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Synthesis of novel pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives and their inhibition against growth of A549 and H322 lung cancer cells

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ABSTRACT

A series of substituted pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one was synthesized by the reaction of ethyl 1-(2-oxo-2-phenylethyl)-3-phenyl-1*H*-pyrazole-5-carboxylate derivatives and 2-(2-aminoethoxy)ethanol or 2-morpholinoethanamine in the condition of microwave-assisted one-step and solvent-free in a good yield. The structures of the compounds were determined by IR, ^{1}H NMR and mass spectroscopy. In addition, a representative single-crystal structure was characterized by using X-ray diffraction analysis. Preliminary biological evaluation showed that the compounds could inhibit the growth of A549 and H322 cells in dosage-dependent manners.

Nitrogen-containing heterocycles have always constituted a subject of great interest due to their ubiquity in nature and extensive presence as part of the skeletal backbone of many therapeutic agents. Of these heterocycles, pyrazole derivatives possess important biological activity such as anti-tumor activity,[1] herbicidal activity,[2] antimicrobial activity,[3] γ-secretase inhibitor,[4] anti-HCV activity.[5] And more, pyrazole-fused heterocycles including pyrazolo[3,4-*d*]pyrimidine,[6–8] pyrazolo[1,5-*a*]pyrimidine,[9] pyrazolo[4,3-*d*]pyrimidin-7-one,[10] pyrazolo[1,5-*a*]pyridine,[11] and pyrazolo[3,4-*b*]pyrrolo[3,4-*d*]pyridine[12] have attracted considerable attention. As part of ongoing research directed toward the development of pyrazole-based compounds with structure diversity, we have already reported efficient methods for the construction of pyrazole-fused heterocycles, for example, pyrazolo[5,1-*c*][1,4]oxazin-4-one[13,14] and pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one.[15–18] The evaluation of biological activity showed that these compounds can inhibit A549 lung cancer cell growth. However, because the substituent in pyrazinone moiety was generally more hydrophobic the modification of structure is needed to extend the diversity of fused-pyrazole skeleton and screen anticancer agents.

Microwave-assisted organic synthesis is an increasingly popular field. The advantages of using microwave irradiation over conventional heating are often a reduction in reaction times and cleaner reactions leading to improved yields. In addition, the use of microwave irradiation minimizes the formation of unwanted by-products and reduces the need for organic solvents to a minimum, or even solvent-free.[19–24] In previous papers, we reported that some pyrazole-fused heterocycle compounds could be synthesized by microwave irradiation strategy.[13,17] These results encouraged us to adopt microwave irradiation for the synthesis of pyrazole derivatives with structure diversity. Herein, we would like to report the synthesis and preliminary biological evaluation of pyrazolo[1,5-*a*]pyrazin-4(5*H*)-one modified with hydrophilic group, such as hydroxyl group.

Synthesis of proposed compounds. In our previous paper,[15] we described that ethyl 1-(2-aryl-2-oxoethyl)-3-ferrocenyl-1*H*-pyrazole-5-carboxylate reacted with aryl ethanamine in toluene in a sealed tube at 160–190 °C for 6–16 h to afford pyrazolo-pyrazinones derivatives in yield 20–44%. Thus, as a model, we began with the investigation of the reaction of **1i** and **2a** by which solubility of target compound should be expected to be improved. According to above method, the reaction of **1i** and **2a** under the condition in xylene in a sealed tube at 160 °C for 6 h thus gave target compound **3i**, but in only 29% yield.

We wanted to determine whether the reaction could be carried out under reflux condition in a polar solvent and catalyst to improve yield. Initially, we performed the reaction in *n*-butanol without catalyst, and no product was found after refluxing 6 h. However, in the presence of acetic acid, the same reaction took place to afford target compound in 12% yield. The obvious

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shortcoming of above procedures prompted us to explore more effective methods. We adopted microwave strategy considering the success in construction of pyrazole-fused heterocycles.[17] We found, fortunately, that **1i** reacted cleanly with **2a** in the condition of solvent free and microwave irradiation for 4 min to afford purpose product **3i** in 82% yield. Encouraged by this result, a series of reactions of **1a–i** with **2a–b** was performed under microwave irradiation and the satisfactory results for the synthesis of compounds **3a–l** were obtained as shown in Scheme 1 and Table 1. The structures of the synthesized compounds were determined by IR, ^{1}H NMR and mass spectroscopy.[25]

A suggested pathway for the formation of compounds **3** under these conditions is outlined in Scheme 2. The nucleophilic addition of primary amine **2** to the carbonyl carbon of ketone in compound **1** formed an imine intermediate (**A**) that should isomerize to an enamine (**B**) because the later isomer was more stable conjugated structure. A final irreversible lactamization occurred most rapidly from enamine (**B**). Similar to our previous report,[15] the annulation reaction should be a tandem reaction of enamine formation and lactamization.

Crystallography. Single crystals of **3i** were grown by slow evaporation from ethanol solution at room temperature.[26] The molecular structure with the atom-numbering scheme is shown in Figure 1, and important hydrogen bonds are given in Table 2. X-ray diffraction analysis reveals that **3i** is crystallized into triclinic space group, P_1^-. The molecular structure is dominated by the arrangement of the rings of the pyrazole, pyrazinone, and two substituted benzenes. Atoms in pyrazole ring and pyrazinone moiety are coplanar with maximum mean plane deviation of 0.034(3) Å. From the bond lengths, it is observed that there is a double bond formed between C8 and C9 with the distance of 1.338(3) Å. Torsion angles C12–C13–O2–C14 and C13–O2–C14–C15 are −176.8(2)° and −176.2(3)° in the structure, respectively. These values clearly suggest that C12, C13, O2, C14 and C15 in ethoxyethanol moiety are almost in the same plane. The dihedral angle between the benzene ring [C18–C19–C20–C21–C22–C23] and the pyrazolopyrazine core is 7.67(9)°, which is smaller than 54.75(7)° created by the other benzene ring [C2–C3–C4–C5–C6–C7] and the core structure. In addition, the two benzene rings make a dihedral angle of 62.42(9)°.

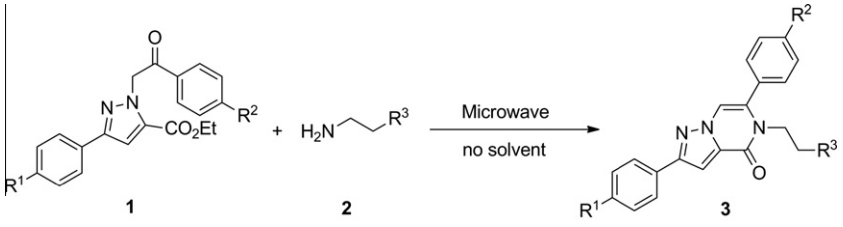
Apparently, weak interactions in **3i** influence significantly the arrangement of the molecule structure. In the crystal structure, it is observed that a $S(6)$ pseudo-ring closed by C13–H13$_B$···O4 intramolecular hydrogen bonding is created. The crystal structure is stabilized by the intermolecular hydrogen bonds and these data are determinant for the crystal packing type. O3–H3$_A$···O5 hydrogen bond self-assembles molecules into $C(17)$ chains, and pairs of C9–H9···O1 hydrogen bonds join two molecules into centrosymmetric dimers of $R_2^2(16)$ type. The combination of these two motifs forms tetramers through $R_4^4(48)$ rings which are further linked by O3–H3$_A$···O5 hydrogen bonds into infinite chain of tetramers. Each molecule forms parallel layers with C23–H23···O4 intermolecular hydrogen bonded polymeric chains in the crystal network. In addition, another type of interaction is weak C4–H4···π [N2–N3–C16–C17–C10] electrons interaction which is important in 3D ordering of molecules (Fig. 2).

Table 1
Results and reaction time for **3a–l** under microwave solvent-free condition

Entry	Reactant **1**	Reactant **2**	Products	Time (min)	Yield (%)
1	**1a**	**2a**	**3a**	12	61
2	**1b**	**2a**	**3b**	4	74
3	**1c**	**2a**	**3c**	6	82
4	**1d**	**2a**	**3d**	4	84
5	**1e**	**2a**	**3e**	4	78
6	**1f**	**2a**	**3f**	4	82
7	**1g**	**2a**	**3g**	4	72
8	**1h**	**2a**	**3h**	4	64
9	**1i**	**2a**	**3i**	4	82
10	**1b**	**2b**	**3j**	7	62
11	**1e**	**2b**	**3k**	12	51
12	**1h**	**2b**	**3l**	12	56

*Inhibitory effects of compounds **3a–l** on the proliferation of A549 and H322 lung cancer cells*. All the compounds synthesized were evaluated for their cytotoxicity against A549 and H322 lung cancer cells lines. Firstly, the assay incubated with compounds at the concentration of 40 μM for 48 h was carried out, and the cells were treated with SRB to measure their growth/viability (% of the untreated control) using a spectrophotometer as described previously.[17,27–29] The results showed that the compounds **3a–d**, **3f**, **3g** and **3i** displayed more effects on the growth of A549 and H322 cells. Among them, **3a**, **3b**, and **3i** showed a most potent inhibitory effect on A549 and H322 cells growth in a dose-dependent manner as shown in Figure 3. Growth inhibitory properties IC_{50} (μM) of the compounds at 48 h in A549 and H322 cells were shown in Table 3. It can be observed that the compounds have more inhibitory effect on A549 cells than H322 cells. The activity difference of the compounds against two kinds of cells might be related with p53 because A549 and H322 cells are p53 wild-type and p53-mutant respectively. The inhibition mechanism should be worthy to be investigated in the future.

Effects of the compounds on the morphology of A549 and H322 cells. We observed the morphological image of the cells treated with compounds **3a**, **3b** and **3i** at 40 μM for 48 h under an inverted microscope. We found that the quantities of the cells treated with compounds **3a**, **3b** and **3i** were decreased greatly for two kinds of cells. In addition, the shape of some A549 cells changed to globular. However, in the cases of H322 cells, there were some morpholog-



1

2

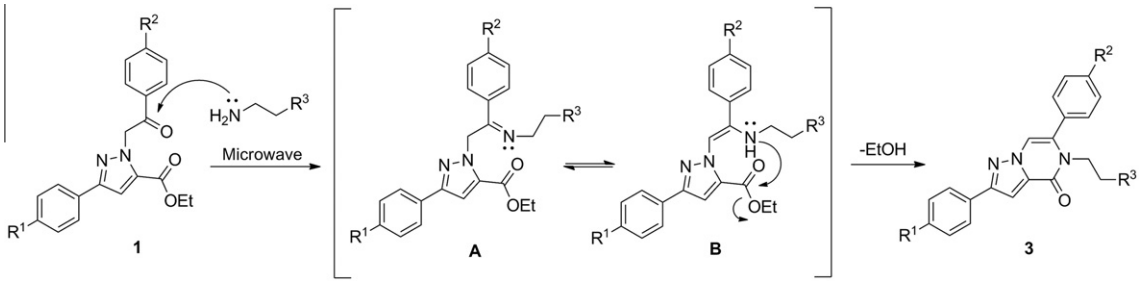
3

1a: $R^1 = R^2 = H$
1b: $R^1 = Cl$, $R^2 = H$
1c: $R^1 = OMe$, $R^2 = H$
1d: $R^1 = H$, $R^2 = Cl$
1e: $R^1 = R^2 = Cl$
1f: $R^1 = OMe$, $R^2 = Cl$

2a: $R^3 = OCH_2CH_2OH$
2b: $R^3 = N(CH_2CH_2)_2O$

1g: $R^1 = H$, $R^2 = OMe$
1h: $R^1 = Cl$, $R^2 = OMe$
1i: $R^1 = R^2 = OMe$

Scheme 1. Synthesis of pyrazolo[1,5-*a*]pyrazin-4(5*H*)-ones.



Scheme 2. Proposed mechanism for the formation of pyrazolo[1,5-*a*]pyrazin-4(5*H*)-ones.

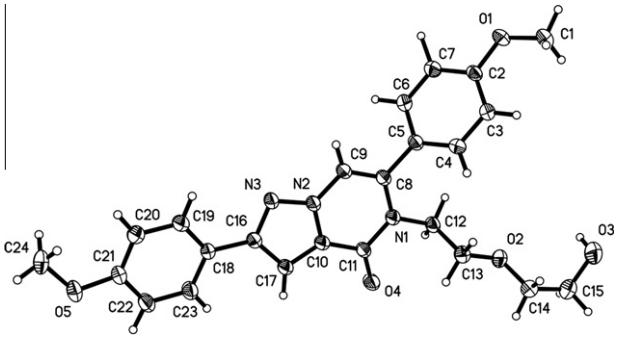


Figure 1. X-ray crystal structure of compound **3i**, with displacement ellipsoids drawn at the 30% probability level.

Table 2
Hydrogen bonding geometry for crystal structure

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)
O3–H3$_A$···O5[a]	0.79(7)	2.15(7)	2.898(4)	159(6)
C9–H9···O1[b]	0.91(3)	2.36(3)	3.254(3)	169(2)
C23–H23···O4[c]	0.89(3)	2.57(3)	3.424(3)	161(3)
C13–H13$_B$···O4	1.01(4)	2.49(3)	3.091(4)	118(2)

[a] Symmetry codes: *x*, *y* − 1, *z* + 1.
[b] −*x* + 1, −y, −z.
[c] −x, −y + 2, −z.

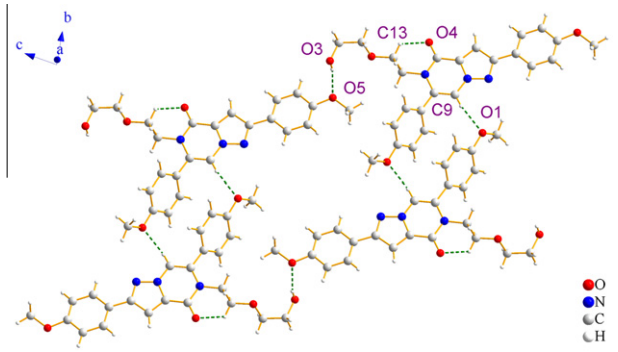


Figure 2. Packing diagram of **3i,** hydrogen bonds are showed with green dashed lines.

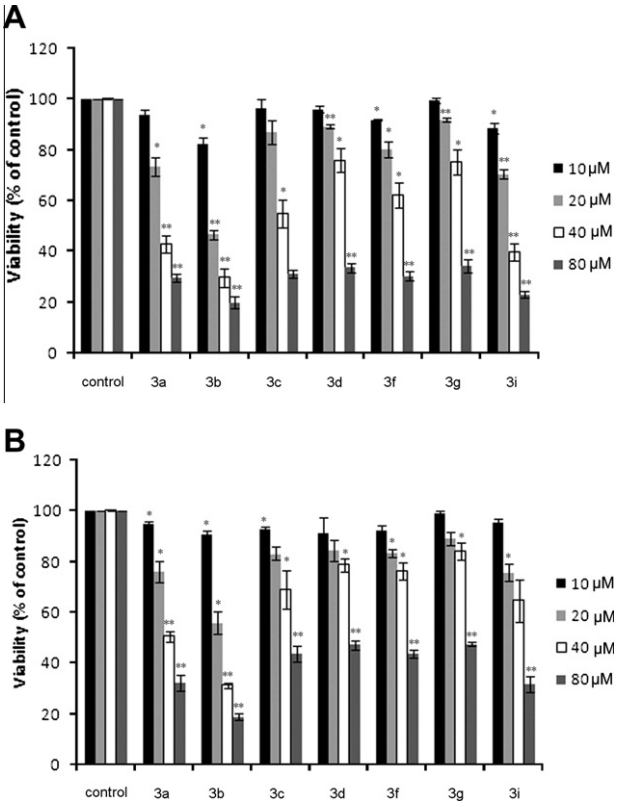


Figure 3. Effects of the compounds on A549 (A) and H322 (B) cell viability at 48 h. A549 and H322 cells were treated with compounds **3a–d**, **3f**, **3g** and **3i** at concentrations of 10, 20, 40 or 80 μM or left untreated (control) for 48 h. Cell viability was analyzed by SRB assay and illustrated in column figures. Results are presented as mean ± SE; (n = 3. $^{*}P < 0.05$ versus control; $^{**}P < 0.01$ versus control).

Table 3
Growth inhibitory properties IC_{50} (μM) for the compounds **3a–d**, **3f**, **3g** and **3i** at 48 h in A549 and H322 cells

Compounds	3a	3b	3c	3d	3f	3g	3i
A549	41.1	24.2	50.3	62.6	49.7	59.0	34.7
H322	45.6	29.4	68.7	91.0	76.9	74.7	50.6

ical changes such as volume shrink, fragment and changing to globular. Such morphological changes were not apparent in the control cells (Fig. 4).

*Compounds **3a**, **3b** and **3i** do not cause necrosis in A549 and H322 lung cancer cells.* In order to determine if the growth inhibitory effects were due to necrosis that is believed to be an unwanted side effect of cancer-fighting agents, LDH assay were carried out on cells treated with compounds **3a**, **3b** and **3i** or 0.1% DMSO (as control). As shown in Figure 5 for A549 and H322 cells, there were no significant differences in LDH release between the cells in the control group and the compounds treatment group. The results demonstrated that the compounds at the test range of concentration did not cause necrosis in the cells.

In conclusion, we have developed an efficient method for the preparation of pyrazolo[1,5-*a*]pyrazin-4(5*H*)-ones in solvent-free

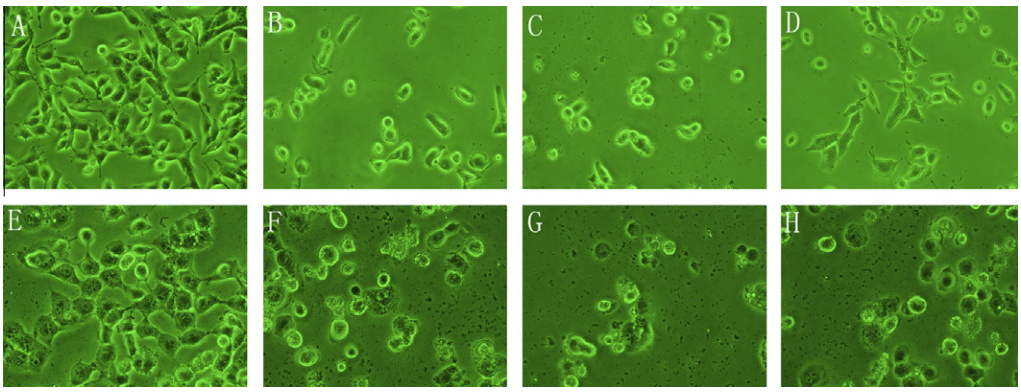


Figure 4. Morphology image of A549 (A–D) and H322 (E–H) cells treated with the compounds **3a**, **3b** or **3i** for 48 h (200×). A and E are control, the cells treated with DMSO 0.1% (v/v) as a vehicle control. B and F, C and G, D and H are treated with the compounds **3a**, **3b** or **3i** at concentration of 40 μM respectively.

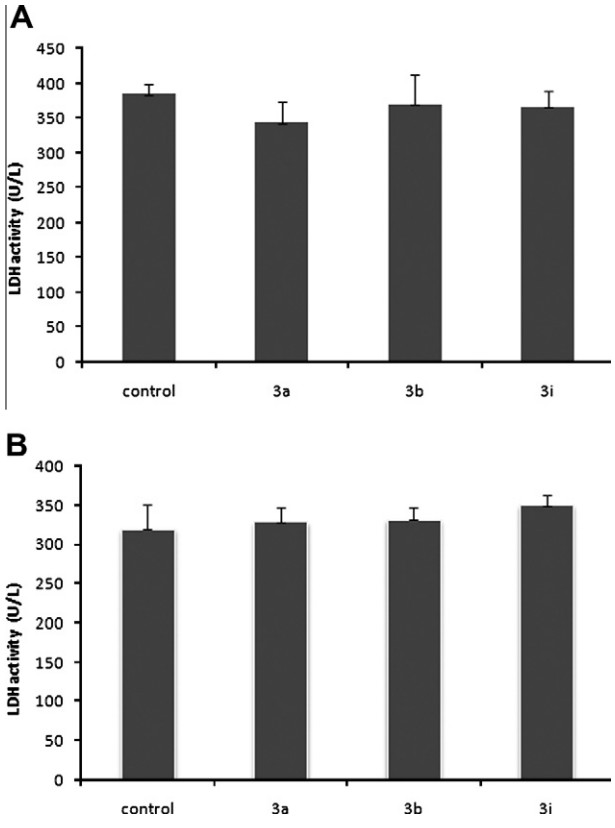


Figure 5. Effects of the compounds **3a**, **3b** or **3i** on LDH release in A549 cells (A) and H322 cells (B). A549 and H322 cells were treated with compounds **3a**, **3b** or **3i** at concentration of 40 μM or left untreated (control) for 48 h. LDH assay results are presented as mean ± SE (n = 3).

microwave-assisted condition. Application of microwave irradiation reduces the reaction time dramatically to 4–12 min and the experimental procedure is operationally easy and leads to high yields in short reaction time without using toxic reagents and solvents. In addition, we determined the structure of a synthesized compound **3i** by X-ray analysis. The overall crystal structures were stabilized through intra- and intermolecular hydrogen bonds and C–H···π networks. Preliminary biological evaluation showed that the compounds **3a**, **3b** and **3i** could inhibit the growth of A549 and H322 cells in dosage-dependent manners and the compounds have more inhibitory effect on A549 cells than H322 cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.035.

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25. *Experimental procedure and a typical structure elucidation*: To a flask ethyl 1-(2-oxo-2-phenylethyl)-3-phenyl-1*H*-pyrazole-5-carboxylate derivatives **1**

(1.0 mmol) and ethanamines **2** (2.0 mmol) was added and mixed. Then, the mixture was irradiated in a focused microwave oven, modified to control the power at 700 W for the time given in Table 1. The crude product was purified by flash chromatography on Silica gel (ethyl acetate/petroleum ether = 1:2) to afford the products **3**. 5-(2-(2-Hydroxyethoxy)ethyl)-2,6-diphenylpyrazolo[1,5-*a*]pyrazin-4(5*H*)-one (**3a**): yellow solid, yield: 61% (229 mg), mp 127–128 °C. IR (KBr, cm^{-1}) ν: 3434 (OH), 1675 (C=O), 1113 (C–N). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 3.42 (t, J = 4.5 Hz, 2H, NCH_2), 3.61 (t, J = 5.7 Hz, 2H, OCH_2), 3.62 (t, J = 4.5 Hz, 2H, OCH_2), 4.15 (t, J = 5.7 Hz, 2H, OCH_2), 7.38 (s, 1H, PyzH), 7.39 (s, 1H, CH), 7.35–7.40 (m, 1H, Ar-H), 7.44–7.48 (m, 4H, Ar-H), 7.49–7.52 (m, 3H, Ar-H), 7.90–7.92 (m, 2H, Ar-H). HR-MS ($C_{22}H_{22}N_3O_3$): calcd for $[M+H]^+$ 376.1661; found 376.1659.

26. *Crystal data of compound* ***3i***: $C_{24}H_{25}N_3O_5$, M = 435.47, Triclinic, crystal dimensions 0.30 × 0.20 × 0.10 mm, space group P_1^- a = 8.4452(17) Å, b = 9.2982(19) Å, c = 15.015(3) Å, α = 79.952(3)°, β = 81.120(3)°, γ = 72.914(4)°, λ = 0.71073 Å, T = 293 K, V = 11.3.1(4) $Å^3$, Z = 2, D_c = 1.311 g cm^{-3}, μ = 0.093 mm^{-1}, F(0 0 0) = 460; 4252 reflections used, 373 refined parameters. The final discrepancy factors were w_R = 0.1440, R = 0.0557, goodness of fit S = 1.051 on F^2, largest difference peak and hole 0.340 and −0.222 e·$Å^{-3}$, CCDC 779358 contains the Supplementary crystallographic data for compound **3i**.
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