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Original article:

ONE POT SYNTHESIS OF PYRROLIDINES TYPE 3,7-DIAZABICYCLO [3.3.0] OCTANE AND BIOLOGICAL ACTIVITY

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ABSTRACT

Pyrrolidines type 2,4-disubstituted (alkyl, aryl or heteroaryl)-6,8-dioxo-3,7-diazabicyclo [3.3.0] octanes (**8a-d**) were successfully synthesized by an efficient one pot 1,3-dipolar cycloaddition of azomethine ylides (*in situ* generated from the reaction of aromatic aldehydes and methyl ester of α-amino acids) with dipolarophile (*N*-phenylmaleimide). The reaction of compounds **8a-d** with hydrazine in ethanol at room temperature took place under nucleophilic substitution which furnished 5-amino-4,6-dioxo-octahydropyrrolo [3,4-b] pyrrole-3-carboxylic acid phenylamides (**12a-d**). Structures of the products were confirmed by IR and ^{1}H NMR. The compounds (**8b** and **12a**) were evaluated for antimicrobial (agar dilution method) and antioxidative (DPPH; 2,2-diphenyl-1-picrylhydrazyl and SOD; superoxide dismutase assays) activities. The results showed that at concentrations of 4-256 μg/mL, the tested compounds exhibited non-significant antimicrobial growth, whereas the **12a** at 200 μg/mL began to exert some antioxidative activity.

Keywords: pyrrolidine, 1-aminopyrrolidine-2,5-dione, antimicrobial and antioxidative activities

INTRODUCTION

Pyrrolidine or tetrahydropyrrole ring is known as cyclic five member amine having nitrogen as part of the ring. The pyrrolidine structure is present in numerous natural alkaloids such as nicotine and hygrine. However, it is found in many drugs, for example, bepridil and procyclidine (**1**) which acts as muscarinic antagonist that is capable of crossing blood-brain barrier (Shorvon, 2001). The compound **1** has been used for treatment of drug-induced extrapyramidal disorders and in Parkinsonism. Pyrrolidones, known as racetams, are a class of nootropic drugs such as piracetam (**2**) which exert its action by activating glutamate receptors that are colocalized with cholinergic receptors, thus increasing the firing of the latter.

Consequently, the racetams increase memory capacity by the same action as acetylcholinesterase inhibitors. Andrimid (**3,** n=2) (Liu et al., 2008) is a hybrid nonribosomal peptide-polyketide antibiotic that blocks the carboxyl-transfer reaction of bacterial acetyl-CoA carboxylase and thereby inhibits fatty acid biosynthesis with submicromolar potency. In addition, benzoylaminocarbothioyl pyrrolidines (**4**) (Dönadas et al., 2006) were screened for *in vitro* antibacterial and antifungal activities including toxicity. Recently, pyrrolidine-2,5-dione derivatives of 1-(2-pyridinyl)-3-substituted (**5**) (Obniska et al., 2005) and *N*-[(4-arylpiperazin-1-yl)methyl **(6)**] (Kamin-

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ski and Obniska, 2008) were evaluated for anticonvulsant activity using the maximum electroshock seizure and pentetrazole seizure threshold test. Moreover, 3-[2′(4-aminophenyl) ethyl] pyrrolidine-2,5-dione exhibited potential anti-tumor activity (Ahmed et al., 1995). The structures of compounds **1**–**6** are presented in Figure 1.

To search for new therapeutics, our rational design based on the reported pyrrolidines constituting drugs and bioactive compounds lead to a combination of pyrrolidine and pyrrolidone rings as a target bicyclicpyrrolidine for potential candidate with interesting biological activities. Such bicyclicpyrrolidine (3,7-diazabicyclo [3,3,0] octane, **8**) had been previously reported by Amornraksa et al. (1987). The synthesis was achieved from cycloaddition reaction of azomethine ylide (**7**), generated through prototropic shift in heating toluene of aryl imines from α-amino acid esters and aromatic aldehydes, with *N*-phenylmaleimide *via* an endo-transition state to give racemic, single diastereoisomeric, 3,7-diazabicyclo [3.3.0] octanes (**8,** R_1 = phenyl, substituted phenyl, 2-furyl, 2-thienyl, 3-pyridyl, R_2 = H, alkyl, benzyl, indol-3-ylmethylene). Structures of **7** and **8** are shown in Figure 2.

The target compounds; type 3,7-diazabicyclo [3.3.0] octanes (**8**) containing both ester and imide functional groups would react with nucleophile in different manners. At least three kinds of products would be obtained from reaction of the functional groups with hydrazine in ethanol. Pathway 1 is nucleophilic substitution on imide, as in the Gabriel synthesis (Solomons and Fryhle, 2008) of primary amine to give pyridazine-3,6-dione (**9**). Pathway 2, initial nucleophilic substitution takes place on ester followed by the second substitution on the imide function to provide pyridazine-3,6-dione (**10**). Pathway 3 is a simple nucleophilic substitution on ester function to furnish pyrrolidine-2,5-dione (**11**) as shown in Figure 3. The present study reports one pot synthesis of 3,7-diazabicyclo [3.3.0] octanes (**8a-d**) *via* [3+2] cycloaddition of azomethine ylide (**7**, precursor of 1,3-dipole species), producing from α-amino acid esters (**a**) and aromatic aldehydes (**b**) with *N*-phenylmaleimide (**c**) (Figure 4) as well as reactions of the title compounds **8a-d** with hydrazine. Antimicrobial and antioxidative activities of the synthesized compounds were also investigated.

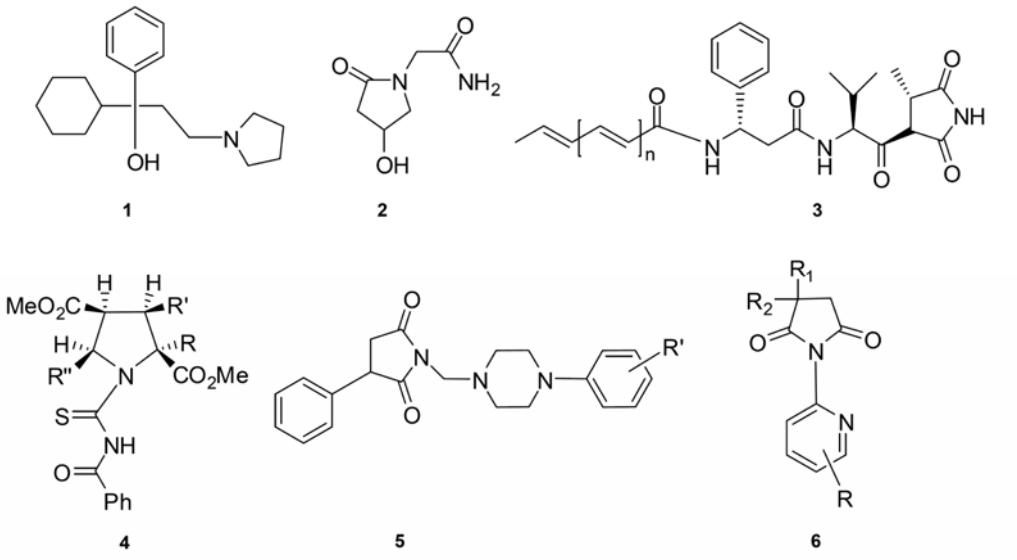

Figure 1: Structures of compounds **1**–**6**

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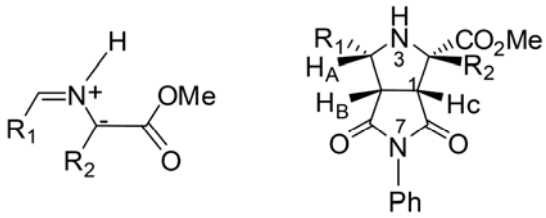

Figure 2: Structures of compounds **7** and **8**

MATERIALS AND METHODS

General

Melting points were determined on the Gallenkamp model Sanyo apparatus and were reported without correction. Nuclear magnetic resonance spectra were determined at 400 MHz on a Bruker spectrometer as specified chemical shifts are given in parts per million (δ) downfield from TMS as internal standard. IR spectra were recorded in the 4000-650 cm^{-1} range on a Perkin Elmer spectrophotometer model 1600. Mass spectra were determined on Thermo-Finnigan model LCQ Advantag.

General procedure for the synthesis of pyrrolidine type 3,7-diazabicyclo [3.3.0] octanes

To a suspend solution of α-amino acid methyl ester hydrochloride salt (5 mmol) in dry toluene (50 mL), triethylamine (5 mmol) was added then the reaction was stirred for further 0.5 h. Aromatic aldehyde (5 mmol) was added and heated up to reflux in order to remove water by Dean-Stark. *N*-phenylmaleimide (4 mmol) was added and the reaction mixture was reflux overnight.

The viscous oil, after removal of all the solvents, was dissolved in methylene chloride and was washed with water. The dried combined methylene chloride layer was evaporated to dryness to give crude precipitate which was recrystalized from methylene chloride/light petroleum.

*Methyl-2-(2-methylpropyl)-6,8-dioxo-4,7-diphenyl-3,7-diazabicyclo [3.3.0] octane-2-carboxylate (**8a**)*

L-leucine methyl ester hydrochloride (0.91 g), triethylamine (0.51 g), benzaldehyde (0.54 g) and *N*-phenylmaleimide (0.86 g) in dry toluene (50 mL) was reflux overnight to yield **8a** 1.37 g (67.82%), m.p. 184-186ºC (dichloromethane-light petroleum). ^{1}H NMR (methanol-d_3 + $CDCl_3$) δ : [0.87 (d, 3H, J = 6.4 Hz, -CH($C\underline{H}_3$)$_2$, 0.98 (d, 3H, J = 6.4 Hz, -CH($C\underline{H}_3$)$_2$], 1.76 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), [1.82 (dd, 1H, J = 8.2, 4.2 Hz, $C\underline{H}_2$CH<), 2.09 (dd, 1H, J = 8.2, 4.2 Hz, $C\underline{H}_2$CH<)], 2.81 (br, 1H, N$\underline{H}$), 3.39 (d, 1H_C, J = 7.2 Hz), 3.65 (t, 1H_B, J = 6.4 Hz), 3.92 (s, 3H, $CO_2C\underline{H}_3$), 4.70(d, 1H_A, J = 7.2 Hz), [7.0 (d, 2H, J = 7.6 Hz), 7.35-7.42 (m, 8H, 2$C_6\underline{H}_5$]; IR υ_{max} (nujol) 3377, 2989, 2717, 1775, 1698, 1456, 1441, 924, 869 cm^{-1}.

*Methyl-2-(methyl ethyl sulfane)-4-(5-methyl-2-furyl)-6,8-dioxo-7-diphenyl-3,7-diazabicyclo [3.3.0] octane-2-carboxylate (**8b**)*

L-methionine methyl ester hydrochloride (0.99 g), triethylamine (0.53 g), 5-methyl-2-furfuraldehyde (0.58 g), and *N*-phenylmaleimide (0.86 g) in dry toluene (50 mL) was reflux overnight to yield **8b** 1.76 g (80.07%), m.p.201-203ºC (dichloromethane-light petroleum). ^{1}H NMR (DMSO-d_6) δ : 2.05 (s, 3H, $C\underline{H}_3$-S), 2.18 (s, 3H, 5-$C\underline{H}_3$), 2.25-2.18 (m, 2H, $C\underline{H}_2$-CH_2-S), 2.58-2.47 (m, 2H, CH_2-$C\underline{H}_2$-S), 3.21 (d, 1H_C, J = 8 Hz), 3.61 (d, 1H_B, J = 8 Hz), 3.72 (s, 3H, $CO_2C\underline{H}_3$), 4.72 (d, 1H_A, J = 4.71 Hz), 5.99 (s, 1H, furyl), 6.28 (d, 1H, J = 4 Hz, furyl), [7.16 (d, 2H), 7.41 (t, 1H), 7.48 (t, 2H, $C_6\underline{H}_5$)]; m/z (%) 429 (M+1, 27), 347[(M+1)-82, 100], 314 [(347-33),16]; IR υ_{max} (nujol) 3319, 2995, 2725, 1745, 1693, 1456, 1441, 928, 863 cm^{-1}.

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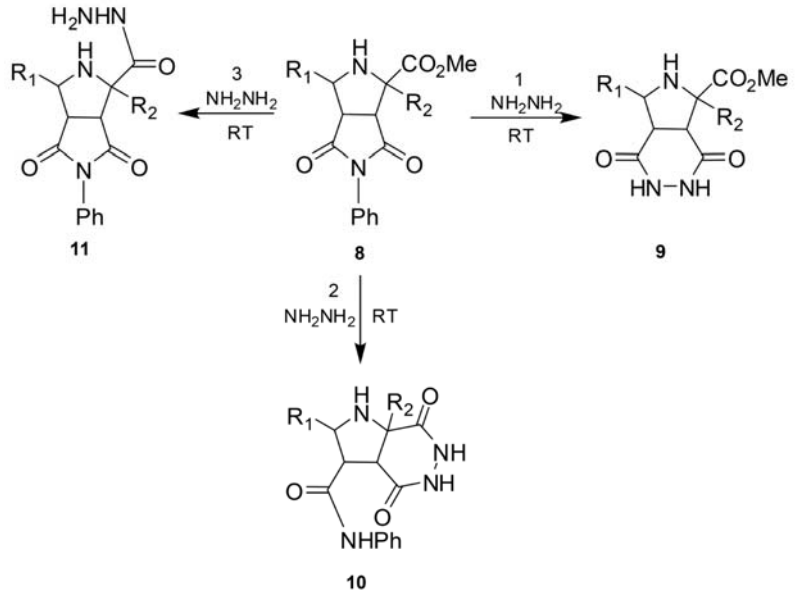

Figure 3: Expected nucleophilic substitution of 3,7diazabicyclo [3.3.0] octanes (**8**)

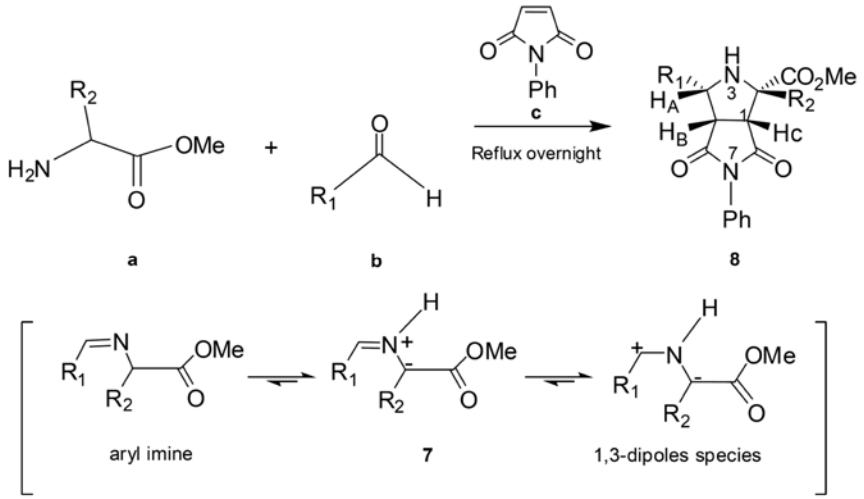

Figure 4: Synthetic route to pyrrolidine type 3,7diazabicyclo [3.3.0] octanes (**8**)

Methyl-2-benzyl-4-(5-methyl-2-furyl)-6,8-dioxo-7-phenyl-3,7-diazabicyclo[3.3.0] octane-2-carboxylate (**8c**)

L-phenylalanine methyl ester hydrochloride (1.07 g), triethylamine (0.78 g), 5-methyl-2-furfuraldehyde (0.86 g) and *N*-phenylmaleimide (0.86 g) in dry toluene (50 mL) was reflux overnight to yield **8c** 1.7036 g (76.88 %), m.p. 241-243 ºC (dichloromethane-light petroleum). ^{1}H NMR (methanol-d_3) δ : 2.19 (s, 3H, 5-C$\underline{H}_3$), 3.07 (d, 2H, J = 13.6 Hz, C$\underline{H}_2$-C_6H_5), 3.37 (d, 1H_C, J = 8 Hz), 3.65 (dd, 1H_B, J = 8 Hz), 3.84 (s, 3H, $CO_2C\underline{H}_3$), 4.86 (d, 1H_A, J = 8 Hz), 5.9 (s, 1H, furyl), 6.31 (d, 1H, J = 4 Hz, furyl), 7.19-7.45 (m, 10H, 2$C_6\underline{H}_5$); IR υ_{max} (nujol) 3419,3134, 2989, 1781, 1717, 1385, 923, 854 cm^{-1}.

*Methyl-2-benzyl-6,8-dioxo-4,7-diphenyl-3,7-diazabicyclo[3.3.0]octane-2-carboxylate (**8d**)*

L-phenylalanine methyl ester hydrochloride (1.08 g), triethylamine (0.50 g), benzaldehyde (0.53 g) and *N*-phenylmaleimide (0.8659 g) in dry toluene (50 mL) was reflux overnight to yield **8d** 1.38 g (62.76 %) m.p. 232-234 ºC (dichloromethane-light petroleum) (lit 232-234°C) (Amornraksa et al., 1987). ^{1}H NMR (methanol-d_3) δ : [3.11 (d, 1H, J = 13.4 Hz, C$\underline{H}$(H)-C_6H_5, 3.14 (d, 1H, J =

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13.4 Hz) CH(H)-C_6H_5)], 3.51 (d, $1H_C$, J = 8 Hz), 3.68 (dd, $1H_B$, J = 7.2, 8 Hz); 3.86 (s, 3H, -CO_2CH_3), 4.95 (d, $1H_A$, J = 7.2 Hz), 7.1-7.55 (m, 15H, $3C_6H_5$); IR υ_{max} (nujol) 3344, 2989, 1780, 1750, 1716, 1377, 916, 852 cm^{-1}.

*General procedure for the reaction of pyrrolidine type 3,7-diazabicyclo [3.3.0] octanes (**8**) with hydrazine in ethanol*

To a suspension solution of pyrrolidine **8a-d** in ethanol (25 mL), hydrazine (mole equivalent) was added and the reaction was stirred at room temperature overnight .The concentrated viscous oil which was dissolved in methylene chloride (30 mL) and extract with water (5 mL). The dried methylene chloride layer was concentrated to give viscous oil which was triturated with ether and filtered. The crude precipitate was crystallized from small amount of ethanol.

*5-amino-6-a-iso-butyl-4, 6-dioxo-2-phenyl-octahydropyrrolo [3,4-b] pyrrole-3-carboxylic acid phenylamide (**12a**)*

Pyrrolidine **8a** (1.50 g) and hydrazine (0.2 g) yielded **12a** 0.82 g (54.76%), m.p. 218.5-220.4ºC (ethanol). ^{1}H NMR (methanol-d_3) δ: 0.95 (d, 3H, J = 6.4 Hz, -CH(CH_3)$_2$), 1.01 (d, 3H, J = 6.4 Hz, -CH(CH_3)$_2$), 1.75 (br m, 1H, -CH_2C<u>H</u>(CH_3)$_2$, [1.89 (dd, 1H, J = 10, 4.4 Hz, C<u>H</u>(H)CH(CH_3)$_2$ and 2.05 (dd, 1H, J = 9.2, 6.4 Hz, CH(<u>H</u>)CH(CH_3)$_2$], 3.46 (d, $1H_C$, J = 8.4 Hz), 3.72 (dd, $1H_B$, J = 8, 6.8 Hz), 4.76 (d, $1H_A$, J = 4.8 Hz), 6.95-7.35 (m, 10H, $2C_6H_5$); IR υ_{max} (nujol) 3369, 3326, 2355, 1772, 1703, 1685, 1682, 1458, 1450 cm^{-1}; m/z (%) 407 (M+1, 42), 314[(M+1)-92, 100], 257(314-57, 12), 230 (314-84, 16).

*5-amino-2-(5-methylfuran-2-yl)-6a-(2-methylsulfanyl-ethyl)-4,6-dioxo-octa-hydropyrrolo [3,4-b] pyrrole-3-carboxylic acid phenylamide (**12b**)*

Pyrrolidine **8b** (0.16 g), hydrazine (0.2 g) yielded **12b** 0.063 g (40.77 %), m.p. 177.4-179.2ºC (ethanol). ^{1}H NMR (DMSO-d_6) δ : 2.05 (s, 3H, CH_3-S), 2.02 (m, 2H, CH_2-CH_2-S), 2.18 (s, 3H, 5-CH_3), 2.47 (m, 2H, CH_2-CH_2-S), 3.39 (d, $1H_C$, J = 7.2 Hz), 3.53 (dd, $1H_B$, J = 7.2, 8.0 Hz), 4.66 (d, $1H_A$, J = 8.0 Hz), 5.98(s, 1H, furyl), 6.38 (s, 1H, furyl), [7.17 [(d, 2H, J = 7.2 Hz and 7.51-7.4 (m, 3H), -C_6H_5]. IR υ_{max} (nujol) 3326, 1783, 1737, 1709, 1455, 1383 cm^{-1}.

*5-amino-6-a-benzyl-2-(5-methylfuran-2-yl)-4,6-dioxooctahydropyrrolo[3,4-b] pyrrole-3-carboxylic acid phenylamide (**12c**)*

Pyrrolidine **8c** (0.25 g), hydrazine (0.2 g) yielded **12c** 0.13 g (52.35 %), m.p. 201.6-203.4 ºC (ethanol). ^{1}H NMR (methanol-d_3) δ : 2.0 (s, 3H, 5-CH_3), 3.10 (d, 2H, $CH_2C_6H_5$, J = 13.2 Hz), 3.55 (d, $1H_C$, J = 7.6 Hz), 3.62 (t, $1H_B$, J = 7.8 Hz), 4.65 (d, $1H_A$, J = 7.6 Hz), 5.85 (s, 1H, furyl), 6.18 (s, 1H, furyl), 7.12-7.25 (m, 10H). IR υ_{max} (nujol) 3341, 3282, 1781, 1713, 1634, 1600, 1458, 1378 cm^{-1}.

*5-amino-6-a-benzyl-4,6-dioxo-2-phenyloctahydropyrrolo [3,4-b] pyrrole-3-carboxylic acid phenylamide (**12d**)*

Pyrrolidine **8d** (0.75 g), hydrazine (0.2 g) yielded **12d** 0.46 g (60.99 %), m.p. 216.5-218.2ºC (ethanol). ^{1}H NMR (methanol-d_3) δ : 3.15 (d, 2H, J = 12 Hz, $CH_2C_6H_5$), 3.50 (d, $1H_C$, J = 8 Hz), 3.62 (t, $1H_B$, J = 7.2 Hz), 4.71 (d, $1H_A$, J= 8.4 Hz), 6.9-7.35 (m, 15H, $3C_6H_5$); IR υ_{max} (nujol) 3533, 3412, 3307, 1773, 1714, 1669, 1600, 1557, 1456, 1377 cm^{-1}.

Antimicrobial activity testing

The antimicrobial activity of the tested compounds was determined against microorganism's growths using the agar dilution method (Prachayasittikul et al., 2008a). The compound dissolved in methanol was mixed with Müller Hinton (MH) broth to final volume of 2 mL. Two fold dilutions were performed and then mixed with MH agar solution that placed onto the plates with the final concentrations (4-256 μg/mL). The microorganisms were cultured in the MH broth at 37 °C for 18-24 h and diluted with 0.9 % normal saline solution to adjust cell density of 10^8 CFU/mL

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compared with 0.5 McFarland. The microorganisms were inoculated onto each plate and incubated at 37 °C for 24-48 h. The inhibition of cell growths was determined. Twenty-seven strains of microorganisms were tested as listed in Table 1.

Antioxidative activity testing

The antioxidative activity screening of the compounds was determined by DPPH radical assay (Prachayasittikul et al., 2008b). The DPPH, a stable purple color, reacts with antioxidant compound for 30 min to produce a light-yellow color of diphenylpicrylhydrazine which was measured by spectrophotometer at 517 nm. The percentage of radical scavenging activity was calculated as following equation:

% Radical scavenging = (1-Abs.sample/Abs.cont)×100

where Abs.cont was the absorbance of the control reaction and Abs.sample was the absorbance of the tested compound.

The SOD activity was performed by measuring inhibition of the photoreduction of nitro blue tetrazolium (NBT) (Piacham et al., 2006). The indirect assay is comprised of several reactions. Briefly, the photochemically excited riboflavin was first reduced by methionine into a semiquinone, which donated an electron to oxygen to form the superoxide source. The superoxide readily converted NBT into a purple formazan product which was detected by spectrophotometer at 550 nm.

Table 1: Microorganisms for antimicrobial activity testing

	Reference strains	Clinical isolate
Gram-negative bacteria	*Escherichia coli* ATCC 25922	*Shigella dysenteriae*
	Klebsiella pneumoniae ATCC 700603	*Salmonella enteritidis* type C
	Serratia marcescens ATCC 8100	*Morganella morganii*
	Salmonella typhimurium ATCC 13311	*Aeromonas hydrophila*
	Shewanella putrefaciens ATCC 8671	*Citrobacter freundii*
	Achromobacter xylosoxidans ATCC 2706	*Plesiomonas shigelloides*
	Pseudomonas aeruginosa ATCC 15442	
	Pseudomonas stutzeri ATCC 17587	
Gram-positive bacteria	*Staphylococcus aureus* ATCC 29213	*Streptococcus pyogenes* II
	Staphylococcus aureus ATCC 25923	*Bacillus cereus*
	Staphylococcus epidermidis ATCC 12228	*Listeria monocytogenes*
	Enterococcus faecalis ATCC 29212	
	Enterococcus faecalis ATCC 33186	
	Micrococcus lutens ATCC 10240	
	Bacillus subtilis ATCC 6633	
	Corynebacterium diphtheriae NCTC 10356	
Yeasts	*Saccharomyces cereviseae* ATCC 2601	
	Candida albicans ATCC 90028	

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RESULTS AND DISCUSSION

Synthesis of pyrrolidine type 3,7-diazabicyclo [3.3.0] octanes

Pyrrolidine type 3,7-diazabicyclo [3.3.0] octanes (**8a-d**) were synthesized by one pot reaction of aromatic aldehyde, α-amino acid ester and *N*-phenylmaleimide *via* non-isolated azomethine ylide intermediate (**7**). Recently, 1,3-dipolar cycloaddition reaction of the azomethine ylide generated from proline and isatin with the dipolarophile (*E*)- 2-arylidine-1-keto carbazoles has been reported for the synthesis of novel spiropyrrolizidines (Periyasami et al., 2008). This [3+2] cycloaddition process involves interaction of either HOMO of 1,3-dipole species with LUMO of dipolarophiles or vice-versa (Figure 5). ^{1}H NMR spectra of methyl-2-(2-methylpropyl)-6,8-dioxo-4,7-diphenyl-3,7-diazabicyclo [3.3.0] octane-2-carboxylate (**8a**) showed two magnetically non equivalent methyls of *iso*-butyl group as two sets of doublet at δ0.87 (d, 3H, J = 6.4 Hz) and 0.98 (d, 3H, J = 6.4 Hz), multiplet one methine proton at δ1.76 and magnetically non equivalent two prochiral methylene protons at δ1.82 and 2.09 as a doublet of doublet with coupling constant of 8.2 and 4.2 Hz. In addition, the ring junction protons, H_B and H_C appeared at δ 3.65 (a triplet, J = 6.4 Hz) and 3.39 (a doublet, J = 7.2 Hz), respectively. H_A was noted at δ 4.70 as a doublet with coupling constant of 7.2 Hz, whereas a singlet of methyl ester appeared at δ 3.92. Aromatic protons (10H) of two phenyl groups showed a doublet of two protons at δ 7.0 and eight protons as a multiplet at 7.35-7.42.

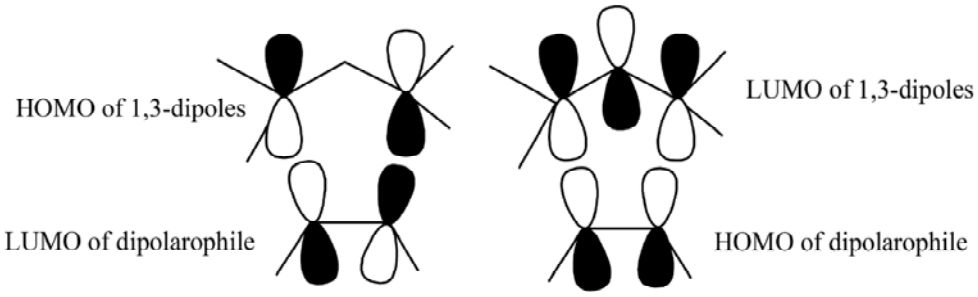
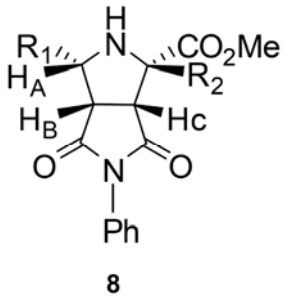


Figure 5: Frontier orbital for [3+2]-cycloaddition



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8a : $R_1 = C_6H_5$, $R_2 = CH_2CH(CH_3)_2$

8b : R_1 = 5-methyl-2-furyl, $R_2 = CH_2CH_2SCH_3$

8c : R_1 = 5-methyl-2-furyl, $R_2 = CH_2C_6H_5$

8d : $R_1 = C_6H_5$, $R_2 = CH_2C_6H_5$

Figure 6: Configuration of H_A, H_B and H_C

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Its IR spectra (cm^{-1}) showed amine stretching vibration at 3377 ester at 1775 and imide at 1698. Compounds **8b-d** also have similar ^1H NMR pattern of the skeleton type 3,7-diazabicyclo [3.3.0] octanes as **8a**, except the pattern of substituents R_1 and R_2. We can conclude that the magnitude of coupling constant less than 12 Hz confirmed the *cis*-configuration of ring junction protons (H_B, H_C) and H_A (Figure 6).

Reaction of pyrrolidine derivatives type 3,7-diazabicyclo [3.3.0] octane

The reaction of 3,7-diazabicyclo [3.3.0] octane (**8a**) with hydrazine in ethanol at room temperature was proposed to give at least three expected products. From the ^1H NMR spectra, carbomethoxy protons were not observed, implying that path way 1 (Figure 3) producing pyridazine-3,6-dione (**9**) *via* nucleophilic substitution is unlikely to occur. Thus, pyridazine-3,6-dione (**10**) and pyrrolidine-2,5-dione (**11**) are possible products. Compounds **10** and **11** have three methine protons which are unambiguously distinguished.

The molecular ion $[M+1]^+$ of either **10** (R_1 = C_6H_5, R_2 = *iso*-C_4H_9) or **11** (R_1 = C_6H_5, R_2 = *iso*-C_4H_9) showed the same value as 407, but the daughter fragmentation patterns should be the data that make possible to distinguish between the structure **10** or **11**. The observed base peak probably represents the loss of aniline molecule at m/e 314 corresponding to [(M^++1)-93], concurrence with the loss of *iso*-butyl group; m/e 257 [314-57]. Fragmentation of 1,2-diazete-3,4-dione molecule is possible at m/e 230 from [314-84]. All of these daughter ion peaks are possible for the structure **10** (R_1 = C_6H_5, R_2 = *iso*-C_4H_9). But, IR υ_{max} (cm^{-1}) at 1772, 1703 supports the structure of five member cyclic imide ring instead of the six member cyclic imide ring (~1710 and ~1700 cm^{-1}). Finally, the ambiguous was proved by X-ray crystallography (unpublished) and revealed the structure of 1-aminopyrrolidine-2,5-dione **(12a)** instead of the structure **10** (pyridazine-3,6-dione).

The formation of **12a** supports the nucleophilicity of nitrogen on hydrazine. Initially, nitrogen attacked the ester group of **8a** to furnish intermediate **11** (as proposed in pathway 3, Figure 3) and then the same nitrogen attacked the imide function. Finally cleavage of C-N linkage from pyrrolidine-2,5-dione ring (**11**) simultaneously formed the pyrrolidine-2,5-dione (**12a**) as outlined in Figure 7. Similary, the bicyclics **8b-d** were transformed in ethanolic hydrazine solution to furnish the corresponding 1-aminopyrrolidine-2,5-diones (**12b-d**) as shown in Figure 8.

Biological activity

Antimicrobial activity of compounds **8b** and **12a** was evaluated using agar dilution method against 27 strains of microorganisms. Results showed that at the tested concentrations (4-256 μg/mL), no growth inhibition was significantly detected. Antioxidative activity of **8b** and **12a** was tested using DPPH (2,2-diphenyl-1-picrylhydrazyl and SOD (superoxide dismutase) assays. It was found that at 200 μg/mL compound **12a** displayed weak antioxidant, 0.3 % radical scavenging activity (DPPH assay) and 3.94 % inhibition of superoxide anion (SOD).

CONCLUSION

The one pot synthesis of pyrrolidine derivatives type 3,7-diazabicyclo [3.3.0] octanes (**8a-d**) has been successfully achieved from the reaction of α-amino acid ester with aromatic aldehyde and *N*-phenylmaleimide. The reaction of **8a-d** with hydrazine in ethanol at room temperature gave derivatives of 1-aminopyrrolidine-2,5-diones (**12a-d**) *via* double *N*-nucleophilic substitution on ester and imide moieties, respectively. Such transformation of **8a-d** demonstrates the ease of forming bicyclic pyrrolidine analogs **12** as well as potential use of other nucleophilic reagents, in stead of hydrazine, to form a vast array of bicyclic compounds with medicinal values. The antimicrobial and anti-

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oxidative activities of **8b** and **12a** were tested, but found to be inactive.

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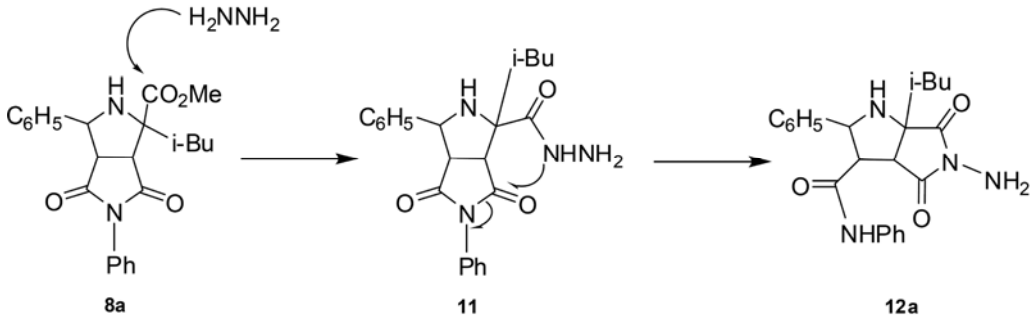
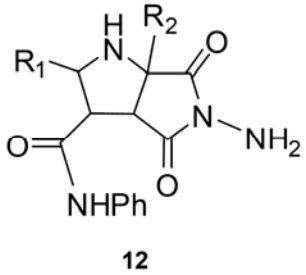


Figure 7: Mechanism for the formation of compound **12a**



12a : $R_1 = C_6H_5$, $R_2 = CH_2CH(CH_3)_2$

12b : R_1 = 5-methyl-2-furyl, $R_2 = CH_2CH_2SCH_3$

12c : R_1 = 5-methyl-2-furyl, $R_2 = CH_2C_6H_5$

12d : $R_1 = C_6H_5$, $R_2 = CH_2C_6H_5$

Figure 8: Structures of compounds **12a**-**d**

REFERENCES

Ahmed S, Smith JH, Nicolls PJ, Whomsley R, Cariuk P. Synthesis and biological evaluation of novel pyrrolidine-2,5-dione inhibitor potential anti-tumor agents. Drug Des Discov 1995;12:275-87.

Amornraksa K, Grigg R, Gunaratine HQ, Nimal, Kemp J, Sridharn V. X=Y-ZH Systems as potential 1,3-dipoles. Part 8. [1]Pyrrolidines and Δ^5-pyrrolines (3,7-diazabicyclo [3.3.0] octenes) from the reaction of imines of α-amino acids and their esters with cyclic dipolarophiles. Mechanism of racemisation of α-amino acids and their esters in the presence of aldehydes. J Chem Soc Perkin Trans 1987;I:2285-96.

Dönadas HA, Nural Y, Duran N, Kilner C. Synthesis, crystal structure and antifungal/antibacterial activity of some novel highly functionalized benzoylaminocarbothioyl pyrrolidines. Turk J Chem 2006;30:573-83.

Kaminski K, Obniska J. Synthesis and anticonvulsant properties of new 1-(2-pyridinyl)-3-substituted pyrrolidine-2,5-dione derivatives. Acta Pol Pharm Drug Res 2008;65:457-65.

Liu X, Fortin PD, Walsh CT. Andrimid producers encode an acetyl-CoA carboxyltransferase subunit resistant to the action of the antibiotic. Proc Natl Acad Sci USA 2008;105:13321-6.

Obniska J, Jurczyk S, Zejc A, Kamiński K, Tatarczyńska E, Stachowicz K. Anticonvulsant properties of N-(4-methylpiperazin-1-yl)- and N-[3-(4-methyl-piperazin-1-yl)propyl] derivatives of 3-aryl- and 3-spirocycloalkyl-pyrrolidine-2,5-dione. Pharmacol Rep 2005; 57:170-5.

Periyasami G, Raghunathan R, Surendiran G, Mathivanan N. Synthesis of novel spiropyrrolizidines as potent antimicrobial agents for human and plant pathogens. Bioorg Med Chem Lett 2008;18:2342–5.

Piacham T, Isarankura-Na-Ayudhya C, Nantasenamat C, Yainoy S, Ye L, Prachayasittikul V. Metalloantibiotic Mn(II)-bacitracin complex mimicking manganese superoxide dismutase. Biochem Biophys Res Comm 2006;341:925-30.

Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidative activities of bioactive constituents from *Hydnophytum formicarum* Jack. Molecules 2008a;13:904-21.

Prachayasittikul S, Suksrichavalit T, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidative activities of 1-adamantylthio derivatives of 3-substituted pyridines. EXCLI J 2008b;7:63-70.

Shorvon S. Pyrrolidone derivatives. Lancet 2001;358:1885-92.

Solomons TWG, Fryhle CB. Organic chemistry, 9th ed. Hoboken, NJ: John Wiley & Sons, 2008, p. 913.