A New Route to Synthesis of 3,6-Diaryl-1,2,4-triazolo[3,4-*b*]1,3,4-oxadiazoles

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Five new 3,6-diaryl-1,2,4-triazolo[3,4-*b*]1,3,4-oxadiazole derivatives were synthesized by 9 steps from aromatic acids and evaluated for their activities of anticancer and antibacteria. The structures of all new compounds synthesized were elucidated by MS, IR, ^{1}HNMR and HRMS.

Keywords: 1,2,4-Triaole; 1,2,4-Triazolo[3,4-b]1,3,4-oxadiazoles; Anticancer and antibacterial test.

INTRODUCTION

Various 1,2,4-triazoles and 1,3,4-thiadiazoles have been reported to possess diverse biological activities such as antimicrobial, insecticidal, herbicidal, and plant growth regulative effects.[1-4] In addition to the above biological activities 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives obtained by coupling these two bioactive rings together had strong CNS depressant, mild hypocholesterolemia, and hypotensive properties.[5,6] Some 3,6-disubstituented-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives showed anti-HIV-1 activity at concentrations slightly below cytotoxic levels.[7]

1,3,4-Oxadiazole derivatives display quite a broad spectrum of biological activities too, such as HIV-activity,[8] antifungal capability,[9] antibacterial,[10] plant growth promoter activities[11] and herbicidal effects.[12] We thought of coupling 1,2,4-triazoles and 1,3,4-oxadiazoles together and screening the biological activity of these fused heterocycles.

This ring system was first reported in 1961[13] and synthesized in 1971.[14] There are only a few reports on the synthesis and biological activity research of this ring system due to the instability of this ring system in acidic and basic conditions, as well as the difficulty of preparing them. Here we present a new and useful synthesis route and have synthesized five new 1,2,4-triazolo[3,4-*b*]1,3,4-oxadiazole derivatives.

RESULTS AND DISCUSSION

Aromatic acids (**1**) were esterified to get esters (**2**) and then hydrazinolysised to give aroylhydrazides (**3**), which reacted with carbon disulfide and potassium hydroxide in boiling ethanol to yield oxadiazole (**4**). Compound **4** was methylated in aqueous sodium hydroxide using dimethyl sulfate to get methyl sulfide (**5**), then oxidized by potassium permanganate in glacial acetic acid to afford sulfonate (**6**). Treatment of compound **6** with hydrazine hydrate in dioxane and ether afforded 2-hydrazino-oxadiazoles (**7**). Compound **7** reacted with acyl chloride to get compound **8**. On treatment with phosphorus oxychloride under refluxing for 5~10 min, the desired compounds, 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives (**9**) (Scheme I), were yielded.

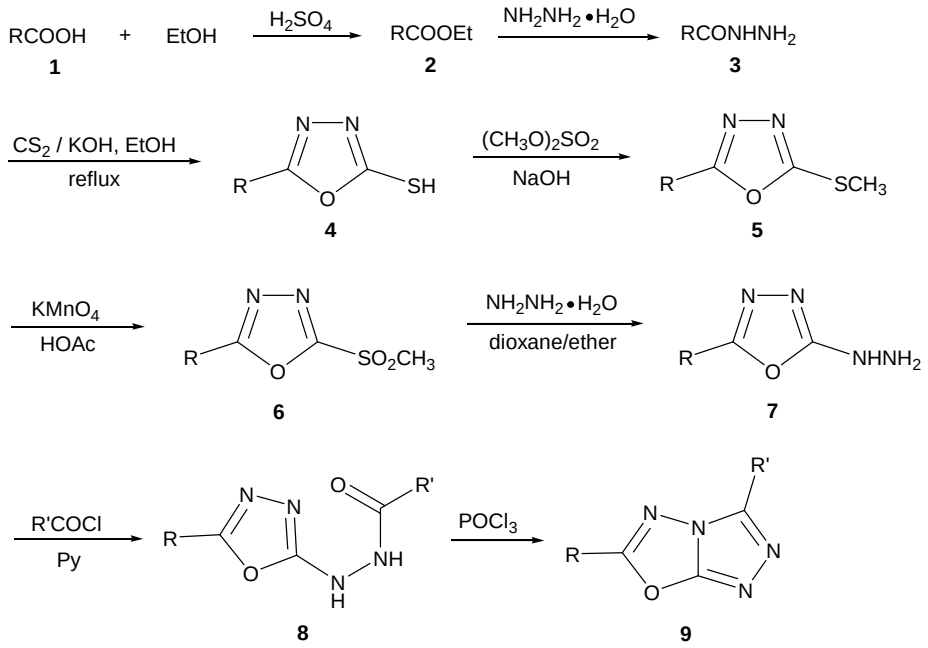
In the process of preparing compound **9**, it is important to select a suitable refluxing time. It was choosen by such a method of compound **8** 100 mg was added into 4 mL phosphorus oxychloride, then stirred and refluxed. After the mixture began to reflux, about 0.3 mL solution was taken out from the mixture by injector per minute to follow this reaction. The mixture of solution was dropped into 20 g crushed ice with hard stirring to yield some white or pale yellow solids. These solids were filtered, washed with water and dissolved in THF. We found a suitable time of 5~10 minutes for this reaction by TLC plate.

Compounds **9a~e** were screened for their anticancer and antibacterial activities by testing their inhibitory rate against *cdc25* and *eMetAP* but no efficient activity was found.

EXPERIMENTAL

All reagents of AR grade were used without purification. The melting points were determined on a Yanaco MP

Scheme I



1~7 a: R = 4-Br-Ph; **b**: R = 4-Cl-Ph; **c**: R = 4-Cl-Ph; **d**: R = 4-Br-Ph; **e**: R = Ph
8~9 a: R = 4-Br-Ph, R′ = Ph; **b**: R = 4-Cl-Ph, R′ = 2-Cl-Ph; **c**: R = 4-Cl-Ph, R′ = Ph;
d: R = 4-Br-Ph, R′ = 2-Cl-Ph; **e**: R = Ph, R′ = 2-Cl-Ph

Table 1. Inhibition effect of *cdc25* and *eMetAP* by compounds **9a~e**

Compd.	*cdc25* (%, C = 250 μM)	*eMetAP* (%, C = 500 μM)	*eMetAP* (%, C = 50 μM)
9a	77.1	*	3.0
9b	6.1	36.1	-
9c	37.7	*	2.1
9d	0	90.5	30.2
9e	0	89.4	17.9

* Not tested because some solid precipitated.

microscropic melting point apparatus and are uncorrected. Elemental analysis was carried out on a Vario E1 Elementar. IR spectra were obtained in KBr discs on a Nicolet AVATA 360FT-IR spectrometer. Mass spectra were performed on a HP-5988A spectrometer (EI at 70 ev). ^{1}H NMR spectra ($CDCl_3$ or DMSO-d_6) were recorded on a FT-Ac200M or FT-Ac80M instrument at room temperature with TMS as an internal standard. HRMS were run on a FT-ICRMS APEXII 47e spectrometer.

Phosphorus oxychloride was redistilled (b.p. 105 °C). Compounds **2**, **3**, and **4** were prepared according to the methods in the literature.[15-16]

Preparation of 2-methylsulfonyl-5-aryl-[1,3,4]-oxadiazoles (6)

Dimethyl sulfate (0.1 mol) was added dropwise to a solution of **4** (0.1 mol) in aqueous NaOH (1 M, 200 mL) with stirring at room temperature for 6 h. The precipitate was filtered, washed with water and recrystallized from ethanol to give compound **5**. To a solution of compound **5** (0.1 mol) in 150 mL glacial acetic acid, 30 g $KMnO_4$ dissolved in 300 mL water was added slowly. This procedure would last about 1 h. After $KMnO_4$ was added over, the resulting suspended solid was stirred for 6 h, cooled to 0 °C and reduced by $NaHSO_3$. The precipitate was filtered, washed with water, dried and recrystallized from ethanol to get compound **6**. The melting points of the compounds synthesized here were the same as those in the literature.[17]

Preparation of 2-hydrazino-5-aryl-[1,3,4]-oxadiazoles (7)

To a solution of compound **6** (50 mmol) in 50 mL dioxane and 30 mL ether, 5 equiv. of hydrazine hydrate (85%) was added with stirring in an ice bath. After 0.5 h there is lots of white precipitate in this solution which was stirred for another 4 h and filtered. The solid was washed with water and dried. It needed no futher purification and could be used in

the next step directly.

Preparation of 2-acylhydrazino-5-aryl-[1,3,4]-oxadiazoles (8)

Acyl chloride (10 mmol) was added dropwise to a mixture of compound **7** (10 mmol) and 10 mL dried pyridine. The solution was stirred for 0.5 h with an ice bath, and then stirred at room temperature for 4 h. After the solvent was distilled under reduced pressure, the residue was poured in 200 mL water. The precipitate was filtered, washed with water, and recrystallized from ethanol to give compound **8**.

8a Yield 90%; mp 198~200 °C. ^{1}H NMR (DMSO-d_6): δ 7.51~7.96 (m, 9H), 10.05 (s, 1H), 10.89 (s, 0.4H), 10.97 (s, 0.6H). MS: 358 (M^+, 16), 360 (16), 183 (39), 185 (36), 155 (12), 157 (11), 105 (100), 77 (40). IR: 3211, 3040, 1676, 1625. Anal. Calcd. for $C_{15}H_{11}N_4O_2Br$: C, 50.16; H, 3.09; N, 15.60; Found: C, 50.11; H, 3.09; N, 15.60.

8b Yield 92%; mp 156~159 °C. ^{1}H NMR (DMSO-d_6): δ 7.46~7.93 (m, 8H), 10.11 (s, 0.4H), 10.21 (s, 0.6H), 10.81 (s, 0.6H), 10.96 (s, 0.4H). MS: 348 (M^+, 13), 350 (7), 313 (2), 179 (4), 139 (100), 141 (33), 111 (29), 113 (9), 75 (16). IR: 3188, 3011, 1675, 1621. Anal. Calcd. for $C_{15}H_{10}N_4O_2Cl_2$: C, 51.60; H, 2.89; N, 16.05; Found: C, 51.55; H, 2.93; N, 15.96.

8c Yield 86%; mp 216~218 °C. ^{1}H NMR ($CDCl_3$): δ 7.45~7.96 (m, 9H), 10.10 (s, 0.6H), 10.20 (s, 0.4H), 10.83 (s, 0.6H), 10.98 (s, 0.4H). MS: 314 (M^+, 8), 316 (3), 139 (74), 141 (23), 111 (33), 113 (10), 105 (100), 77 (77), 75 (26). IR: 3210, 3028, 1675, 1602. Anal. Calcd. for $C_{15}H_{11}N_4O_2Cl$: C, 57.24; H, 3.52; N, 17.80; Found: C, 57.13; H, 3.52; N, 17.89.

8d Yield 89%; mp 155~157 °C. ^{1}H NMR (DMSO-d_6): δ 7.48~7.85 (m, 8H), 10.10 (s, 0.6H), 10.19 (s, 0.4H), 10.79 (s, 0.4H), 10.94 (s, 0.6H). MS: 392 (M^+, 2), 394 (3), 357 (1), 359 (1), 196 (3), 198 (3), 183 (23), 185 (19), 139 (100), 141 (28), 111 (19), 75 (18). IR: 3184, 3010, 1675, 1621. Anal. Calcd. for $C_{15}H_{10}N_4O_2BrCl$: C, 45.77; H, 2.56; N, 14.23; Found: C, 45.58; H, 2.50; N, 14.15.

8e Yield 90%; mp 148~150 °C. ^{1}H NMR (DMSO-d_6): δ 7.44~7.97 (m, 9H), 10.07 (s, 0.3H), 10.14 (s, 0.7H), 10.79 (s, 0.7H), 10.86 (s, 0.3H). MS: 314 (M^+, 8), 316 (3), 279 (2), 139 (100), 141 (29), 111 (23), 105 (40), 77 (25). IR: 3186, 2975, 1701, 1618. Anal. Calcd. for $C_{15}H_{11}N_4O_2Cl$: C, 57.24; H, 3.52; N, 17.80; Found: C, 57.18; H, 3.57; N, 17.69.

Synthesis of 3,6-dialkyl-1,2,4-triazolo[3,4-*b*]1,3,4-oxadiazoles (9)

A mixture of 1 mmol compound **8** and 5 mL phosphorus oxychloride was heated to reflux for 5~10 min. The cooled reaction mixture was poured into 200 g crushed ice with hard stirring. The solid was filtered and washed with water then purified by column chromatography using PE-EA (4:1 v/v) as an eluant to furnish compound **9**.

9a Yield 71%; mp 162~164 °C. ^{1}H NMR (DMSO-d_6): δ 7.53~8.20 (m, 9H). MS: 340 (M^+, 26), 342 (28), 215 (37), 217 (37), 181 (27), 183 (31), 102 (31), 103 (32), 105 (14), 77 (32), 36 (100). IR: 1578, 1537, 1467, 1450, 1068, 1044. HRMS (M+H): 341.0031 (341.0033).

9b Yield 65%; mp 214~216 °C. ^{1}H NMR (DMSO-d_6): δ 7.54~8.19 (m, 8H). MS: 330 (M^+, 9), 332 (4), 137 (100), 139 (37), 102 (39), 75 (28). IR: 1583, 1548, 1472, 1426, 1088, 1017, 955, 823, 721. HRMS (M+H): 331.0150 (331.0148).

9c Yield 60%; mp 192~194 °C. ^{1}H NMR ($CDCl_3$): δ 7.52~8.28 (m, 9H). MS: 296 (M^+, 49), 298 (16), 137 (84), 139 (35), 103 (100), 76 (32). IR: 1582, 1541, 1480, 1456, 1090, 1046, 955, 838, 718. HRMS (M+H): 297.0536 (297.0538).

9d Yield 62%; mp 171~173 °C. ^{1}H NMR (DMSO-d_6): δ 7.41~8.29 (m, 8H). MS: 374 (M^+, 14), 376 (18), 183 (81), 181 (84), 137 (44), 139 (25), 102 (100), 75 (43). IR: 1585, 1545, 1481, 1458, 1071, 1034, 944, 829, 731. HRMS (M+H): 374.9638 (374.9643).

9e Yield 58%; mp 165~167 °C. ^{1}H NMR ($CDCl_3$): δ 7.48~8.00 (m, 9H). MS: 296 (M^+, 35), 298 (11), 261 (4), 137 (100), 139 (75), 103 (98), 76 (33). IR: 1548, 1539, 1468, 1443, 1061, 1030, 946, 759, 684. HRMS (M+H): 297.0533 (297.0538).

Bioactivity Test

Cdc25 Inhibition Assay

cdc25s phosphate can resolve the phosphate of fluorescent substrate OMFP. Excitation wavelength was 485 nm; emission was monitored at 530 nm. The reaction time was in proportion to the enhancement of absorbance. Then reaction beginning speed of enzyme can be tested by the slope of absorbance enhancement line.[18]

eMetAP Activity Assay

eMetAP can hydrolysis the thiopeptolide bond of the Met-S-C-Phe substrate. The product of Met-SH reacts quickly with excessive DTNB; the 3-hydroxy-4-nitro-thiophenolate that was generated has an absorbance at 412 nm. We can determine the activity of enzyme by examining the absorbance change.[19]

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