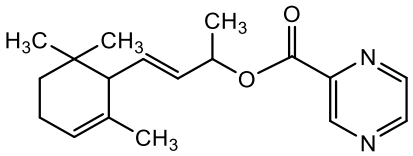
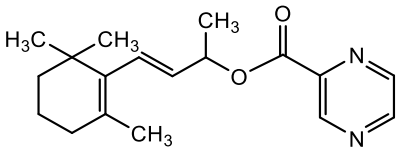
Synthesize 2-Pyrazinoic Acid Monoterpene Esters (3q, 3r, 3s, 3t, 3u, 3v)

In a 20 mL round-bottom flask, 2-pyrazinoic acid (124 mg, 1 mmol) and dichloromethane or dimethylformamide (2 mL) were combined (Table 2). Dimethylaminopyridine (37 mg, 0.3 mmol) was added, and after 10 minutes, the terpene compound (0.7 mmol) was introduced. The reaction mixture was placed in an ice bath to cool to zero degrees Celsius. Following this, ZnBr2 (22 mg, 0.1 mmol) and EDC.HCl (173 mg, 0.9 mmol) were gradually added to the reaction mixture. After 30 minutes, the cooling source was removed, and the reaction mixture was stirred at room temperature for 4 hours. The progress of the reaction was monitored using thin-layer chromatography (TLC) with an ethyl acetate/hexane mixture in a 3:1 ratio. Finally, the mixture was saturated with a sodium bicarbonate solution and then washed with a 0.5 M HCl solution. The organic phase was dried using magnesium sulfate, and the solvent was evaporated under reduced pressure to obtain the ester compounds. The oil obtained from the chromatographic column was purified (Scheme 6). The 3R compound was obtained as pure crystalline material.



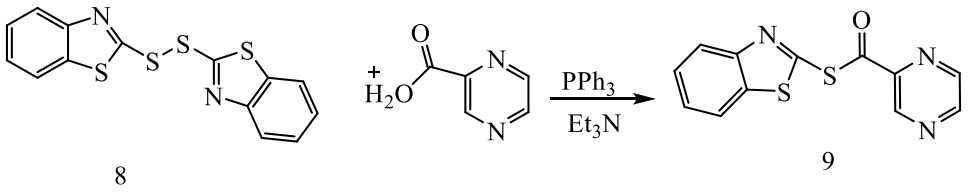
Scheme 6. The oil obtained from the chromatographic column.

Table 2. Synthesis of 2-Pyrazinoic Acid Monoterpene Esters.

Compound Name	Structure	Appearance / Characteristics	Yield	Melting Point (°C)
3,7-Dimethylacta-6,1-DN-3-Il pyrazine-2-carboxylate (3q)		Purification with chromatographic column	40%	-
2-(1R,2S,5R)-2-Isopropyl-5-Methylcyclohexylpyrazine-2-carboxylate (3R)		White needle crystals	95%	68-69
7,3-Dimethylacta-6-N-1-Il pyrazine-2-carboxylate (3s)		Purification with chromatographic column	70%	-
(E)-7,3-Dimethylaceta-2,6-Dianylpyrazine-2-carboxylate (geranylpyrazinoate) and (Z)-3,7-Dimethylaceta-6,2-Dianylpyrazine-2-carboxylate (renylpyrazinoate) (3t)		Purification with chromatographic column	65%	-
(E)-4-(2,6,6-Trimethylcyclohex-2-Anil) boot-3-in-2-Il pyrazine-2-carboxylate (3u)		Purification with chromatographic column	70%	-
(E)-4-(2,6,6-Trimethylcyclohex-2-Anil) boot-3-in-2-Il pyrazine-2-carboxylate (3v)		Purification with chromatographic column	40%	-

Synthesis of S-Benzothiazole-2-yl Pyrazine-2-Carboxylate (9)

Benzothiazole (2, 2-DTB) (0.332g, 1 mmol) and triphenylphosphine (0.263g, 1 mmol) were dissolved in 10 mL of acetone and stirred for 30 minutes. Then, 2-pyrazinoic acid (0.124g, 1 mmol) was added, and gradually, 1.0 g (1 mmol) of trimethylamine was introduced. The reaction was allowed to proceed at room temperature for an additional 4 hours, and the progress of the reaction was monitored by thin-layer chromatography (TLC) using an ethyl acetate/hexane mixture in a 1:3 ratio. The precipitate that formed during the reaction was separated by filtration, washed with a small amount of acetone, and yielded yellow needle-like crystals with a melting point of 145-148 °C and a 90% yield (Scheme 8 and 9).

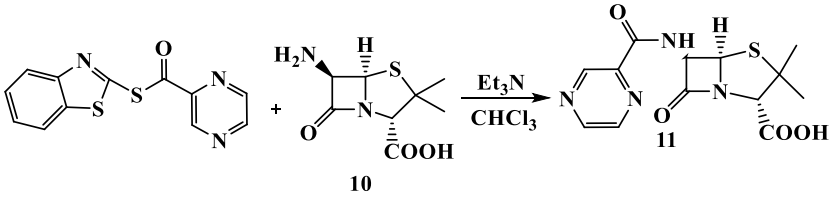


8 9

Scheme 8 and 9. Synthesis Thio ester 2-Pyrazinoic acid from 2, 2 Di Thio Base (2, 2-DTB) Benzothiazole

Synthesis of (6S, 5S, 2)-3, 3-dimethyl-7-OXO-6-(pyrazine-2-carboxamido)-4-TIA-1-cycloazabi [3, 2, 0] heptane-2-carboxylic acid (11)

A spinning suspension of 6-aminopenicillanic acid (6-APA) (0.216g, 1 mmol) in chloroform (10 mL) was maintained at a temperature of 5°C (0.101g, 1 mmol). Triethylamine was added, followed by the addition of 2-pyrazinoic acid 2-benzothiazoyl thio ester (0.273 mg, equivalent to 1 mmol). The reaction mixture was stirred at ambient temperature for 4 hours and then extracted with 20 mL of water. The resulting solution was adjusted to a pH of 3 by adding a hydrochloric acid solution and then extracted with chloroform. After evaporating the solvent at reduced temperature, white gel-like product was obtained with an 80% yield (Scheme 10 and 11).



10 11

Scheme 10 and 11. Synthesis of (11) from 6APA and Thio ester 2-Pyrazinoic acid

Determination of anti-tuberculosis activity in small molecules using Mycobacterium tuberculosis containing Green Fluorescent Protein1 2-bacteria (GFP)

Mycobacterium tuberculosis (H37Rv) containing green fluorescent protein (GFP) was cultured in a complete 7H9 environment using BD Difco (cat# 271310, Becton Dickinson) with oleic acid-albumin-dextrose-catalase (OADC) at a 10% concentration, glycerol at 0.02% - 0.05%, and Tween 80 at 0.5%. The culture was allowed to grow until the optical density (OD) reached 0.4, and then it was frozen at -80 degrees Celsius.

Preparation of Compounds and Measurement of Antimycobacterial Properties

45 ml of 7H9 medium without glycerol and Tween 80 was mixed with 5 ml of OADC. A solution of each compound at a concentration of 10 mg/ml was prepared. 1 microliter of each compound was diluted with 800 microliters of 7H9 test medium. Then, 80 microliters of each concentration were added to 96-well plates

(Corning). Mycobacteria were cultivated at 37°C, then centrifuged for ten minutes at 3700 × G using a Heraeus centrifuge with a swingout rotor. After removing the supernatant, the bacterial pellet was resuspended in a 7H9 test medium to reach a concentration of $2 \times 10^6/20$ µL. Subsequently, 20 microliters of the bacterial suspension were added to the wells, either with or without the tested compounds. In each plate, rifampicin was used as a reference drug with known inhibitory activity against mycobacteria. The plates were sealed with air-permeable membranes (Porvair Science) and incubated with gentle agitation (Heidolph). Bacterial growth was assessed on the seventh day by measuring fluorescence intensity using a Microplate Reader.

In Vitro Toxicity Measurement of Compounds on Human Macrophage Cells

The in vitro toxicity of synthetic compounds on human macrophage cells was assessed using the xCELLigence RTCA system, following established protocols as reported in scientific sources (Ke et al., 2011).

Determining the amount of antimicrobial properties

The Minimum Inhibitory Concentration (MIC) of the synthesized compounds was determined by dilution. Tested compounds were dissolved in dimethyl sulfoxide to obtain a stock solution of 100 micrograms per milliliter for each compound. Consecutive dilutions were prepared in Mueller-Hinton medium for bacteria and Sabouraud dextrose medium for fungi to achieve concentrations of 2, 4, 8, 16, 32, 62.5, 125, 250, 500, and 1000 micrograms per milliliter. A control test was performed to ensure that the solvent itself had no antimicrobial effect, using a dimethyl sulfoxide-sampled medium at the same concentration as in the test. Gentamicin and nystatin were similarly diluted for antibacterial and antifungal control tests, respectively. Microorganisms were added to each microplate to reach a concentration of CFU/mL 5×10^2. After 24 hours of incubation at 35 degrees Celsius, growth was assessed by microplate reading, and the lowest compound concentration that inhibited microbial growth was considered the MIC.

Measurement of DPPH free radical inhibition activity

The free radical inhibitory strength of DPPH (2,2-diphenyl-1-picrylhydrazyl) was measured by adding 1 mL of different concentrations of each synthetic material, dissolved in methanol, to 1 mL of DPPH• solution (1.0 mmol in methanol). The reaction commenced, and absorption was measured at 517 nm (As) after 30 minutes of incubation at 37°C in the dark. As a control, the absorption value of DPPH• in 1 mL of methanol (Ac) at 517 nm was measured. The DPPH•

RESULTS AND DISCUSSION

The results of anti-mycobacterial properties are somewhat agreed with the assumption that 2-pyrazinoic acid esters that have a bioavailable factor group in their alkyl part are likely to have higher anti-mycobacterial activity than similar ones without a bioavailable center due to the involvement of the enzyme phythic acid synthase in a lateral regenerative reaction. Previously, the Setz group had linked the higher inhibition of the pyrazinovate derivative containing 4 - stoxybenzyl to the self-lmmolative activity of this reservoir and its synergic effect. It has also been found3-ketohexanoic acid stops the biosynthesis of fatty acids in the mycobacteria smegmatis (Msm) (Seitz *et al*., 2002).

Considering that the entire Ester molecule is responsible for anti-mycobacterial activity, it is still unclear whether these results are due to the double inhibitory effect of the alkyl part of the pyrazinoic acid Ester

molecule on phytynetase or due to the greater penetration of molecules into the cell wall and the increased bioactivity due to the presence of polar factor groups. The output of the measurement of antibacterial activity shows the importance of factor groups in changing the antibacterial power of 2 - pyrazinoic acid Ester. The 3e compound has more antibacterial activity against both Gram-positive and Gram-negative bacteria than the 3d compound, which indicates the importance of the epoxide group in increasing antibacterial properties.

In the case of phosphomycin-an antibiotic containing the epoxide group-it has been found that the nucleophilic attack of amino acid cysteine on the epoxide center on the drug causes irreversible inactivation of the enzyme involved in the construction of the cell wall of the bacterium (Zhu *et al*., 2012).

The MTT measurement also agrees with the hypothesis of this study. For example, pyrazinoic acid derived from benzoin, which contains alpha-hydroxy ketone, shows higher toxicity on cell line K562. Another confirmatory example is pyrazinoate containing the oxyran group, which has a greater toxicity effect than similar without the epoxide factor group. A firm interaction with a cellular target caused by increased nucleophilic activity of the epoxide ring may be a possible reason for these observations (Ngo *et al*., 2012).

Due to the similarity between cancer cells and pathogenic bacteria, some anti-tumor drugs also appear to have an effective inhibitory effect on bacterial growth. Daunorubicin and Dexorubicin were originally isolated as antibiotics, but their high toxicity prevented them from being used as antibiotics, and several years later their anti-cancer properties were discovered and are now used as anti-cancer drugs. For this reason, this research has tried to investigate the effectiveness of compounds against cancer cells and bacteria simultaneously (Mahboub *et al*., 2012).

Geraniol and other monoterpenes are a new class of compounds for chemotherapy for various cancers as a component of plants and fruits the mechanism of action of monoterpenes is by affecting the function of the enzyme redactase 3 - hydroxy-3 - methylglutaryl – coenzyme A (HMGCoA), who's concentration in cancerous tissue increases and becomes irregular. Limiting enzyme activity will reduce the products of the molonate pathway, a pathway that organismal cells, for proteinization and growth, urgently need for its products. Biosynthesis of cholesterol and other sterols is also performed as an essential component for cell growth through this pathway of molonate. Molonate also plays a role in DNA formation.

In this study, Alpha-ionol and beta-ionol pyrazinovates show higher efficiency than the values reported in previous studies in the fight against cancer cells. Previous studies have shown that geraniol is more efficient than its CIS derivative, renol, in the face of cancer cells, because the pyrazinoate mixture of geraniol and renol is used on the cancerous cell, and no specific conclusions can be drawn. The conversion of the alcoholic end of terpenes into pyrazinoic acid Ester appears to prevent it from oxidizing, resulting in higher efficiency of terpenes against cancerous tumors (Mo *et al*., 2004).

CONCLUSION

Due to the effectiveness of terpenes in crossing the cell wall of bacteria due to proper friendship fat and changes in the permeability of their cell walls it seems that terpenes pyrazinoic acid esters easily pass through the cell wall but antibacterial properties can be the result of the total function of pyrazinoic acid Ester because if the ester is hydrolyzed into two parts pyrazinoic acid and terpenes because most bacteria have a strong eflax system to excrete 2 - pyrazinoic acid are out of the cytoplasm, the pyrazinoic acid segment practically cannot play a role in the occurrence of antibacterial properties. So the aggravated antibacterial properties of Turpen 2 - pyrazinoic acid esters can be attributed to both parts of the building. Interestingly, the appropriate efficacy of the

beta - lactam unit derivative in preventing the growth of the tuberculosis agent mycobacteria, because penam-containing antibiotics such as penicillins do not usually have favorable efficacy against Mycobacterium tuberculosis, which is mainly caused by their inability to cross the mycobacterial wall. However, the penam 2-pyrazinoic acid derivative showed in this experiment that mycobacteria have a high sensitivity to this Phnom derivative. The important point of this finding is that this sensitivity is shown while mycobacteria tuberculosis is among the bacteria with the enzyme beta-lactamase, which has resisted drugs containing the beta-lactam ring.

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