Synthesis and Molecular Docking Study of Pyrazine-2-carboxylic acid Derivatives

Muhammad Zulqurnain¹, M. Riza Ghulam Fahmi², Arif Fadlan¹, Mardi Santoso¹*¹

¹Departemen of Chemistry, Faculty of Science, Institut Teknologi Sepuluh Nopember, Kampus ITS Sukolilo Surabaya 60111, Indonesia
²Ma Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, Villa Puncak Tidar N-01, Malang 65151, Indonesia

*Corresponding author: tsv09@chem.its.ac.id

Abstract. The pyrazine-2-carboxylic acid derivatives 1a-c with aromatic, cyclic, and aliphatic side chain were successfully synthesized. The structures of derivatives were confirmed by spectroscopic methods (FTIR, NMR, HRMS). The molecular docking was performed to determine the possible binding interaction between 1a-c with Mycobacterium tuberculosis InhA protein. The derivative 1e showed the lowest rerank score (-86.4047 kcal mol⁻¹) and it might correspond to the lowest experimental MIC value.

1. Introduction

Tuberculosis (TB), an old infectious disease caused by organisms of Mycobacterium tuberculosis, contaminates one-third of population and leads the cause of death worldwide [1]. About 10 million new TB were found all over the world in 2018 and 8% of these cases were experienced by Indonesian. TB cases in Indonesia have decreased from 2000 to 2018, but there has been an increase in new TB cases and relapses [2]. The TB cases have been complicated by the emersion of multi- and extensively-drug resistance (MDR, XDR) of the first-line TB medication including isoniazid, rifampicin, ethambutol, and pyrazinamide which usually consume in six months’ duration [3], [4]. The development of potential and promising lead compounds with better activity for TB treatment then is needed.

The first-line of TB medication especially pyrazinamide gained special attention for the development of lead compounds for preventing drug resistance and shorten the treatment duration [5]. Pyrazinamide derivatives with substitution at C2 and C3 position in pyrazine unit have been synthesized and showed promising activity [6]. Further installation of different groups such as ethylphenyl, cycloheptyl, and octyl on the pyrazine carboxamide structure by utilization of thionyl chloride for amidation gave diverse derivatives with higher activity against M. tuberculosis [7]–[10]. Due to the safety of thionyl chloride in organic synthesis, several researchers then reported new systems including cerium catalyst in water, N-heterocyclic carbene (NHC) in toluene, and Fe-mont catalyst for amidation [11]–[14]. In order to find lead compounds for TB treatment and in continuation of our interest in anti-TB agents [15], the present study reported synthesis of pyrazine-2-carboxylic acid derivatives 1a-e using Yamaguchi esterification and study their interaction with M. tuberculosis InhA protein by molecular docking.
Figure 1. Structure of pyrazine-2-carboxylic acid derivatives 1a-c.

2. Experimental Section

2.1. General information
Pyrazine-2-carboxylic acid, 2,4,6-trichlorobenzoyl chloride, 4-dimethylaminopyridine, 4-ethylaniline, cycloheptylamine, n-octylamine, triethylamine, and tetrahydrofuran were purchased from Sigma-Aldrich, Tokyo Chemical Industry, and Merck. Melting point analysis was performed on Fisher-John melting point apparatus. FT-IR Shimadzu 8400S was used for FTIR spectra measurement. NMR spectra were recorded in deuterated chloroform using TMS as standard. The mass spectroscopy analysis was achieved from a Waters Q-TOF Xevo instrument.

2.2. General method for synthesis 1a-c
1a-c was obtained by applying esterification method [15] with pyrazine-2-carboxylicacid (1 eq), triethylamine (1 eq), 2,4,6-trichlorobenzoyl chloride (1 eq), 4-dimethylaminopyridine (1 eq). The mixture was stirred at rt for 20 minutes, and after that 4-dimethylamino-pyridine (1 eq) and aniline or amine (0.25 eq) were added. The mixture was cooled after refluxed for 1 h, filtered, and extracted with dichloromethane. The organic part was washed with 5% HCl, 5% NaOH, 5% Na$_2$CO$_3$, water, and dried. 1b as yellow solid (86%) was obtained after evaporation and 1a, c were yielded as white solid (50% and 6%) after further purification of the crude with chromatotron.

2.3. Computational details
The interaction of pyrazine-2-carboxylic acid derivatives 1a-c with M. tuberculosis InhA protein (PDB ID: 4DRE) using molecular docking were studied. Geometry of ligands were optimized with semi-Empirical (AM1) on Hyperchem 8.0.7. and protein and ligand reference (NAI) were prepared using Molegro Virtual Docking (MVD) 5.0. Docking of pyrazine-2-carboxylic acid 1a-c was performed on MVD using MolDOck SE Algorithm to search the preferred docking pose (Number of LGA runs: 50). The ligands were docked on the same site as the standard ligand (NAI) and the docking results were visualized using MVD.

3. Result and Discussion

3.1. Chemistry
A critical issue in tuberculosis medication is the long duration of treatment (6 months) with five different types of drugs. Pyrazinamide derivatives have attracted attention and been developed as lead compounds to overcome these problems. Many pyrazinamide derivatives have been synthesized and showed promising activity against M. tuberculosis. In the present study, compounds 1a-c were prepared by using Yamaguchi esterification to avoid the use of thionyl chloride.
**Scheme 1. Synthesis of 1a-c.**

1a-c were obtained by Yamaguchi esterification according to previous paper [15] (Scheme 1). In the Yamaguchi esterification, a mixed anhydride is produced from the reaction of carboxylic acid and Yamaguchi reagent (2,4,6-trichlorobenzoyl chloride). Further reaction of the anhydride with an alcohol in the presence of stoichiometric amount of DMAP generate the esters. In the previous paper we reported the synthesis of 2-phenylethyl esters from reaction of various carboxylic acids with 2,4,6-trichlorobenzoyl chloride to form aliphatic aromatic anhydrides which upon nucleophilic acyl substitution with 2-phenylethanol furnished the desired esters [15]. Here in this paper we utilized this method to generate pyrazine 2-carboxylic acid derivatives 1a-c from a reaction of pyrazine-2-carboxylic acid with 2,4,6-trichlorobenzoyl chloride followed by reaction of the resulting anhydride with 4-dimethylamino-pyridine (1 eq) and aniline or amine. The pyrazine 2-carboxylic acid derivative 1b was yielded after evaporation of the reaction mixture whereas the derivatives 1a,c were produced after purification of the crude. The pyrazine-2-carboxylic acid derivatives (1a-c) was detected as [M+H]^+ species in ESI high resolution mass spectrometry (1a calcd 228.1137, found 228.1135; 1b calcd 220.1450, found 220.1461; 1c calcd 236.1763, found 236.1762). The IR spectra showed bands at 1659-1678 cm\(^{-1}\) and 3310-3358 cm\(^{-1}\) indicated amide carbonyl, NH-secondary groups, and the absence of hydroxyl group (Figure 2). The \(^1\)H NMR spectra recorded in CDCl\(_3\) confirmed the structure of 1a-c which showed singlet signals for NH proton at 7.75-9.62 ppm and the carbonyl carbon appeared at 160.6-162.9 ppm in \(^13\)C NMR spectra. The NMR data in details are presented in Table 1-2.

![Figure 2. FTIR of pyrazine-2-carboxylic acid derivatives 1a-c.](image-url)
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### 3.2. Molecular Docking Study

Molecular docking is an important method in drug discovery area due to its ability to model the interaction between small molecules with proteins at the atomic level. This method can be used to study the characteristics of small molecules at target binding sites as well as to explain the fundamental biochemical processes [16]. In this paper, the computational calculation was done to study the possible interaction of 1a-c with *M. tuberculosis* InhA protein (PDB ID: 4DRE). Good binding interaction of 1a-c with amino acid residues via hydrogen bonding and π-π interaction on the active site of protein was observed. Compound 1c showed best activity with lowest rerank score (-86.4047 kcal/mol) as can be seen in Table 3. The derivative 1c has three correlations with protein through hydrogen bonding (Glycine14, Gly14; Throneine39, Thr39; and Phenylalanine41, Phe41) and five connections via π-π interaction (Phe41; Leucine63, Leu63; Gly14; Thr39; and Isoleucin95, Ile95). The number of

| Table 1. 1H-NMR data of pyrazine-2-carboxylic acid derivatives (1a-c). |
|------------------|-----------------------------|-----------------------------|
| **Proton**       | **N-(4-ethylphenyl)pyrazine-2-carboxamide (1a)** | **N-cycloheptylpyrazine-2-carboxamide (1b)** | **N-octylpyrazine-2-carboxamide (1c)** |
| -CH₃             | 1.22 (t, J = 7.6 Hz, 3H)    | -                          | 0.85 (t, J = 6.8 Hz, 3H)            |
| -CH₂             | 2.63 (q, J = 7.6 Hz, 2H)    | 1.54-1.69,1.96-2.02 (m,12H) | 1.24-1.40, 1.58-1.65 (m,12H)       |
| ArH              | 7.21 (d, J = 8.4 Hz, 2H)    | 8.48 (dd, J = 2.4, 1.3 Hz, 1H) | 8.50 (dd, J = 2.4, 1.4 Hz, 1H)    |
|                  | 7.65 (d, J = 8.4 Hz, 2H)    | 8.71 (d, J = 2.4 Hz, 1H)    | 8.72 (d, J = 2.4 Hz, 1H)            |
|                  | 8.56 (dd, J = 2.4, 1.4 Hz, 1H) | 9.37 (d, J = 1.3 Hz, 1H) | 9.39 (d, J = 1.4 Hz, 1H)            |
|                  | 8.77 (d, J = 2.4 Hz, 1H)    | 9.49 (d, J = 1.4 Hz, 1H)    |                                      |
| -CH⁻              | -                           | 4.10-4.15 (m, 1H)          | -                                      |
| NH                | δ 9.62 (bs, 1H)             | 7.75 (bs, 1H)              | 7.80 (s, 1H)                          |

| Table 2. 13C-NMR data of pyrazine-2-carboxylic acid derivatives (1a-c). |
|---------------------|-----------------------------|-----------------------------|
| **Carbon**          | **N-(4-ethylphenyl)pyrazine-2-carboxamide (1a)** | **N-cycloheptylpyrazine-2-carboxamide (1b)** | **N-octylpyrazine-2-carboxamide (1c)** |
| -CH₃                | 15.8                        | 14.2                        |                                      |
| -CH₂                | 28.5                        | 24.1                        | 22.7                                 |
|                    | 28.1                        | 29.3                        | 29.3                                 |
|                    | 35.1                        | 29.3                        | 29.6                                 |
|                    | 29.3                        | 31.9                        | 39.6                                 |
| -CH⁻                | -                           | 50.5                        | -                                    |
| ArCH               | 120.0                       | 142.5                       | 142.6                                |
|                    | 128.6                       | 144.5                       | 144.5                                |
|                    | 142.5                       | 147.1                       | 147.2                                |
|                    | 144.7                       | 147.5                       |                                      |
| ArC                | 134.9                       | 144.9                       | 144.7                                |
|                    | 141.1                       | 144.6                       |                                      |
| C=O                | 160.6                       | 161.6                       | 162.9                                |

The number of
interactions of 1c is greater than with 1a-b both through hydrogen bonding and π-π interaction. Compound 1c could interact with the amino acid Thr39 of the protein through hydrogen bonding and π-π. This mode also showed by the amino acids Gly14 and Phe41. This interactions may be responsible for the lower rerank score of 1c which might correlate with the MIC value in the experimental study.

Table 3. Docking results of 1a-c with *M. tuberculosis* InhA protein (PDB ID: 4DRE).

| Compounds | Rerank Score (kcal mol⁻¹) | Interaction                      |
|-----------|---------------------------|---------------------------------|
| 1a        | -79.3431                  | H-Bond: Val65                  |
|           |                           | π-π: Gly96, Ile16, and Val65    |
| 1b        | -73.5657                  | H-Bond: Gly96                  |
|           |                           | π-π: Val65, Phe41, Gly96, and Ile95 |
| 1c        | -86.4047                  | H-Bond: Gly14, Thr39, and Phe41|
|           |                           | π-π: Phe41, Leu63, Gly14, Thr39, and Ile95 |

Figure 3. Interaction of 1c with *M. tuberculosis* InhA protein (PDB ID: 4DRE).
4. Conclusion
Pyrazine-2-carboxylic acid derivatives 1a-c were successfully synthesized by Yamaguchi method. Molecular docking study on *M. Tuberculosis* InhA protein (PDB ID: 4DRE) indicated that 1c have the lowest rerank score (-86.407 kcal mol⁻¹) and it might correspond with experimental MIC value.

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