

Regular Article

A Novel Methodology for Synthesis of 1,5,6-Trisubstituted 2(1H)-Pyrazinones of Biological Interest

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In this report, we describe a new method for the synthesis of densely functionalized 2(1H)-pyrazinones. Treatment of mesoionic 1,3-oxazolium-5-olates with carbanions derived from activated methylene isocyanides (*p*-toluenesulfonylmethyl isocyanide (TosMIC) and ethyl isocyanoacetate) causes a novel ring transformation affording 2(1H)-pyrazinones in moderate to high yields. The cytotoxicity and antibacterial activity of some of the obtained products were studied and some of the products exhibited tumor-specific cytotoxicity.

Key words ring transformation; mesoionic; pyrazinone; isocyanide

The 2(1H)-pyrazinone is an important privileged scaffold in medicinal chemistry, as well as in several alkaloids with diverse biological activity, including antitumor and antiviral properties.^{1–6} Therefore, several methods for the synthesis of 2(1H)-pyrazinones have been described in the literature: (a) condensation of α -aminoamides with α -diketones,^{7,8} (b) condensation of α -aminonitrile with oxalyl halides,^{6,9,10} and (c) Ugi four-component reactions.¹¹ Whereas these methods have proven very useful for the synthesis of 2(1H)-pyrazinones, they generally involve multistep synthetic operations and/or a hazardous cyanide source that limit the scope of these reactions. Indeed, it is still desirable to develop an efficient method for the synthesis of 2(1H)-pyrazinones.

As a continuation of our studies on the synthetic potential of mesoionic 4-trifluoroacetyl-1,3-oxazolium-5-olates (**1**) for heterocyclic synthesis,¹² we herein report a reaction of mesoionic oxazoles (**1**) with carbanions derived from activated methylene isocyanides (*p*-toluenesulfonylmethyl isocyanide (TosMIC) and ethyl isocyanoacetate). The mesoionic oxazoles (**1**) are easily prepared from *N*-acyl-*N*-alkylglycines (**2**) in a one step through the cyclodehydration by trifluoroacetic anhydride followed by trifluoroacetylation at C-4 position of an intermediary mesoionic 1,3-oxazolium-5-olates¹² (Chart 1).

TosMIC is a versatile, widely applicable reagent bearing an active methylene group and readily forms a stabilized, nucleophilic carbanion, which will react with a variety of electrophiles, such as aldehydes, ketones and imines.¹³ This class of reagent has most commonly been used in heterocyclic ring construction, in particular, of oxazole, pyrrole and imidazole moieties.

We have preliminarily reported that the unexpected reaction of mesoionic oxazoles (**1**) with TosMIC proceeds *via* an initial attack of TosMIC anions on the C-2 position of the ring followed by autooxidation to afford 2(1H)-pyrazinones in good yields.¹⁴ The origin of C-2 carbonyl oxygen in the 2(1H)-pyrazinones was molecular oxygen.

We report here our detailed studies of the reactions of **1** with carbanions derived from activated methylene isocyanides such as TosMIC and ethyl isocyanoacetate, and the behavior

of **1** with TosMIC from the comparison with the reaction of the related mesoionic 1,3-thiazolium-5-olates (**7**) with TosMIC in order to elucidate the presence of 4-trifluoroacetyl group essential for the reaction; herein, efficient syntheses of multiply substituted 2(1H)-pyrazinones and their anti-bacterial and tumor-cytotoxic activities are reported.

Results and Discussion

In our previous communication,¹⁴ we examined the reaction of **1a** with TosMIC under varying reaction conditions to determine the optimum conditions and reported the isolated yields of **3a–i** which were shown in Table 1. The best conditions to obtain the 2-pyrazinones **3a–i** were achieved using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base in *N,N*-dimethylformamide (DMF) at 0°C under O₂ atmosphere with bubbling by O₂ throughout the reaction. It was found that the reaction was very sensitive to the dioxygen and the yield of **3a** increased to 72% in the presence of dioxygen. The pathway of the reaction was elucidated to conduct the reaction under an atmosphere of ¹⁸O₂ and the carbonyl group of amide in **3a** contained more than 67% of the ¹⁸O label.¹⁴

In this study, two new substrates **1j** and **1k** were subjected to the reaction (Table 1, entries 10 and 11). In addition, imidazo[1,5-*a*]pyrazin-8(7H)-one derivatives (**4a–h**, **4j**, **4k**) were also isolated as a side product as shown in Table 1. The reaction of TosMIC with aldimines to form imidazoles is known as the van Leusen imidazole synthesis.¹⁵ The imidazo[1,5-*a*]pyrazine core was reported to be synthesized *via* condensation with TosMIC with pyrazin-2-one derivative.¹⁶ Therefore, the formation of imidazo[1,5-*a*]pyrazin-8(7H)-one derivatives (**4**) could be explained by the further reaction of the **3** with excessive TosMIC. Indeed, the reaction of **3a** with TosMIC

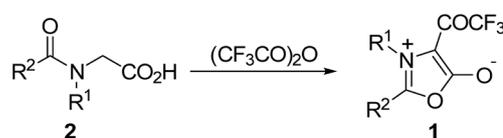
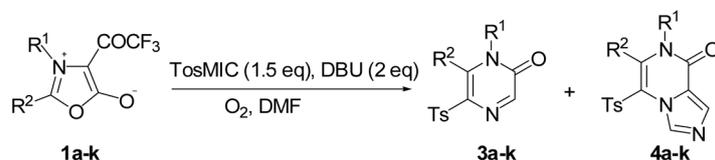


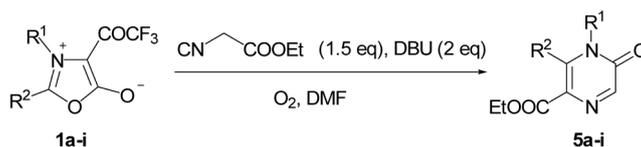
Chart 1. Synthesis of Mesoionic **1** from *N*-Alkyl-*N*-acylglycines (**2**)

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Table 1. Reactions of Mesoionic **1** with TosMIC^{a)}

Entry	1	R ¹	R ²	Conditions	Products (Yield, %) ^{b)}
1	a	Me	Ph	0°C, 3 h	3a (72), 4a (16)
2	b	Ph	Ph	0°C, 3 h	3b (65), 4b (10)
3	c	Ph	Me	-40°C, 12 h	3c (42), 4c (3)
4	d	Me	4-BrC ₆ H ₄	0°C, 1 h	3d (70), 4d (20)
5	e	Me	4-MeOC ₆ H ₄	0°C, 1 h	3e (78), 4e (5)
6	f	C ₆ H ₅ CH ₂	Ph	0°C, 2 h	3f (55), 4f (36)
7	g	4-MeOC ₆ H ₄ CH ₂	Ph	0°C, 2 h	3g (53), 4g (34)
8	h	C ₆ H ₅ CH ₂	4-MeOC ₆ H ₄	0°C, 2 h	3h (58), 4h (35)
9	i	Me	<i>t</i> -Bu	0°C, 5 h	3i (11), 4i (0)
10	j	Ph	4-NO ₂ C ₆ H ₄	0°C, 3 h	3j (25), 4j (44)
11	k	Ph	4-MeOC ₆ H ₄	0°C, 1 h	3k (35), 4k (36)

a) Reactions were carried out using **1** (0.50 mmol), TosMIC (0.75 mmol) and DBU (1.00 mmol) in DMF (3 mL) under atmosphere of oxygen. b) Isolated yield.

Table 2. Reactions of Mesoionic **1** with Ethyl Isocyanoacetate^{a)}

Entry	1	R ¹	R ²	Conditions	Products (Yield, %) ^{b)}
1	a	Me	Ph	0°C, 3 h	5a (92)
2	b	Ph	Ph	0°C, 3 h	5b (88)
3	c	Ph	Me	-40°C, 12 h	5c (52)
4	d	Me	4-BrC ₆ H ₄	0°C, 1 h	5d (85)
5	e	Me	4-MeOC ₆ H ₄	0°C, 1 h	5e (85)
6	f	C ₆ H ₅ CH ₂	Ph	0°C, 2 h	5f (90)
7	g	4-MeOC ₆ H ₄ CH ₂	Ph	0°C, 2 h	5g (91)
8	h	C ₆ H ₅ CH ₂	4-MeOC ₆ H ₄	0°C, 2 h	5h (91)

a) Reactions were carried out using **1** (0.50 mmol), TosMIC (0.75 mmol) and DBU (1.00 mmol) in DMF (3 mL) under atmosphere of oxygen. b) Isolated yield.

was reported to give **4a** in high yield.¹⁷⁾ The C3-position is electrophilically reactive position in the pyrazinone core^{18–20)} and could undergo nucleophilic attack by nucleophiles such as being part of an imine system.

On the basis of the above results, we tried to react **1a–h** with ethyl isocyanoacetate, using the same conditions for the reaction with TosMIC.¹⁴⁾ Gratifying, we found that the ring transformation reaction could be efficiently occurred in high yields. Thus, the substituents on C2 and N3 of the mesoionic ring on the reaction were investigated and the results are shown in Table 2. In the reaction of ethyl isocyanoacetate, an imidazo[1,5-*a*]pyrazin-8(*7H*)-one derivative was not obtained. In separate experiment, the reaction of **5a** with ethyl isocyanoacetate failed to afford the expected imidazo[1,5-*a*]pyrazin-8(*7H*)-one derivative under the comparable reaction conditions. Also, the reaction of **3a** with ethyl isocyanoacetate failed to afford the corresponding imidazo[1,5-*a*]pyrazine derivative. On the other hand, the reaction of **5a** with TosMIC gave **6** in 65% yield (Chart 2). Overall, the yields of the 2-pyrazinone products are generally better for the reaction with ethyl isocyanoacetate than the reaction with TosMIC (Tables

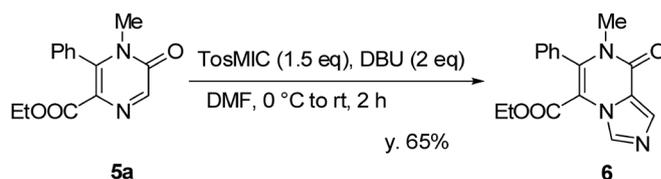
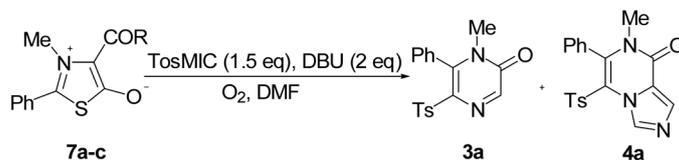


Chart 2. Synthesis of Imidazo[1,5-*a*]pyrazin-8(*7H*)-one (**6**) from Pyrazin-2(*1H*)-one (**5a**)

1, 2).

We next examined the effect of 4-trifluoroacetyl group in mesoionic oxazoles **1**. In our laboratory, we have synthesized 4-trifluoroacetyl- (**7a**), 4-acetyl- (**7b**)²¹⁾ and 4-formyl-1,3-thiazolium-5-olates (**7c**). Notably, there is much less information about the reactivity of 1,3-thiazolium-5-olates in the literature compared to 1,3-oxazolium-5-olates. A comparative study of the suitability of 4-acyl group in the reaction of **7a–c** with TosMIC under the same reaction conditions with **1** was undertaken. The results were shown in Table 3. The trifluoroacetyl derivative **7a** worked well (entry 1). On the other hand, 4-acetyl (**7b**) and 4-formyl (**7c**) derivatives afforded poor con-

Table 3. Reactions of Mesoionic Thiazoles with TosMIC^{a)}

Entry	7	R	Conditions	Products (Yield, %) ^{b)}	
				3a	4a
1	a	CF ₃	0°C, 2 h	68	2
2	b	CH ₃	0°C, 12 h	10	11
3	c	H	0°C, 12 h	13	15

a) Reactions were carried out using **1** (0.50 mmol), TosMIC (0.75 mmol) and DBU (1.00 mmol) in DMF (3 mL) under atmosphere of oxygen. b) Isolated yield.

Table 4. Cytotoxicity of Selected Compounds against Human Oral Malignant and Non-malignant Cells with CC₅₀ (μM)^{a)}

Compounds	Human oral squamous cell carcinoma cell lines					Human oral normal cells				SI ^{b)}
	Ca9-22	HSC-2	HSC-3	HSC-4	Mean (A) ^{c)}	HGF	HPLF	HPC	Mean (B) ^{d)}	
3f	70	93	95	85	86±11	275	362	>400	>346	>4.0
3g	49	47	180	50	82±66	358	>400	>400	>386	>4.7
3j	75	71	199	101	112±60	173	138	62	124±57	1.1
5g	163	87	95	87	108±37	228	225	216	223±6	2.1
5-FU	80	9.5	7.8	<7.8	26±36	>1000	>1000	>1000	>1000	>38.5
Melphalan	57	11	12	9.8	22±23	111	192	145	149±41	6.8

a) The CC₅₀ figure is the concentration of compound required to kill 50% of the cells and is the average of two independent determinations. b) The letter SI refer to the selectivity index. c) Mean A is average CC₅₀ value of the compound toward Ca9-22, HSC-2, HSC-3, and HSC-4 squamous cell carcinoma cell lines. d) Mean B is average CC₅₀ value of the compound toward human non-malignant HGF gingival fibroblasts, HPLF periodontal ligament fibroblasts and HPC pulp cells.

version (entries 2 and 3).

The molecular structures of **3a**¹⁴⁾ and **4a**¹⁷⁾ were previously confirmed by X-ray diffraction study of single crystals. A tentative mechanism which accounts for the formation of **3** is described in the communication.¹⁴⁾ Thus, initial nucleophilic attack by the TosMIC anion on C-2 of **1** gave rise to an adduct which is converted to the 2-pyrazinones (**3**) via a ring closure followed by auto-oxidation.

The anti-microbial activities of new pyrazinones (**3a-j** and **5a-h**) for six Gram-negative bacteria and six Gram-positive bacteria (listed in Experimental section) were measured by disc diffusion method.²²⁾ All the tested pyrazinones (**3a-j** and **5a-h**) showed no inhibition zone same as that of solvent control, whereas Gentamicin and Kanamycin showed inhibition zone (≥11 mm) against tested Gram-negative bacteria except for *Pseudomonas aeruginosa* 01 and Vancomycin showed inhibition zone (≥10 mm) against Gram-positive bacteria (data not shown). The tested compounds (**3a-j** and **5a-h**) were not active against the all tested microorganisms. Additional compounds in this series will be prepared to try to improve activity.

The compounds **3a-j** and **5a-h** were evaluated for their cytotoxicity against human oral squamous cell carcinoma cell lines (Ca9-22 derived from gingival tissue, and HSC-2, HSC-3 and HSC-4 derived from tongue), and human normal oral cells (HGF gingival fibroblasts, HPLF periodontal ligament fibroblasts and HPC pulp cells), using 5-fluorouracil (5-FU) and melphalan as the positive controls. These data for the selected compounds are presented in Table 4. Among ten **3** series and eight **5** series, **3g** showed the highest cytotoxicity, followed by **3f**, **5g**, and **3j**, whereas the other compounds showed

much lower cytotoxicity. Out of these four compounds, **3g** showed the highest tumor-specificity (TS=4.7), followed by **3f** (TS=4.0). When tumor-selectivity was calculated by Ca9-22 and HGF cells, both derived from gingival tissues, **3g** showed higher tumor-specificity [TS=7.3 (358/49)], as compared with melphalan [TS=1.9 (111/57)] (Table 4). The rest of the assayed compounds did not show any cytotoxicities against the studied tumor cells (CC₅₀>400 μM). The compounds in **3** series are more potent than the analogs in **5** series in which the same substituents are present at 1 and 6 positions (data not shown). Compounds **3g**, **f** and **5g**, with 1-(4-methoxyphenyl)methyl or 1-benzyl and 6-phenyl groups, were found to be the derivatives with the highest cytotoxic activity of the two series. **3j** with 4-nitrophenyl group showed moderate cytotoxicity against Ca9-22 and HSC-2 cells. These results suggest that further modification of these scaffolds might lead to compounds with enhanced cytotoxic activities.

Conclusion

We described herein a novel type of ring transformation of mesoionic oxazoles **1** into 2(1*H*)-pyrazinones **3** and **5** via an initial attack of TosMIC or ethyl isocyanoacetate on the C-2 position of the ring, opening of the oxazole ring, subsequent cyclization, and autoxidation. This unique ring transformation reaction of mesoionic oxazoles with TosMIC was not anticipated but the reaction is general as evidenced by the examples indicated in Tables 1 and 2. The method appears to be useful and convenient in terms of the ready accessibility of the starting materials, operational simplicity and mild condition. In addition, the 2(1*H*)-pyrazinones showed promising anticancer potency through preliminary biological studies.

Experimental

General Remarks Melting points (mp) were measured with a Yanaco MP-J3 melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AVANCE500 (500 MHz for ^1H and 126 MHz for ^{13}C) with tetramethylsilane (Me_4Si) as an internal reference and CDCl_3 as the solvent. ^1H - and ^{13}C -NMR spectral data are reported in parts per million (δ) relative to Me_4Si . IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. Mass spectra were recorded on JEOL JMS-GC mate II spectrometer with a direct inlet system at 70 eV and a Bruker micrOTOF-Q mass spectrometer with methanol as the solvent. Elemental analyses were carried out on a Yanaco CHN Corder MT-5 at the Integrated Center for Science, Ehime University. Standard work-up means that the organic layers were finally dried over anhyd. Na_2SO_4 , filtered, and concentrated *in vacuo* below 37°C using a rotary evaporator.

Materials The following compounds were prepared by employing the reported method.

N-Benzoyl-*N*-methylglycine (**2a**)

mp 101–104°C (mp²³) 102–104°C.

N-Benzoyl-*N*-phenylglycine (**2b**)

mp 126–128°C (mp²³) 127–129°C.

N-Acetyl-*N*-phenylglycine (**2c**)

mp 196–198°C (mp²⁴) 193–195°C.

N-(4-Bromobenzoyl)-*N*-methylglycine (**2d**)

mp 143–145°C (mp²⁵) 147–150°C.

N-(4-Methoxybenzoyl)-*N*-methylglycine (**2e**)

mp 151–154°C (mp²³) 155–160°C.

N-Benzoyl-*N*-benzylglycine (**2f**)

mp 103–104°C (mp²⁶) 106–107°C.

N-Benzoyl-*N*-(4-methoxyphenylmethyl)glycine (**2g**)

mp 157–158°C (mp²⁷) 157–158°C.

N-Benzyl-*N*-(4-methoxybenzoyl)glycine (**2h**)

mp 149–151°C (mp²⁵) 149–151°C.

N-Methyl-*N*-pivaloylglycine (**2i**)

mp 65–68°C (mp²⁸) 75–76°C.

N-(4-Nitrobenzoyl)-*N*-phenylglycine (**2j**)

mp 178–180°C (mp²³) 172–175°C.

N-(4-Methoxybenzoyl)-*N*-phenylglycine (**2k**)

mp 135–137°C (mp²³) 158–163°C.

4-Trifluoroacetyl-3-methyl-2-phenyl-1,3-oxazolium-5-olate (**1a**)

Pale yellow crystals, 95% yield. mp 161–163°C (mp²⁹) 162–163°C.

4-Trifluoroacetyl-2,3-diphenyl-1,3-oxazolium-5-olate (**1b**)

Yellow crystals, 94% yield. mp 194–196°C (mp²⁹) 194–196°C.

4-Trifluoroacetyl-2-methyl-3-phenyl-1,3-oxazolium-5-olate (**1c**)

White crystals, 90% yield. mp 200–203°C (mp²⁹) 211–212°C. 2-(4-Bromophenyl)-4-trifluoroacetyl-3-methyl-1,3-oxazolium-5-olate (**1d**)

White crystals, 90% yield. mp 188–191°C (mp²⁵) 188–191°C.

4-Trifluoroacetyl-2-(4-methoxyphenyl)-3-methyl-1,3-oxazolium-5-olate (**1e**)

Pale yellow crystals, 82% yield. mp 142–143°C (ethyl acetate/hexane). (mp¹⁷) 142–143°C.

3-Benzyl-4-trifluoroacetyl-2-phenyl-1,3-oxazolium-5-olate (**1f**)

White crystals, 83% yield. mp 143–145°C (mp²⁷) 143–145°C.

4-Trifluoroacetyl-3-(4-methoxybenyl)-2-phenyl-1,3-oxazolium-5-olate (**1g**)

White crystals, 75% yield. mp 147–148°C (mp²⁷) 147–148°C.

3-Benzyl-4-trifluoroacetyl-2-(4-methoxyphenyl)-1,3-oxazolium-5-olate (**1h**)

White crystals, 89% yield. mp 158–160°C (mp²⁶) 158–160°C.

2-*tert*-Butyl-4-trifluoroacetyl-3-methyl-1,3-oxazolium-5-olate (**1i**)

White crystals, 67% yield. mp 120–121°C (mp²⁸) 120–121°C.

4-Trifluoroacetyl-2-(4-nitrophenyl)-3-phenyl-1,3-oxazolium-5-olate (**1j**)

Yellow crystals, 70% yield. mp 175–178°C (ethyl acetate/hexane). (mp¹⁷) 175–178°C.

4-Trifluoroacetyl-2-(4-methoxyphenyl)-3-phenyl-1,3-oxazolium-5-olate (**1k**)

Pale yellow crystals, 93% yield. mp 203–205°C (mp²⁵) 182–185°C.

General Procedure for Synthesis of 2(1*H*)-Pyrazinones (**3**) and Imidazo[1,5-*a*]pyrazin-8(7*H*)-ones (**4**)

To a stirred solution of TosMIC (146 mg, 0.75 mmol) in DMF (3 mL) was added DBU (152 mg, 1.00 mmol) at 0°C, and the mixture was stirred for 1 h under atmosphere of oxygen. To the mixture was added 4-trifluoroacetyl-1,3-oxazolium-5-olate **1** (0.50 mmol), and the whole was stirred at 0°C for an additional several hours. After workup with aq. Na_2CO_3 , the mixture was extracted with AcOEt ($\times 3$). The combined organic layers were washed with brine, dried over anhyd. Na_2SO_4 , and evaporated. The residue was purified by column chromatography (silica gel, hexane–AcOEt=4:1 to 1:1) to give the products **3** and **4**.

1-Methyl-5-[(4-methylphenyl)sulfonyl]-6-phenylpyrazin-2(1*H*)-one (**3a**)

White crystals, 72% yield. mp 147–149°C (CHCl_3 –hexane).

IR (KBr) cm^{-1} : 3057, 1674, 1662, 1487, 1322, 575. ^1H -NMR (500 MHz, CDCl_3) δ : 2.41 (s, 3H, Ar CH_3), 3.13 (s, 3H, N CH_3), 7.21–7.27 (m, 4H, Ar H), 7.51–7.59 (m, 5H, Ar H), 8.13 (s, 1H, H -3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.5 (CH_3), 33.3 (N CH_3), 128.3 (CH), 128.6 (CH), 128.9 (CH), 129.0 (C), 129.4 (CH), 130.6 (CH), 134.0 (C-5), 137.3 (C), 144.3 (C), 145.0 (C-6), 145.8 (C-3), 155.5 (CO). MS m/z : 313 (M^+ , 66), 58 (100). *Anal.* Calcd for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_3\text{S}$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.32; H, 5.03; N, 8.09.

5-[(4-Methylphenyl)sulfonyl]-1,6-diphenylpyrazin-2(1*H*)-one (**3b**)

Yellow crystals, 65% yield. mp 228–230°C (CHCl_3 –hexane).

IR (KBr) cm^{-1} : 3059, 3050, 1685, 1485, 1328, 1214, 1169, 1160, 806, 692, 681, 651, 585, 553. ^1H -NMR (500 MHz, CDCl_3) δ : 2.43 (s, 3H, Ar CH_3), 6.89–6.91 (m, 2H, Ar H), 7.02–7.04 (m, 2H, Ar H), 7.17–7.27 (m, 8H, Ar H), 7.62 (d, $J=8.0$ Hz, 2H, Ar H), 8.24 (s, 1H, H -3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.7 (CH_3), 127.7 (CH), 128.3 (CH), 128.6 (CH), 128.7 (C), 129.2 (C), 129.2 (CH), 129.5 (CH), 129.8 (CH), 130.0 (CH), 134.0 (C), 135.3 (C), 137.3 (C), 144.5 (CH), 144.8 (C), 147.6 (C-3), 155.2 (CO). MS m/z : 402 (M^+ , 5.6), 180 (100). *Anal.* Calcd for $\text{C}_{23}\text{H}_{18}\text{N}_2\text{O}_3\text{S} \cdot 0.5\text{H}_2\text{O}$: C, 67.14; H, 4.65; N, 6.81. Found: C, 66.80; H, 4.73; N, 6.75.

6-Methyl-5-[(4-methylphenyl)sulfonyl]-1-phenylpyrazin-2(1*H*)-one (**3c**)

Orange crystals, 42% yield. mp 212–215°C (CHCl_3 –hexane).

IR (KBr) cm^{-1} : 2926, 1680, 1508, 1491, 1305, 1159, 696, 675, 586, 556. ^1H -NMR (500 MHz, CDCl_3) δ : 2.46 (s, 6H, CH_3 and Ar CH_3), 7.13 (d, $J=7.5$ Hz, 2H, Ar H), 7.37 (d, $J=8.0$ Hz, 2H,

ArH), 7.52–7.59 (m, 3H, ArH), 7.91 (d, $J=8.5$ Hz, 2H, ArH), 8.03 (s, 1H, *H*-3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 17.3 (CH_3), 21.7 (CH_3), 127.2 (CH), 128.5 (CH), 129.8 (CH), 130.2 (C), 130.5 (CH), 133.7 (C), 135.5 (C), 137.1 (C), 143.6 (C), 144.8 (C), 145.4 (C-3), 155.5 (CO). MS m/z : 340 (M^+ , 11.7), 118 (100). *Anal.* Calcd for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_3\text{S}\cdot 0.5\text{H}_2\text{O}$: C, 61.87; H, 4.90; N, 8.02. Found: C, 62.21; H, 4.61; N, 8.24.

6-(4-Bromophenyl)-1-methyl-5-[(4-methylphenyl)sulfonyl]pyrazin-2(1*H*)-one (**3d**)

Pale yellow crystals, 70% yield. mp 178–180°C (CHCl_3 –hexane). IR (KBr) cm^{-1} : 3050, 2953, 2922, 1676, 1593, 1483, 1323, 1136, 1010, 850, 655, 582. ^1H -NMR (500 MHz, CDCl_3) δ : 2.42 (s, 3H, ArCH_3), 3.14 (s, 3H, NCH_3), 7.13 (dd, $J=8.4$, 1.9 Hz, 2H, ArH), 7.26–7.27 (m, 2H, ArH), 7.60 (dd, $J=8.3$, 1.7 Hz, 2H, ArH), 7.69 (dd, $J=8.5$, 1.9 Hz, 2H, ArH), 8.13 (s, 1H, *H*-3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.7 (CH_3), 33.4 (NCH_3), 125.3 (C), 127.9 (C), 128.3 (CH), 129.6 (CH), 130.3 (CH), 132.3 (CH), 134.0 (C), 137.1 (C), 143.8 (C), 144.7 (C), 146.2 (C-3), 155.4 (CO). MS m/z : 418 (4.2)+420 (4.3) (M^+ , 1:1), 196 (100). *Anal.* Calcd for $\text{C}_{18}\text{H}_{15}\text{BrN}_2\text{O}_3\text{S}\cdot 0.5\text{H}_2\text{O}$: C, 50.48; H, 3.77; N, 6.54. Found: C, 50.47; H, 3.77; N, 6.68.

6-(4-Methoxyphenyl)-1-methyl-5-[(4-methylphenyl)sulfonyl]pyrazin-2(1*H*)-one (**3e**)

Pale yellow crystals, 78% yield. mp 135–138°C (CHCl_3 –hexane). IR (KBr) cm^{-1} : 2955, 2833, 1671, 1497, 1257, 1178, 1152, 848, 658. ^1H -NMR (500 MHz, CDCl_3) δ : 2.40 (s, 3H, ArCH_3), 3.15 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 7.02 (d, $J=8.7$ Hz, 2H, ArH), 7.13 (d, $J=8.6$ Hz, 2H, ArH), 7.23 (d, $J=8.2$ Hz, 2H, ArH), 7.57 (d, $J=8.2$ Hz, 2H, ArH), 8.11 (s, 1H, *H*-3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.6 (CH_3), 33.3 (NCH_3), 55.4 (OCH_3), 114.3 (CH), 120.8 (C), 128.2 (CH), 129.4 (CH), 130.2 (CH), 134.4 (C), 137.5 (C), 144.3 (C), 145.3 (C), 145.5 (C-3), 155.8 (CO), 161.2 (C). MS m/z : 370 (M^+ , 5.1), 148 (100). *Anal.* Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}_4\text{S}\cdot 0.5\text{H}_2\text{O}$: C, 60.14; H, 5.05; N, 7.38. Found: C, 60.02; H, 5.06; N, 7.35.

1-Benzyl-5-[(4-methylphenyl)sulfonyl]-6-phenylpyrazin-2(1*H*)-one (**3f**)

White crystals, 55% yield. mp 164–165°C (CHCl_3 –hexane). IR (KBr) cm^{-1} : 3063, 3046, 3030, 1668, 1327, 1162, 704, 654, 576. ^1H -NMR (500 MHz, CDCl_3) δ : 2.41 (s, 3H, ArCH_3), 4.95 (s, 2H, NCH_2Ar), 6.75 (d, $J=7.0$ Hz, 2H, ArH), 7.00 (d, $J=7.0$ Hz, 2H, ArH), 7.05–7.30 (m, 5H, ArH), 7.37 (t, $J=8.0$ Hz, 2H, ArH), 7.54 (m, 3H, ArH), 8.21 (s, 1H, *H*-3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.6 (CH_3), 48.7 (NCH_2), 127.3 (CH), 128.0 (CH), 128.1 (C), 128.3 (CH), 128.4 (CH), 128.5 (CH), 129.4 (CH), 129.5 (CH), 130.6 (CH), 134.4 (C), 134.7 (C), 137.3 (C), 144.5 (C), 145.0 (C), 146.8 (C-3), 155.6 (CO). MS m/z : 416 (M^+ , 22.8), 91 (100). *Anal.* Calcd for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_3\text{S}$: C, 69.21; H, 4.84; N, 6.73. Found: C, 69.21; H, 4.79; N, 6.86.

1-(4-Methoxyphenylmethyl)-5-[(4-methylphenyl)sulfonyl]-6-phenylpyrazin-2(1*H*)-one (**3g**)

Pale yellow oil, 53% yield. IR (neat) cm^{-1} : 3062, 2957, 3014, 1674, 1514, 1327, 1250, 1162, 795. ^1H -NMR (500 MHz, CDCl_3) δ : 2.40 (s, 3H, ArCH_3), 3.75 (s, 3H, NCH_3), 4.89 (s, 2H, NCH_2Ar), 6.69 (s, 4H, ArH), 7.03 (d, $J=8.3$ Hz, 2H, ArH), 7.22 (d, $J=8.1$ Hz, 2H, ArH), 7.41 (t, $J=8.1$ Hz, 2H, ArH), 7.53–7.56 (m, 3H, ArH), 8.19 (s, 1H, *H*-3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.6 (CH_3), 48.3 (NCH_2), 55.3 (OCH_3), 113.8 (CH), 126.7 (C), 128.1 (C), 128.3 (CH), 128.4 (CH), 129.1 (CH), 129.5 (CH), 129.5 (CH), 130.6 (C), 134.3 (C),

137.3 (C), 144.5 (CH), 145.0 (C), 146.7 (C-3), 155.7 (CO), 159.3 (C). MS m/z : 446 (M^+ , 15.4), 121 (100). HR-MS (EI) for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_4\text{S}$ (M^+): Calcd, 446.1300. Found, 446.1307.

1-Benzyl-6-(4-methoxyphenyl)-5-[(4-methylphenyl)sulfonyl]pyrazin-2(1*H*)-one (**3h**)

Yellow oil, 58% yield. IR (neat) cm^{-1} : 3063, 3030, 2964, 1695, 1681, 1651. ^1H -NMR (500 MHz, CDCl_3) δ : 2.40 (s, 3H, ArCH_3), 3.89 (s, 3H, ArOCH_3), 4.96 (s, 2H, NCH_2Ar), 6.79 (d, $J=8.2$ Hz, 2H, ArH), 6.85–6.93 (m, 4H, ArH), 7.17–7.26 (m, 5H, ArH), 7.55 (d, $J=8.3$ Hz, 2H, ArH), 8.19 (s, 1H, ArH). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.6 (CH_3), 48.7 (NCH_2), 55.4 (OCH_3), 113.7 (CH), 120.1 (C), 127.3 (CH), 128.0 (C), 128.4 (CH), 128.6 (CH), 129.5 (CH), 130.9 (CH), 134.8 (CH), 134.9 (C), 137.3 (C), 144.4 (C), 145.2 (C), 146.5 (C-3), 155.8 (CO), 161.2 (C). MS m/z : 446 (M^+ , 40.4), 91 (100). HR-MS (EI) for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_4\text{S}$ (M^+): Calcd, 446.1281. Found, 446.1300.

6-*tert*-Butyl-1-methyl-5-[(4-methylphenyl)sulfonyl]pyrazin-2(1*H*)-one (**3i**)

Pale yellow crystals, 11% yield. mp 128–130°C (CHCl_3 –hexane). IR (KBr) cm^{-1} : 2981, 1668, 1482, 1301, 1162, 658. ^1H -NMR (500 MHz, CDCl_3) δ : 1.80 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.43 (s, 3H, ArCH_3), 3.64 (s, 3H, NCH_3), 7.29 (d, $J=8.0$ Hz, 2H, ArH), 7.62 (s, 1H, *H*-3), 7.77 (d, $J=8.0$ Hz, 2H, ArH). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.6 (CH_3), 31.3 ($\text{C}(\text{CH}_3)_3$), 38.3 ($\text{C}(\text{CH}_3)_3$), 39.6 (NCH_3), 129.2 (CH), 129.2 (CH), 138.0 (C), 139.0 (C), 139.8 (CH), 144.2 (C), 156.6 (C), 156.8 (C). MS m/z : 320 (M^+ , 1.6), 240 (100). *Anal.* Calcd for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_3\text{S}\cdot 0.5\text{H}_2\text{O}$: C, 58.34; H, 6.43; N, 8.50. Found: C, 58.03; H, 6.14; N, 8.32.

5-[(4-Methylphenyl)sulfonyl]-6-(4-nitrophenyl)-1-phenylpyrazin-2(1*H*)-one (**3j**)

Pale orange crystals, 25% yield. mp >300°C (dec.) (CHCl_3 –hexane). IR (KBr) cm^{-1} : 3065, 1693, 1520, 1489, 1350, 1329, 1166, 862, 701. ^1H -NMR (500 MHz, CDCl_3) δ : 2.45 (s, 3H, ArCH_3), 6.93 (dd, $J=8.0$, 2.2 Hz, 2H, ArH), 7.25–7.34 (m, 7H, ArH), 7.69 (d, $J=8.3$ Hz, 2H, ArH), 8.09 (d, $J=8.7$ Hz, 2H, ArH), 8.25 (s, 1H, *H*-3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.7 (CH_3), 122.9 (CH), 128.1 (CH), 128.7 (CH), 129.7 (CH), 129.8 (CH), 129.9 (C), 131.1 (CH), 133.8 (C), 134.7 (C), 135.2 (C), 136.6 (C), 142.1 (C), 145.2 (CH), 148.2 (C), 148.7 (C-3), 154.6 (CO). MS m/z : 447 (M^+ , 1.5), 77 (100). *Anal.* Calcd for $\text{C}_{23}\text{H}_{17}\text{N}_3\text{O}_5\text{S}\cdot 0.33\text{H}_2\text{O}$: C, 61.10; H, 4.33; N, 9.32. Found: C, 61.14; H, 4.22; N, 9.26.

6-(4-Methoxyphenyl)-5-[(4-methylphenyl)sulfonyl]-1-phenylpyrazin-2(1*H*)-one (**3k**)

Pale orange crystals, 35% yield. mp 202–206°C (dec.) (CHCl_3 –hexane). IR (KBr) cm^{-1} : 3026, 1676, 1596, 1446, 1359, 1340, 1258, 1162, 1087, 885, 810, 789, 658. ^1H -NMR (500 MHz, CDCl_3) δ : 2.42 (s, 3H, ArCH_3), 3.75 (s, 3H, OCH_3), 6.69 (d, $J=8.7$ Hz, 2H, ArH), 6.90 (dd, $J=8.3$, 1.7 Hz, 2H, ArH), 6.93 (d, $J=8.7$ Hz, 2H, ArH), 7.22–7.27 (m, 5H, ArH), 7.62 (d, $J=8.3$ Hz, 2H, ArH), 8.21 (s, 1H, *H*-3). ^{13}C -NMR (126 MHz, CDCl_3) δ : 21.6 (CH_3), 55.2 (OCH_3), 113.2 (CH), 120.7 (C), 128.2 (CH), 128.5 (CH), 129.1 (C), 129.3 (CH), 129.5 (CH), 131.5 (CH), 134.4 (C), 135.4 (C), 137.4 (C), 144.4 (CH), 144.9 (C), 147.2 (C-3), 155.4 (CO), 160.5 (C). MS m/z : 432 (M^+ , 7.8), 210 (100). *Anal.* Calcd for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_4\text{S}\cdot 0.25\text{H}_2\text{O}$: C, 65.96; H, 4.73; N, 6.41. Found: C, 65.95; H, 4.64; N, 6.46.

7-Methyl-5-[(4-methylphenyl)sulfonyl]-6-phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4a**)

Pale yellow crystals, 16% yield. mp 178–180°C (CHCl_3 –hexane).¹⁷⁾

5-[(4-Methylphenyl)sulfonyl]-6,7-diphenylimidazo[1,5-*a*]-pyrazin-8(7*H*)-one (**4b**)

Pale yellow crystals, 10% yield. mp 263–266°C (dec.) (CHCl₃–hexane).¹⁷⁾

6-Methyl-5-[(4-methylphenyl)sulfonyl]-7-phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4c**)

A yellow solid, 3% yield. mp 232–235°C (dec.) (CHCl₃–hexane).¹⁷⁾

6-(4-Bromophenyl)-7-methyl-5-[(4-methylphenyl)sulfonyl]-imidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4d**)

White crystals, 20% yield. mp 180–183°C (acetone–hexane).²¹⁾

6-(4-Methoxyphenyl)-7-methyl-5-[(4-methylphenyl)sulfonyl]-imidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4e**)

Ocher crystals, 5% yield. mp 173–176°C (CHCl₃–hexane).¹⁷⁾

7-Benzyl-5-[(4-methylphenyl)sulfonyl]-6-phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4f**)

Pale yellow crystals, 36% yield. mp 232–235°C (dec.) (CHCl₃–hexane).¹⁷⁾

7-(4-Methoxyphenylmethyl)-5-[(4-methylphenyl)sulfonyl]-6-phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4g**)

Pale yellow crystals, 34% yield. mp 183–185°C (CHCl₃–hexane).¹⁷⁾

7-Benzyl-6-(4-methoxyphenyl)-5-[(4-methylphenyl)sulfonyl]imidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4h**)

A pale yellow oil, 35% yield.¹⁷⁾

5-[(4-Methylphenyl)sulfonyl]-6-(4-nitrophenyl)-7-phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4j**)

Yellow crystals, 44% yield. mp 244–245°C (CHCl₃–hexane).¹⁷⁾

6-(4-Methoxyphenyl)-5-[(4-methylphenyl)sulfonyl]-7-phenylimidazo[1,5-*a*]pyrazin-8(7*H*)-one (**4k**)

Pale yellow crystals, 36% yield. mp 253–256°C (dec.) (CHCl₃–hexane).¹⁷⁾

General Procedure for Synthesis of Ethyl 5-Oxo-3-phenylpyrazine-2-carboxylates (5) To a stirred solution of ethyl isocyanacetate (85 mg, 0.75 mmol) in DMF (3 mL) was added DBU (152 mg, 1.00 mmol) at 0°C, and the mixture was stirred for 1 h under atmosphere of oxygen. To the mixture was added 4-trifluoroacetyl-1,3-oxazolium-5-olate **1** (0.50 mmol), and the whole was stirred at 0°C for an additional several hours. After workup with aq. Na₂CO₃, the mixture was extracted with AcOEt (×3). The combined organic layers were washed with brine, dried over anhyd. Na₂SO₄, and evaporated. The residue was purified by column chromatography (silica gel, hexane–AcOEt=4:1 to 1:1) to give the product **5**.

Ethyl 4,5-Dihydro-4-methyl-5-oxo-3-phenylpyrazine-2-carboxylate (**5a**)

Yellow crystals, 92% yield. mp 111–113°C (CHCl₃–hexane). IR (KBr) cm⁻¹: 2986, 1713, 1671, 1491, 1376, 1317, 1137, 1025, 866, 760, 711. ¹H-NMR (500 MHz, CDCl₃) δ: 1.05 (t, *J*=7.0 Hz, 3H, CH₂CH₃), 3.23 (s, 3H, NCH₃), 4.09 (q, *J*=7.0 Hz, 2H, OCH₂), 7.26–7.28 (m, 2H, ArH), 7.52–7.55 (m, 3H, ArH), 8.24 (s, 1H, *H*-6). ¹³C-NMR (126 MHz, CDCl₃) δ: 13.8 (CH₂CH₃), 33.5 (NCH₃), 61.3 (OCH₂), 124.4 (C), 127.9 (CH), 129.0 (CH), 129.9 (CH), 131.9 (C), 146.1 (C-3), 146.2 (C), 156.1 (CO), 164.0 (CO). MS *m/z*: 258 (M⁺, 99), 185 (100). HR-MS (EI) for C₁₄H₁₄N₂O₃: Calcd, 258.1004. Found: 258.1006. *Anal.* Calcd for C₁₄H₁₄N₂O₃: C, 65.11; H, 5.46; N, 10.85. Found: C, 65.14; H, 5.43; N, 10.79.

Ethyl 4,5-Dihydro-5-oxo-3,4-diphenylpyrazine-2-carboxylate (**5b**)

Yellow crystals, 88% yield. mp 198–201°C (CHCl₃–hexane). IR (KBr) cm⁻¹: 3001, 2983, 1712, 1681, 1569, 1484, 1389, 1378, 1323, 1126, 1004, 928, 754, 710. ¹H-NMR (500 MHz, CDCl₃) δ: 1.03 (t, *J*=7.0 Hz, 3H, CH₂CH₃), 4.11 (q, *J*=7.0 Hz, 2H, OCH₂), 6.96–6.98 (m, 2H, ArH), 7.02–7.07 (m, 2H, ArH), 7.17–7.28 (m, 6H, ArH), 8.36 (s, 1H, *H*-6). ¹³C-NMR (126 MHz, CDCl₃) δ: 13.8 (CH₂CH₃), 61.4 (OCH₂), 124.5 (C), 127.8 (CH), 128.4 (CH), 129.0 (CH), 129.1 (CH), 129.2 (CH), 129.2 (CH), 131.5 (C), 135.8 (C), 145.7 (C), 147.8 (C-3), 155.7 (CO), 164.3 (CO). MS *m/z*: 320 (M⁺, 100). HR-MS (EI) for C₁₉H₁₆N₂O₃: Calcd, 320.1161. Found: 320.1149. *Anal.* Calcd for C₁₉H₁₆N₂O₃·0.33H₂O: C, 69.94; H, 5.15; N, 8.59. Found: C, 69.95; H, 4.98; N, 8.41.

Ethyl 4,5-Dihydro-3-methyl-5-oxo-4-phenylpyrazine-2-carboxylate (**5c**)

Yellow crystals, 52% yield. mp 150–153°C (CHCl₃–hexane). IR (KBr) cm⁻¹: 3060, 2976, 1705, 1671, 1517, 1385, 1306, 1230, 1095, 776, 709. ¹H-NMR (500 MHz, CDCl₃) δ: 1.43 (t, *J*=7.0 Hz, 3H, CH₂CH₃), 2.40 (s, 3H, 6-CH₃), 4.43 (q, *J*=7.0 Hz, 2H, OCH₂), 7.16–7.18 (m, 2H, ArH), 7.52–7.60 (m, 3H, ArH), 8.20 (s, 1H, *H*-6). ¹³C-NMR (126 MHz, CDCl₃) δ: 14.4 (CH₂CH₃), 18.2 (CH₃), 61.7 (OCH₂), 123.7 (C), 127.2 (CH), 129.9 (CH), 130.3 (CH), 136.1 (C), 145.3 (C-3), 146.0 (C), 156.0 (CO), 165.0 (CO). MS *m/z*: 258 (M⁺, 100). HR-MS (EI) for C₁₄H₁₄N₂O₃: Calcd, 258.1004. Found: 258.1006. *Anal.* Calcd for C₁₄H₁₄N₂O₃·0.33H₂O: C, 63.64; H, 5.59; N, 10.60. Found: C, 63.73; H, 5.42; N, 10.26.

Ethyl 3-(4-Bromophenyl)-4,5-dihydro-4-methyl-5-oxopyrazine-2-carboxylate (**5d**)

Crystals, 85% yield. mp 183–185°C (CHCl₃–hexane). IR (KBr) cm⁻¹: 2982, 2901, 1723, 1662, 1485, 1423, 1379, 1313, 1177, 1013, 876, 833, 803, 677. ¹H-NMR (500 MHz, CDCl₃) δ: 1.12 (t, *J*=7.0 Hz, 3H, CH₂CH₃), 3.23 (s, 3H, NCH₃), 4.13 (q, *J*=7.0 Hz, 2H, OCH₂), 7.16 (d, *J*=8.5 Hz, 2H, ArH), 7.69 (d, *J*=8.5 Hz, 2H, ArH), 8.24 (s, 1H, *H*-6). ¹³C-NMR (126 MHz, CDCl₃) δ: 13.9 (CH₂CH₃), 33.5 (NCH₃), 61.5 (OCH₂), 124.2 (C), 124.5 (C), 129.5 (CH), 130.7 (C), 132.4 (CH), 145.2 (C), 146.5 (C-3), 155.9 (CO), 163.8 (CO). MS *m/z*: 338 (M⁺, 55.7), 263 (100). HR-MS (EI) for C₁₄H₁₃BrN₂O₃: Calcd, 336.0110. Found: 336.0131. *Anal.* Calcd for C₁₄H₁₃BrN₂O₃: C, 49.87; H, 3.89; N, 8.31. Found: C, 49.94; H, 3.89; N, 8.55.

Ethyl 4,5-Dihydro-3-(4-methoxyphenyl)-4-methyl-5-oxopyrazine-2-carboxylate (**5e**)

Crystals, 85% yield. mp 148–150°C (CHCl₃–hexane). IR (KBr) cm⁻¹: 2983, 2931, 2836, 1714, 1669, 1504, 1315, 1247, 1182, 1029, 871, 816, 694. ¹H-NMR (500 MHz, CDCl₃) δ: 1.11 (t, *J*=7.0 Hz, 3H, CH₂CH₃), 3.25 (s, 3H, NCH₃), 3.88 (s, 3H, OCH₃), 4.13 (q, *J*=7.0 Hz, 2H, OCH₂), 7.04 (d, *J*=8.5 Hz, 2H, ArH), 7.19 (d, *J*=9.0 Hz, 2H, ArH), 8.21 (s, 1H, *H*-6). ¹³C-NMR (126 MHz, CDCl₃) δ: 13.9 (CH₂CH₃), 33.4 (NCH₃), 55.4 (OCH₃), 61.3 (OCH₂), 114.5 (CH), 123.8 (C), 124.9 (C), 129.4 (CH), 145.9 (C-3), 146.1 (C), 156.3 (CO), 160.7 (C), 164.2 (CO). MS *m/z*: 288 (M⁺, 75.8), 215 (100). HR-MS (EI) for C₁₅H₁₆N₂O₄: Calcd, 288.1110. Found: 288.1112. *Anal.* Calcd for C₁₅H₁₆N₂O₄·0.33H₂O: C, 61.23; H, 5.71; N, 9.52. Found: C, 61.24; H, 5.38; N, 9.54.

Ethyl 4-Benzyl-4,5-dihydro-5-oxo-3-phenylpyrazine-2-carboxylate (**5f**)

Yellow crystals, 90% yield. mp 116–119°C (CHCl₃–hexane).

IR (KBr) cm^{-1} : 3032, 2984, 1715, 1664, 1567, 1519, 1398, 1307, 1197, 1130, 1020, 905, 767, 700. $^1\text{H-NMR}$ (500MHz, CDCl_3) δ : 1.01 (t, $J=7.0\text{Hz}$, 3H, CH_2CH_3), 4.06 (q, $J=7.0\text{Hz}$, 2H, OCH_2), 5.04 (s, 2H, NCH_2Ar), 6.78–6.80 (m, 2H, ArH), 7.03–7.05 (m, 2H, ArH), 7.17–7.19 (m, 3H, ArH), 7.35–7.38 (m, 2H, ArH), 7.46–7.49 (m, 1H, ArH), 8.33 (s, 1H, $H-6$). $^{13}\text{C-NMR}$ (126MHz, CDCl_3) δ : 13.7 (CH_2CH_3), 48.7 (NCH_2), 61.3 (OCH_2), 124.9 (C), 127.1 (CH), 127.8 (CH), 128.4 (CH), 128.5 (CH), 129.9 (CH), 131.0 (C), 135.1 (C), 146.0 (C), 147.0 (C-3), 156.0 (CO), 164.0 (CO). MS m/z : 334 (M^+ , 82.3), 91 (100). HR-MS (EI) for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3$: Calcd, 334.1317. Found: 334.1312. Anal. Calcd for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.54; H, 5.40; N, 8.27.

Ethyl 4,5-Dihydro-4-(4-methoxyphenylmethyl)-5-oxo-3-phenylpyrazine-2-carboxylate (**5g**)

Crystals, 91% yield. mp 75–77°C (CHCl_3 –hexane). IR (KBr) cm^{-1} : 2966, 2935, 2837, 1723, 1661, 1510, 1297, 1250, 1132, 1028, 805, 764, 712. $^1\text{H-NMR}$ (500MHz, CDCl_3) δ : 0.99 (t, $J=7.2\text{Hz}$, 3H, CH_2CH_3), 3.74 (s, 3H, OCH_3), 4.04 (q, $J=7.2\text{Hz}$, 2H, OCH_2), 4.98 (s, 2H, NCH_2Ar), 6.39–6.74 (m, 4H, ArH), 7.07 (d, $J=7.2\text{Hz}$, 2H, ArH), 7.40 (t, $J=7.5\text{Hz}$, 2H, ArH), 7.49 (t, $J=7.5\text{Hz}$, 1H, ArH), 8.29 (s, 1H, $H-6$). $^{13}\text{C-NMR}$ (126MHz, CDCl_3) δ : 13.7 (CH_2CH_3), 48.2 (NCH_2), 55.2 (OCH_3), 61.2 (OCH_2), 113.8 (CH), 124.9 (C), 127.2 (C), 128.4 (CH), 128.7 (CH), 128.8 (CH), 129.8 (CH), 131.1 (C), 145.9 (C), 147.0 (C-3), 156.0 (CO), 159.2 (C), 164.0 (CO). MS m/z : 364 (M^+ , 60.5), 121 (100). HR-MS (EI) for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4$: Calcd, 364.1423. Found: 364.1411. Anal. Calcd for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 68.99; H, 5.42; N, 7.87.

Ethyl 4-Benzyl-4,5-dihydro-3-(4-methoxyphenyl)-5-oxopyrazine-2-carboxylate (**5h**)

Crystals, 91% yield. mp 78–81°C (CHCl_3 –hexane). IR (KBr) cm^{-1} : 2979, 2843, 1727, 1666, 1609, 1504, 1311, 1250, 1129, 1023, 857, 729, 695. $^1\text{H-NMR}$ (500MHz, CDCl_3) δ : 1.07 (t, $J=7.1\text{Hz}$, 3H, CH_2CH_3), 3.84 (s, 3H, OCH_3), 4.09 (q, $J=7.1\text{Hz}$, 2H, OCH_2), 5.06 (s, 2H, NCH_2Ar), 6.83–6.84 (m, 2H, ArH), 6.88 (d, $J=8.8\text{Hz}$, 2H, ArH), 6.96 (d, $J=8.5\text{Hz}$, 2H, ArH), 7.20–7.22 (m, 3H, ArH), 8.30 (s, 1H, $H-6$). $^{13}\text{C-NMR}$ (126MHz, CDCl_3) δ : 13.9 (CH_2CH_3), 48.6 (NCH_2), 55.3 (OCH_3), 61.3 (OCH_2), 113.8 (CH), 123.1 (C), 125.4 (C), 127.1 (CH), 127.8 (CH), 128.5 (CH), 130.0 (CH), 135.3 (C), 145.9 (C), 146.9 (C-3), 156.2 (CO), 160.7 (C), 164.2 (CO). MS m/z : 364 (M^+ , 63), 135 (100). HR-MS (EI) for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4$: Calcd, 364.1423. Found: 364.1411. Anal. Calcd for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.01; H, 5.60; N, 7.59.

Conversion of 2(1H)-Pyrazinone (5a) to Imidazo[1,5-a]pyrazin-8(7H)-one (6) To a stirred solution of TosMIC (57 mg, 0.290 mmol) in DMF (1 mL) was added DBU (88 mg, 0.581 mmol) at 0°C, and the mixture was stirred for 1 h under atmosphere of argon. To the mixture was added **5a** (50 mg, 0.194 mmol), and the whole was stirred at room temperature (r.t.) for an additional 24 h. After workup with aq. Na_2CO_3 , the mixture was extracted with AcOEt ($\times 3$). The combined organic layers were washed with brine, dried over anhyd. Na_2SO_4 , and evaporated. The residue was purified by column chromatography (silica gel, hexane–AcOEt=1:1) to give ethyl 7,8-dihydro-7-methyl-8-oxo-6-phenylimidazo[1,5-a]pyrazine-5-carboxylate **6** as colorless crystals, 65% yield. mp 163–165°C (CHCl_3 –hexane). IR (KBr) cm^{-1} : 3146, 2979, 2934, 1709, 1667, 1336, 1321, 1207, 1065, 719. $^1\text{H-NMR}$ (500MHz, CDCl_3) δ : 0.78 (t, $J=7.2\text{Hz}$, 2H, CH_2CH_3), 3.11 (s,

3H, NCH_3), 3.96 (q, $J=7.2\text{Hz}$, 2H, OCH_2), 7.30–7.32 (m, 2H, ArH), 7.49–7.54 (m, 3H, ArH), 8.06 (s, 1H, $H-1$), 8.83 (s, 1H, $H-3$). $^{13}\text{C-NMR}$ (126MHz, CDCl_3) δ : 13.2 (CH_2CH_3), 32.2 (NCH_3), 61.5 (OCH_2), 109.0 (C), 122.0 (C), 128.8 (CH), 129.6 (CH), 131.4 (CH), 133.2 (CH), 135.0 (CH), 139.2 (C), 155.6 (CO), 161.9 (CO). MS m/z : 297 (M^+ , 100). HR-MS (EI) for $\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}_3$: Calcd, 297.1113. Found: 297.1104. Anal. Calcd for $\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 64.66; H, 5.07; N, 13.88.

Procedures for Synthesis of 1,3-Thiazolium-5-olates (7a–c)

4-Trifluoroacetyl-3-methyl-2-phenyl-1,3-thiazolium-5-olate (**7a**)

To a stirred suspension of *N*-methyl-*N*-thiobenzoylglycine (1.00 g, 4.78 mmol) in *tert* butyl methyl ether (10 mL) was added trifluoroacetic anhydride (TFAA) (2 mL, 14.3 mmol) at 0°C, and the solution was stirred at 0°C for 3 h. After workup with aq. Na_2CO_3 , the mixture was extracted with AcOEt ($\times 3$). The combined organic layers were washed with brine, dried over anhyd. Na_2SO_4 , and evaporated. The residue was washed with diethyl ether, and recrystallized from hexane–ethyl acetate to give the product **7a** as yellow crystals (1.17 g, 85% yield). mp 131–133°C (AcOEt–hexane). IR (KBr) cm^{-1} : 3062, 3044, 2970, 1673, 1620, 1451, 1396, 1327, 1310, 1261, 1237, 1081, 1049, 1025, 997, 946, 791, 766, 736, 700. $^1\text{H-NMR}$ (500MHz, CDCl_3) δ : 4.04 (s, 3H, NCH_3), 7.50 (d, $J=7.5\text{Hz}$, 2H, ArH), 7.59 (t, $J=7.5\text{Hz}$, 2H, ArH), 7.63–7.66 (m, 1H, ArH). $^{13}\text{C-NMR}$ (126MHz, CDCl_3) δ : 42.5 (NCH_3), 115.5 (C), 116.9 (q, $^1J=288.5\text{Hz}$, CF_3), 127.1 (C), 129.3 (CH), 129.7 (CH), 132.6 (CH), 158.5 (C), 168.6 (q, $^2J=38.1\text{Hz}$, CCF_3), 174.2 (C). MS m/z : 287 (M^+ , 67.5), 121 (100). HR-MS (EI) for $\text{C}_{12}\text{H}_8\text{F}_3\text{NO}_2\text{S}$: Calcd, 287.0228. Found: 287.0217.

4-Acetyl-3-methyl-2-phenyl-1,3-thiazolium-5-olate (**7b**)

A mixture of *N*-methyl-*N*-thiobenzoylglycine (1.00 g, 4.78 mmol) and acetic anhydride (15 mL) was heated at 120°C for 4 h. After workup with aq. Na_2CO_3 , the mixture was extracted with AcOEt ($\times 3$). The combined organic layers were washed with brine, dried over anhyd. Na_2SO_4 , and evaporated. The residue was purified by column chromatography (silica gel, hexane–AcOEt=4:1 to 1:1) to give the product **7b** as yellow crystals (0.903 g, 81% yield). mp 131–132°C (AcOEt–hexane) (mp²¹) 133–134°C. IR (KBr) cm^{-1} : 3057, 3000, 2920, 1648, 1622, 1375, 1341, 1252, 1173, 1083, 946, 764, 695. $^1\text{H-NMR}$ (500MHz, CDCl_3) δ : 2.53 (s, 3H, CCH_3), 4.06 (s, 3H, NCH_3), 7.46 (dd, $J=8.0, 1.5\text{Hz}$, 2H, ArH), 7.53–7.60 (m, 3H, ArH). $^{13}\text{C-NMR}$ (126MHz, CDCl_3) δ : 29.7 (CH_3), 42.1 (NCH_3), 119.0 (C), 127.7 (C), 129.4 (CH), 129.5 (CH), 131.7 (CH), 151.3 (C), 176.5 (C), 187.7 (C). MS m/z : 233 (M^+ , 36.7), 118 (100). HR-MS (EI) for $\text{C}_{12}\text{H}_{11}\text{NO}_2\text{S}$: Calcd, 233.0510. Found: 233.0504.

4-Formyl-3-methyl-2-phenyl-1,3-thiazolium-5-olate (**7c**)

To a mixture of pyridine (0.805 mL, 10 mmol) and DMF (1.925 mL, 25 mmol) in toluene (15 mL) was added phosphoryl chloride (1.2 mL, 12.5 mmol) at 0°C, and the solution was stirred at 0°C for 1 h. To the mixture was added *N*-methyl-*N*-thiobenzoylglycine (0.522 g, 2.5 mmol) and the whole was heated at 80°C for an additional 3 h. After workup with aq. Na_2CO_3 , the mixture was extracted with AcOEt ($\times 3$). The combined organic layers were washed with brine, dried over anhyd. Na_2SO_4 , and evaporated. The residue was recrystallized from hexane–ethyl acetate to give **7c** as amber crystals

(0.318 g, 58% yield). mp 160–162°C (AcOEt–hexane). IR (KBr) cm^{-1} : 3056, 3027, 2829, 2782, 2757, 1670, 1604, 1365, 1326, 1307, 1239, 1152, 1051, 859, 774, 759, 697. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 4.11 (s, 3H, NCH_3), 7.47–7.49 (m, 2H, ArH), 7.55–7.63 (m, 3H, ArH), 9.57 (s, 1H, CHO). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) δ : 40.2 (NCH_3), 118.1 (C), 127.1 (C), 129.4 (CH), 129.6 (CH), 132.1 (CH), 152.0 (C), 176.2 (C), 178.4 (C). MS m/z : 219 (M^+ , 30.8), 118 (100). HR-MS (EI) for $\text{C}_{11}\text{H}_9\text{NO}_2\text{S}$: Calcd, 219.0354. Found: 219.0346.

Biology

Anti-microbial Activity

The preliminary anti-microbial activities of new compounds were measured in a concentration of 50 mg/L by disc diffusion method.²²⁾ Gram-negative bacteria of *Acinetobacter baumannii* ATCC17978, *Escherichia coli* TGI, *Klebsiella pneumonia* IID5209, *Pseudomonas aeruginosa* 01, *Serratia marcescens* (clinical isolate), and *Vibrio parahaemolyticus* RIMD221051 and Gram-positive bacteria of *Bacillus subtilis* ATCC6633, *Enterococcus faecalis* IID622, *Staphylococcus aureus* FDA209P, methicillin-resistant *Staphylococcus aureus* (MRSA) (clinical isolate), *Streptococcus pneumonia* IID555, and *S. pyogenes* 124 were used. Briefly, 100 μL of the middle-logarithmic phase bacteria cells (10^8 CFU/mL) were spread on surface of the Muller–Hinton agar plate and discs containing 5 μg of new compounds were put on the agar plate, and then incubated at 37°C. The inhibition zone were measured in millimeters at the end of an incubation period of 18–24 h. Dimethyl sulfoxide (DMSO) was used as solvent control and Gentamicin, Kanamycin and Vancomycin were used as standard drugs.

Cytotoxicity Determinations

A literature procedure was employed when examining the lethal effects of series 3 and 5 compounds on Ca9–22, HSC-2, HSC-3, HSC-4 (purchased from Riken Cell Bank, Tsukuba, Japan), HGF, HPLF and HPC cells (established in Meikai University School of Dentistry according to the guideline of intramural ethic committee) except the time of incubation was extended from 24 to 48 h.³⁰⁾ In brief, different concentrations of test compounds or positive control such as 5-FU (Wako Pure Chemical Industries, Ltd., Osaka, Japan) or melphalan (Sigma-Aldrich Inc., St. Louis, MO, U.S.A.) were incubated at 37°C with the cells in Dulbecco's modified Eagle's medium (DMEM) medium supplemented with 10% heat-inactivated fetal bovine serum. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used in determining cell viability.³⁰⁾

Acknowledgments The authors gratefully acknowledge Ms. Ayaka Uematsu and Mr. Daichi Kameda for conducting *in vitro* antibacterial assay. This work was partially supported by KAKENHI from the Japan Society for the Promotion of Science (JSPS) (Challenging Exploratory Research 25670897; Scientific Research (C) 16K11519).

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

References

- Mehta V. P., Sharma Ak., Modha S. G., Sharma S., Meganathan T., Parmar V. S., Van der Eycken E., *J. Org. Chem.*, **76**, 2920–2925 (2011).
- Belfrage A. K., Abdurakhmanov E., Kerblom E., Brandt P., Oshalim A., Gising J., Skogh A., Neyts J., Danielson U. H., Sandström A., *Bioorg. Med. Chem.*, **24**, 2603–2620 (2016).
- Ahuja V. T., Hartz R. A., Molski T. F., Mattson G. K., Lentz K. A., Grace J. E. Jr., Lodge N. J., Bronson J. J., Macor J. E., *Bioorg. Med. Chem. Lett.*, **26**, 2184–2187 (2016).
- Taggi A. E., Stevenson T. M., Berezna J. F., Sharpe P. L., Gutteridge S., Forman R., Bisaha J. J., Cordova D., Crompton M., Geist L., Kovacs P., Marshall E., Sheth R., Stavits C., Tseng C.-P., *Bioorg. Med. Chem.*, **24**, 435–443 (2016).
- Young W. B., Barbosa J., Blomgren P., Bremer M. C., Crawford J. J., Dambach D., Gallion S., Hymowitz S. G., Kropf J. E., Lee S. H., Liu L., Lubach J. W., Macaluso J., Maciejewski P., Maurer B., Mitchell S. A., Ortwine D. F., Di Paolo J., Reif K., Scheerens H., Schmitt A., Sowell C. G., Wang X., Wong H., Xiong J.-M., Xu J., Zhao Z., Currie K. S., *Bioorg. Med. Chem. Lett.*, **25**, 1333–1337 (2015).
- Pawar V. G., De Borggraeve U. H., *Synthesis*, **17**, 2799–2814 (2006).
- Tota Y. A., Elderfield R. C., *J. Org. Chem.*, **7**, 313–319 (1942).
- Karmas G., Spoerri P. E., *J. Am. Chem. Soc.*, **74**, 1580–1584 (1952).
- Vekemans J., Pollers-Wieers C., Hoornaert G. J., *J. Heterocycl. Chem.*, **20**, 919–923 (1983).
- Gising J., Ortqvist P., Sandstrom A., Larhed M., *Org. Biomol. Chem.*, **7**, 2809–2815 (2009).
- Azuaje J., El Maatougui A., Perez-Rubio J. M., Coelho A., Fernandez F., Sotelo E., *J. Org. Chem.*, **78**, 4402–4409 (2013).
- Saijo R., Kawase M., *Tetrahedron Lett.*, **53**, 2782–2785 (2012).
- Saijo R., Uno H., Mori S., Kawase M., *Chem. Commun.*, **52**, 8006–8009 (2016).
- Saijo R., Kurihara K., Akira K., Uno H., Kawase M., *Tetrahedron Lett.*, **54**, 4418–4421 (2013).
- Van Leusen D., Van Leusen A. M., *Org. React.*, **57**, 417–666 (2001).
- Mukaiyama H., Nishimura T., Kobayashi S., Ozawa T., Kamada N., Komatsu Y., Kikuchi S., Oonota H., Kusama H., *Bioorg. Med. Chem.*, **15**, 868–885 (2007).
- Saijo R., Uno H., Kawase M., *Heterocycles*, **92**, 2016–2058 (2016).
- Pawar S. V., Pawar V. G., Dehaen W., De Borggraeve W. M., *Org. Lett.*, **10**, 4473–4476 (2008).
- Mehta V. P., Sharma A. K., Modha S. G., Sharma S., Meganathan T., Parmar V. S., Van der Eycken E., *J. Org. Chem.*, **76**, 2920–2925 (2011).
- Mishra N. M., Peshkov V. A., Pereshivko O. P., Modha S. G., Van der Eycken E., *Tetrahedron Lett.*, **53**, 4676–4678 (2012).
- Ohta M., Shin C.-G., *Bull. Chem. Soc. Jpn.*, **38**, 704–706 (1965).
- Kirkpatrick W. R., Turner T. M., Fothergill A. W., McCarthy D. I., Redding S. W., Rinaldi M. G., Patterson T. F., *J. Clin. Microbiol.*, **36**, 3429–3432 (1998).
- DeRuiter J., Davis R. A., Wandrekar V. G., Mayfield C. A., *J. Med. Chem.*, **34**, 2120–2126 (1991).
- DeRuiter J., Swearingen B. E., Wandrekar V., Mayfield C. A., *J. Med. Chem.*, **32**, 1033–1038 (1989).
- Kawase M., Koiwai H., *Chem. Pharm. Bull.*, **56**, 433–438 (2008).
- Kawase M., Saito S., Kurihara T., *Chem. Pharm. Bull.*, **49**, 461–464 (2001).
- Saijo R., Hagimoto Y., Kawase M., *Org. Lett.*, **12**, 4776–4779 (2010).
- Kawase M., Saito S., *Chem. Pharm. Bull.*, **48**, 410–414 (2000).
- Greco C. V., Gray R. P., Grosso V. G., *J. Org. Chem.*, **32**, 4101–4103 (1967).
- Sakagami H., Shimada C., Kanda Y., Amano O., Sugimoto M., Ota S., Soga T., Tomita M., Sato A., Tanuma S., Takao K., Sugita Y., *Toxicol. Rep.*, **2**, 1281–1290 (2015).