NEIHUMICIN, A NEW CYTOTOXIC ANTIBIOTIC FROM *MICROMONOSPORA NEIHUENSIS*

III. STRUCTURE-ACTIVITY RELATIONSHIPS

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Structure-cytotoxicity relationships studies have indicated that the C-3 and C-6 disubstituted piperazine-2,5-diones are structurally required for significant cytotoxicity, and the neihumicin-like C-3 and C-6 disubstituted unsymmetrical piperazine derivatives are, in general, more cytotoxic than the corresponding symmetrical piperazine-2,5-diones. Several synthetic analogs including 3,6-di-(2,4,5-trimethoxybenzylidene)piperazine-2,5-dione, 3,6-dibenzylidene-2-ethoxy-3,6-dihydropyrazine-5-one, 3-benzylidene-6-(*m*-chlorobenzylidene)-2-methoxy-3,6-dihydropyrazin-5-one, and 3,6-di-(*m*-chlorobenzylidene)-2-methoxy-3,6-dihydropyrazin-5-one, have been shown to be more cytotoxic than neihumicin.

Neihumicin produced by *Micromonospora neihuensis* Wu, sp. nov. is a new cytotoxic antibiotic. The isolation, structural determination and total synthesis have been described in the preceding papers[1,2]. In this paper, we report on the structure-cytotoxicity relationships of neihumicin and related compounds.

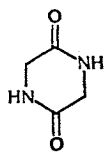
Synthesis of Target Compounds

A survey of the literature revealed no record of the systematic structure-cytotoxic antitumor activity relationships study among the piperazine-2,5-dione derivatives. Fig. 1 lists the target compounds we have synthesized for a systematic structure-cytotoxicity correlation among neihumicin (**6**) related compounds. These compounds were divided into three groups. Group I is piperazinedione without substitution at either C-3 or C-6, such as **2**, or with monosubstitution at C-3, such as **3**~**5** and **7**~**12**. Compounds **2**~**5** are intermediates involved in the total synthesis of **6**[2]. An evaluation of this group of compounds will clarify whether absence of a disubstitution at both C-3 and C-6 of the symmetrical piperazine-2,5-dione skeleton will still lead to potential cytotoxic agents. Group II corresponds to compounds (**13**~**22**) with substitution at C-3 and C-6 on piperazine-2,5-diones moieties. Evaluation of this group of compounds will delineate the importance of a disubstitution at C-3 and C-6 for potential cytotoxicity of the symmetrical piperazinedione derivatives. Group III refers to compounds (**6** and **23**~**26**) with substitutions C-3 and C-6 on 2-methoxy-3,6-dihydropyrazin-5-one. This will elucidate whether the disubstitution at C-3 and C-6 on an unsymmetrical piperazinedione is the structural requirement for the potent cytotoxicity of **6** and related compounds. The general synthetic scheme of the target compounds is shown in the Fig. 2.

Fig. 1. Target compounds.

Group I: Compounds without substitution at C-3 and C-6 or with monosubstitutions at C-3.
Group II: Compounds with substitution at C-3 and C-6 on piperazine-2,5-diones.
Group III: Compounds with substitution at C-3 and C-6 on 2-methoxy(or 2-ethoxy)-3,6-dihydropyrazin-5-one.

Group I



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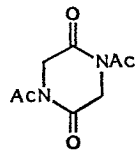

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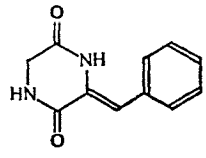

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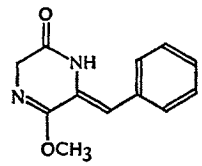

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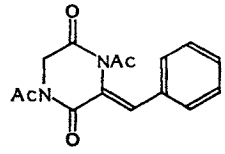

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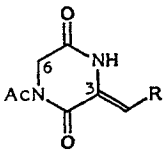

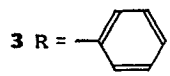

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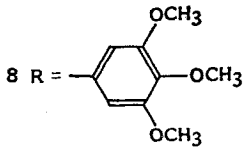

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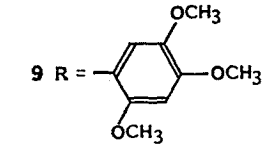

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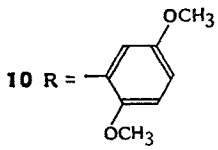

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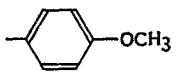

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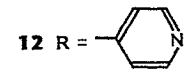

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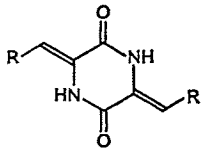

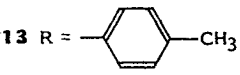

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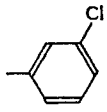

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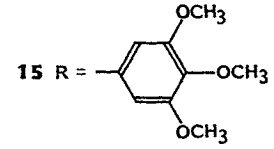

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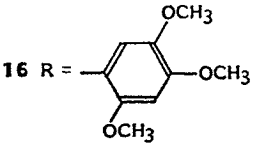

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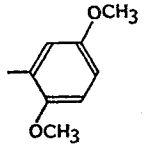

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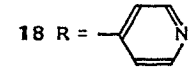

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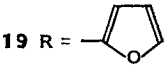

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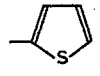

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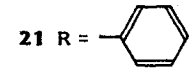

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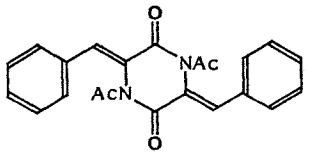

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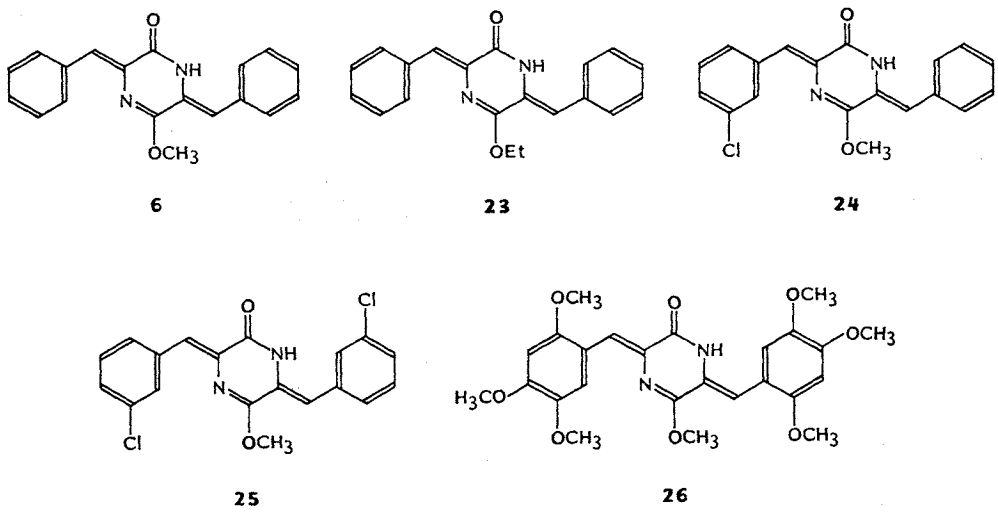
Fig. 1. (Continued)

Group III

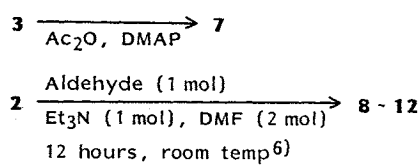


6 23 24

25 26

Fig. 2. The synthetic scheme of the target compounds.

Group I



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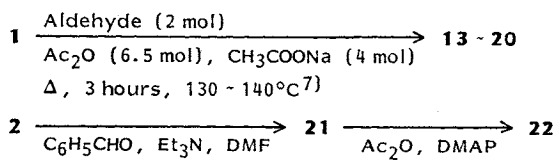

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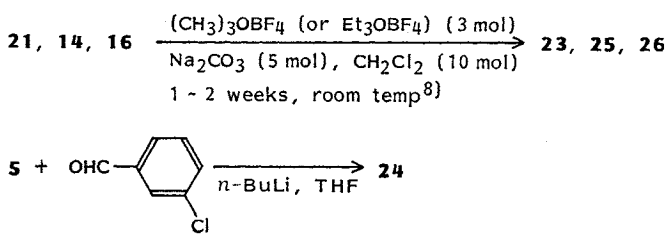

Table 1. Cytotoxicity of compounds (**2**~**26**).

Group I		Group II		Group III	
No.	ED_{50} (μg/ml)†	No.	ED_{50} (μg/ml)	No.	ED_{50} (μg/ml)
2	>4.0	**13**	>4.0	**6**	0.94
3	>4.0	**14**	>4.0	**23**	0.08
4	>4.0	**15**	>4.0	**24**	0.29
5	>4.0	**16**	0.27	**25**	0.40
7	>4.0	**17**	>4.0	**26**	3.60
8	>4.0	**18**	>4.0		
9	>4.0	**19**	>4.0		
10	>4.0	**20**	>4.0		
11	>4.0	**21**	>4.0		
12	>4.0	**22**	>4.0		

† KB cells.

Biological Evaluation and Conclusion

All compounds prepared in this study were assayed for their *in vitro* cytotoxicity against KB tissue culture cells according to a modification of the method of GERAN *et al.*[3,4] as discussed previously[1]. A comparison of the ED_{50} values of compounds **2**~**26** (Table 1) clearly indicated the importance of the disubstitution at C-3 and C-6 of the piperazine-2,5-dione as the monosubstituted compounds in Group I were all inactive (MIC>4 μg/ml). The C-3, C-6 disubstituted unsymmetrical 2-methoxy-(or 2-ethoxy-)-3,6-dihydropyrazin-5-ones, such as **6** and **25**, are in general more cytotoxic than the corresponding symmetrical piperazine-2,5-dione, such as **21** and **14**, respectively, except for compounds **26** and **16**, in which the latter is more cytotoxic than the former. The degree of cytotoxicity is also obviously dependent on the type of C-3 or C-6 substituents. Thus, among compounds in Group II and Group III only, such as those found in **16**, **23**, **24**, **25** and **26**, were most cytotoxic. The nature of the enolized alkyl ether is also important in contributing to the potent cytotoxicity. Thus, **23** is approximately 10-fold more cytotoxic than **6**. *In vivo* screening of these cytotoxic antitumor agents is currently in progress.

Expeirmental

General

The general experimental details have been reported previously[2].

1,4-Diacetyl-3-benzylidenepiperazine-2,5-dione (7)

A mixture of **3** (1.22 g, 5 mmol) and 4-dimethylaminopyridine (DMAP) (61 mg) in acetic anhydride (24 ml) was stirred under nitrogen for 24 hours at 40°C. The reaction mixture was allowed to cool to room temperature, poured into ice-water, and extracted with ethyl acetate (3×15 ml). The combined organic layers were washed consecutively with cold 5% aqueous sodium carbonate solution (to neutralize the acetic acid), brine, dried over anhydrous sodium sulfate, and evaporated under vacuum to yield a residue. Chromatography of the residue on silica gel column (22 g, 2×17 cm) in chloroform gave fractions which contained **7**. Recrystallization from ethanol furnished **7** as colorless needles (1.12 g, 78.6%): MP 150~152°C (literature[5] 151~152°C); IR (KBr) cm^{-1} 1695, 1625; UV λ_{max}^{EtOH} nm (log ε) 302 (4.13); NMR (60 MHz) δ 2.50 (3H, s, NAc), 2.62 (3H, s, NAc), 4.67 (2H, s, NCH_2CO), 7.58 (1H, s, vinyl H), 7.40 (5H, s, ArH).

1-Acetyl-3-(3,4,5-trimethoxybenzylidene)piperazine-2,5-dione (8)

A mixture of **2** (1.04 g, 5 mmol), 3,4,5-trimethoxybenzaldehyde (1.03 g, 5 mmol), triethylamine (0.7 ml, 5 mmol) and DMF (10 ml) was stirred at room temperature for 3 days. The mixture was

neutralized with acetic acid and poured into water. The product was extracted with ethyl acetate. The organic phase was washed with water, brine, dried over anhydrous magnesium sulfate, evaporated under vacuum, and gave **8** as pale yellow crystals (1.03 g, 62%) after recrystallization from methanol: MP 164~166°C; IR (KBr) cm^{-1} 3200, 1680, 1620; UV λ_{max}^{EtOH} nm (log ε) 332 (4.24); NMR (250 MHz) δ 2.66 (3H, s, NAc), 3.88 (6H, s, 2×OCH_3), 3.89 (3H, s, OCH_3), 4.52 (2H, s, NCH_2CO), 6.57 (2H, s, ArH), 7.10 (1H, s, vinyl H), 8.01 (1H, br, NH); *Anal* calcd for $C_{16}H_{16}N_2O_6$: C 57.48, H 5.43, N 8.38; found: C 57.55, H 5.57, N 8.41.

1-Acetyl-3-(2,4,5-trimethoxybenzylidene)piperazine-2,5-dione (**9**)

A mixture of **2** (1.04 g, 5 mmol), 2,4,5-trimethoxybenzaldehyde (1.03 g, 5 mmol), triethylamine (0.7 ml, 5 mmol) and DMF (10 ml) was stirred at room temperature for 2 days. The reaction mixture was neutralized with acetic acid and poured into water. The product was extracted with ethyl acetate. The organic phase was washed with water, brine, dried over anhydrous magnesium sulfate, evaporated, and yielded **9** (2.51 g, 75%) after recrystallization from methanol: MP 200~202°C; IR (KBr) cm^{-1} 3180, 1665, 1605; UV λ_{max}^{EtOH} nm (log ε) 360 (4.24); NMR (250 MHz) δ 2.65 (3H, s, NAc), 3.86, 3.94, 3.97 (9H, s, 3×OCH_3), 4.48 (2H, s, NCH_2CO), 6.58~7.05 (3H, m, ArH and vinyl H), 8.76 (1H, br, NH); *Anal* calcd for $C_{16}H_{18}N_2O_6$: C 57.48, H 5.43, N 8.38; found: C 57.86, H 5.37, N 8.43.

1-Acetyl-3-(2,5-dimethoxybenzylidene)piperazine-2,5-dione (**10**)

Compound **10** was prepared from **2** and 2,5-dimethoxybenzaldehyde (0.83 g, 5 mmol) by the same procedure which was used for the synthesis of **9**. Compound **10** was obtained as pale yellow crystals (1.42 g, 94%): MP 215~217°C; IR (KBr) cm^{-1} 3160, 1670, 1625; UV λ_{max}^{EtOH} nm (log ε) 348 (3.95), 305 (4.06); NMR (250 MHz) δ 2.66 (3H, s, NAc), 3.79 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.48 (2H, s, NCH_2CO), 6.82~7.09 (4H, m, ArH and vinyl H), 8.60 (1H, br, NH).

1-Acetyl-3-(*p*-methoxybenzylidene)piperazine-2,5-dione (**11**)

A mixture of **2** (0.99 g, 5 mmol), *p*-anisaldehyde (0.6 ml, 5 mmol), triethylamine (0.7 ml, 5 mmol) and DMF (10 ml) was stirred at 120~130°C for 8 hours. The reaction mixture was neutralized with acetic acid and poured into water. Compound **11** was prepared following the same procedure described for **9**. The crude **11** was recrystallized from ethanol to give yellow crystals (1 g, 78%): MP 178~180°C (literature[6] 179~180°C); IR (KBr) cm^{-1} 3240, 1670, 1620, 1600; UV λ_{max}^{EtOH} nm (log ε) 331 (4.27), 230 (4.17); NMR (250 MHz) δ 2.65 (3H, s, NAc), 3.86 (3H, s, OCH_3), 4.52 (2H, s, NCH_2CO), 6.98 (2H, d, ArH), 7.14 (1H, s, vinyl H), 7.36 (2H, d, ArH), 7.80 (1H, br, NH).

1-Acetyl-3-isonicotinylidenepiperazine-2,5-dione (**12**)

A mixture of **2** (1.15 g, 5.8 mmol), isonicotinaldehyde (0.6 ml, 5.8 mmol), triethylamine (0.8 ml, 5.8 mmol), and DMF (12 ml) was stirred at room temperature for 20 hours. The mixture was neutralized with acetic acid and poured into water. Compound **12** was prepared by the sequence used for the preparation of **9**. An analytical sample was recrystallized from methanol to give 488 mg (35%) of **12**: MP 214°C (dec); IR (KBr) cm^{-1} 3360, 1680, 1625, 1600; UV λ_{max}^{EtOH} nm (log ε) 303 (4.08); NMR (200 MHz, DMSO-d_6) δ 3.30 (3H, s, NAc), 4.37 (2H, s, NCH_2CO), 6.87 (1H, s, vinyl H), 7.49 (2H, d, ArH), 8.60 (2H, d, ArH), 10.61 (1H, br, NH).

3,6-Di-(*p*-methylbenzylidene)piperazine-2,5-dione (**13**)

A mixture of **1** (2.28 g, 20 mmol), *p*-tolualdehyde (4.8 g, 40 mmol), anhydrous sodium acetate (6.6 g, 80 mmol) and acetic anhydride (12 ml, 127 mmol) was heated for 3 hours at 130~140°C until a solid cake was formed. The reaction mixture was filtered, washed with hot water, ethanol and ether. Recrystallization from acetic acid yield 1.4 g (24%) of **13**[7] as yellow crystalline powders: MP 303~305°C; IR (KBr) cm^{-1} 3200, 1670, 1615; UV λ_{max}^{EtOH} nm 346, 246, 240; NMR (250 MHz) δ 2.40 (6H, s, 2×CH_3), 7.00 (2H, s, vinyl H), 7.25~7.32 (8H, m, ArH), 8.13 (2H, br, 2×NH).

3,6-Di-(*m*-chlorobenzylidene)piperazine-2,5-dione (**14**)

Compound **14** was prepared from *m*-chlorobenzaldehyde (4.5 ml, 40 mmol) by the same procedure

used to prepare **13**. Compound **14** (3.76 g, 52%) was obtained as pale yellow crystals after recrystallization from acetic acid: MP 283~284°C (literature[7] 280°C); IR (KBr) cm^{-1} 3200, 1670, 1615; UV λ_{max}^{EtOH} nm (log ε) 337 (4.21), 268 (3.69), 238 (3.78); NMR (250 MHz, DMSO-d_6) δ 6.74 (2H, s, vinyl H), 6.74~7.61 (8H, m, ArH), 10.63 (2H, br, 2×NH); *Anal* calcd for $C_{18}H_{12}N_2O_2Cl_2$: C 60.19, H 3.37, N 7.80; found: C 60.14, H 3.42, N 7.83.

3,6-Di-(3,4,5-trimethoxybenzylidene)piperazine-2,5-dione (**15**)

This compound was prepared from 3,4,5-trimethoxybenzaldehyde (7.85 g, 40 mmol) using the same procedure as for **13**. Compound **15** (4.05 g, 43%) was obtained as pale yellow crystals after recrystallization from acetic acid: MP 260~262°C; IR (KBr) cm^{-1} 3200, 1670, 1630; UV λ_{max}^{EtOH} nm (log ε) 359 (4.59), 263 (4.22); NMR (250 MHz) δ 3.89 (18H, s, 6×OCH_3), 6.59 (4H, s, ArH), 6.96 (2H, s, vinyl H), 8.22 (2H, br, 2×NH); *Anal* calcd for $C_{24}H_{26}N_2O_8$: C 61.27, H 5.57, N 5.96; found: C 61.27, H 5.59, N 5.79.

3,6-Di-(2,4,5-trimethoxybenzylidene)piperazine-2,5-dione (**16**)

Compound **16** was prepared from 2,4,5-trimethoxybenzaldehyde (7.85 g, 40 mmol) using the same procedure as for **13**. Compound **16** (2.44 g, 26%) was obtained as crystals: MP 234~235°C; IR (KBr) cm^{-1} 3180, 1665, 1620; UV λ_{max}^{EtOH} nm (log ε) 394 (4.13), 276 (3.71); NMR (250 MHz, DMSO-d_6) δ 3.75, 3.84, 3.86 (3×6H, s, 2×OCH_3), 6.73~7.05 (6H, m, ArH and vinyl H), 9.95 (2H, s, 2×NH); *Anal* calcd for $C_{24}H_{26}N_2O_8$: C 61.27, H 5.57, N 5.95; found: C 61.19, H 5.57, N 6.03.

3,6-Di-(2,5-dimethoxybenzylidene)piperazine-2,5-dione (**17**)

Compound **17** was prepared in the same method as that used for **13** using 2,5-dimethoxybenzaldehyde (6.65 g, 40 mmol) as the starting material. Compound **17** (1.58 g, 19.3%) was obtained as yellow needles after recrystallization from acetic acid: MP 282~283°C; IR (KBr) cm^{-1} 3180, 1670, 1620; UV λ_{max}^{EtOH} nm (log ε) 372 (4.21), 323 (4.14); NMR (400 MHz) δ 3.79 (6H, s, 2×OCH_3), 3.91 (6H, s, 2×OCH_3), 6.84~6.95 (8H, m, ArH and vinyl H), 8.89 (2H, br, 2×NH).

3,6-Diisonicotinylidenepiperazine-2,5-dione (**18**)

A mixture of **2** (1.98 g, 10 mmol), isonicotinaldehyde (2 ml, 20 mmol), triethylamine (2.8 ml, 20 mmol), and DMF (20 ml) was stirred for 3 days at room temperature. The reaction mixture was concentrated under vacuum. After cooling, the solid was filtered, washed with water, ethanol and dried to give **18** (2.58 g, 88.4%) after recrystallization from acetic acid: MP 302~304°C (dec); IR (KBr) cm^{-1} 3210, 1675, 1625; UV λ_{max}^{DMF} nm 335; NMR (250 MHz, DMSO-d_6) δ 6.73 (2H, s, vinyl H), 7.48 (2H, d, ArH), 8.58 (2H, d, ArH), 10.73 (2H, s, 2×NH); *Anal* calcd for $C_{16}H_{12}N_4O_2$: C 65.57, H 4.14, N 19.17; found: C 65.49, H 4.41, N 19.07.

3,6-Difurfurylidenepiperazine-2,5-dione (**19**)

Compound **19** was prepared by use of a procedure identical to that described for preparation of **13**. Using furfural (3.3 ml, 40 mmol) as the starting material. Recrystallization from methanol yielded **19** as yellow crystals (3.01 g, 55.8%): MP 289~290°C; IR (KBr) cm^{-1} 3380, 1670, 1635; UV λ_{max}^{EtOH} nm (log ε) 383 (4.65), 287 (3.93), 276 (4.03); NMR (250 MHz) δ 6.54 (2H, dd, furan H), 6.59 (2H, d, furan H), 7.06 (2H, d, furan H), 9.29 (2H, br s, furan H); *Anal* calcd for $C_{14}H_{10}N_2O_4$: C 66.22, H 3.68, N 10.28; found: C 62.22, H 3.73, N 10.37.

3,6-Di-(2-thiophenylidene)piperazine-2,5-dione (**20**)

Compound **20** was prepared by the same procedure used to obtain **13**. In this case, 2-thiophenecarboxaldehyde (3.7 ml, 40 mmol) was used as the starting material. Recrystallization from acetic acid gave 604 mg (10%) of **20** as yellow crystals: MP 304~306°C (dec); IR (KBr) cm^{-1} 3110, 1670, 1610; UV λ_{max}^{DMF} nm (log ε) 378 (4.42); NMR (250 MHz, DMSO-d_6) δ 7.18 (2H, dd, thiophene H), 7.55 (2H, d, thiophene H), 7.73 (2H, d, thiophene H), 8.01 (2H, br, 2×NH); *Anal* calcd for $C_{14}H_{10}N_2O_2S_2$: C 55.61, H 3.33, N 9.26; found: C 55.44, H 3.42, N 9.11.

3,6-Dibenzylidenepiperazine-2,5-dione (**21**)

A mixture of **2** (4.95 g, 25 mmol), benzaldehyde (6 ml, 59 mmol) and triethylamine (10 ml) in

DMF (2.5 ml) was refluxed for 5 hours. The reaction mixture was cooled and the deposited solid broken up, and the mixture was refluxed for 15 hours more. The reaction mixture was then cooled, ethyl acetate added, the crystalline product filtered and washed with more solvent. The pale yellow crystals were obtained by recrystallization from acetone to give **21** (6.54 g, 90.2%): MP 293~295°C; IR (KBr) cm^{-1} 3215, 1677, 1620; UV λ_{max}^{EtOH} nm 325, 228; NMR (60 MHz, DMSO-d_6) δ 6.78 (2H, s, vinyl H), 7.44~7.50 (10H, m, ArH), 10.33 (2H, br, 2×NH).

1,4-Diacetyl-3,6-dibenzylidenepiperazine-2,5-dione (**22**)

A mixture of **21** (10.7 g, 3.69 mmol), DMAP (451 mg), in acetic anhydride (24.4 ml) was heated at 130°C for 10 minutes and then stirred under nitrogen at 40°C for 12 hours. The reaction mixture was cooled, poured into aqueous chipped ice and extracted with ethyl acetate. The combined organic phases were washed with 2.5% sodium bicarbonate solution, water, brine, dried over anhydrous sodium sulfate, evaporated under reduced pressure, and gave **22** as colorless needles (81.1 mg, 5.95%) after recrystallization from chloroform - ether: MP 238~239°C; IR (KBr) cm^{-1} 1710, 1620; UV λ_{max}^{EtOH} nm (log ε) 289 (4.31); NMR (200 MHz) δ 2.53 (6H, s, 2×NAc), 7.41~7.42 (10H, m, ArH), 7.68 (2H, s, vinyl H); *Anal* calcd for $C_{22}H_{18}N_2O_4$: C 70.58, H 4.85, N 7.48; found: C 70.60, H 5.00, N 7.59.

3,6-Dibenzylidene-2-ethoxy-3,6-dihydropyrazin-5-one (**23**)

A mixture of **21** (743 mg, 2.56 mmol), 0.1 M triethyloxonium tetrafluoroborate in dichloromethane (10 ml, 10 mmol) and dichloromethane (10 ml) was stirred at room temperature for 4 days. The reaction mixture was poured into sodium carbonate solution and extracted with chloroform. The combined organic layers were washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness. The residue (559 mg) was purified by preparative TLC (silica gel, chloroform - *n*-hexane, 1 : 1) and gave **23** (80 mg, 10%) as yellow crystals after recrystallization from ethanol: MP 158~159°C; IR (KBr) cm^{-1} 3160, 1670, 1600, 1580; UV λ_{max}^{EtOH} nm (log ε) 365 (4.64), 241 (4.11); NMR (200 MHz) δ 1.51 (3H, t, CH_3), 4.50 (2H, q, CH_2), 6.61 (1H, s, vinyl H), 7.25~7.48 (10H, m, ArH), 8.07~8.12 (3H, br, NH and vinyl H); *Anal* calcd for $C_{20}H_{18}N_2O_2$: C 75.45, H 5.70, N 8.80; found: C 75.60, H 5.87, N 8.77.

3-Benzylidene-6-(*m*-chlorobenzylidene)-2-methoxy-3,6-dihydropyrazin-5-one (**24**)

To a solution of **5** (108 mg, 0.5 mmol) in dry THF (15 ml) was added *n*-butyllithium (0.41 ml, 0.65 mmol) at −78°C. After the solution was stirred under argon for 30 minutes, a solution of 3-chlorobenzaldehyde (73.6 μl, 0.65 mmol) in THF (20 ml), was added dropwise. After the mixture was stirred at −78°C for an additional 1 hour, it was allowed to warm up to 0°C for 20 minutes. The reaction was quenched with saturated aqueous ammonium chloride (until pH<7) and extracted with ethyl acetate - ether (3×20 ml). The combined organic layers were washed with brine, water, dried over anhydrous sodium sulfate, evaporated under vacuum, and gave **24** (8.6 mg, 5.1%) as pale yellow crystals after recrystallization from ethanol: MP 161~163°C; IR (KBr) cm^{-1} 3250, 1670, 1620, 1580; UV λ_{max}^{EtOH} nm (log ε) 364 (4.55), 246 (3.94); NMR (200 MHz) δ 4.08 (3H, s, OCH_3), 6.63 (1H, s, vinyl H), 7.21~7.45 (11H, m, ArH and vinyl H), 8.41 (1H, br, NH); MS *m/z* 338.0815 (M^+), 304, 116.

3,6-Di-(*m*-chlorobenzylidene)-2-methoxy-3,6-dihydropyrazin-5-one (**25**)

A mixture of **14** (1.08 g, 3 mmol) and trimethyloxonium tetrafluoroborate (2.7 g, 18 mmol) was stirred in dichloromethane (90 ml). After 2 weeks at room temperature, excess of sodium carbonate solution was added and the reaction mixture was extracted with chloroform. The combined organic extracts were washed with brine, water, dried over anhydrous magnesium sulfate, and evaporated under vacuum to yield a residue. This residue was purified by preparative TLC, and gave **25** (60 mg, 5.4% yield) after recrystallization from chloroform: MP 226~228°C; IR (KBr) cm^{-1} 3110, 1670, 1600; UV λ_{max}^{EtOH} nm (log ε) 365 (4.49), 247 (4.02); NMR (200 MHz) δ 4.09 (3H, s, OCH_3), 6.56 (1H, s, vinyl H), 7.24~7.43 (8H, m, ArH), 7.97 (1H, s, vinyl H), 8.41 (1H, br, NH).

3,6-Di-(2,4,5-trimethoxybenzylidene)-2-methoxy-3,6-dihydropyrazin-5-one (**26**)

A mixture of **16** (1.7 g, 3.7 mmol) and trimethyloxonium tetrafluoroborate (2 g, 14 mmol) was stirred in dichloromethane (150 ml). After 12 days at room temperature, the reaction mixture was

poured into a sodium carbonate solution and extracted with chloroform. The combined organic layers were washed with brine, water, dried over anhydrous magnesium sulfate and evaporated to dryness. The resulting residue was purified by preparative TLC (silica gel, chloroform - *n*-hexane, 7 : 3) to give, after crystallization from ethanol, 80 mg (6% yield) of **26** as orange-red crystals: MP 240°C; IR (KBr) cm^{-1} 3190, 1655, 1600, 1575; UV λ_{max}^{EtOH} nm (log ε) 427 (4.63), 327 (3.78); NMR (250 MHz) δ 3.86, 3.88, 3.90, 3.91, 3.93, 4.05, (each 3H, s, OCH_3), 6.45, 6.51, 6.58, 6.77, 7.26, 7.80, 8.54, (each 1H, s, ArH, vinyl H and NH); *Anal* calcd for $C_{25}H_{28}N_2O_8$: C 61.97, H 5.83, N 5.78; found: C 61.66, H 5.75, N 5.32.

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