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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF AZO AND ASPIRIN-AZO DERIVATIVES

(Sintesis dan Aktiviti Antibakteria Terhadap Azo dan Terbitan Azo-Aspirin)

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Abstract

A series of azo derivatives (**1a-i**) were synthesized *via* coupling reaction with of overall yield 58 – 72% while aspirin-azo derivatives (**2a-i**) were prepared by esterification reaction of aspirin and azo derivatives (**1a-i**) with overall yield 38 – 75%. In this study, the structures of synthesized compounds were characterized using elemental analysis (CHN), nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) and Fourier Transform Infrared (FTIR) spectroscopy. The synthesized compounds were tested on antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* S48/81 *via* turbidimetric kinetic method. The azo derivative–substituted fluorine, **1f** showed the highest antibacterial activities against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* S48/81 compared with other synthesized compounds. However, synthesized aspirin–azo derivatives (**2a-i**) showed weak antibacterial activity against tested bacteria due to bulky molecular structure thus hindered the penetration into bacterial cell wall.

Keywords: aspirin, azo derivatives, turbidimetric kinetic, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* S48/81

Abstrak

Satu siri terbitan azo **(1a-i)** telah dihasilkan melalui tindak balas gandingan dengan hasil keseluruhan 58 – 72% dan terbitan azo-aspirin **(2a-i)** telah disediakan melalui tindak balas esterifikasi aspirin dan terbitan azo **(1a-i)** dengan hasil keseluruhan 38 – 75%. Dalam kajian ini, struktur sebatian yang dihasilkan dicirikan menggunakan analisis unsur (CHN), resonans magnetik nukleus (^{1}H NMR dan ^{13}C NMR) dan spektroskopi inframerah transformasi Fourier . Kesemua sebatian yang dihasilkan telah diuji pada aktiviti anti-bakteria terhadap *Escherichia coli* ATCC 25922 dan *Staphylococcus aureus* S48 / 81 melalui kaedah kinetik turbidimetrik. Terbitan azo tertukarganti fluorin **1f** menunjukkan aktiviti antibakteria tertinggi terhadap *Escherichia coli* ATCC 25922 dan *Staphylococcus aureus* S48/81 berbanding dengan sebatian lain. Walau bagaimanapun, terbitan azo aspirin **(2a-i)** menunjukkan aktiviti anti-bakteria yang lemah terhadap bakteria diuji disebabkan oleh struktur molekul yang besar itu telah menghalang penembusan ke dalam dinding sel bakteria.

Kata kunci: aspirin, terbitan azo, kinetik turbidimetrik, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* S48/81

Introduction

Aspirin is a white crystalline weak acidic product that has analgesic and anti-inflammatory properties [1, 2]. It is also used to prevent cardiovascular disease and cancer. The compound has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels [3]. However, aspirin may cause some side effects such as vomiting and

stomach bleeding after prolonged usage [4]. Research on aspirin has been studied extensively. Chemical modification of aspirin and its derivatives have improved its pharmacological properties with less gastrointestinal toxicity [5]. Aspirin derivatives were also reported to apply many biological activities such as antibacterial [6], antithrombic [7], antiplatelet and anticancer properties [8].

Azo is a compound which consist of either an aryl or alkyl group functional group (R-N=N-R'). The most stable azo drivatives contain two aryl groups. The presence of N=N functional group is claimed to contribute to various applications in food [9], paints [10] and cosmetics [11]. In addition, azo derivatives have been reported to play important roles in many biological processes such as antibacterial [12], antifungal [13], antiviral [14] and anticancer activities [15], which stimulate the interest in the synthesis of a series compound containing aspirin with azo moiety.

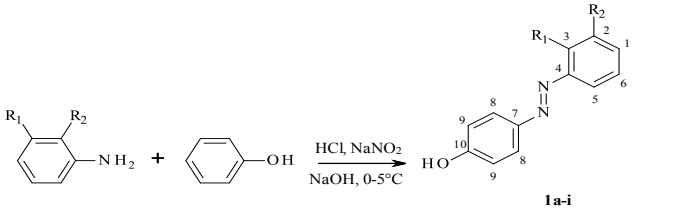
In this paper, we report the synthesis of aspirin-azo derivatives **2a-i** by reaction of aspirin with azo derivatives **1a-i** *via* esterification. The preparation of halogenated azo derivatives **1a-i** was carried out *via* diazotiation followed by coupling reaction. The antibacterial activity of the synthesized azo derivatives **1a-i** and aspirin-azo derivatives **2a-i** against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* S48/81 have also been characterized.

Materials and Methods

Melting point was measured by Stuart SMP3 melting point apparatus and the elemental analysis was determined by flash EA1112 analyzer. IR spectra (ν/cm^{-1}) were recorded on a Perkin Elmer GX spectrometer with potassium bromide pellet (KBr). ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz on a JEOL.ECA NMR spectrometer.

General method to synthesis of azo derivatives 1a-i

The general method for synthesizing azo derivatives **1a-i** was shown in (Scheme 1). A mixture of HCl (2 M, 6 mL) and aniline derivatives (0.46 g, 5 mmol) was added with sodium nitrite solution (1 M, 2 mL) in water at 0 –5 °C slowly. Sodium hydroxide (0.4 g, 10 mmol) and phenol (0.47 g, 5 mmol) dissolved in water (10 mL) stirred and cooled to 0 – 5 °C. The diazo salt compound was added slowly into phenol solution at 0 – 5 °C and stirred for 40 minutes. The mixture was acidified by adding 2 M HCl. The precipitate formed was filtered and washed with cold water. The crude product was purified by crystallization from hot ethanol to give **1a-i**.



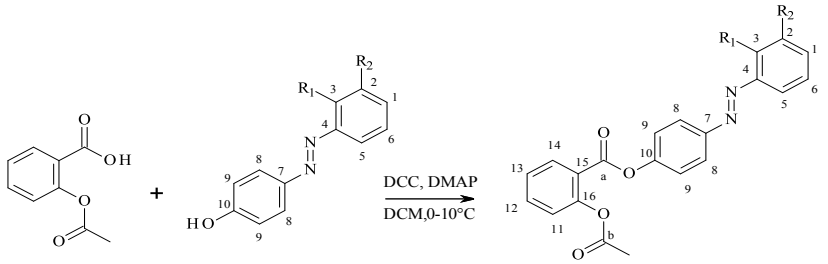
1a, R_1=H, R_2=H
1b, R_1=F, R_2=H
1c, R_1=Cl, R_2=H
1d, R_1=Br, R_2=H
1e, R_1=I, R_2=H
1f, R_1=H, R_2=F
1g, R_1=H, R_2=Cl
1h, R_1=H, R_2=Br
1i, R_1=H, R_2=I

Scheme 1. Synthesis of azo derivatives **1a-i**

General method to synthesis of aspirin-azo derivatives 2a-i

The aspirin-azo derivatives **2a-i** can be synthesized from the reaction of aspirin and azo derivatives **1a-i** *via* esterification reaction (Scheme 2). Azo derivatives **1a-i**, (3 mmol) was dissolved in dichloromethane (20 mL) and added into the flask a solution containing ***acetylsalicylic acid (0.54 g, 3 mmol) in*** dichloromethane (20 mL) under ice batch. The mixture was added with dicyclohexylcarbodiimide (DCC) (0.62, 3 mmol) followed by N,N-dimethyl-4-aminopyridine (DMAP) (0.37 g, 3 mmol). The reaction mixture was stirred for 4 hours at 0 – 10 ˚C and product formation was accompanied by thin layer chromatography (TLC) analysis using ethyl acetate/hexane (1:4).

The white precipitate of by-product (dicyclohexylurea) was filtered off under suction. The filtrate was allowed to be evaporated under vacuum to form precipitate. The precipitate was purified by flash column chromatography on silica gel, using hexane as eluent. The product formed was recrystallized from hot ethanol to give **2a-i**.



1a-i

1a, R_1=H, R_2=H
1b, R_1=F, R_2=H
1c, R_1=Cl, R_2=H
1d, R_1=Br, R_2=H
1e, R_1=I, R_2=H
1f, R_1=H, R_2=F
1g, R_1=H, R_2=Cl
1h, R_1=H, R_2=Br
1i, R_1=H, R_2=I

2a-i

2a, R_1=H, R_2=H
2b, R_1=F, R_2=H
2c, R_1=Cl, R_2=H
2d, R_1=Br, R_2=H
2e, R_1=I, R_2=H
2f, R_1=H, R_2=F
2g, R_1=H, R_2=Cl
2h, R_1=H, R_2=Br
2i, R_1=H, R_2=I

Scheme 2. Synthesis of aspirin-azo derivatives **2a-i**

Antibacterial screening

The antibacterial activities of the synthesized compounds were studied against *E. coli* and *S. aureus*. *Escherichia coli* was cultured on Luria–Bertani (LB) broth and incubated at 37 ^{o}C for 24 hours with shaking at 250 rpm in order to be used as inoculums. Transmittances (T) were recorded in UV-Visible spectrophotometer. Erlenmeyer flasks containing 100 ml of culture medium added with 50 ppm, 80 ppm and 100 ppm concentrations of compounds and inoculated with 0.99 ml of inoculums and stirred in a culture chamber at 37 ºC with 180 rpm. Aliquots were extracted at 1 hour intervals for 6 hours and transmittances (T) were recorded in a UV-Visible spectrophotometer at 560 nm wavelength. T values were extrapolated to the number of cfu/ml (colony forming units/ml) for *E. coli* expressed in ln N_t. The antibacterial screening was repeated by replaced with *S. aureus* [16].

Results and Discussion

Characterization study

The structures of **1a-i** and **2a-i** were confirmed by CHN elemental analysis, FTIR, ^{1}H and ^{13}C NMR spectroscopy. The FTIR spectra of **1a-i** and **2a-i** showed that the peak observed at 1491 – 1450 cm^{-1} was associated to ν(N=N). The peak observed at 1605 – 1583 cm^{-1} was attributed to aromatic (C=C), while the peak at 764 – 749 cm^{-1} and 918 – 752 cm^{-1} were corresponded to *ortho*-substituted of halogen atom and *meta*-substituted of halogen atom, respectively [17]. In addition, **2a-i** showed that disappearance of ν(O-H) peak at 3300 – 3000 cm^{-1} and two new strong absorption peaks found at 1772 – 1732 cm^{-1} indicated the presence of ν(C=O) stretching of ester bond proved that the reaction was completed.

The ^{1}H and ^{13}C NMR spectroscopy were further confirmed the targeted structures of **1a-i** and **2a-i**. In the ^{1}H NMR spectra of **1a-i** and **2a-i**, the aromatic protons were observed at δ 8.2 – 6.9 ppm. The ^{1}H NMR spectra of **1a-i** showed the presence of OH group was resonated as singlet at δ 10.4 – 10.3 ppm (DMSO-d_6) and δ 5.8 – 5.6 ppm ($CDCl_3$), while ^{1}H NMR spectra of **2a-i** showed the presence of -CH_3 appeared as singlet was observed at δ 2.28 – 2.26 ppm. The ^{13}C NMR spectra of **2a-i** revealed the -CH_3 at δ 20.8 – 20.7 ppm. Whereas, the C=O was observed at δ 169.5 – 162.0 ppm. Other the resonance of aromatic carbon **1a-i** and **2a-i** were observed at δ 163.5 – 95.6 ppm.

[(phenyl)diazenyl]phenol (1a)
(0.67 g, 68%) yellow solid m.p. 169-171℃. (Found: C, 72.59; H, 5.02; N, 14.02 % $C_{12}H_{10}N_2O$ Requires C, 72.73; H, 5.05; N, 14.14 %); R_f 0.55 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3068 (OH), 1583 (C=C aromatic), 1454 (N=N), 1218 (C-N), 1137 (C-O), 678, 763, 835 (C-H), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 10.31 (s, 1H, OH) 7.82-7.80 (m, 4H, Ar-$H_{3,7}$) 7.57-7.48 (m, 3H, Ar-$H_{1, 2, 5}$) 6.94 (d, J = 8.6 Hz, 2H, Ar-H_8), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 160.9 (Ar-C_9), 152.1 (Ar-C_4), 145.2 (Ar-C_6), 130.5 (Ar-C_1), 129.3 (Ar-C_2), 124.9 (Ar-C_7), 122.1 (Ar-C_3), 115.9 (Ar-C_8).

3-[(E)-(Florophenyl)diazenyl]phenol (1b)
(0.70 g, 65%) yellow solid m.p. 128 – 129℃. (Found: C, 66.53; H, 4.30; N, 12.87% $C_{12}H_9N_2OF$ requires C, 66.67; H, 4.17; N, 12.96%); R_f 0.58 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3157 (OH), 1588 (C=C aromatic), 1450 (N=N), 1239 (C-N), 1145 (C-O), 840, 785, 673 (C-F), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 10.42 (s, 1H, OH) 7.81 (d, J = 8.6 Hz, 2H, Ar-H_8) 7.61 – 7.54 (m, 3H, Ar-$H_{1, 3, 5}$) 7.33 (t, J = 7.2 Hz, 1H, Ar-H_6) 6.95 (d, J = 8.6 Hz, 2H, Ar-H_9), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 163.5 (Ar-C_2), 161.6 (Ar-C_{10}), 153.5 (Ar-C_4), 144.8 (Ar-C_7), 130.9 (Ar-C_6), 125.0 (Ar-C_8), 119.6 (Ar-C_5), 116.8 (Ar-C_1), 115.9 (Ar-C_9), 106.9 (Ar-C_3).

3-[(E)-(Chlorophenyl)diazenyl]phenol (1c)
(0.79 g, 68%) red solid m.p. 140 – 141℃. (Found: C, 61.51 ; H, 3.86; N, 12.01% $C_{12}H_9N_2OCl$ requires C, 61.94; H, 3.87; N, 12.04%); R_f 0.54 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3253 (OH), 1596 (C=C aromatic), 1476 (N=N), 1253 (C-N), 1151 (C-O), 833, 788, 675 (C-Cl), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 10.44 (s, 1H, OH) 8.08 (s, 1H, Ar-H_3) 7.86 – 7.81 (m, 4H, Ar-$H_{1, 5, 8}$) 7.36 (t, J =7.7 Hz, 1H, Ar-H_6) 6.94 (d, J = 9.2 Hz, 2H, Ar-H_9), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 161.8 (Ar-C_{10}), 153.4 (Ar-C_4), 145.3 (Ar-C_7), 134.3 (Ar-C_2), 131.4 (Ar-C_1), 130.2 (Ar-C_6), 125.5 (Ar-C_8), 122.3 (Ar-C_3), 120.7 (Ar-C_5), 116.3 (Ar-C_9).

3-[(E)-(Bromophenyl)diazenyl]phenol (1d)
(0.99 g, 72%) red solid m.p. 146 – 147℃. (Found: C, 51.66; H, 3.15; N, 10.06% $C_{12}H_9N_2OBr$ requires C, 51.99; H, 3.25; N, 10.11%); R_f 0.56 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3196 (OH), 1592 (C=C aromatic), 1474 (N=N), 1246 (C-N), 1141 (C-O), 833, 789, 675 (C-Br), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 10.46 (s, 1H, OH), 7.89 – 7.84 (m, 3H, Ar-$H_{3, 8}$), 7.59 (d, J = 8.1 Hz, 1H, Ar-H_5) 7.50 (t, J = 8 Hz, 1H, Ar-H_6), 7.42 (d, J = 8 Hz, 1H, Ar-H_1), 6.97 (d, J = 8.6 Hz, 2H, Ar-H_9), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 161.5 (Ar-C_{10}), 153.2 (Ar-C_4), 144.8 (Ar-C_7), 132.8 (Ar-C_6), 131.4 (Ar-C_1), 125.2 (Ar-C_3), 123.2 (Ar-C_8), 122.6 (Ar-C_5), 122.4 (Ar-C_2), 116.0 (Ar-C_9).

3-[(E)-(Iodophenyl)diazenyl]phenol (1e)
(1.01 g, 62%) yellow solid m.p. 149 – 151℃. (Found: C, 44.38; H, 2.63; N, 8.61% $C_{12}H_9N_2OI$ requires C, 44.44; H, 2.78; N, 8.64%); R_f 0.54 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3109 (OH), 1586 (C=C aromatic), 1469 (N=N), 1244 (C-N), 1143 (C-O), 833, 776, 679 (C-I), ^{1}H NMR (500 MHz, $CDCl_3$) $^\delta$H (ppm): 8.20 (s, 1H, Ar-H_3), 7.90 – 7.85 (m, 3H, Ar-$H_{5, 8}$), 7.75 (d, J = 8 Hz, 1H, Ar-H_1), 7.22 (t, J = 8 Hz, 1H, Ar-H_6), 6.94 (d, J = 9.2 Hz, 2H, Ar-H_9), 5.72 (s, 1H, OH), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 161.4 (Ar-C_{10}), 153.0 (Ar-C_4), 145.0 (Ar-C_7), 138.7 (Ar-C_1), 131.4 (Ar-C_3), 129.1 (Ar-C_6), 125.2 (Ar-C_8), 123.0 (Ar-C_5), 116.0 (Ar-C_9), 95.4 (Ar-C_2).

2-[(E)-(Florophenyl)diazenyl]phenol (1f)
(0.67g, 62%) orange solid m.p. 104 – 105℃. (Found: C, 65.99; H, 4.10; N, 12.88% $C_{12}H_9N_2OF$ requires C, 66.67; H, 4.17; N, 12.96%); R_f 0.56 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3303 (OH), 1585 (C=C aromatic), 1480 (N=N), 1212 (C-N), 1142 (C-O), 753 (C-F), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 10.43 (s, 1H, OH), 7.81 (d, J = 8.6 Hz, 2H, Ar-H_5), 7.66 (t, J= 7.7 Hz, 1H, Ar-H_1), 7.53 (d, J = 8.2 Hz, 1H, Ar-H_8), 7.45 (d, J = 8 Hz, 1H, Ar-H_2), 7.31 (t, J = 7.2 Hz, 1H, Ar-H_6), 6.95 (d, J = 8.6 Hz, 2H, Ar-H_9), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 115.9 (Ar-C_9), 117.0 (Ar-C_2), 117.1(Ar-C_8), 117.9 (Ar-C_5), 124.1 (Ar-C_6), 125.5 (Ar-C_1), 131.8 (Ar-C_4), 140.9 (Ar-C_7), 147.6 (Ar-C_3), 159.1 (Ar-C_{10}).

2-[(E)-(Chlorophenyl)diazenyl]phenol (1g)
(0.67g, 58%) orange solid m.p. 112 – 113℃. (Found: C, 61.32; H, 3.76; N, 11.95% $C_{12}H_9N_2OCl$ requires C, 61.94; H, 3.87; N, 12.04%); R_f 0.53 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3301 (OH), 1588 (C=C aromatic), 1483

(N=N), 1238 (C-N), 1139 (C-O), 754 (C-Cl), ^{1}H NMR (500 MHz, $CDCl_3$) $^{\delta}$H (ppm): 7.92 (d, J = 8.6 Hz, 2H, Ar-H_8), 7.66 (d, J = 7.4 Hz, 1H, Ar-H_5), 7.53 (d, J = 7.4 Hz, 1H, Ar-H_2), 7.34 (m, 2H, Ar-$H_{1,6}$), 6.95 (d, J = 8.6 Hz, 2H, Ar-H_9), 5.72 (s, 1H, OH), ^{13}C NMR (125 MHz, DMSO-d_6) $^{\delta}$C (ppm): 161.6 (Ar-C_{10}), 148.1 (Ar-C_4), 145.5 (Ar-C_7), 133.1 (Ar-C_1), 131.6 (Ar-C_2), 130.6 (Ar-C_6), 127.9 (Ar-C_3), 125.4 (Ar-C_5), 117.5 (Ar-C_8), 116.1 (Ar-C_9).

2-[(E)-(Bromophenyl)diazenyl]phenol (1h)
(0.93g, 67%) red solid m.p. 121 – 123℃. (Found: C, 51.46; H, 3.15; N, 10.01% $C_{12}H_9N_2OBr$ requires C, 51.99; H, 3.25; N, 10.11%); R_f 0.54 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3294 (OH), 1586 (C=C aromatic), 1467 (N=N), 1225 (C-N), 1140 (C-O), 749 (C-Br), ^{1}H NMR (500 MHz,$CDCl_3$) $^{\delta}$H (ppm): 7.92 (d, J = 8.6 Hz, 2H, Ar-H_8), 7.72 (d, J= 6.3 Hz, 1H, Ar-H_5), 7.63 (d, J = 8.1 Hz, 1H, Ar-H_2), 7.36 (t, J = 6.9 Hz, 1H, Ar-H_6), 7.28 (t, J = 6.9 Hz, 1H, Ar-H_1), 6.94 (d, J = 8.6 Hz, 2H, Ar-H_9), 5.78 (s, 1H, OH), ^{13}C NMR (125 MHz, DMSO-d_6) $^{\delta}$C (ppm): 161.4 (Ar-C_{10}), 148.8 (Ar-C_4), 145.3 (Ar-C_7), 133.4 (Ar-C_2), 131.7 (Ar-C_1), 128.4 (Ar-C_6), 125.2 (Ar-C_5), 123.7 (Ar-C_8), 117.5 (Ar-C_9), 115.9 (Ar-C_3).

2-[(E)-(Iodophenyl)diazenyl]phenol (1i)
(1.04g, 64%) orange solid m.p. 132 – 133℃. (Found: C, 43.33; H, 2.75; N, 8.55% $C_{12}H_9N_2OI$ requires C, 44.44; H, 2.78; N, 8.64%); R_f 0.53 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 3192 (OH), 1587 (C=C aromatic), 1472 (N=N), 1249 (C-N), 1144 (C-O), 750 (C-I), ^{1}H NMR (500 MHz, $CDCl_3$) $^{\delta}$H (ppm): 8.01 (d, J = 9.2 Hz, 1H, Ar-H_2), 7.93 (d, J= 9.2 Hz, 2H, Ar-H_8), 7.60 (d, J = 6.9 Hz, 1H, Ar-H_5), 7.41 (t, J = 6.9 Hz, 1H, Ar-H_1), 7.12 (t, J = 8.3 Hz, 1H, Ar-H_6), 6.95 (d, J = 9.2 Hz, 2H, Ar-H_9), 5.65 (s, 1H, OH), ^{13}C NMR (125 MHz, $CDCl_3$) $^{\delta}$C (ppm): 158.9 (Ar-C_{10}), 151.5 (Ar-C_4), 147.2 (Ar-C_7), 139.9 (Ar-C_2), 131.8 (Ar-C_1), 129.0 (Ar-C_6), 125.9 (Ar-C_5), 117.4 (Ar-C_8), 116.1 (Ar-C_9), 102.1 (Ar-C_3).

[(phenyl)diazenyl]phenylaspirinate (2a)
(0.81 g, 75%) pale yellow solid m.p. 168 – 170℃. (Found: C, 69.97; H, 4.46; N, 7.67% $C_{12}H_9N_2OI$ requires C, 70.0; H, 4.17; N, 7.78%); R_f 0.64 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1736, 1763 (C=O), 1604 (C=C aromatic), 1481 (N=N), 1249 (C-N), 1183 (C-O), 762, 686 (C-H) ^{1}H NMR (500 MHz, DMSO-d_6) $^{\delta}$H (ppm): 8.20 (d, J = 9.2 Hz, 1H, Ar-H_{14}), 8.01 (d, J = 9.2 Hz, 2H, Ar-H_7), 7.91 (d, J = 8.6 Hz, 2H, Ar-H_3), 7.79 (t, J= 7.7 Hz, 1H, Ar-H_{13}), 7.64 – 7.58 (m, 3H, Ar-$H_{1, 2, 5}$), 7.51 (t, J = 7.8Hz, 1H, Ar-H_{13}), 7.48 (d, J= 8.6 Hz, 2H, Ar-H_8), 7.35 (d, J = 8 Hz, 1H, Ar-H_{11}), 2.27 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^{\delta}$C (ppm): 169.3 (C_b=O), 162.4 (C_a=O), 152.4 (Ar-C_4), 151.9 (Ar-C_9), 149.8 (Ar-C_6), 150.5 (Ar-C_{10}), 135.3 (Ar-C_{12}), 131.9 (Ar-C_1), 131.7 (Ar-C_{14}), 129.5 (Ar-C_2), 126.6 (Ar-C_{13}), 124.2 (Ar-C_{11}), 124.0 (Ar-C_7), 122.9 (Ar-C_3), 122.6 (Ar-C_8), 121.9 (Ar-C_{15}), 20.8 (-CH_3).

3-[(E)-(Florophenyl)diazenyl]phenylaspirinate (2b)
(0.62 g, 55%) pale yellow solid. m.p. 150 – 151℃. (Found: C, 66.61; H, 4.04; N, 7.33% $C_{21}H_{15}N_2O_4F$ requires C, 66.67; H, 3.97; N, 7.41%); R_f 0.65 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1768, 1742 (C=O), 1592 (C=C aromatic), 1453 (N=N), 1244 (C-N), 1186 (C-O), 914, 884, 785 (C-F), ^{1}H NMR (500 MHz, DMSO-d_6) $^{\delta}$H (ppm): 8.20 (d, J = 7.5 Hz, 1H, Ar-H_{15}), 8.03 (d, J = 8.6 Hz, 2H, Ar-H_8), 7.83 – 7.79 (m, 2H, Ar-$H_{6, 14}$), 7.71-7.66 (m, 2H, Ar-$H_{3, 5}$), 7.54 – 7.45 (m, 4H, Ar-$H_{1, 9, 13}$) 7.35 (d, J = 8.0 Hz, 1H, Ar-H_{12}), 2.27 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^{\delta}$C (ppm): 169.3 (C_b=O), 163.7 (C_a=O), 162.4 (Ar-C_2), 161.6 (Ar-C_4), 153.4 (Ar-C_{10}), 152.8 (Ar-C_{11}), 150.5 (Ar-C_7), 149.9 (Ar-C_{13}), 135.4 (Ar-C_{15}), 131.9 (Ar-C_6), 131.4 (Ar-C_{14}), 126.6 (Ar-C_{12}), 124.3 (Ar-C_8), 123.1 (Ar-C_9), 121.9 (Ar-C_{16}), 120.51 (Ar-C_5), 117.9 (Ar-C_1), 107.6 (Ar-C_3), 20.8 (-CH_3).

3-[(E)-(Chlorophenyl)diazenyl]phenylaspirinate (2c)
(0.57 g, 48%) pale yellow solid m.p. 153 – 154℃. (Found: C, 63.39; H, 3.78; N, 7.04% $C_{21}H_{15}N_2O_4Cl$ requires C, 63.88; H, 3.80; N, 7.10%); R_f 0.62 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1760, 1733 (C=O), 1605 (C=C aromatic), 1484 (N=N), 1248 (C-N), 1184 (C-O), 785, 885, 918 (C-Cl), ^{1}H NMR (500 MHz, DMSO-d_6) $^{\delta}$H (ppm): 8.20 (d, J = 7.6 Hz, 1H, Ar-H_{15}), 8.03 (d, J = 8.60 Hz, 2H, Ar-H_8), 7.92 – 7.90 (m, 2H, Ar-$H_{3, 14}$), 7.79 (t, J = 7.7 Hz, 1H, Ar-H_6), 7.68 – 7.66 (m, 2H, Ar-$H_{1, 5}$), 7.55 – 7.50 (m, 3H, Ar-$H_{9, 13}$), 7.35 (d, J = 8.0 Hz, 1H, Ar-H_{12}), 2.27 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^{\delta}$C (ppm): 169.2 (C_b=O), 162.4 (C_a=O), 152.8 (Ar-C_4), 150.5 (Ar-C_{10}), 149.7(Ar-C_{11}), 135.4 (Ar-C_7), 134.2 (Ar-C_{13}), 131.9 (Ar-C_2), 131.3 (Ar-C_1), 131.2 (Ar-C_{15}), 126.6 (Ar-C_6), 124.3 (Ar-C_{14}), 124.1 (Ar-C_{12}), 123.1 (Ar-C_8), 122.6 (Ar-C_3), 121.9 (Ar-C_9), 121.6 (Ar-C_5), 121.0 (Ar-C_{16}), 20.7 (-CH_3).

3-[(E)-(Bromophenyl)diazenyl]phenylaspirinate (2d)
(0.58 g, 44%) pale yellow solid m.p. 162 – 163℃. (Found: C, 57.21; H, 3.51; N, 6.21% $C_{21}H_{15}N_2O_4Br$ requires C, 57.40; H, 3.42; N, 6.38%); R_f 0.61 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1759, 1732 (C=O), 1600 (C=C aromatic), 1487 (N=N), 1242 (C-N), 1183 (C-O), 752, 884, 918 (C-Br), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 8.20 (d, J = 7.3 Hz, 1H, Ar-H_{15}), 8.06 – 8.03 (m, 3H, Ar-$H_{3, 8}$), 7.95 (d, J = 8.6 Hz, 1H, Ar-H_5), 7.83 – 7.77 (m, 3H, Ar-$H_{1, 6, 14}$), 7.59 (t, J = 7.2 Hz, 1H, Ar-H_{13}), 7.48 (d, J = 6.9 Hz, 2H, Ar-H_9), 7.35 (d, J = 8.0 Hz, 1H, Ar-H_{12}), 2.27 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 169.3 (C_b=O), 162.2 (C_a=O), 152.6 (Ar-C_4), 150.5 (Ar-C_{10}), 149.4 (Ar-C_{11}), 135.9 (Ar-C_7), 135.3 (Ar-C_{13}), 131.9 (Ar-C_{15}), 129.7 (Ar-C_6), 128.9 (Ar-C_1), 126.6 (Ar-C_3), 124.3 (Ar-C_{14}), 124.2 (Ar-C_{12}), 124.1 (Ar-C_8), 123.0 (Ar-C_2), 122.3 (Ar-C_5), 121.9 (Ar-C_9), 121.5 (Ar-C_{16}), 20.7 (-CH_3).

3-[(E)-(Iodophenyl)diazenyl]phenylaspirinate (2e)
(0.78 g, 54%) pale yellow solid m.p. 172 – 173℃. (Found: C, 51.61; H, 3.06; N, 5.68% $C_{21}H_{15}N_2O_4I$ requires C, 51.85; H, 3.09; N, 5.76%); R_f 0.62 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1772, 1742 (C=O), 1603 (C=C aromatic), 1481 (N=N), 1246 (C-N), 1184 (C-O), 915, 887, 769 (C-I), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 8.20 (d, J = 8.0 Hz, 1H, Ar-H_{15}), 8.19 (s, 1H, Ar-H_3), 8.02 (d, J = 8.6 Hz, 2H, Ar-H_8), 7.97-7.94 (m, 2H, Ar-$H_{1, 5}$), 7.79 (t, J = 7.7 Hz, 1H, Ar-H_{14}), 7.52 (t, J = 7.7 Hz, 1H, Ar-H_6), 7.48 (d, J = 8.6 Hz, 2H, Ar-H_9), 7.42 (t, J = 7.7 Hz, 1H, Ar-H_{13}), 7.35 (d, J = 8.0 Hz, 1H, Ar-H_{12}), 2.26 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 169.3 (C_b=O), 162.4 (C_a=O), 152.8 (Ar-C_4), 152.7 (Ar-C_{10}), 150.6 (Ar-C_{11}), 149.6 (Ar-C_7), 139.9 (Ar-C_1), 135.4 (Ar-C_{13}), 131.9 (Ar-C_3), 131.7 (Ar-C_{15}), 129.5 (Ar-C_6), 126.6 (Ar-C_{14}), 124.3 (Ar-C_{12}), 124.1 (Ar-C_8), 123.6 (Ar-C_9), 123.1 (Ar-C_5), 121.9 (Ar-C_{16}), 95.7 (Ar-C_2), 20.8 (-CH_3).

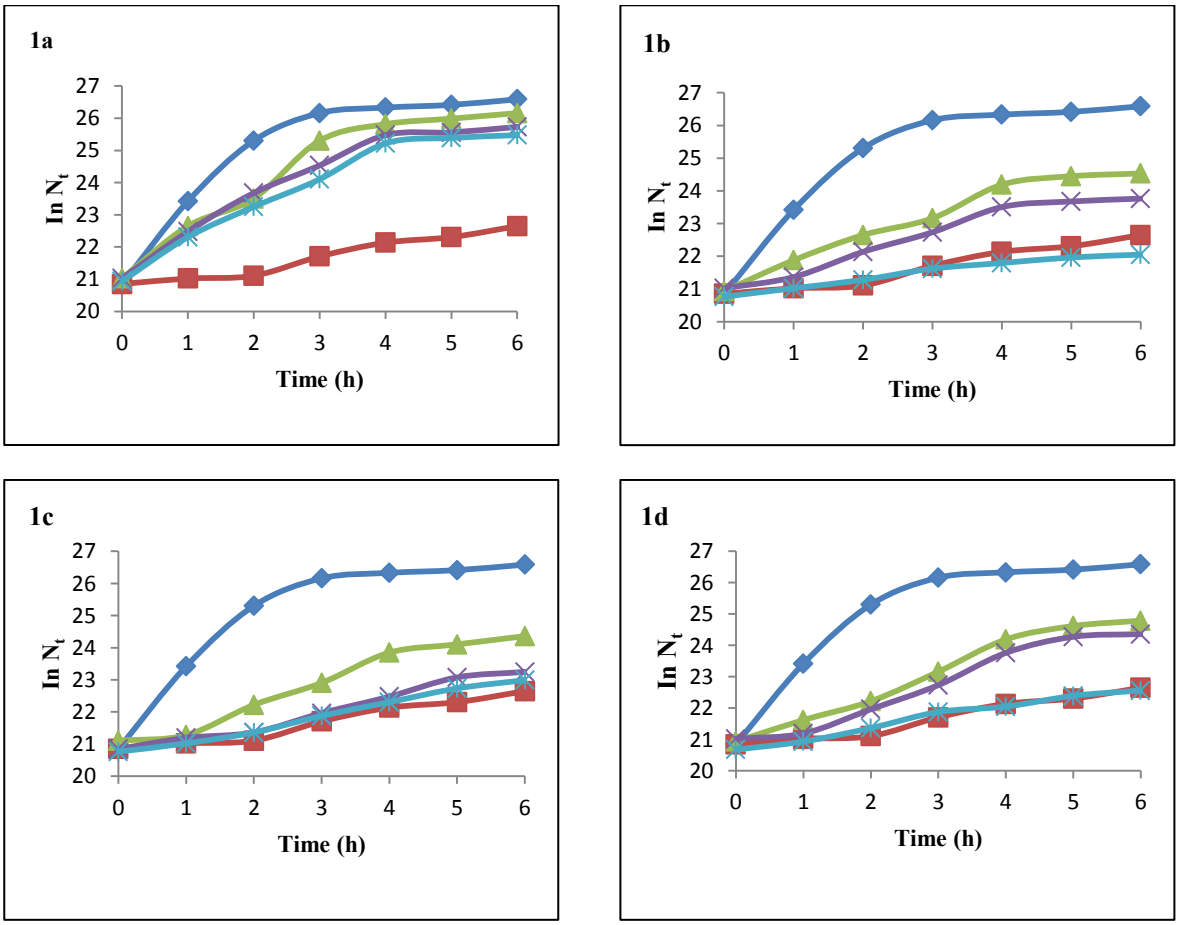
2-[(E)-(Florophenyl)diazenyl]phenylaspirinate (2f)
(0.45 g, 40%) pale yellow 123 – 124℃. (Found: C, 66.52; H, 3.91; N, 7.22% $C_{21}H_{15}N_2O_4F$ requires C, 66.67; H, 3.97; N, 7.41%); R_f 0.62 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1755, 1732 (C=O), 1588 (C=C aromatic), 1481(N=N), 1250 (C-N), 1181 (C-O), 762 (C-F), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 8.20 (d, J = 8.6 Hz, 1H, Ar-H_{15}), 8.02 (d, J = 6.9 Hz, 2H, Ar-H_8), 7.79 (1H, t, J= 8.6 Hz, Ar-H_1), 7.74 (t, J= 8.6 Hz, 1H, Ar-H_{14}), 7.62 (t, J = 6.9 Hz, 1H, Ar-H_{13}), 7.54 – 7.49 (m, 4H, Ar-$H_{5, 6, 9}$), 7.39 – 7.35 (m, 2H, Ar-$H_{2, 12}$), 2.27 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 169.3 (C_b=O), 162.4 (C_a=O), 160.4 (Ar-C_3), 158.4 (Ar-C_{10}), 152.8 (Ar-C_{11}), 150.6 (Ar-C_7), 150.0 (Ar-C_4), 139.7 (Ar-C_{13}), 135.4 (Ar-C_1), 133.8 (Ar-C_{15}), 131.9 (Ar-C_{14}), 126.6 (Ar-C_6), 125.1 (Ar-C_5), 124.3 (Ar-C_{12}), 123.1 (Ar-C_8), 121.9 (Ar-C_9), 117.5 (Ar-C_{16}), 117.3 (Ar-C_2), 20.8 (-CH_3).

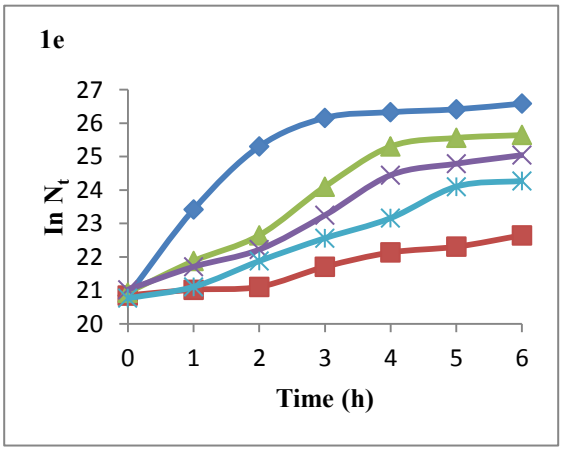
2-[(E)-(Chlorophenyl)diazenyl]phenylaspirinate (2g)
(0.54 g, 46%) orange solid m.p. 119 – 120℃. (Found: C, 63.66; H, 3.76; N, 6.98% $C_{21}H_{15}N_2O_4Cl$ requires C, 63.88; H, 3.80; N, 7.10%); R_f 0.62 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1770, 1742 (C=O), 1591 (C=C aromatic), 1482(N=N), 1244 (C-N), 1184 (C-O), 753 (C-Cl), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 8.21 (d, J = 8.0 Hz, 1H, Ar-H_{15}), 8.04 (d, J = 8.6 Hz, 2H, Ar-H_8) 7.80 (t, J = 7.8 Hz, 1H, Ar-H_{14}), 7.74 (d, J = 8.0 Hz, 1H, Ar-H_5), 7.70 (1H, d, J = 8.0 Hz, Ar-H_2), 7.58 (t, J = 7.8 Hz, 1H, Ar-H_{13}), 7.55 – 7.48 (m, 4H, Ar-$H_{1, 6, 9}$), 7.36 (d, J = 7.5 Hz, 1H, Ar-H_{12}), 2.28 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 169.4 (C_b=O), 162.1 (C_a=O), 152.6 (Ar-C_4), 150.2 (Ar-C_{10}), 149.5 (Ar-C_{11}),), 147.4 (Ar-C_7), 135.4 (Ar-C_{13}), 133.9 (Ar-C_1), 132.8 (Ar-C_{15}), 131.9 (Ar-C_2), 130.9 (Ar-C_3), 128.2 (Ar-C_6), 126.6 (Ar-C_{14}), 124.5 (Ar-C_{12}), 124.3 (Ar-C_5), 123.2 (Ar-C_8), 121.5 (Ar-C_9), 117.6 (Ar-C_{16}), 20.8 (-CH_3).

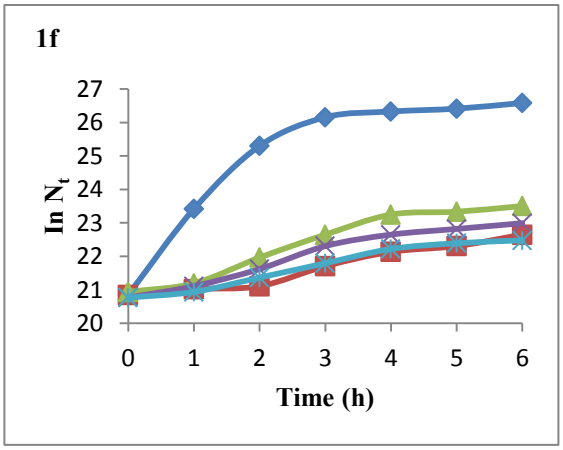
2-[(E)-(Bromophenyl)diazenyl]phenylaspirinate (2h)
(0.59 g, 45%) pale red solid m.p. 121-123℃. (Found: C, 57.52; H, 3.41; N, 6.21 % $C_{21}H_{15}N_2O_4Br$ Requires C, 57.40; H, 3.42; N, 6.38 %); R_f 0.63 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1767, 1741 (C=O), 1590 (C=C aromatic), 1483 (N=N), 1243 (C-N), 1183 (C-O), 749 (C-Br), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 8.20 (d, J = 7.5 Hz, 1H, Ar-H_{15}), 8.02 (d, J = 7.5 Hz, 2H, Ar-H_8), 7.88 (d, J = 7.5 Hz, 1H, Ar-H_5), 7.78 (t, J = 8.6 Hz, 1H, Ar-H_{14}), 7.64 (t, J = 7.5 Hz, 1H, Ar-H_{13}), 7.49-7.54 (5H, m, Ar-$H_{1, 2, 6, 9}$), 7.34 (1H, d, J = 7.5 Hz, Ar-H_{12}), 2.27 (3H, s, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6), $^\delta$C (ppm): 169.6 (C_b=O), 162.7 (C_a=O), 153.2 (Ar-C_4), 150.8 (Ar-C_{10}), 150.1 (Ar-C_{11}), 149.0 (Ar-C_7), 135.7 (Ar-C_{13}), 134.1 (Ar-C_2), 133.4 (Ar-C_{15}), 132.2 (Ar-C_1), 129.0 (Ar-C_6), 126.9 (Ar-C_{14}), 125.0 (Ar-C_5), 124.72(Ar-C_{12}), 124.3 (Ar-C_8), 123.4 (Ar-C_9), 122.1 (Ar-C_{16}), 118.1 (Ar-C_3), 21.0 (-CH_3).

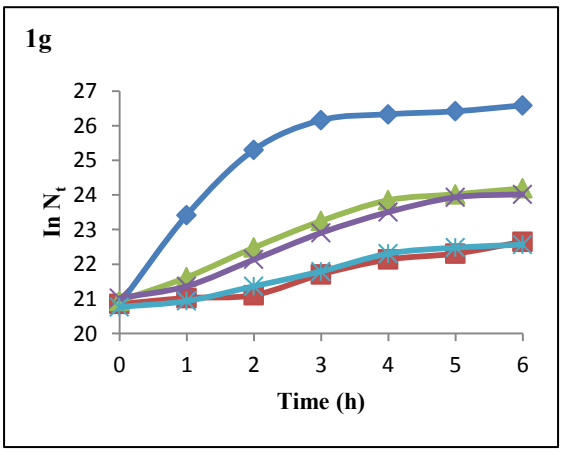
2-[(E)-(Iodophenyl)diazenyl]phenylaspirinate (2i)
(0.55 g, 38%) pale red solid m.p. 128 – 129℃. (Found: C, 51.76; H, 3.01; N, 5.69% $C_{21}H_{15}N_2O_4I$ requires C, 51.85; H, 3.09; N, 5.76%); R_f 0.62 (1:4 EA/ hexane) IR: ν_{max} (thin film/cm^{-1}) 1758, 1740 (C=O), 1588 (C=C aromatic), 1491 (N=N), 1248 (C-N), 1185 (C-O), 753 (C-I), ^{1}H NMR (500 MHz, DMSO-d_6) $^\delta$H (ppm): 8.21 (d, J = 8.0 Hz, 1H, Ar-H_{15}), 8.12 (d, J = 7.5 Hz, 1H, Ar-H_2), 8.04 (d, J = 8.6 Hz, 2H, Ar-H_8), 7.80 (t, J = 8.3 Hz, 1H, Ar-H_1), 7.62 – 7.52 (m, 5H, Ar-$H_{6, 9, 13, 14}$), 7.36 (d, J = 8.0 Hz, 1H, Ar-H_5), 7.32 (d, J = 8.0 Hz, 1H, Ar-H_{12}), 2.28 (s, 3H, -CH_3), ^{13}C NMR (125 MHz, DMSO-d_6) $^\delta$C (ppm): 169.3 (C_b=O), 162.4 (C_a=O), 152.9 (Ar-C_4), 150.8 (Ar-C_{10}), 150.6 (Ar-C_{11}), 149.6 (Ar-C_7), 139.9 (Ar-C_2), 135.4 (Ar-C_{13}), 133.2 (Ar-C_1), 131.9 (Ar-C_{15}), 129.4 (Ar-C_6), 126.6 (Ar-C_{14}), 124.5 (Ar-C_5), 124.3 (Ar-C_{12}), 123.2 (Ar-C_8), 121.9 (Ar-C_9), 117.2 (Ar-C_{16}), 102.7 (Ar-C_3), 20.8 (-CH_3).

Antibacterial screening
The inhibition activity of **1a-i** and **2a-i** against *E. coli* and *S. aureus* are shown in Figure 1 and Figure 2, respectively. Most halogenated azo derivatives **1b-i** showed good antibacterial activities against *E. coli* and *S. aureus* at all concentration (50, 80 and 100 ppm). However, **2a-i** only showed good inhibition activity against bacteria at high concentrations (100 ppm). The effects of synthesized compounds tested at three different concentrations (50, 80 and 100 ppm) are further revealed by minimum inhibitory concentration (MIC) values. The MIC value of **1a-i** and **2a-i** were determined by extrapolating the concentration to zero growth rates of *E. coli* and *S. aureus* [16].




1e
ln Nt
Time (h)


1f
ln Nt
Time (h)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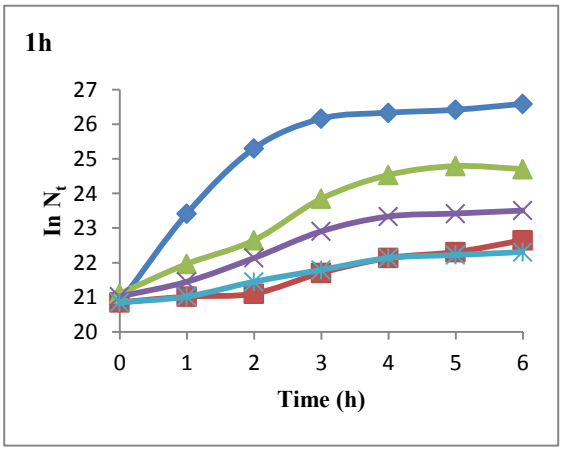
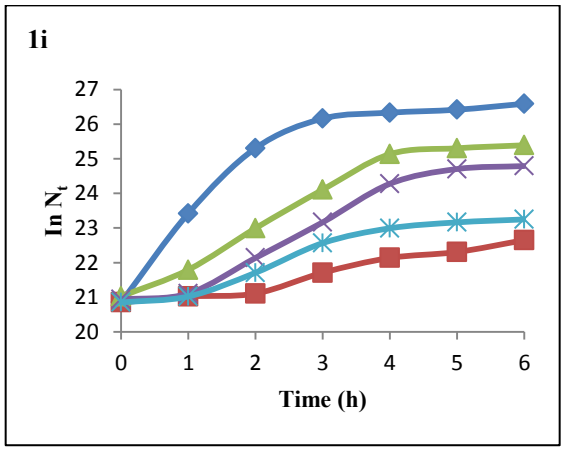
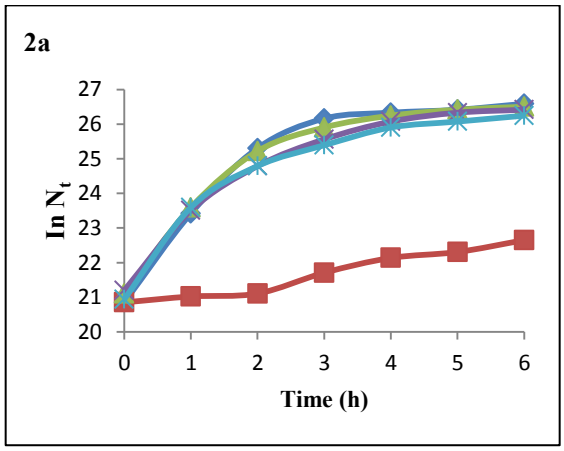
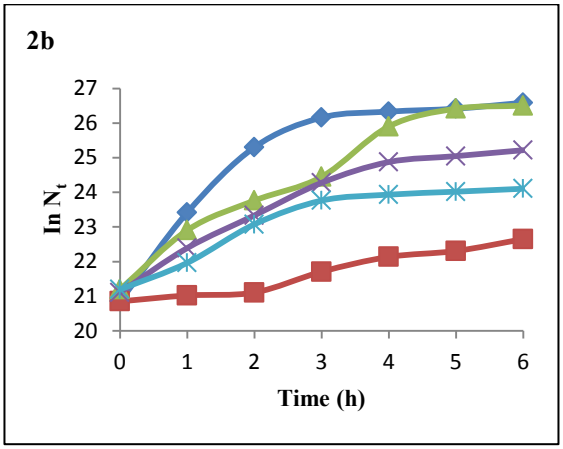
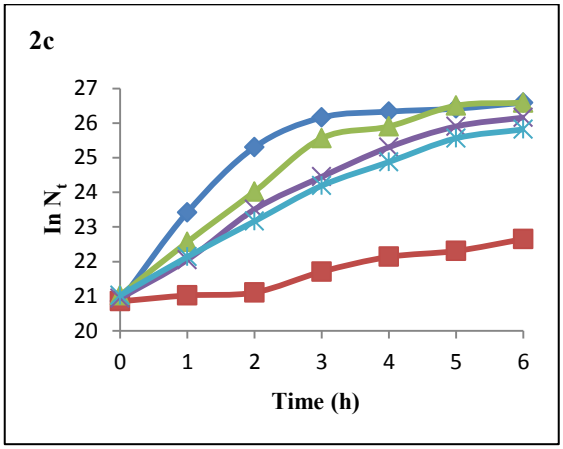
ln Nt
Time (h)

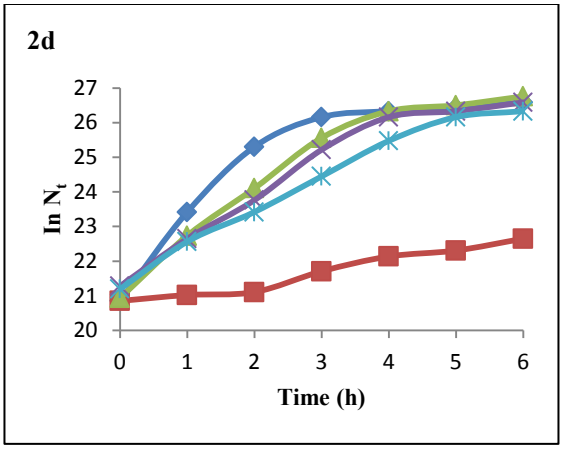

1h
ln Nt
Time (h)

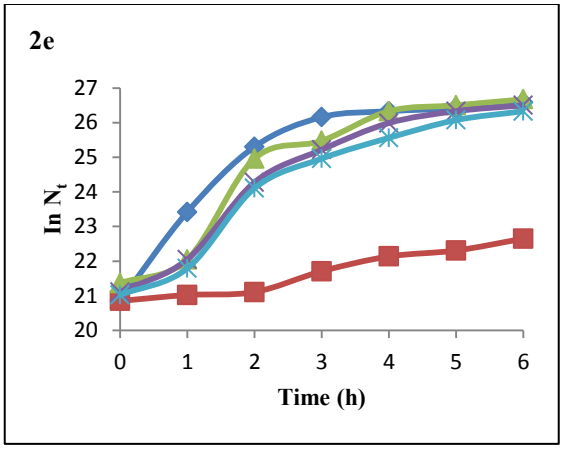

1i
ln Nt
Time (h)

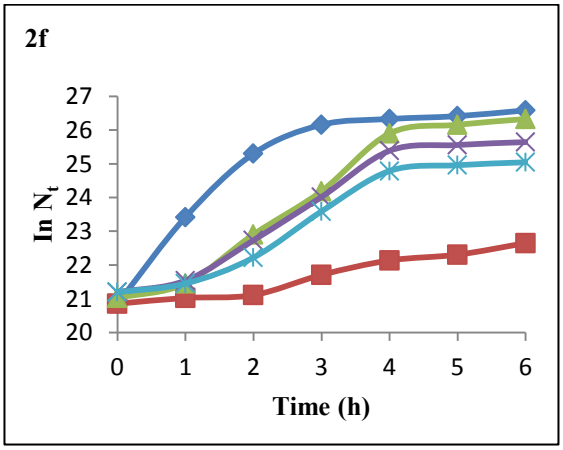

2a
ln Nt
Time (h)

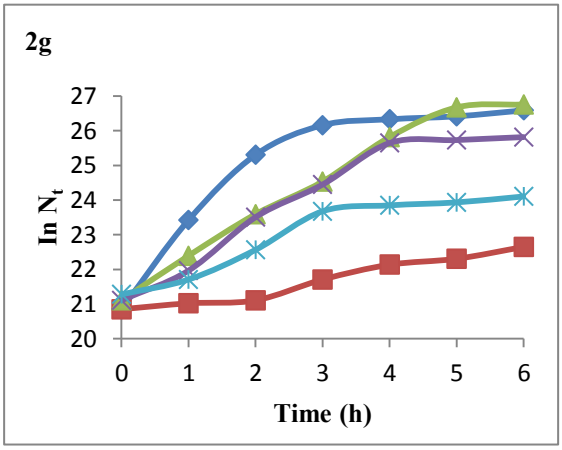

2b
ln N_t
Time (h)


2c
ln N_t
Time (h)


2d
ln N_t
Time (h)


2e
ln N_t
Time (h)


2f
ln N_t
Time (h)


2g
ln N_t
Time (h)

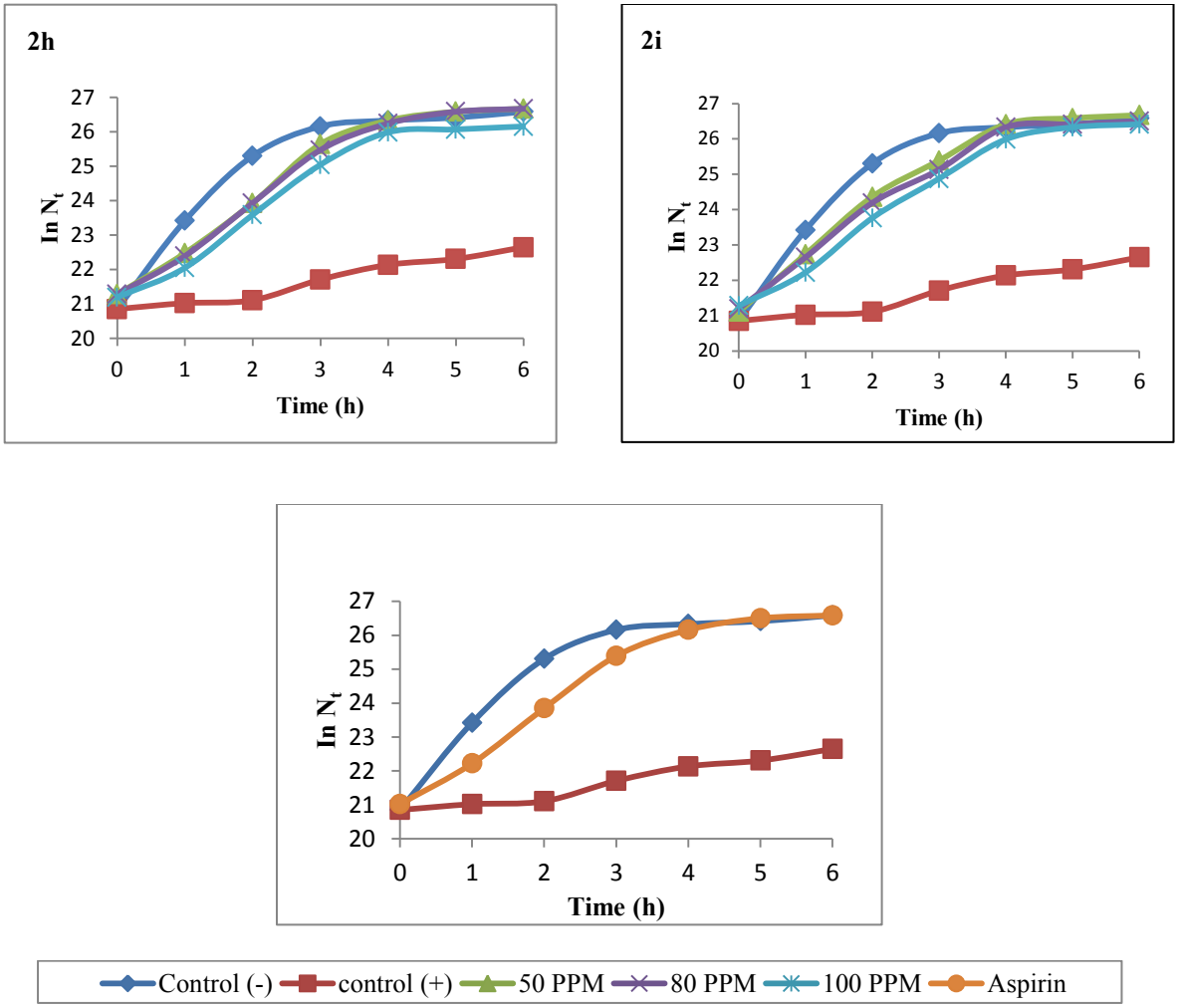
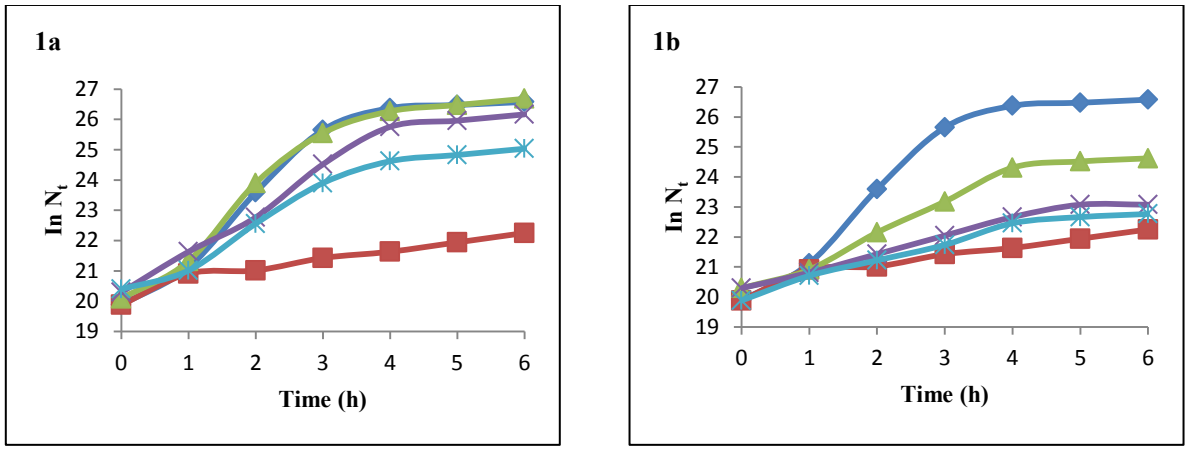
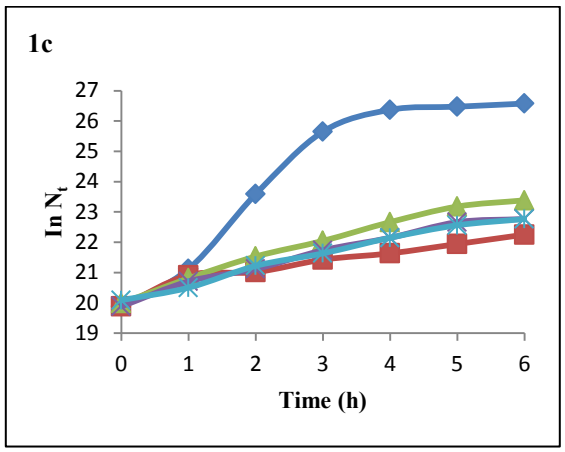
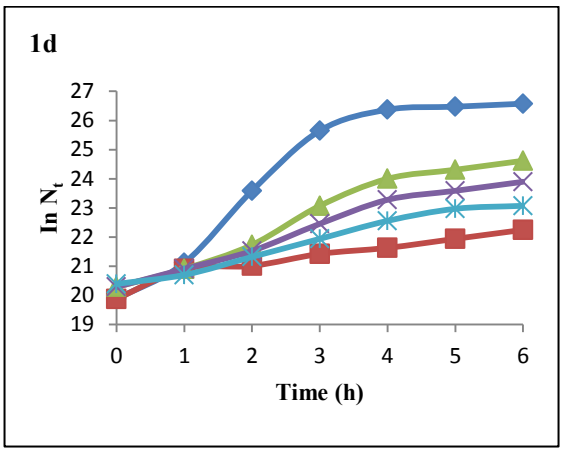
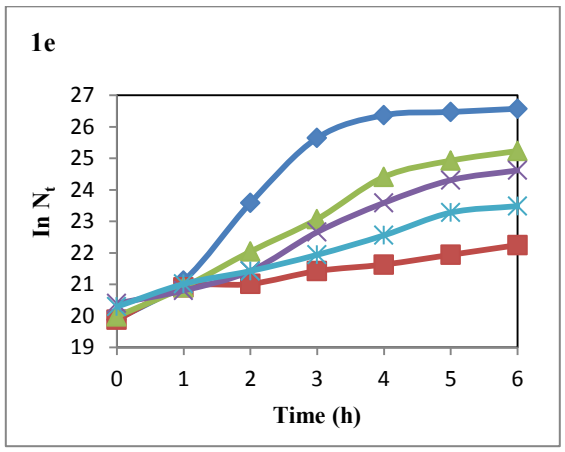


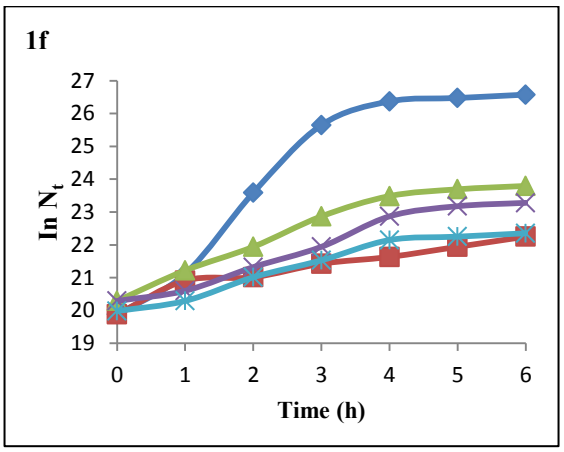
Figure 1. Inhibition activity of **1a-i** and **2a-i** against *E. coli* shown as In N_t for *E. coli* growth versus time.

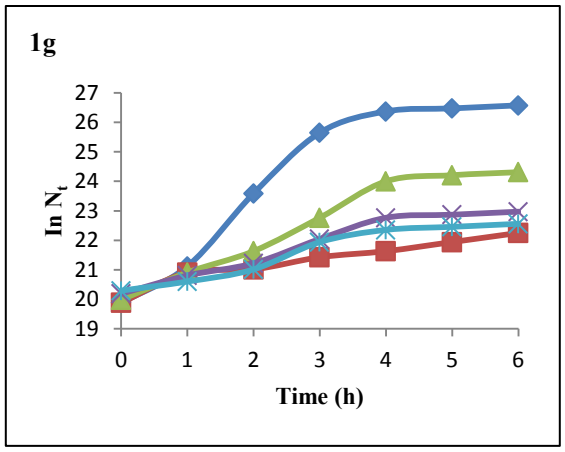


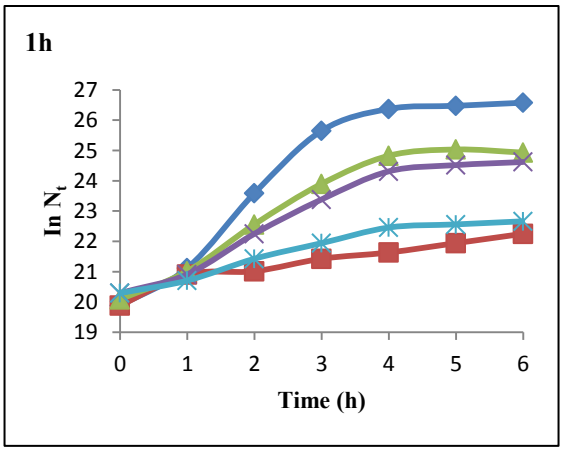

1c
ln N_t
Time (h)

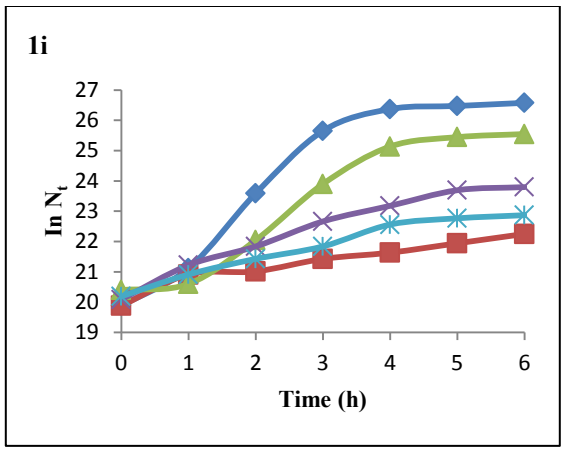

1d
ln N_t
Time (h)

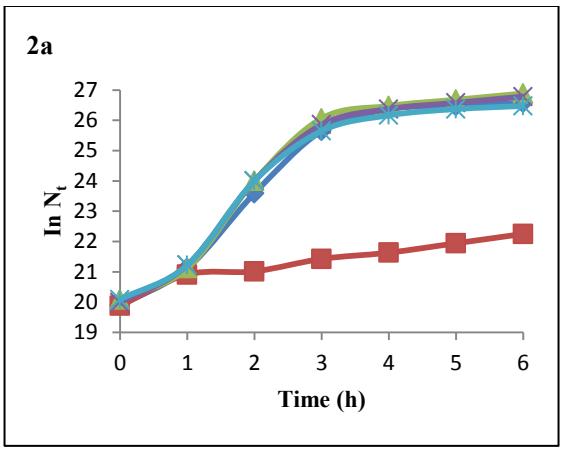

1e
ln N_t
Time (h)

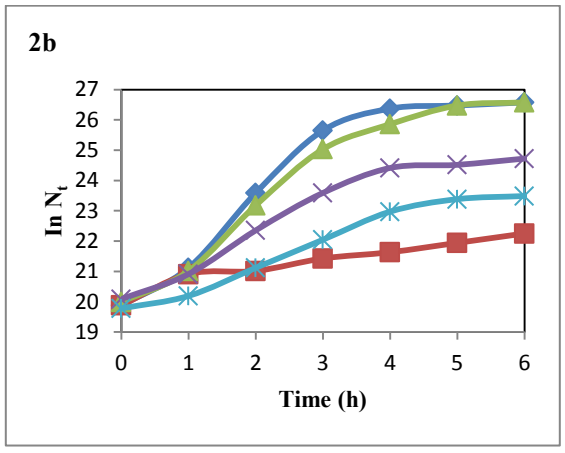

1f
ln N_t
Time (h)

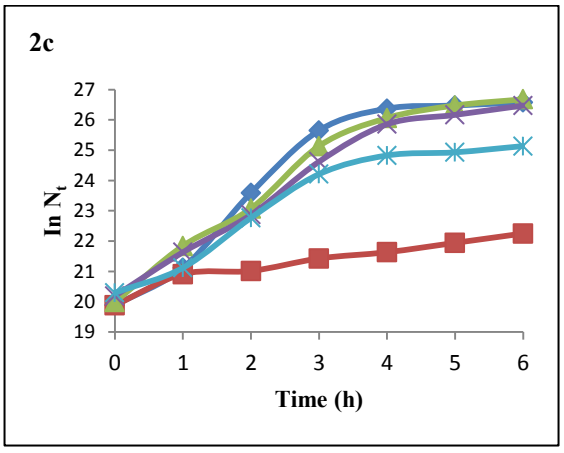

1g
ln N_t
Time (h)

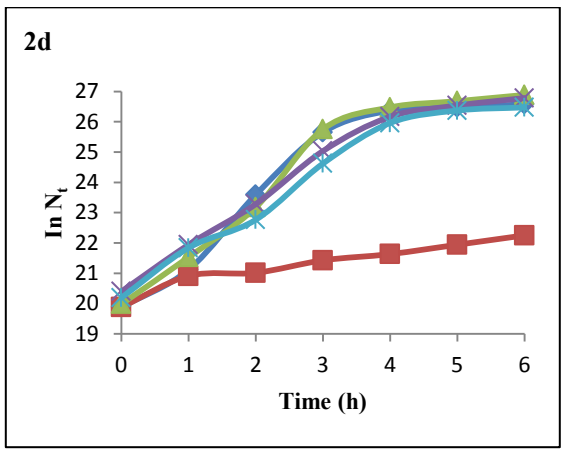

1h
ln N_t
Time (h)

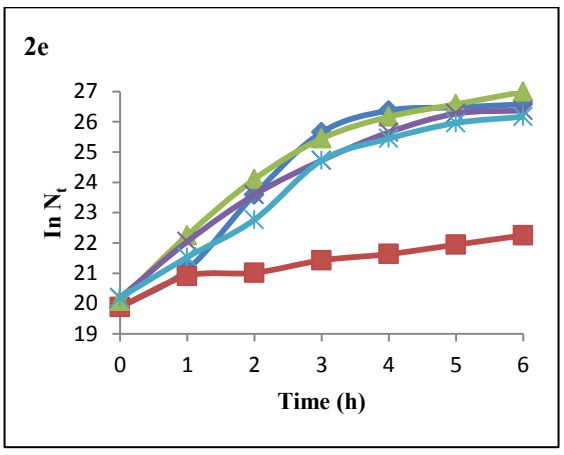

1i
ln N$_t$
Time (h)


2a
ln N$_t$
Time (h)


2b
ln N$_t$
Time (h)


2c
ln N$_t$
Time (h)


2d
ln N$_t$
Time (h)


2e
ln N$_t$
Time (h)

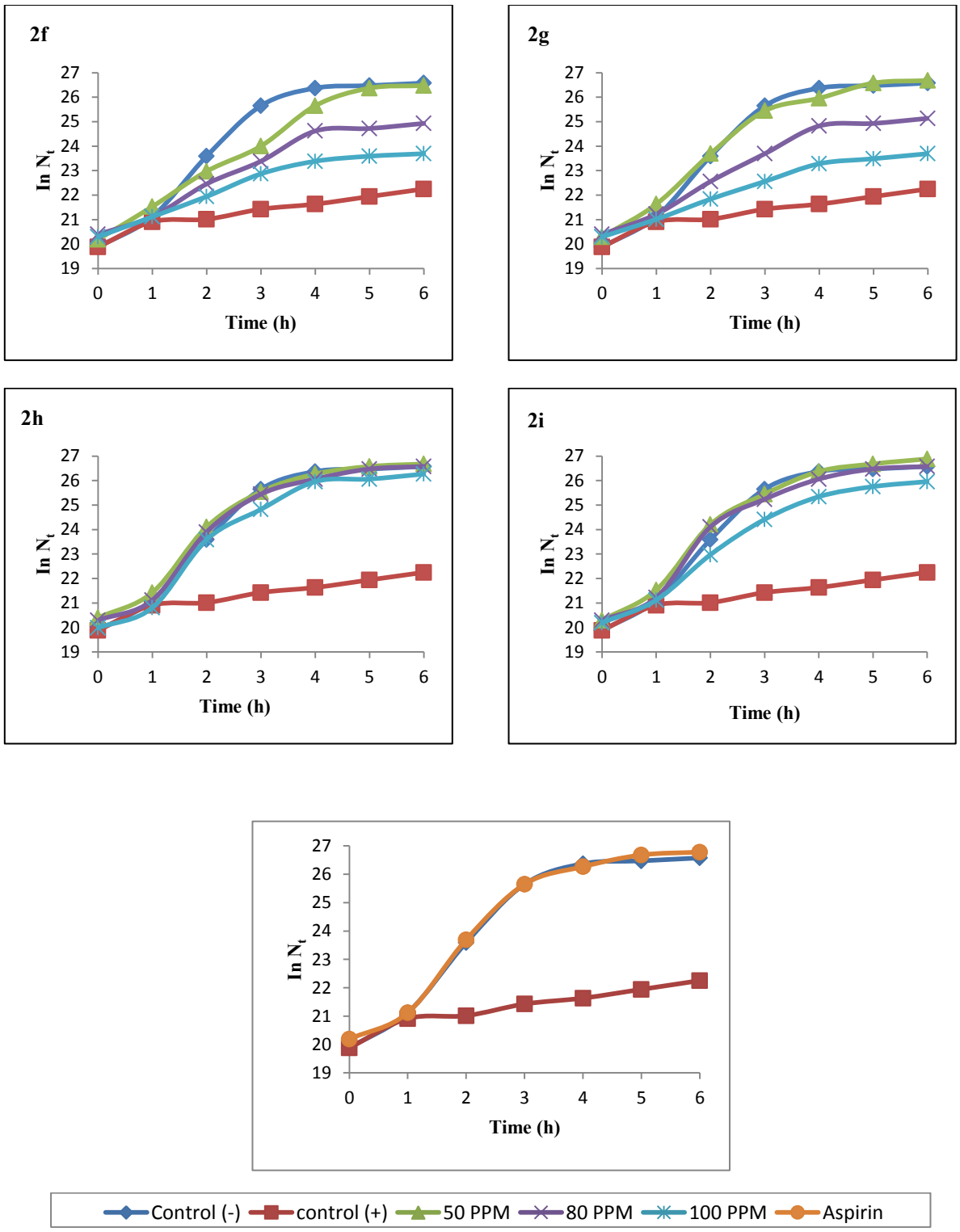


Figure 2. Inhibition activity of aspirin, **1a-i** and **2a-i** against *S. aureus* shown as In N_t for *S. aureus* growth versus time

Based on the MIC values shown in Table 1, the MIC values showed that all halogenated azo derivatives **1b-i** exhibited very good bacteriostatic activities against *E. coli* and *S. aureus* compared to **1a** (without halogen substituent). All the synthesized halogenated azo derivatives **1b-i** demonstrated good inhibition against *E. coli* (MIC < 220 ppm) and *S. aureus* (MIC < 220 ppm), which indicated that these compounds are a potential antibacterial agent [18]. Among these compounds, **1f** (*ortho* fluorine atom substituent) gave the best inhibition with MIC values of 108 ppm and 132 ppm against *E. coli* and *S. aureus*, respectively. These results in agreement with Saeed et al. and Yarovenko et al. which reported that the halogen group and N=N played important roles in antibacterial activities [19, 20]. Furthermore, compounds consist of phenyl groups and halogen atoms have also revealed that those with more lipophilic character could easily penetrates the cell wall of microorganism [21, 22]. The azo derivatives **1b-i** consists of -N=N- group can be protonated under acidic condition to react with the phosphate group on the polysaccharide peptidoglycan layer of bacteria, which hinder the formation of cell wall [23, 24]. Patrick also reported that the halogenated compound was involved in inhibition of bacteria *via* interaction of halogen with receptor of enzyme [25]. In addition, The -N=N- and OH group in the synthesized halogenated azo derivatives **1b-i** interacts with active site on the enzyme of *E. coli* and *S. aureus* through hydrogen bond formation, thus inhibit the growth of bacteria [26].

For compound **2a-i**, only **2b** and **2f-g** exhibited good antibacterial activity against *E. coli* and *S. aureus* compared to **2a** (without halogen substituent) and aspirin. It is because the presence of halogen substituent and N=N in molecular structure has contributed to antibacterial activities. The **2f** and **2g** substituted with fluorine and chlorine atoms, respectively at *ortho* position are more active to inhibit towards *E. coli* and *S. aureus* compared to compounds substituted with halogen atom at *meta* position. These results also revealed the same finding in which the halogen substituted at different position may have effect on the antibacterial activity as reported Lee et al. [27]. Other compounds showed weak antibacterial activity against *E. coli* and *S. aureus* due to larger size of bromine atom and iodine atom which resulted in bulkiness and occurrence of steric hindrance, thus avoid the compound from binding to the active site of bacteria [28]. Furthermore, presence of more phenyl ring in molecular structure might also result in bulkiness. Therefore, it is more difficult to penetrate into bilayer of phospholipid *E. coli* and *S. aureus* [29].

Table 1. Minimum inhibitory concentration (MIC ppm) for compound **1a-i** and **2a-i**

Compounds	**MIC (ppm)**	
	E. coli	***S. aureus***
Aspirin	>220	>220
1a	>220	>220
1b	113.4	150.2
1c	111.3	139.0
1d	118.3	140.9
1e	145.1	155.3
1f	108.5	132.3
1g	117.3	138.3
1h	111.7	159.5
1i	130.5	164.2
2a	>220	>220
2b	180.9	194.1
2c	> 220	> 220
2d	> 220	> 220
2e	>220	>220
2f	156.3	199.5
2g	167.0	198.9
2h	>220	>220
2i	>220	>220
Ampicillin (+)	92.9	124.2

Conclusion

In conclusion, there are two series of azo derivatives **1b-i** and aspirin-azo derivatives **2b-i** were synthesized and screened for their antibacterial activities. The results showed that all halogenated azo derivatives **1b-i** exhibited better antibacterial activities against both gram-negative bacteria and gram-positive bacteria when compared to all aspirin- azo derivatives **2b-i**. The esterification of aspirin with azo derivatives **1b-i** had increase the bulkiness of the molecular structure causing the steric hindrance, thus avoid the molecular structure from binding to the active site of bacteria. In addition, the bulky structure was caused difficulty to penetrate into the cell wall of bacteria. Azo derivatives **1f** substituted with fluorine atom were found the best among the rest with the lowest MIC values due to the smaller size of fluorine.

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