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Synthesis, structure, and bioactivity of N′-substituted benzylidene-3,4,5-trimethoxybenzohydrazide and 3-acetyl-2-substituted phenyl-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1,3,4-oxadiazole derivatives

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Abstract—Some 3-acetyl-2-substituted phenyl-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1,3,4-oxadiazole derivatives were synthesized by cyclization reaction of N′-substituted benzylidene-3,4,5-trimethoxybenzohydrazide in acetic anhydride. Their structures were verified by elemental analysis, IR, ^{1}H NMR, and ^{13}C NMR. Compound **3i** was provided with X-ray crystallographic data. The compounds were evaluated for their antiproliferative activities against some cancer cells in vitro by MTT method. Among them, **2a**, **2b**, **2c**, **2f**, **3l**, and **3m** were highly effective against PC3 cells and **2a**, **2c**, and **2f** showed moderate activities against Bcap37 and BGC823 cells. The IC_{50} values of high active compounds **2a**, **2b**, **2c**, **2f**, **3l**, and **3m** against PC3 cells were 0.2, 1.8, 0.2, 1.2, 1.7, and 0.3 μM, respectively.

Hydrazones are well acknowledged to possess a diverse range of bioactivities in pharmaceutical and agrochemical field.[1–3] Several hydrazone compounds are concerned because of their special biological catalyst-enzyme activity.[4] Furthermore, substituted 1,3,4-oxadiazole derivatives also have been reported to show broad spectrum bioactivities including insecticidal,[5] antibacterial,[6] anticancer,[7] and anti-inflammatory activities.[8] On the other hand, recently the synthesis and bioactivity of gallic acid derivatives have attracted more and more attention, among which some hydrazone and 1,3,4-oxadiazole derivatives containing 3,4,5-trimethoxyphenyl moiety with certain antimicrobial activity were reported.[9] However, in our previous work, some 1,3,4-thiadiazole derivatives bearing 3,4,5-trimethoxyphenyl moiety were proved having good antitumor bioactivity.[10] As a continuation of our research for finding new anticancer agents, we designed a series of new compounds by replacing the 1,3,4-thiadiazole moiety in those anticancer compounds[10] mentioned above with hydrazone and 1,3,4-oxadiazole moiety. Hence, a series of new aroyl hydrazones together with 1,3,4-oxadiazole derivatives containing 3,4,5-trimethoxyphenyl moiety were synthesized starting from gallic acid. The structures of new compounds were confirmed by spectral analysis. The antitumor activity of the new compounds was also evaluated by MTT method. The synthetic route to target compounds is shown in Scheme 1.

In order to optimize the reaction condition for preparation of compound **2**, the synthesis of **2a** was carried out under different conditions. The effects of different solvents, reaction time, and temperature are summarized in Table 1. It was found that the yield was up to 81.4% when the reaction mixture was refluxed for 30 min in anhydrous ethanol.

We also studied the substituent effects together with influence of substituent position (**2a**–**2o**).[11] The result showed that *ortho*-substituted and *para*-substituted benzaldehyde afforded hydrazones in higher yields than *meta*-substituted benzaldehyde. The reason may be that

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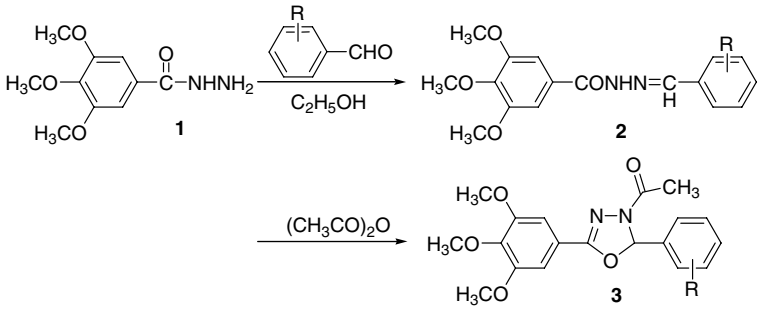

R= **a:** 2-F; **b:** 3-F; **c:** 4-F; **d:** 2-CF_3; **e:** 3-CF_3; **f:** 4-CF_3; **g:** 3,4-2Cl; **h:** 2,5-2OCH_3; **i:** 3,4-2F; **j:** 2,3-2OCH_3; **k:** 4-Cl-3-NO_2; **l:** 3,5-2Cl; **m:** 2,4-2OCH_3; **n:** 2,6-2Cl; **o:** 3,4,5-3OCH_3

Scheme 1.

Table 1. Yields of **2a** at different reaction conditions

Entry	Solvent	Time (h)	Temp. (°C)	Yield (%)
1	Methanol	3	Reflux	56.7
2	Methanol	5	Reflux	77.0
3	Ethanol	5	rt	70.0
4	Ethanol	0.5	Reflux	81.4
5	Ethanol	1.0	Reflux	82.5
6	Ethanol	0.2	Reflux	78.2
7	Ethanol	0.5	40–50	58.8
8	Ethanol	0.5	60–65	72.8
9	—	0.5	75–80	78.0

the electron-negative substituents polarize the carbonyl through conjugation effect, which makes the carbonyl easier to be attacked by the nucleophilic group $–NH_2$. It was found that electron-releasing group, such as $–OCH_3$, could hinder the reaction and then properly extended refluxing time or even catalytic acetic acid was needed.

Cyclization of the hydrazones **2** by refluxing in acetic anhydride afforded series of oxadiazole **3**. The reaction conditions for the transformation of **2a**–**3a** were optimized. It was found that **3a** could be obtained in the yield of 71.5% after refluxing for 2 h. No product was obtained when the temperature was below 100 °C. Additionally, the hydrazones could not be converted into target product when the refluxing time was less than 30 min. However, due to the strong dehydration efficacy of acetic anhydride, the by-product was evidently increased when the reaction time was prolonged to more than 120 min, which was detected by thin-layer chromatography (petroleum ether/ethyl acetate, 1:1).

Using the above optimal condition,**3a**–**3o** were prepared by the cyclization reaction of N′-substituted benzylidene-3,4,5-trimethoxybenzohydrazide (**2a**–**2o**) at refluxing temperature in acetic anhydride for 2 h (Table 2).

The single-crystal structure of **3i** was determined by X-ray crystallography[13] as illustrated in Figure 1.[14] In **3i**, the bond length of N(1)–C(10) (1.269(2) Å) is shorter than that of typical C=N (1.34 Å),[15] while N(2)–C(11) (1.355(3) Å) is remarkably shorter than normal C–N (1.47 Å),[15] which is indicative of significant double bond character. Though the C(11) carbon of the oxadiazole

Table 2. Synthesis of **3a**–**3o**[12]

Entry[a]	Compound	R	Yield[a] (%)
1	**3a**	2-F	71.5
2	**3b**	3-F	62.9
3	**3c**	4-F	67.5
4	**3d**	2-CF_3	66.5
5	**3e**	3-CF_3	57.5
6	**3f**	4-CF_3	59.4
7	**3g**	3,4-di-Cl	58.8
8	**3h**	2,5-di-MeO	60.0
9	**3i**	3,4-di-F	68.0
10	**3j**	2,3-di-MeO	60.0
11	**3k**	4-Cl-3-NO_2	57.4
12	**3l**	3,5-di-Cl	70.5
13	**3m**	2,4-di-MeO	72.0
14	**3n**	2,6-di-Cl	78.3
15	**3o**	3,4,5-tri-MeO	67.3

[a] All reactions were refluxed in acetic anhydride.

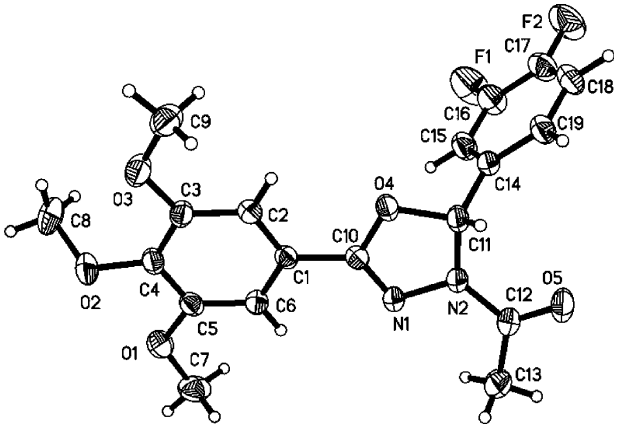

Figure 1. ORTEP drawing of **3i**.

ring is sp^3 hybridized, the oxadiazole ring [C(10), N(1), N(2), C(11), O(4)] is fairly planar, and the deviations from the least-squares plane through the ring atoms are all less than or equal to 0.0063 nm. While for the two benzene rings [C(1), C(2), C(3), C(4), C(5), C(6)] and [C(14), C(15), C(16), C(17), C(18), C(19)], the deviations from the least squares plane through the ring atoms are smaller than 0.0034 and 0.0013 nm, respectively. The dihedral angle between the plane of oxadia-

zole ring and the plane of 3,4-difluorobenzene is 87°, which is reasonable considering the sp^3 configuration of C(11). For the plane of oxadiazole ring and the plane of the trimethoxybenzene ring, the dihedral angle is 7.5°. There is an intermolecular hydrogen bond between C(18)–H(18)...O(5) (symmetry code, $-x$, $-y$, $-z$ +1), with C(18)–H(18) = 0.930 Å, H(18)...O(5) = 2.43, C(18)...O(5) = 3.347(3) Å, and ∠C(18)–H(18)...O(5) = 169.2°.

The antitumor activities in vitro of these compounds were evaluated against PC3, BGC823, and Bcap-37 cells by MTT method.[16] The results for hydrazones **2** and oxadiazoles **3** are summarized in Tables 3 and 4, respectively.

It can be found from Table 3 that compounds **2c**, **2a**, **2b**, and **2f** have strong inhibitory activity against PC3 cells. The data given in Table 3 indicate that the change of substituent of the phenyl ring affects the antitumor activity. When the benzene ring is monosubstituted by F atom or CF_3 group, the compounds generally have potential anticancer bioactivity, such as **2a**–**2f**, with antiproliferative activity of 70.6%, 53.5%, 76.0%, 45.0%, 51.0%, and 59.9% at 1 μM, respectively. Among these compounds, **2a** (R = 2-F) and **2c** (R = 4-F) are much more active against PC3 cells than the other ones, with inhibition rate of 70.6% (**2a**, 1 μM), 81.8% (**2a**, 5 μM) and 76.0% (**2c**, 1 μM), 82.80% (**2c**, 5 μM), respectively. While for the di- or tri-substituted hydrazones, their inhibitory activities are generally low, as could be seen from the bioassay data of compounds **2g**–**2o**, except for compound **2j**, which was di-substituted by 2,3-dimethoxy groups. For the high active compounds **2a**, **2b**, **2c**, and **2f**, further bioassay was conducted and their IC_{50} values against PC3 cells were 0.2, 1.8, 0.2, and 1.2 μM, respectively.

The data given in Table 4 indicate that the changes of substituents also affected the antitumor activity of title compounds **3a**–**3o**. Highest inhibitory activity was achieved when R = 2,4-dimethoxy (**3m**), with inhibition rates of 69.5% and 92.0% at 1 and 5 μM, respectively. Compound **3l** (R = 3,5-dichloro) could inhibit the proliferation of PC3 cells up to 52.7% and 93.9% at 1 and 5 μM. The other compounds **3a**–**3k** and **3n**–**3o** have relatively lower antitumor activities than those of **3l** and **3m**, whose IC_{50} values were 1.7 and 0.3 μM, respectively.

It is well known that the biological activity associated with the hydrazone compounds is attributed to the presence of the active pharmacophore (–CONH–N=C–). Hence many hydrazone compounds **2** containing the active moiety (–CONH–N=C–) showed good anticancer bioactivities as indicated in Table 3. The interesting thing is, the conversion of the (–CONH–N=C–) moiety in active compounds **2a**–**2f** into 1,3,4-oxadiazoles **3a**–**3f** weakened their anticancer activity (antiproliferation rate −1.5% to 30.1% against PC3, BGC823, and Bcap37 cells at 1 μM, see Table 4). While such conversion of compounds **2l** and **2m** strengthened their activities (see the bioassay data of **3l** and **3m** in Table 4). The reason for such interesting differences is still under investigation.

In summary, we described a practical and efficient procedure for preparing 3-acetyl-2-substituted phenyl-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1,3,4-oxadiazole derivatives through the cyclization of N′-substituted benzylidene-3,4,5-trimethoxybenzohydrazide in refluxing acetic anhydride for 2 h. The reaction is very fast, experimentally simple with moderate yield. In addition, among the synthesized compounds, **2c**, **2a**, **2b**, **2f**, and **3l**, **3m** are highly effective against PC3 cells. Moreover, **2a**, **2c**, and **2f** are moderatively effective against Bcap37 cells. These identified trimethoxyphenyl oxadiazole com-

Table 3. Inhibition rate[a] (%) of compounds **2a**–**2o** against PC3 and A431 cells at 1 and 5 μM in vitro

Compound	PC3 cells		Bcap37		BGC823	
	1 μM	5 μM	1 μM	5 μM	1 μM	5 μM
2a	70.6 ± 4.7*	81.8 ± 2.3*	51.6 ± 0.9*	76.54 ± 1.0*	58.9 ± 1.5*	81.8 ± 1.5*
2b	53.5 ± 1.4*	64.1 ± 1.5*	13.6 ± 1.5*	44.8 ± 1.0*	38.0 ± 2.0*	49.0 ± 1.6*
2c	76.0 ± 2.4*	82.8 ± 2.3*	50.9 ± 2.3*	61.1 ± 3.7*	50.2 ± 2.2*	55.5 ± 2.5**
2d	45.0 ± 3.7*	52.9 ± 2.5*	22.1 ± 4.4*	35.6 ± 1.2*	56.8 ± 1.7*	80.0 ± 1.5**
2e	51.0 ± 2.8*	79.8 ± 2.5*	17.7 ± 2.8*	33.8 ± 2.5*	8.3	15.5 ± 1.9*
2f	59.9 ± 2.8*	61.6 ± 2.7*	47.4 ± 6.1*	56.3 ± 1.5*	61.8 ± 1.0*	65.6 ± 0.9*
2g	13.5 ± 1.1*	31.6 ± 1.0*	29.6 ± 1.6*	32.7 ± 1.5*	0.1	0.6
2h	30.9 ± 1.9*	51.7 ± 1.1*	0.1	10.11	0.0	0.0
2i	35.1 ± 2.1*	43.7 ± 1.6*	33.1 ± 2.6*	51.9 ± 2.4*	58.5 ± 2.8*	68.2 ± 2.1**
2j	53.2 ± 4.2*	67.0 ± 3.9*	−0.1	14.2	−2.3	12.3
2k	32.1 ± 1.4*	42.3 ± 1.0*	−5.8	2.3	−4.4	9.6
2l	22.1	46.3 ± 1.5*	−7.5	9.2	−5.4	3.6
2m	10.0	22.2	−6.9	3.6	−3.0	−0.1
2n	43.0 ± 3.0*	52.1 ± 3.1*	0.9	27.8 ± 4.3*	−7.2	2.9
2o	8.5	48.3 ± 5.5*	−5.0	22.9 ± 3.8*	11.8 ± 2.5*	13.6 ± 2.1*

[a] Inhibition rate (%) = $(A1 - A2)/A1 \times 100\%$. $A1$, the average optical densities of untreated cells; $A2$, the average optical densities of drug treated cells.

* $P < 0.05$.

** $P < 0.01$.

Table 4. Inhibition rate (%)[a] of compounds **3a**–**3o** against PC3, Bcap37, and BGC823 cells at 1 and 5 μM in vitro

Compound	PC3 cells		Bcap37		BGC823	
	1 μM	5 μM	1 μM	5 μM	1 μM	5 μM
3a	13.5 ± 3.1*	31.6 ± 3.6*	29.6 ± 4.0*	32.8 ± 3.7*	−1.5	−2.0
3b	16.5 ± 2.1*	43.5 ± 1.9*	14.7 ± 2.4*	37.7 ± 2.4*	14.0 ± 2.9*	23.8 ± 2.6*
3c	0.0	0.1	−0.4	13.3	−1.4	−0.1
3d	11.0	15.2	31.8 ± 3.4*	34.8 ± 2.9*	−1.2	16.2
3e	30.1 ± 2.5*	43.6 ± 2.2*	27.9 ± 3.8*	41.1 ± 3.0*	10.1 ± 2.1*	35.5 ± 1.8*
3f	−11.0	−0.7	4.2	3.4	−3.5	14.2 ± 4.2*
3g	28.1 ± 2.5*	26.1 ± 2.7*	−0.8	3.1	−3.2	0.0
3h	37.2 ± 2.7*	43.2 ± 2.4*	0.4	18.2 ± 3.6*	−3.8	2.3
3i	35.5 ± 1.9*	46.9 ± 2.0*	18.3 ± 2.6*	21.6 ± 2.1*	−1.3	2.3
3j	35.0 ± 1.4*	58.2 ± 1.1*	3.8	17.5 ± 1.9*	0.1	2.8
3k	43.3 ± 2.8*	61.4 ± 2.5*	−2.3	0.0	−2.9	1.0
3l	52.7 ± 1.6*	93.9 ± 1.7**	0.0	6.6	−1.1	1.3
3m	69.5 ± 2.3*	92.0 ± 1.8**	6.3	−3.1	−0.3	2.5
3n	5.1	−2.2	−1.4	15.9 ± 3.4*	9.3	45.8 ± 3.7*
3o	−1.8	0.1	−1.8	−1.4	5.1	5.4

[a] Inhibition rate (%) = $(A1 - A2)/A1 \times 100\%$. $A1$, the average optical densities of untreated cells; $A2$, the average optical densities of drug treated cells.

* $P < 0.05$.

** $P < 0.01$.

pounds can be very useful in the development of optimization strategies for cancer chemotherapy.

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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.2006.07.048.

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11. General experimental procedure for the synthesis of N′-substituted benzylidene-3,4,5-trimethoxybenzohydrazide **2**. To the mixture of 3,4,5-trimethoxybenzohydrazide **1** (2 mmol) in ethanol (10 mL) was added appropriate aldehyde (2 mmol). Then the mixture was refluxed for 30 min. After cooling, the crude product was obtained by filtration and recrystallized from ethanol–DMF to afford **2a**–**2o** as white solids. Their physico-chemical properties and the spectra data can be found in supporting information.
12. Synthesis of 3-acetyl-2-substituted phenyl-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1,3,4-oxadiazoles (**3**). Compound **2** (1 mmol) was refluxed in acetic anhydride (5 mL) for 2 h. The mixture was cooled, poured into crush ice, and allowed to stand at room temperature over night. The separated solid was washed by water, dried, and recrystallized from acetone-water to give title compound **3a**–**3o**. Their physico-chemical properties and the spectra data can be found in supporting information.
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14. Crystal data of **3i**. $C_{19}H_{18}F_2N_2O_5$, $M = 392.35$, Monoclinic, $a = 7.659(3)$, $b = 15.781(6)$, $c = 15.583(6)$ Å, $\beta = 96°$, $V = 1872.7(13)$ Å^3, $T = 296(2)$ K, space group P2(1)/c, $Z = 4$, $Dc = 1.392$ g/cm^3, μ (Mo–Kα) = 0.114 mm^{-1}, $F(000) = 816$. 10468 reflections measured, 3854 unique (Rint = 0.0449) which were used in all calculation. Fine $R1 = 0.0449$, $wR(F^2) = 0.1243$ (all data). Full crystallographic details of **3i** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 299720.

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16. MTT assay against cancer cell proliferation: All tested compounds were dissolved in DMSO (1–100 μM solution) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density 2×10^3 cells/well/100 μL of the proper culture medium and treated with the compounds at 1 and 5 μM for 72 h. In parallel, the cells treated with 0.1% DMSO served as control. An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay (Roche Molecular Biochemicals, 1465-007) was performed 30 h later according to the instructions provided by Roche. This assay is based on the cellular cleavage of MTT into formazan which is soluble in cell culture medium. And the absorbance caused by formazan was measured at 595 nm with a microplate reader (Bio-Rad, model 680), which is directly proportional to the number of living cells in culture. Three types of cells were used in these assays, PC3 (prostate cancer), BGC823 (human gastric cancer) and Bcap37 (breast cancer) cell lines, provided by ATCC and cultivated in RPMI 1640 (for PC3, BGC823 and Bcap37) supplemented with 10% fetal bovine serum. Tissue culture reagents were obtained from Gibco-BRL Skehan, P.; Storeng, R.; Scadiero, D.; Monks, A.; McMahon, I.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boy, M. R. *Natl. J. Cancer Inst.* **1990**, *82*, 1107.