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EXPERIMENTAL AND DENSITY FUNCTIONAL THEORY INVESTIGATION ON IMINE FORMATION USING MICROWAVE IRRADIATION

(Penyiasatan Secara Eksperimen dan Teori Fungsi Ketumpatan ke atas Penghasilan Imina Menggunakan Penyinaran Gelombang Mikro)

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Abstract

Four starting materials comprising of *N*-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide **1,** 4-amino-*N*-(4,6-dimethylpyridin-2-yl)benzamide **2**, 4-amino-2-methylbenzoic acid **3** and 4-formylbenzoic acid **4** react in a pairwise manner through the condensation reaction to give four imine derivatives, **Imine 5**, **6**, **7** and **8**. A simple method has been developed for the synthesis of these imine derivatives under microwave irradiation. In addition, these compounds were synthesised also by conventional heating procedures for comparison. All the compounds synthesised were characterised by melting point, infrared, mass spectrometry, ^{1}H and ^{13}C NMR spectroscopy. Comparison between conventional and microwave irradiation was done by comparing total reaction time and percentage yield. The results suggest that microwave-irradiation lead to higher yields within very short reaction times. Compounds **1** and **2** crystallised in the orthorhombic ($P2_12_12_1$ space group) and monoclinic ($P2_1/c$ space group) crystal systems, respectively. The nature of minimal replicator of imine **6** *via* autocatalytic reaction was calculated using density functional theory (DFT) with the combination of hybrid functional B3LYP and 6-311G(d,p) basis set. The reaction pathway facilitated with the addition of imine **6** or imine **5** equipped with complementary recognition sites of two carboxylic acids and two 4,6-dimethylamidopyridines were predicted to be thermodynamically favourable.

Keywords: imine, condensation, conventional heating, microwave irradiation, density functional theory

Abstrak

Empat bahan bermula terdiri daripada *N*-(4,6-dimetilpiridin-2-il)-4-formilbenzamida **1**, 4-amino-*N*-(4,6-dimetilpiridin-2-il)benzamida **2**, asid 4-amino-2-metilbenzoik **3** dan asid 4-formilbenzoik **4** bertindak balas secara berpasangan melalui tindak balas kondensasi untuk menghasilkan empat terbitan imina, imina **5**, **6**, **7** dan **8**. Satu kaedah mudah telah dibangunkan untuk menghasilkan terbitan imina di bawah penyinaran gelombang mikro. Di samping itu, terbitan imina ini turut dihasilkan melalui kaedah pemanasan konvensional sebagai perbandingan. Semua sebatian yang di sintesis dicirikan melalui analisis takat lebur, inframerah, spektrometri jisim, spektroskopi ^{1}H dan ^{13}C RMN. Perbandingan antara kaedah konvensional dan penyinaran gelombang mikro dilakukan dengan membandingkan masa tindak balas dan hasil peratusan. Keputusan ini mencadangkan kaedah penyinaran gelombang mikro memberikan peratusan hasil yang lebih tinggi dalam masa tindak balas yang singkat. Sebatian **1** dan **2** masing-masing menghablur dalam sistem ortorombik (kumpulan ruang $P2_12_12_1$) dan monoklinik (kumpulan ruang $P2_1/c$). Sifat replikator minimal imina **6** melalui tindak balas autokatalitik telah dikira menggunakan teori fungsi ketumpatan (DFT) dengan menggunakan gabungan asas fungsi hibrid B3LYP dan set asas 6-311G(d,p). Laluan tindak balas dibantu dengan penambahan sebatian imina **6** atau imina **5** yang dilengkapi dengan tapak pengecaman, terdiri daripada dua moieti asid karboksilik dan dua moieti 4,6-dimetilpiridina yang dijangka berada di bawah kawalan termodinamik.

Kata kunci: imina, kondensasi, pemanasan konvensional, penyinaran gelombang mikro, teori fungsi ketumpatan

Introduction

Imine derivatives typically formed through condensation between an amine and an aldehyde are an essential precursor in the pharmaceutical industry to produce dyes [1], catalysts [2], and pigments [3]. Imine represents a class of biological reactive intermediates that have been the focus of intensive toxicological research in recent years. Hence, these derivatives play a role in various biological activities such as antiviral [4], antibacterial [5], antimicrobial [6], antitumor [7], anti-inflammatory agents [8], antituberculosis [9] and anticonvulsant activity [10]. The standard method for the preparation of imine is the conventional reflux technique, which is carried out in ethanol with or without the presence of a catalyst. The molecular sieves are utilised to remove the water molecules formed in the reaction before tetramethyl orthosilicate [11] or trimethyl orthoformate are introduced as an *in-situ* method to remove the water [12]. The main drawbacks are that it involves large amounts of flammable solvents and relatively time-consuming.

Since 1996, microwave technology has been implemented in many organic synthesis explorations. Microwave irradiation significantly reduces the use of toxic solvents, as well as, the reaction time, ensuring high yields and clean product formations [13]. The ease of preparation also helps to encourage more research in preparing the imine derivatives. Souza et al. used ethanol to synthesise thiazol-2-imines *via* a one-pot reaction [14], while others used water as the solvent [15]. Additionally, a new one-pot acid-catalysed to furnish aromatic imines using a minimum amount of methanol as solvent under microwave irradiation. The reaction yields a substantial amount of imine derivatives and can also be extended to include various substrates [16]. This technique allows the condensation reactions such as imine synthesis to complete the reaction by heating the water generated [17]. The formation of imine derivatives that consists of two aromatic aldehydes and amines incorporating a recognition site of 4,6-dimethylamidopyridines and carboxylic acid has been carried out previously in our research group [18-20] utilising the classical heating, which resulted in longer reaction time and excessive use of organic solvents.

In this approach, the formation of imine derivatives was carried out under microwave irradiation in a minimum amount of organic solvents and compared with conventional synthesis based on the percentage yield and time of reaction. **Imine 5** were formed when aldehyde **1** reacted with amine **3** while **Imine 6** were obtained through condensation reaction between amine

2 and aldehyde **4** as shown in Figure 1. On the other hand, **Imine 7** utilised the condensation between aldehyde **1** and amine **2**. Similarly, aldehyde **4** was reacted with similar amine **3** to furnish **Imine 8**. Subsequently, we validated the minimal replication capability [20] of **Imine 5** and **6** using density functional theory calculation with the combination of hybrid functional B3LYP at 6-311G(d,p) basis set.

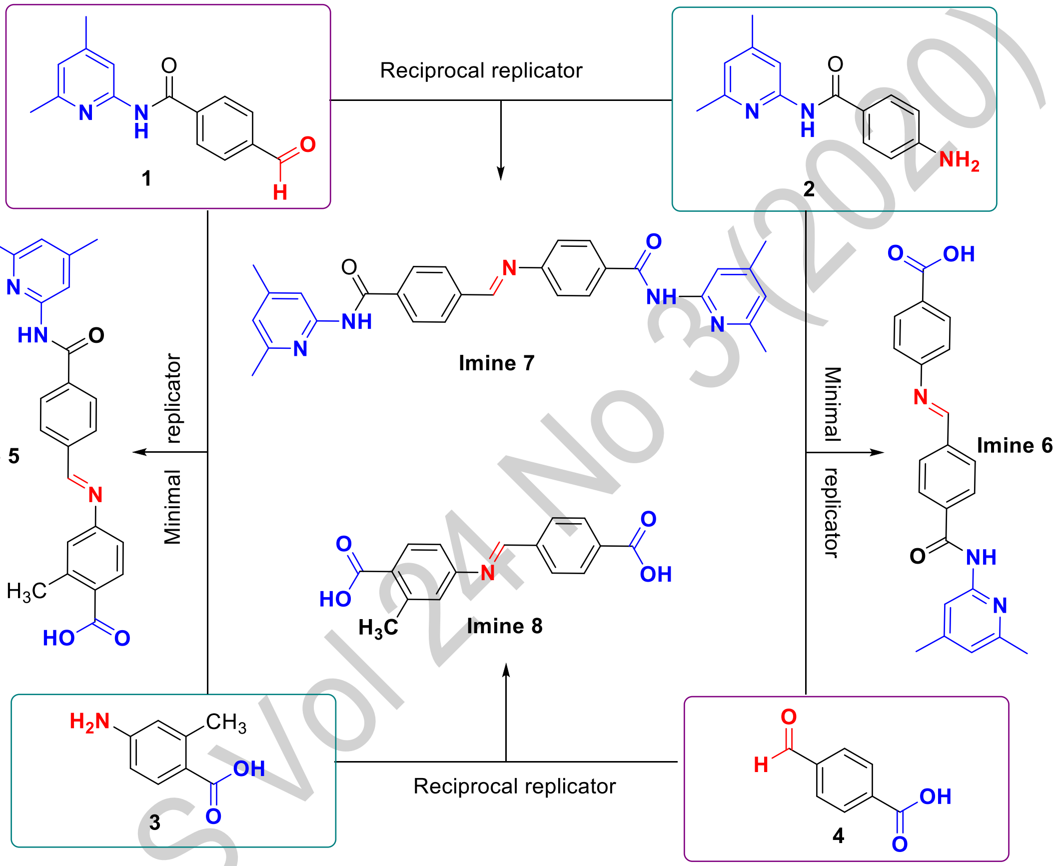


Figure 1. The schematic reaction consists of two aldehydes (**1** and **4 –** purple boxes) and two amines (**2** and **3** – emerald boxes) to obtain four imine derivatives, namely Imine **5** and **6** (minimal replicator) and Imine **7** and **8** (reciprocal replicator)

Materials and Methods

All solvents and reagents used for synthesis were of general reagent grade and used as received or otherwise purified by standard techniques. 4-amino-2-methylbenzoic acid (**3**), 4-formylbenzoic acid (**4**), 2-amino-4,6-dimethylpyridine, 4-nitrobenzoyl chloride, $Ni(OAc)_2 \cdot 4H_2O$, triethylamine, sodium borohydride ($NaBH_4$), toluene, cyclohexane, ethyl acetate, acetonitrile, dichloromethane, acetone, ethanol, hydrochloric acid, sodium hydrogen bicarbonate, magnesium sulphate, and thionyl chloride were purchased from Sigma Aldrich, Bodensen and Merck. Anhydrous dichloromethane was prepared in the presence of calcium hydride at 40 °C for 6 h before undergoing distillation under inert condition. Synthesis was carried out using modified reported procedures for microwave-assisted synthesis [21].

For comparison, conventional synthesis was also carried out using the reported procedures [19, 20]. The reactions were monitored by thin layer chromatography (TLC) with precoated silica plates (POLYGRAM® SIL G/UV254). Column chromatography was carried out on silica gel (230-400 mesh) eluting with solvents as supplied under positive pressure or compressed air. Melting points were recorded in open capillaries tube apparatus and are uncorrected. Infrared (IR) was recorded (KBr disc method) on Perkin-Elmer spectrum GX spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts were reported in ppm downfield from tetramethylsilane (TMS), which was used as the internal standard. Mass spectra were recorded on a Bruker MICROTOF-Q 86 spectrometer equipped with electrospray ionization (ESI) module in the positive ion mode. Single crystal X-ray experiments were performed on a Bruker D8 QUEST diffractometer with MoK α radiation. Microwave-assisted reactions were carried out in a domestic microwave oven (Electrolux, EMM2017X) at 360 W.

Computational methods

The quantum chemical calculations carried out in the present work were performed with the Gaussian09 program package. The molecular structure of the imine compounds as well as their duplex and ternary complex structures were fully optimised using the combination of hybrid functional B3LYP with 6-311G(d,p) basis set [22-26]. The vibrational frequency calculations were performed to ensure that the optimised structures represent the local minima where only positive real number force constants (eigen values) were present [27, 28]. The enthalpy of reaction (ΔH) and thermal energy corrections were obtained with the consideration of the vibrational mode calculation [29]. Gaussian09 program provides the sum of internal electronic energy (ε_0) and thermal enthalpies, H_{corr} (H_{corr} = E_{tot} + k_BT; k_B = Boltzmann constant = 1.380662×10^{-23} J/K). The E_{tot} expression gives the internal thermal energy due to translation (E_t), rotational (E_r), vibrational (E_v) and electronic (E_e) motions. Thus, the ΔH is simply the difference of the sums of ε_0 and H_{corr} for the reactants and the product, as given in Equation 1.

$$\Delta H\ (298\ \mathrm{K}) = \sum(\varepsilon_0 + H_{corr})\mathrm{products} - \sum(\varepsilon_0 + H_{corr})\mathrm{reactants} \tag{1}$$

Synthesis of N-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide - 1

4-formylbenzoic acid (**4**) (4.0 g, 26.6 mmol, 1.0 eq) and thionyl chloride (3.0 mL, 40.0 mmol, 1.5 eq) were added at 110 °C for 24 hours in toluene (100 mL) to obtain brownish precipitate. The precipitate was subsequently washed with toluene for three times to remove the remaining chloride before dried under vacuum to yield brownish solid, 4-formylbenzoyl chloride (4.4 g, 97%). The 4-formylbenzoyl chloride (1.0 g, 5.9 mmol, 1.0 eq) obtained was then used without any purification to react with 2-amino-4,6-dimethylpyridine (0.7 g, 5.9 mmol, 1.0 eq) in the presence of Et_3N (900 µL, 6.53 mmol, 1.1 eq) at 0 °C. The reaction was then left stirred at room temperature for 48 hours in anhydrous CH_2Cl_2 (14 mL) under N_2 gas. The resulting brownish solution obtained was then quenched with distilled water. The organic layer was then extracted with 1 M HCl and saturated $NaHCO_3$ solution before dried with anhydrous $MgSO_4$, filtered and dried under vacuum before purified using column chromatography (CHex:EtOAc, 3:2) to obtain colourless powder, *N*-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide **1** (67%). Colourless solid; m.p.: 109 - 110 °C; FT-IR (KBr, cm^{-1}) 3331, 2920, 2857, 1678, 1543, 1425 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.13 (1H, s, C*H*O), 8.67 (1H, br s, CON*H*), 8.11 (2H, d, $^3J_{HH}$ 8.0 Hz, 2×Ar*H*), 8.06 (1H, s, Ar*H*), 8.03 (2H, d, $^3J_{HH}$ 8.0 Hz, 2×Ar*H*), 6.83 (1H, s, Ar*H*), 2.45 (3H, s, CH_3), 2.40 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 191.5 (*C*HO), 164.5 (*C*=O), 156.4 (Ar*C*), 150.6 (Ar*C*), 150.4 (Ar*C*), 139.5 (Ar*C*), 138.6 (Ar*C*), 130.0 (Ar*C*H), 127.9 (Ar*C*H), 121.1 (Ar*C*H), 111.8 (Ar*C*H), 23.8 (*C*H_3), 21.4 (*C*H_3); MS (ESI) m/z calculated for $C_{15}H_{14}N_2O_2$ $[M + Na]^+$ 277.1055, found 277.1022.

Synthesis of 4-amino-N-(4,6-dimethylpyridin-2-yl)benzamide - 2

2-amino-4,6-dimethylpyridine reacted with 4-nitrobenzoyl chloride (3.0 g, 16.2 mmol) to yield *N*-(4,6-dimethylpyridin-2-yl)-4-nitrobenzamide (4.2 g, 15.5 mmol, 97 %) as intermediate compound. Subsequently, the nitro compound obtained (0.3 g, 1 mmol, 1.0 eq) was then dissolved in wet acetonitrile (CH_3CN:H_2O ; 3.0 mL:0.3 mL) before the addition of $Ni(OAc)_2{\cdot}4H_2O$ (0.049 g, 0.2 mmol, 0.2 eq). The reaction was stirred for 5 minutes before further reduced using $NaBH_4$ (0.151 g, 4.0 mmol). The black precipitate was immediately deposited and left stirred for 20 minutes while being monitored using thin layer chromatography (TLC) before quenched with distilled water. The reaction mixture extracted with CH_2Cl_2 (8 mL × 3), dried using anhydrous $MgSO_4$ and purified using column chromatography (CHex:EtOAc; 3:2) to yield 4-amino-*N*-(4,6-dimethylpyridin-2-yl)benzamide **2** (54%). Yellowish solid; m.p.: 132 - 133°C; FT-IR (KBr, cm^{-1}) 3461, 3350, 1606, 1446, 1283 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.43 (1H, s; CON*H*), 8.05 (1H, s; Ar*H*), 7.78 (2H, d, $^3J_{HH}$ 8.4 Hz; 2×Ar*H*), 6.76 (1H, s; Ar*H*), 6.72 (2H, d, $^3J_{HH}$ 8.4 Hz; 2×Ar*H*), 4.09 (2H, s; NH_2), 2.44 (3H, s; CH_3), 2.36 (3H, s; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.3 (*C*=O), 155.9 (Ar*C*), 151.2 (Ar*C*), 150.4 (Ar*C*), 150.3 (Ar*C*), 129.2 (Ar*C*H), 123.5 (Ar*C*), 120.2 (Ar*C*H), 114.2 (Ar*C*H), 111.6 (Ar*C*H), 23.7 (*C*H$_3$), 21.4 (*C*H$_3$); MS (ESI) m/z calculated for $C_{14}H_{15}N_3O$ $[M + Na]^+$ 264.1215, found 264.1169.

Synthesis of (E)-4-((4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene)amino)-2-methylbenzoic acid - Imine 5

N-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide (**1**) (0.05 mmol) and 4-amino-2-methylbenzoic acid (**3**) (0.05 mmol) were sonicated in acetone (1 mL) for 1 minute to mix the mixture well in a reaction vial before it was placed in a microwave oven. The mixture was then irradiated at 360 W for 5 minutes. After the reaction was completed, chilled ethanol (2 mL) was added into the reaction mixture. The resulting precipitate was filtered off and dried under vacuum. Likewise, *N*-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide (**1**) (0.5 mmol) and 4-amino-2-methylbenzoic acid (**3**) (0.5 mmol) were dissolved in acetone (5 mL) and refluxed for 5 hours. The reaction mixture was then evaporated to approximately half its original volume, and the resulting imine **5** was filtered, washed with chilled ethanol and dried under vacuum. The compound was obtained as yellowish powder in 94% yield (microwave irradiation), 93% yield (conventional method); m.p.: 284 - 285 °C; FT-IR (KBr, cm^{-1}) 3273, 2491, 1679, 1629, 1581, 1447 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ_H 12.68 (1H, br s; COO*H*), 10.81 (1H, s; CON*H*), 8.73 (1H, s; C*H*=N), 8.15 (2H, d, $^3J_{HH}$ 8.4 Hz; 2×Ar*H*), 8.05 (2H, d, $^3J_{HH}$ 8.4 Hz; 2×Ar*H*), 7.92 (1H, d, $^3J_{HH}$ 8.0 Hz; Ar*H*), 7.90 (1H, s; Ar*H*), 7.20 (1H, s; Ar*H*), 7.18 (1H, d, $^3J_{HH}$ 8.4 Hz; Ar*H*), 6.90 (1H, s; Ar*H*), 2.58 (3H, s; CH_3), 2.42 (3H, s; CH_3), 2.32 (3H, s; CH_3); ^{13}C NMR (100 MHz, d_6-DMSO) δ_C 168.7 (*C*OOH), 165.6 (*C*ONH), 161.8 (*C*=N), 156.7 (Ar*C*), 152.8 (Ar*C*), 149.6 (Ar*C*), 142.4 (Ar*C*), 141.5 (Ar*C*), 139.7 (Ar*C*), 138.5 (Ar*C*), 133.5 (Ar*C*H), 129.7 (Ar*C*H), 129.2 (Ar*C*H), 124.4 (Ar*C*), 120.8 (Ar*C*H), 116.2 (Ar*C*H), 112.7 (Ar*C*H), 110.9 (Ar*C*H), 23.8 (*C*H$_3$), 22.8 (*C*H$_3$), 21.3 (*C*H$_3$); MS (ESI) m/z calculated for $C_{23}H_{21}N_3O_3$ $[M + H]^+$ 388.1583, found 388.1492.

Synthesis of (E)-4-((4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene)amino)benzoic acid - Imine 6

A mixture of 4-amino-*N*-(4,6-dimethylpyridin-2-yl)benzamide (**2**) (0.05 mmol) and 4-formylbenzoic acid (**4**) (0.05 mmol) was sonicated in acetone (1 mL) for 1 minute to mix it well in a reaction vial before it was placed in a microwave oven. The mixture was then irradiated at 360 W for 5 minutes. After the reaction was completed, chilled ethanol (2 mL) was added into the reaction mixture. The resulting precipitate was filtered off and dried under vacuum. Likewise, 4-amino-*N*-(4,6-dimethylpyridin-2-yl)benzamide (**2**) (0.5 mmol) and 4-formylbenzoic acid (**4**) (0.5 mmol) were dissolved in acetone (5 mL) and refluxed for 5 hours. The reaction mixture was then evaporated to approximately half its original volume, and the resulting imine **6** was filtered, washed with chilled ethanol and dried under vacuum. The compound was obtained as yellowish powder in 93% yield (microwave irradiation), 82% yield (conventional method); m.p.:

294 - 296 °C; FT-IR (KBr, cm^{-1}) 3280, 2457, 1688, 1630, 1602, 1453 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO) δ_H 13.32 (1H, br s; COO*H*), 10.63 (1H, s; CON*H*), 8.78 (1H, s; C*H*=N), 8.13 (2H, d, $^{3}J_{HH}$ 8.0 Hz; 2×Ar*H*), 8.10 (4H, m; 4×Ar*H*), 7.91 (1H, s; Ar*H*), 7.38 (2H, d, $^{3}J_{HH}$ 8.0 Hz; 2xAr*H*), 6.87 (1H, s; Ar*H*), 2.41 (3H, s; C*H*$_3$), 2.32 (3H, s; C*H*$_3$); ^{13}C NMR (100 MHz, d_6-DMSO) δ_C 167.1 (*C*OOH), 165.9 (*C*ONH), 161.9 (*C*=N), 156.4 (Ar*C*), 152.9 (Ar*C*), 152.5 (Ar*C*), 149.1 (Ar*C*), 139.3 (Ar*C*), 136.2 (Ar*C*), 132.2 (Ar*C*), 130.4 (Ar*C*H), 130.1 (Ar*C*H), 130.0 (Ar*C*H), 121.4 (Ar*C*), 113.0 (Ar*C*H), 112.3 (Ar*C*H), 23.9 (*C*H$_3$), 21.3 (*C*H$_3$).

Synthesis of (E)-N-(4,6-dimethylpyridin-2-yl)-4-((4-((4,6-dimethylpyridin-2-yl)carbamoyl)benzylidene) amino)benzamide - Imine 7

N-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide (**1**) (0.05 mmol) and 4-amino-*N*-(4,6-dimethylpyridin-2-yl)benzamide (**2**) (0.05 mmol) were sonicated in acetone (1 mL) for 1 minute to mix the mixture well in a reaction vial before it was placed in a microwave oven. The mixture was then irradiated at 360 W for 5 minutes. After the reaction was completed, chilled ethanol (2 mL) was added into the reaction mixture. The resulting precipitate was filtered off and dried under vacuum. Likewise, a mixture of 4-amino-*N*-(4,6-dimethylpyridin-2-yl)benzamide (**2**) (0.5 mmol) and *N*-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide **1** (0.5 mmol) was dissolved in acetone (5 mL) and refluxed for 5 hours. The reaction mixture was then evaporated to approximately half its original volume, and the resulting imine **7** was filtered, washed with chilled ethanol and dried under vacuum. The compound was obtained as yellowish powder in 71% yield (microwave irradiation), 64% yield (conventional method); m.p.: 211 - 212 °C; FT-IR (KBr, cm^{-1}) 3413, 1686, 1680, 1575, 1449 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO) δ_H 10.83 (1H, s; CON*H*), 10.66 (1H, s; CON*H*), 8.77 (1H, s; C*H*=N), 8.16 (2H, d, $^{3}J_{HH}$ 8.0 Hz; 2×Ar*H*), 8.13 (1H, s; Ar*H*), 8.11 (1H, s; Ar*H*), 8.07 (2H, d, $^{3}J_{HH}$ 8.0 Hz; 2×Ar*H*), 7.90 (2H, m), 7.39 (2H, d, $^{3}J_{HH}$ 8.0 Hz; 2×Ar*H*), 6.89 (1H, s; Ar*H*), 6.88 (1H, s; Ar*H*), 2.41 (3H, s; C*H*$_3$), 2.31 (3H, s; C*H*$_3$); ^{13}C NMR (100.6 MHz, d_6-DMSO): δ_C 165.8 (*C*=O), 165.7 (*C*=O), 161.9 (*C*=N), 156.7 (Ar*C*), 156.6 (Ar*C*), 154.6 (Ar*C*), 152.1 (Ar*C*), 151.9 (Ar*C*), 149.5 (Ar*C*), 149.4 (Ar*C*), 138.9 (Ar*C*), 137.2 (Ar*C*), 132.1 (Ar*C*H), 129.8 (Ar*C*H), 129.1 (Ar*C*H), 128.9 (Ar*C*H), 121.4 (Ar*C*H), 120.7 (Ar*C*H), 120.5 (Ar*C*H), 112.7 (Ar*C*H), 23.9 (*C*H$_3$), 21.3 (*C*H$_3$); MS (ESI) m/z calculated for $C_{29}H_{27}N_5O_2$ $[M + Na]^+$ 500.2165, found 500.1868.

Synthesis of (E)-4-(((4-carboxyphenyl)imino)-methyl)-2-methylbenzoic acid - Imine 8

A mixture of 4-amino-2-methylbenzoic acid **3** (0.05 mmol) and 4-formylbenzoic acid (**4**) (0.05 mmol) was sonicated in acetone (1 mL) for 1 minute to mix it well in a reaction vial before it was placed in a microwave oven. The mixture was then irradiated at 360 W for 5 minutes. After the reaction was completed, chilled ethanol (2 mL) was added into the reaction mixture. The resulting precipitate was filtered off and dried under vacuum. Likewise, a mixture of 4-amino-2-methylbenzoic acid **3** (0.5 mmol) and 4-formylbenzoic acid (**4**) (0.5 mmol) was dissolved in acetone (5 mL) and refluxed for 5 hours. The reaction mixture was then evaporated to approximately half its original volume, and the resulting imine **5** was filtered, washed with chilled ethanol and dried under vacuum. The compound was obtained as yellowish powder in 93% yield (microwave irradiation), 80% yield (conventional method); m.p.: 334 - 337 °C; FT-IR (KBr, cm^{-1}) 2667, 1693, 1645, 1584, 1455 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO) δ_H 12.93 (2H, br s; 2×COO*H*), 8.73 (1H, s; C*H*=N), 8.08 – 8.07 (4H, m; 4×Ar*H*), 7.91 (1H, d, $^{3}J_{HH}$ 8.0 Hz; Ar*H*), 7.20 (1H, s; Ar*H*), 7.18 (1H, d, $^{3}J_{HH}$ 8.4 Hz; Ar*H*), 2.58 (3H, s; C*H*$_3$); ^{13}C NMR (100 MHz, d_6-DMSO) δ_C 167.3 (*C*OOH), 167.0 (*C*OOH), 161.8 (*C*=N), 152.8 (Ar*C*), 141.5 (Ar*C*), 133.5 (Ar*C*H), 132.3 (Ar*C*), 130.4 (Ar*C*H), 130.2 (Ar*C*H), 130.0 (Ar*C*H), 129.4 (Ar*C*H), 124.4 (Ar*C*), 118.8 (Ar*C*H), 21.9 (*C*H$_3$); MS (ESI) m/z calculated for $C_{16}H_{13}NO_4$ $[M + H]^+$ 284.0845, found 284.0809.

Results and Discussion

Four imine derivatives were prepared under microwave irradiation and the percentage yield was compared with conventional heating methods as indicated in Table 1. The yields of the synthesised imine **5**, **6** and **8** have been increased nearly 10% under microwave irradiation within 5 min of reaction time. However, the percentage yield of imine **7** gave the lowest yield, presumably due to steric factors [30]. From the ^1H NMR data for all of the imine derivatives, there was a characteristic proton signal at around 8.73 to 8.78 ppm indicated the imine group's proton (*H*C=N), while ^{13}C NMR showed the presence of the carbon signal at 161.2 ppm from the imine group (C=N).

Compounds **1** and **2** have been described earlier [19], though no information on the crystal structure has been reported so far. The molecular structure of *N*-(4,6-dimethylpyridin-2-yl)-4-formylbenzamide **1** and 4-amino-*N*-(4,6-dimethylpyridin-2-yl)benzamide **2** with the atomic numbering schemes are shown in Figure 2(a) and 2(b), respectively. The asymmetric unit of **1** contains a water solvent molecule, which was omitted for clarity purposes. The crystals were crystallised separately from CH_2Cl_2 through a slow evaporation technique. Compound **1** and **2** crystallised in the orthorhombic ($P2_12_12_1$ space group) and monoclinic ($P2_1/c$ space group) crystal system, respectively. The crystal data and parameter of structure refinement for **1** and **2** are given in Table 2.

In the molecular structure of **1**, the plane of pyridine (N2/C9/C10/C11/C12/C13) and the benzene moieties (C1/C2/C3/C4/C5/C6) are substantially planar and slightly twisted to each other by a minor deviation angle of 2.9 (2)º. The former aromatic ring adopted a *cis* configuration for the carbonyl, O atom, across the N1−C8 bond. Besides, both the aromatic rings are positioned *trans* against each other along with the same reference bond, N1−C8. The *co*-planar situation with regards to both of the aromatic rings was likely attributed to the presence of intramolecular hydrogen bond formation *via* C10−H10···O2, which led to the construction of a pseudo-six-membered ring (C10/H10/O2/C8/N1/C9). Concurrently, an inter-molecular hydrogen bonding connecting **1** with a water molecule, was also observed in the crystal lattice, N1−H1A···O1W, forming a one-dimensional column viewed along the *a*-axis [Figure 2(a)]. The presence of water adducts was due to the loose packing arrangement of the molecules [31].

Based on Figure 2(b), the asymmetric unit consists of two **2** molecules and two water solvent molecules (omitted for clarity). Similar to compound **1**, the two molecules of **2** possessed both the pyridine and benzene rings. However, the mean plane angles of both aromatic rings for molecule A [17.8268 (6)°] and B [16.6744 (7)°] are deviated much more compared to that of compound **1**. Congruently, the bond length of intramolecular connections *via* C1A-H1A···O1A [2.307 Å (molecule A)] and C1B-H1B···O1B [2.347 Å (molecule B)] are also much longer compared to C10-H10···O2 (2.264 Å) of compound **1**. Nevertheless, both the aromatic rings are still positioned *trans* against each other along the N2A-C8A and N2B-C8B bond for molecule A and B, respectively. Water molecules are involved in the construction of the supramolecular structure **2** that propagates in a network viewed along the *b*-axis [Figure 3(b)]. The bond lengths and angles corresponding to the secondary amide linkage in compounds **1** and **2** agree with related structures, for example, *R*-fluoro-*N*-(pyridyl)benzamide (*R* = *para*-/*meta*-/*ortho*-) [32].

It has been shown that similar imine compounds bearing both amidopyridine and carboxylic acid active sites tend to self-generate *via* a series of dynamic replication cycle [20]. Besides, such a reaction mechanism was also shown to be kinetically favourable compared to the direct bimolecular formation of imine [19, 33]. Herein, we explore the enthalpies and geometrical characteristics involved through the quantum chemical calculation to validate the replicating pathways. Figure 4 demonstrates a typical autocatalytic reaction cycle, which involves the self-replication of imine **6** *via* its own-template or the use of imine **5**-template.

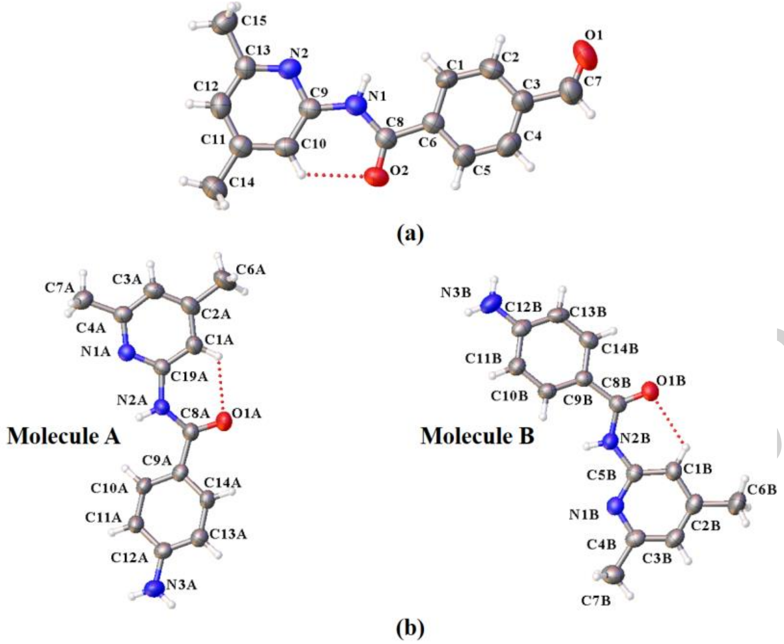
Table 1. The comparison in yield and reaction time between conventional heating method and microwave irradiation

Imine	**Conventional Method**		**Microwave Irradiation**		
	Time (hours)	**Yield (%)[a]**	**Time (minutes)**	**Power (W)**	**Yield (%)**
5	5	93	5	360	94
6	5	82	5	360	93
7	5	64	5	360	71
8	5	80	5	360	93

[a]the yield of the reflux method is comparable to the previous literature [19-20]

Table 2. Crystal data and parameter of structure refinement for compounds **1** and **2**

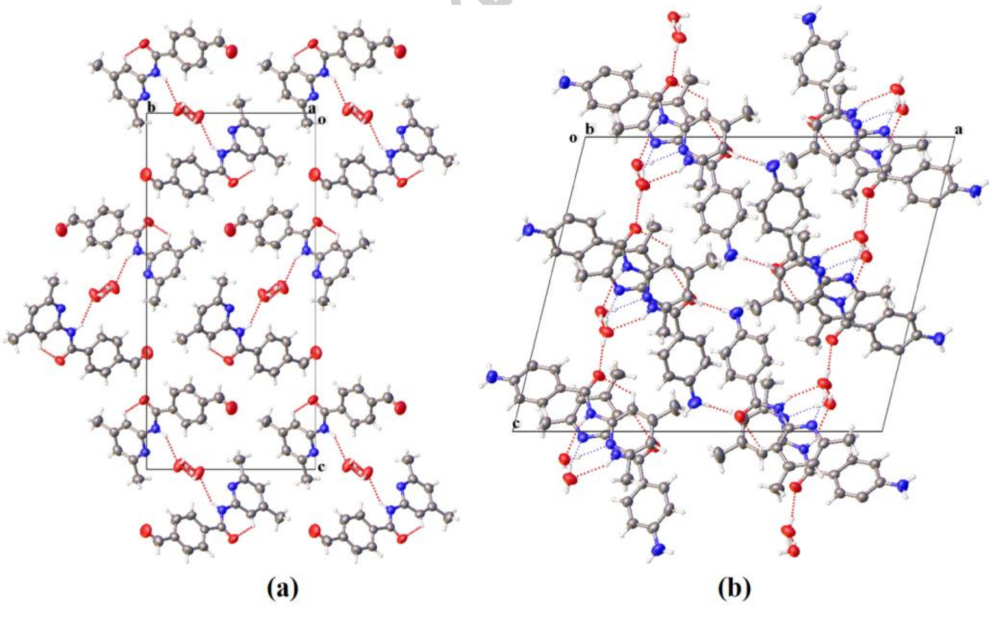
Parameter	**1**	**2**
Empirical formula	$C_{15}H_{16}N_2O_3$	$C_{28}H_{33}N_6O_4$
Molar mass	272.30	517.60
Temperature, T	302(2)K	303(2)K
Crystal system	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$P2_1/c$
Unit cell dimension	a = 4.548(2) Å b = 12.012(6) Å c = 25.347(11) Å	a = 21.2704(9) Å b = 7.6084(3) Å, β = 103.871(2) ° c = 17.4597(7) Å
Volume	1384.8(11) $Å^3$	2743.17(19) $Å^3$
Density, Z	1.306 g/cm^3, 4	1.253 g/cm^3, 4
Absorption coefficient	0.092 mm^{-1}	0.086 mm^{-1}
F(000)	584	1100
Theta range for data collection	2.95 to 25.49 °	2.85 to 28.33°
Index ranges	-5<=h=<4, -9<=k=<14, -19<=l=<30	-26<=h=<-28, -10<=k=<10, -23<=l=<23
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	1550 / 2 / 195	6780 / 4 / 363
Final R indices [I>2sigma(I)]	R1 = 0.0600, wR2 = 0.1563	R1 = 0.0633, wR2 = 0.1332



(a)

(b)

Figure 2. The asymmetric unit of compound (a) **1** and (b) **2**, showing the atom-labelling scheme. Displacement ellipsoids are drawn at 50 % probability level. Intramolecular interactions are shown as dotted lines.



(a)

(b)

Figure 3. A partial view of the crystal packing of compound (a) **1** and (b) **2**, displaying the crystal packing, viewed along with the *a*- and *b*-axis, respectively. The hydrogen-bonding interactions are shown as dotted lines

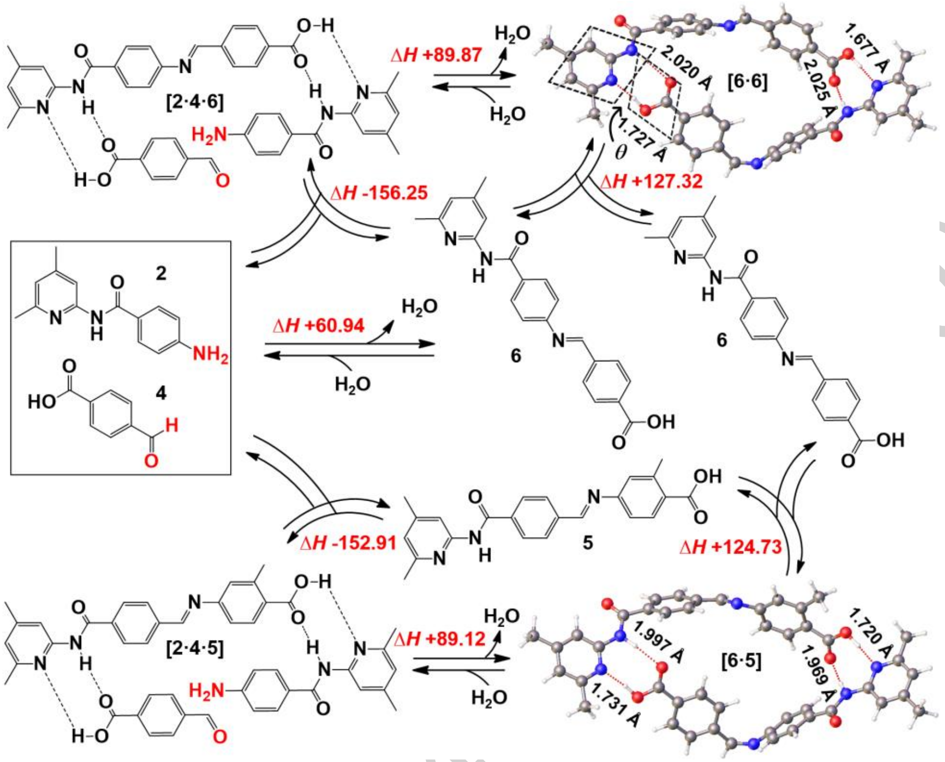


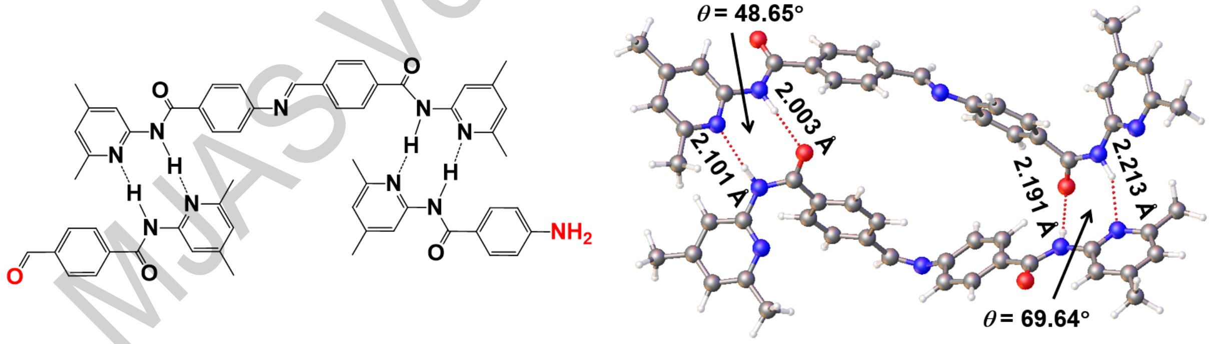
Figure 4. The formation of imine **6** *via* direct condensation of **2** and **4**, as well as through thermodynamically [Δ*H* (kJ/mol)] favourable autocatalytic cycles mediated by ternary complexes **[2·4·6]** or **[2·4·5]**. The optimised [B3LYP/6-311G(d,p)] duplex complexes **[6·6]** and **[6·5]** are shown along with the measured intermolecular hydrogen bond (red dotted lines) lengths. The angle, *θ* represents the degree of deviation between the mean planes of the amidopyridines and carboxylic acids

Conventionally, compounds **2** and **4** can undergo a reversible reaction to form imine **6,** and it is deemed to have taken place at the initial stages of the reaction. Our theoretical investigation on the condensation process suggests that such a pathway entails an endothermic process with the enthalpy of reaction (Δ*H* = +60.94 kJ/mol). Then, the synthesised imine **6** can act as a template for its subsequent formation. The scouring and binding of compounds **2** and **4** *via* the reciprocal sites on the imine **6** engender the formation of a ternary complex **[2·4·6]** (Δ*H* = -156.25 kJ/mol) with less net energy than the reactants did at the start of the process.

It has been reported that the lowest energy geometry is related to the *co*-planar arrangement of the two binding fragments (*θ* = 0°), which creates a strong intermolecular hydrogen bonding between the recognition sites [23]. Based on the **[6·6]** complex structure, the mean plane angles of the two associated binding sites involving the amidopyridines and carboxylic acids are not planar and are twisted by an average angle of (*θ* = 47.06°). The distorted duplex complex ultimately dissociates itself from the steric conformation and completes the autocatalytic cycle.

We also envisaged that imine **5** might act as a similar template for the autocatalytic formation of amine 6 shown in Figure **4**. The optimised geometry of the imine **5** compound revealed that both recognition sites, amidopyridine, and carboxylic acid are placed within the suitable spacer length as of imine **6**, to enable the interaction between the two recognition sites through intermolecular hydrogen bonding and facilitate the formation of imine **6**. In this case, the formation of a ternary complex **[2·4·5]** is also feasible, and the exothermic reaction accounts for ΔH = -152.91 kJ/mol. The eventual condensation step (ΔH = +89.12 kJ/mol) develops into a duplex complex **[6·5]**. Likewise, the mean plane angles of the corresponding recognition fragments are also similarly twisted with the average angle (θ = 46.37°). Noteworthily, the net enthalpy involved in the template-mediated pathway, either with imine **6** or **5** as the template, adds up to the same with the direct bimolecular mechanism, which is an endothermic process. The strong intermolecular hydrogen bond interactions observed in the duplex complexes following the hydrogen bond criterion [34]. Nevertheless, the use of a catalytic template-**5** in the synthesis of imine **6** is considered redundant since the latter can undertake a self-replicating route and catalyses its formation, corroborated by a high percentage yield (82-93%) for the reaction.

Imine **7** is devoid of the carboxylic group, which can complement and attract an amidopyridine group. Interestingly, the synthesis of imine **7** reflects the lowest percentage yield (64-71%) compared to the rest, regardless of the synthetic methods used. To access the likelihood of imine **7** adapting a self-replicating mechanism for its formation, we have performed DFT calculation to identify the possible binding modes. We found that the first possible binding mode involves only the N-H···N, hydrogen bond of amidopyridine (Figure 5a), while the other feasible mode is *via* the amide [C(O)NH] linkage (Figure 5b). Unfortunately, the former mode promotes a counter-positioned aldehyde and amine group of the reactants that impede the condensation process to form imine **7**. The second mode of binding is also undesirable since the intermolecular hydrogen bonds are elongated, and the distances are extended further as compared to the situation involving imine **6**. Moreover, the mean planes of similar binding sites are severely twisted, 48.65-69.64° suggesting that the autocatalytic mechanism is not preferably viable for an efficient self-replicating reaction.



(a) (b)

Figure 5. The chemical structure of the ternary complex (a) and the DFT optimised [B3LYP/6-311G(d,p)] duplex complex (b) involving the formation of imine **7**. The former assumes only the N-H···N intermolecular hydrogen bonding, while the latter participates in a reciprocal interaction *via* N-H···N and N-H···O

Conclusion

In summary, we have demonstrated the synthesis of four imine derivatives utilising both microwave irradiation and conventional heating procedure. The microwave irradiation method is proven to increase the yield by approximately 10% higher than conventional synthesis within very short reaction times. The improvement in yield is significant considering acceleration of the chemical reaction can be accelerated from hours to minutes, thus providing quick results. Besides, the molecular structures of compounds **1** and **2**, possessing an orthorhombic and monoclinic system, respectively, were successfully described with the X-ray crystallographic method. Based on the DFT studies, the capability of imine **5** and **6** to establish an autocatalytic reaction with the participation of recognition sites involving the aminopyridine and carboxylic acid is corroborated. On the contrary, imine **7** has a low percentage yield, which is justified by the lack of reciprocal recognition sides for an efficient self-replicating reaction.

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