ACS Combinatorial Science

ARTICLE

pubs.acs.org/acscombsci

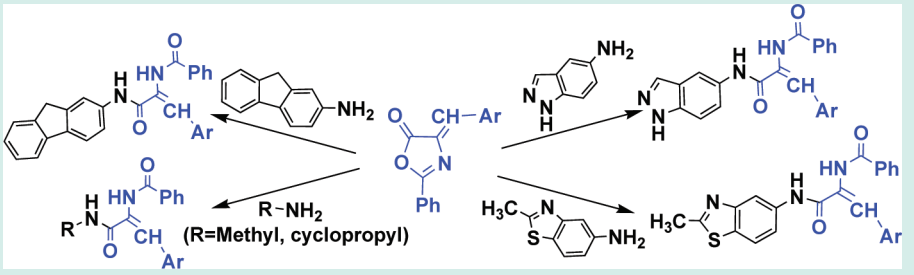
Facile Diversity-Oriented Synthesis of Novel Dipeptide Mimetic Compounds Containing Bioactive Molecular Skeletons under Microwave Irradiation

Feng Shi,[†,‡] An-Xiao Dai,[‡] Xiao-Hong Zhang,[‡] Bo Jiang,[‡] and Shu-Jiang Tu*[,†,‡]

[†]College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215000, P.R. China

[‡]School of Chemistry and Chemical Engineering, Xuzhou Normal University, Xuzhou 221116, P.R. China

Supporting Information



ABSTRACT: The diversity-oriented synthesis of dipeptide mimetic compounds embedded with bioactive molecular skeletons have been successfully established via microwave-assisted reactions between various 4-arylidene-2-phenyloxazol-5(4*H*)-ones and a broad scope of amines including aliphatic, aromatic, and heteroaromatic ones. This synthetic approach has prominent features of short reaction time, high yields, operational simplicity, as well as widespread applications, leading to a facile and straightforward access to structurally diverse dipeptide analogues with potential bioactivities. Moreover, the preliminary evaluation on the cytotoxic activity of this type of dipeptide derivatives has resulted in the finding of five compounds with stronger cytotoxicity than doxorubicin hydrochloride at the concentration of 10 μg/mL.

KEYWORDS: diversity-oriented synthesis, dipeptide mimetic compounds, bioactive molecular skeletons, microwave-assisted reactions

INTRODUCTION

Peptides play crucial roles in the human body and other organisms.[1] Among them, dipeptides and their analogues have exhibited a wide spectrum of important bioactivities such as antibacterial,[2] antitumor,[3] anticarcinogenic,[4] neuroprotective,[5] antidiabetic,[6] antiplatelet and anticoagulative[7] activities. Because of the good affinity of peptides toward cells and nucleic acids, the introduction of a peptide segment to drugs can facilitate their actions to cells and tissues and thereby provide a robust strategy to design new drugs or lead compounds. On the other hand, heterocyclic compounds have distinguished themselves from other small molecules because of their profound bioactivities. The practice of attaching heterocyclic skeletons and dipeptides into one molecule has received much attention from synthetic and medicinal chemists for the discovery of novel compounds with unknown or improved pharmacological properties.[8,4b] However, the scopes of bioactive small molecules, especially heterocyclic molecules used for incorporation with dipeptide segments are rather limited. As a result, it is a formidable and urgent task to synthesize a new class of dipeptide mimetic compounds containing bioactive skeletons.

Indazole derivatives, an important family of heterocycles,[9] show diverse pharmacological properties as exemplified by antimicrobial,[10] anti-inflammatory,[11] and cytostatic[12] activities. Additionally, benzothiazole derivatives have received considerable attention over the past decades because of their wide range of bioactivity profiles including cytotoxic,[13] antitumor,[14] anticancer,[15,16a] antimicrobial,[16] immunosuppressive and antiviral properties.[17] Moreover, fluorene derivatives have not only been used as antitumor,[18] anticancer,[19] and antiviral agents,[20] but have also been proven to be apoptosis inducers[21] and insulin secretagogues.[22]

Considering the versatile bioactivities of the above-mentioned structures, we hypothesize that the integration of indazole, benzothiazole, or fluorene scaffold with a dipeptide segment

Received: October 25, 2010
Revised: November 25, 2010
Published: December 17, 2010

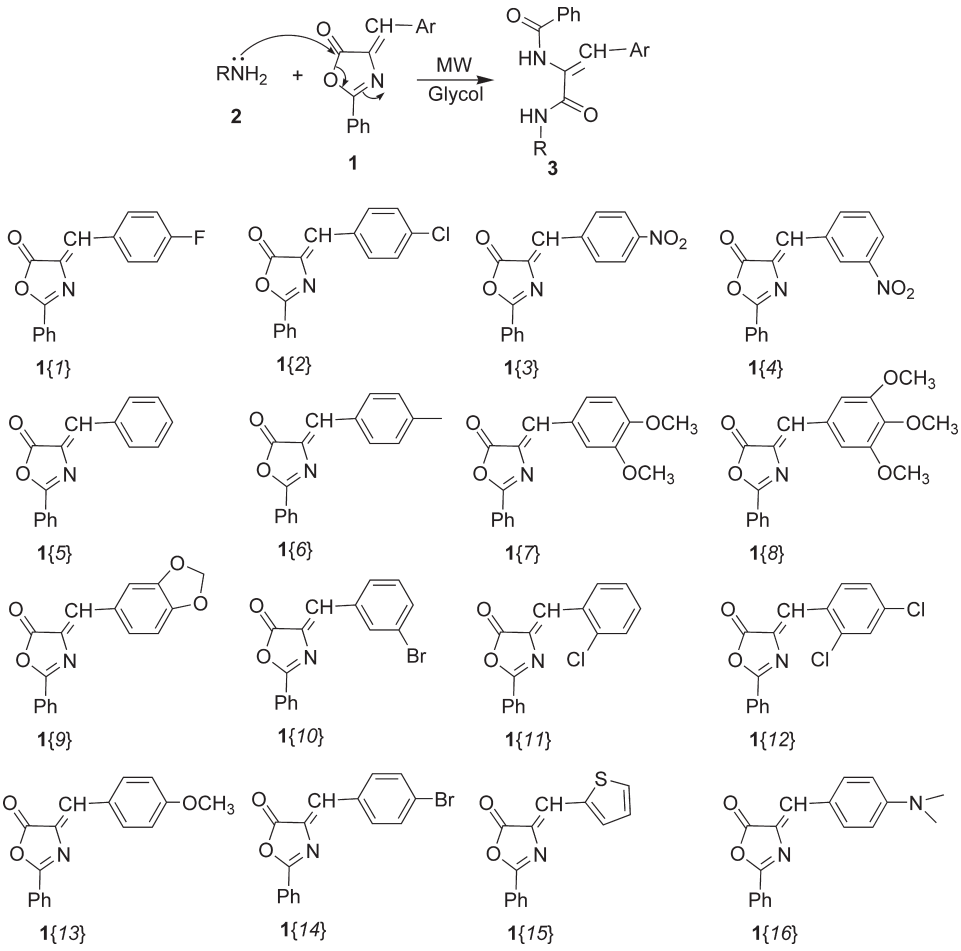


Figure 1. Diversity of 4-arylidene-2-phenyloxazol-5(4*H*)-ones **1**{*1*−*16*}.

may result in the discovery of new drug candidates with unknown bioactivities. However, the design of dipeptide mimetic compounds implanted with indazole, benzothiazole, and fluorene frameworks for medicinal purpose has been less recognized, and only very few reports describe the synthesis of related compounds.[23] Therefore, the development of a facile approach to access these novel targets with structural diversity is highly desirable and valuable for medicinal chemistry and drug discovery.

In view of the prominent merits of diversity-oriented synthesis (DOS)[24] and microwave-assisted reactions (MW),[25] we have a great interest in the synthesis of small molecules with potential bioactivities under microwave irradiation.[26] As a continuous effort, we report a facile diversity-oriented synthesis of dipeptide mimetic compounds **3** embedded with bioactive structural motifs via microwave-assisted reactions between 4-arylidene-2-phenyloxazol-5(4*H*)-ones **1** (Figure 1) and various amines **2** to provide a library of novel peptide mimetic molecules (Scheme 1).

RESULTS AND DISCUSSION

An appropriate reaction media is of crucial importance in the successful microwave-promoted organic transformations. Thus, the initial attempt to perform a microwave-assisted condensation reaction of 4-(4-chlorobenzylidene)-2-phenyloxazol-5(4*H*)-one **1**{*2*} with 1*H*-indazol-5-amine **2**{*1*} was focused on the optimization of the reaction conditions (Table 1). Screening of the solvents for the synthesis of **3**{*2,1*} revealed that glycol turned out to be an appropriate media, which provided not only a faster reaction time, but also a higher yield than other solvents examined (Entries 1−5). Interestingly, the use of either acetic acid or water as the reaction media produced only a trace amount of the desired products, probably because acetic acid deactivated the nucleophilicity of the amine by protonation and water might undergo hydrolysis with **1**{*2*}. The examination of the reaction temperature led to a regulation that the reaction of **1**{*2*} and **2**{*1*} proceeded more smoothly at an elevated temperature because the reaction became much faster and cleaner after increasing the temperature from 90 to 120 °C (Entries 5−8). However, no improvement in the yield was realized when the temperature was further increased to 130 °C (Entry 9). Thus, the most suitable reaction temperature was assigned to be 120 °C.

With the optimal reaction conditions in hand, the generality of the protocol was explored (Table 2). A variety of structurally diverse 4-arylidene-2-phenyloxazol-5(4*H*)-ones **1** bearing different substituents, including electron-withdrawing and electron-donating groups on the aromatic ring, were reacted with 1*H*-indazol-5-amine **2**{*1*} to furnish *N*-(1-(1*H*-indazol-5-ylcarbamoyl)-2-arylvinyl)benzamide **3**{*n,1*}, a novel class of dipeptide mimetic compounds containing the indazole moiety. As illustrated in Table 2 (Entries 1−9), substrates **1** with either electronically poor or rich aryl substituents were well tolerated and participated in the clean reactions within 10−18 min, giving rise to the desired dipeptide derivatives **3**{*n,1*} in high yields ranging from 70% to 83%.

dx.doi.org/10.1021/co1000458 |*ACS Comb. Sci.* 2011, 13, 147–153

Scheme 1. Synthesis of Dipeptide Mimetic Compounds 3

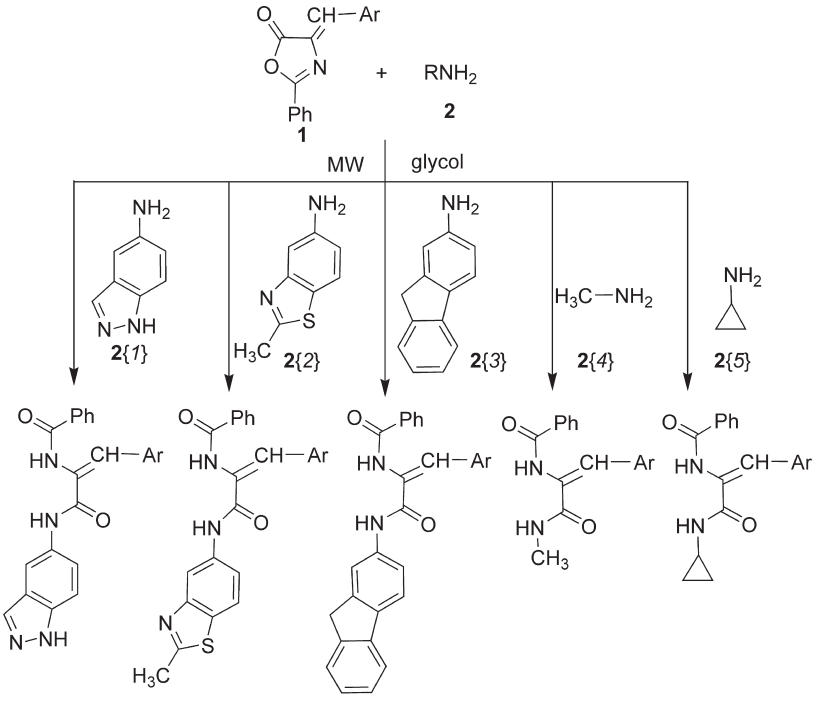


Table 1. Optimization of Reaction Conditions for the Synthesis of 3{2,1}[a]

Entry	Solvent	*T*/°C	Time/min	Yield[b]/%
1	EtOH	110	20	38
2	HOAc	110	30	trace
3	water	110	30	trace
4	DMF	110	18	55
5	glycol	110	15	73
6	glycol	90	20	48
7	glycol	100	20	62
8	glycol	120	12	81
9	glycol	130	12	80

[a] Unless otherwise specified, all the reactions were carried out in the presence of 1 mmol of **1**{*2*}, 1 mmol of **2**{*1*} in 2 mL of solvent under MW at the maximum power of 350 W. [b] Isolated yields.

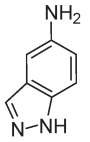
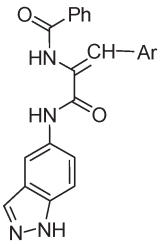
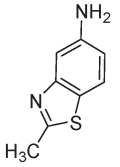
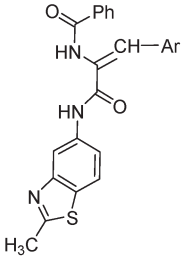
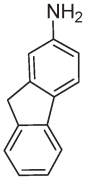
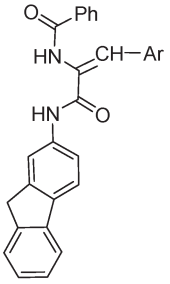
Moreover, 2-methylbenzo[*d*]thiazol-5-amine **2**{*2*} and 9*H*-fluoren-2-amine **2**{*3*} were employed to react with a variety of substrates **1** bearing either an electronically poor or rich aromatic ring for the synthesis of another two series of dipeptide derivatives containing thiazole and fluorine motifs. Delightfully, the two series of *N*-(1-(2-methylbenzo[*d*]thiazol-5-ylcarbamoyl)-2-arylvinyl)benzamide **3**{*n,2*} (Table 2, entries 10−17) and *N*-(1-(9*H*-fluoren-7-ylcarbamoyl)-2-arylvinyl)benzamide **3**{*n,3*} (Table 2, entries 18−28) were readily generated under the optimized conditions. Notably, in all these cases, the reactions proceeded smoothly to give the corresponding dipeptide derivatives **3**{*n,2*} and **3**{*n,3*} in good yields of 68−81% within 12−18 min. As such, this protocol enables a library of novel dipeptide mimetic compounds containing indazole, benzothiazole, and fluorene frameworks to be easily prepared in a structurally diverse manner.

To further examine the scope of the microwave-assisted condensation reaction, and to obtain more structurally diverse dipeptide mimetic compounds, two aliphatic amines including methanamine **2**{*4*} and cyclopropanamine **2**{*5*} were employed to react with representative substrates of type **1** (Table 3). As we anticipated, a number of *N*-(1-(methylcarbamoyl)-2-arylvinyl)-benzamide **3**{*n,4*} and *N*-(1-(cyclopropylcarbamoyl)-2-arylvinyl)-benzamide **3**{*n,5*} were successfully prepared under the similar reaction conditions in high yields of 65−71% within 10−18 min. These results, together with those presented in Table 2, lead to a conclusion that a wide scope of substrates could be accommodated in this reaction. In comparison with the similar reaction types reported previously,[8,27] which were conducted either under traditional heating conditions or with limited amine substrates, thereby suffered from narrow substrate scope, long reaction time, and unsatisfactory conversion in some cases, this approach offers a global and efficient shortcut to dipeptide analogues containing small molecular skeletons accompanied by the prominent advantages of a wider substrate scope, shorter reaction time, and higher yields.

To survey the possible bioactivity of these novel dipeptide mimetic analogues, a few of the synthesized compounds **3** containing indazole, benzothiazole, and fluorene skeletons were subjected to the preliminary evaluation on their in vitro cytotoxic activity, which was represented as the inhibition rate of the tested compounds to colon carcinoma cell line SW1116. As illustrated in Table 4, all the tested compounds at the concentration of 10 μg/mL exhibited significant or moderate cytotoxicity to SW1116 cells with an inhibition rate varing from 6.8% to 52.8%. Notably, five of the tested compounds which include **3**{*6,1*}, **3**{*3,3*}, **3**{*11,3*}, **3**{*5,3*}, and **3**{*13,3*} (Entries 4, 14−15 and 17−18) showed higher inhibition rates than doxorubicin hydrochloride (Entry 22), a powerful anticancer drug that was utilized as a positive control.

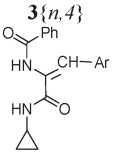
dx.doi.org/10.1021/co1000458 |*ACS Comb. Sci.* 2011, 13, 147–153

Table 2. DOS of Dipeptide Mimetic Compounds 3 from Amine 2{*1-3*}[a]

Entry	2	Product 3		Ar=	Time /min	Yield[b]/%
			3{*1,1*}	4-FC_6H_4	12	77
2			**3**{2,1}	4-ClC_6H_4	12	81
3			**3**{*3,1*}	4-$NO_2C_6H_4$	10	83
4			**3**{*4,1*}	3-$NO_2C_6H_4$	12	75
5			**3**{*5,1*}	C_6H_5	12	73
6			**3**{*6,1*}	4-$CH_3C_6H_4$	12	74
7	**2**{*1*}		**3**{*7,1*}	3,4-$(CH_3O)_2C_6H_3$	15	71
8		**3**{*n,1*}	**3**{*8,1*}	3,4,5-$(CH_3O)_3C_6H_2$	18	70
9			**3**{*9,1*}	Benzo[*d*][1,3]dioxol-5-yl	15	74
10			**3**{*2,2*}	4-ClC_6H_4	12	80
11			**3**{*3,2*}	4-$NO_2C_6H_4$	12	81
12			**3**{*10,2*}	3-BrC_6H_4	12	73
13			**3**{*11,2*}	2-ClC_6H_4	15	72
14			**3**{*12,2*}	2,4-$Cl_2C_6H_3$	12	73
15			**3**{*6,2*}	4-$CH_3C_6H_4$	15	70
16	**2**{*2*}		**3**{*13,2*}	4-$CH_3OC_6H_4$	15	74
17		**3**{*n,2*}	**3**{*8,2*}	3,4,5-$(CH_3O)_3C_6H_2$	15	72
18			**3**{*2,3*}	4-ClC_6H_4	12	78
19			**3**{*14,3*}	4-BrC_6H_4	12	80
20			**3**{*3,3*}	4-$NO_2C_6H_4$	12	77
21			**3**{*11,3*}	2-ClC_6H_4	15	73
22			**3**{*12,3*}	2,4-$Cl_2C_6H_3$	15	71
23			**3**{*5,3*}	C_6H_5	15	74
24			**3**{*6,3*}	4-$CH_3C_6H_4$	15	70
25	**2**{*3*}		**3**{*13,3*}	4-$CH_3OC_6H_4$	15	71
26			**3**{*7,3*}	3,4-$(CH_3O)_2C_6H_3$	18	72
27		**3**{*n,3*}	**3**{*9,3*}	Benzo[*d*][1,3]dioxol-5-yl	15	70
28			**3**{*15,3*}	Thiophen-2-yl	18	68

[a] Unless otherwise specified, all the reactions were carried out in the presence of 1 mmol of **1**, 1 mmol of **2**{*1*−*3*} in 2 mL of glycol under MW at 120 °C with the maximum power of 350 W. [b] Isolated yields.

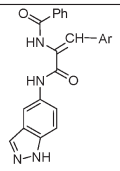
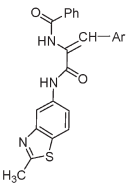
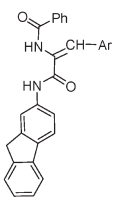
Table 3. DOS of Dipeptide Mimetic Compounds 3 from Amine 2{*4-5*}[a]

Entry	2	Product 3		Ar=	Time / min	Yield[b]/%
1			**3**{*2,4*}	4-ClC_6H_4	12	69
2	H_3C-NH_2		**3**{*12,4*}	2,4-$Cl_2C_6H_3$	15	67
3	**2**{*4*}		**3**{*9,4*}	Benzo[*d*][1,3]dioxol-5-yl	18	65
4		**3**{*n,4*}	**3**{*16,4*}	4-$(CH_3)_2NC_6H_4$	15	66
5			**3**{*2,5*}	4-ClC_6H_4	10	68
6			**3**{*14,5*}	4-BrC_6H_4	12	70
7			**3**{*5,5*}	C_6H_5	15	69
8	**2**{*5*}		**3**{*16,5*}	4-$(CH_3)_2NC_6H_4$	15	71
		3{*n,5*}				

[a] Unless otherwise specified, all the reactions were carried out in the presence of 1 mmol of **1**, 1 mmol of **2**{*4*} or **2**{*5*} in 2 mL of glycol under MW at 120 °C with the maximum power of 350 W. [b] Isolated yields.

dx.doi.org/10.1021/co1000458 |*ACS Comb. Sci.* 2011, 13, 147–153

Table 4. Preliminary Evaluation on the Cytotoxicity of the Selected Compounds 3[a]

Entry	Compounds[b]		Ar=	Inhibition rate /%
1		**3**{*1,1*}	4-FC_6H_4	19.1±2.2
2		**3**{*3,1*}	4-$NO_2C_6H_4$	32.6±2.3
3		**3**{*4,1*}	3-$NO_2C_6H_4$	34.1±2.0
4		**3**{*6,1*}	4-$CH_3C_6H_4$	**40.1**±3.3
5	**3**{*n,1*}	**3**{*8,1*}	3,4,5-$(CH_3O)_3C_6H_2$	24.3±1.3
6		**3**{*2,2*}	4-ClC_6H_4	12.5±1.2
7		**3**{*10,2*}	3-BrC_6H_4	9.9±0.5
8		**3**{*11,2*}	2-ClC_6H_4	11.1±1.0
9		**3**{*6,2*}	4-$CH_3C_6H_4$	12.9±1.2
10		**3**{*13,2*}	4-$CH_3OC_6H_4$	6.8±0.5
11	**3**{*n,2*}	**3**{*8,2*}	3,4,5-$(CH_3O)_3C_6H_2$	23.5±1.6
12		**3**{*2,3*}	4-ClC_6H_4	19.3±0.5
13		**3**{*14,3*}	4-BrC_6H_4	24.3±2.7
14		**3**{*3,3*}	4-$NO_2C_6H_4$	**52.8**±1.1
15		**3**{*11,3*}	2-ClC_6H_4	**37.3**±3.5
16		**3**{*12,3*}	2,4-$Cl_2C_6H_3$	34.2±2.8
17		**3**{*5,3*}	C_6H_5	**43.1**±1.7
18		**3**{*13,3*}	4-$CH_3OC_6H_4$	**52.1**±0.9
19	**3**{*n,3*}	**3**{*7,3*}	3,4-$(CH_3O)_2C_6H_3$	28.2±3.1
20		**3**{*9,3*}	Benzo[*d*][1,3]dioxol-5-yl	25.1±1.5
21		**3**{*15,3*}	Thiophen-2-yl	33.6±2.3
22	Doxorubicin hydrochloride[c]			37.0±1.3

[a] The cytotoxicity of the tested compounds was represented as inhibition rate (mean ± S.D., n = 3) on SW1116 cells. [b] The concentration of the tested compounds is 10 μg/mL. [c] Doxorubicin hydrochloride was used as a positive control.

To further understand the dose-activity relationships, nine selected active compounds **3** were evaluated on their cytotoxicity toward SW1116 cells at three different concentrations of 0.1, 1, and 10 μg/mL (Table 5). The results suggested that nearly all the selected compounds in the three concentrations inhibited the proliferation of SW1116 cells. Most of the selected compounds at the concentration of 10 μg/mL had the highest cytotoxic activity for SW1116 cells. Among them, compounds **3**{*4,1*}, **3**{*6,1*}, **3**{*5,3*}, **3**{*13,3*}, and **3**{*15,3*} exhibited a tendency of dose-dependent cytotoxic activity since their inhibition rates went up with the increase of dosage. On the contrary, the inhibition rates of compounds **3**{*3,3*} and **3**{*12,3*} went down as the concentration was increased from 0.1 to 10 μg/mL. With regard to the positive control, doxorubicin hydrochloride, it displayed a positive dose-dependent cytotoxicity toward SW1116 cells. It is surprising that most of the selective active compounds, except **3**{*6,1*} and **3**{*15,3*}, exhibited a much stronger inhibitory effect on the growth of SW1116 cells than the positive control at the same concentration of 0.1 μg/mL. It is encouraging that five of the selected active compounds showed more remarkable cytotoxicity for SW1116 cells than doxorubicin hydrochloride at the same concentration of 1 μg/mL. Therefore, these results indicate

Table 5. Dose-Activity Relationships for Selected Active Compounds 3 on Their Cytotoxicity[a]

		Inhibition rate /%		
Entry	Compounds	0.1 μg/mL	1 μg/mL	10 μg/mL
1	**3**{*3,1*}	**19.1** ± 2.4	3.3 ± 2.1	32.6 ± 2.3
2	**3**{*4,1*}	**7.3** ± 0.9	8.8 ± 1.3	34.1 ± 2.0
3	**3**{*6,1*}	0.1 ± 0.0	4.0 ± 0.8	**40.1** ± 3.3
4	**3**{*3,3*}	**57.2** ± 3.1	**49.7** ± 0.7	**52.8** ± 1.1
5	**3**{*11,3*}	**47.5** ± 4.2	**48.1** ± 4.5	**37.3** ± 3.5
6	**3**{*12,3*}	**46.5** ± 3.2	**44.0** ± 4.0	34.2 ± 2.8
7	**3**{*5,3*}	**16.5** ± 3.0	**31.5** ± 2.9	**43.1** ± 1.7
8	**3**{*13,3*}	**21.5** ± 0.5	**35.4** ± 3.6	**52.1** ± 0.9
9	**3**{*15,3*}	3.0 ± 0.8	10.1 ± 1.9	33.6 ± 2.3
10	doxorubicin hydrochloride[b]	5.6 ± 2.7	13.2 ± 2.7	37.0 ± 1.3

[a] The cytotoxicity of the selected active compounds was represented as inhibition rate (mean ± S.D., n = 3) on SW1116 cells. [b] Doxorubicin hydrochloride was used as a positive control.

that these novel dipeptide derivatives, with potent cytotoxicity, might become promising anticancer drug candidates after further investigations on the mechanism of their pharmacological actions.

CONCLUSION

In summary, we have accomplished a diversity-oriented synthesis of dipeptide mimetic compounds **3** embedded with bioactive moieties of small molecules via microwave-assisted reactions between various 4-arylidene-2-phenyloxazol-5(4*H*)-ones **1**, bearing either electronically poor or rich aryl substituents, and a wide range of amines **2** including aliphatic, aromatic, and heteroaromatic ones. This synthetic approach has the prominent features of high efficiency, good yields, operational simplicity, as well as broad scope of substrate tolerance, leading to a facile and straightforward access to dipeptide analogues with potential bioactivities. Moreover, the preliminary evaluation on the cytotoxic activity of this type of dipeptide derivatives **3** has resulted in the finding of five compounds with stronger cytotoxicity than doxorubicin hydrochloride at the concentration of 10 μg/mL. Therefore, this work not only provides plenty of novel dipeptide mimetic compounds with structural diversity for further bioassay, but also enriches the research contents of the related fields.

EXPERIMENTAL SECTION

General Procedure for the Synthesis of Compounds 3. In a 10 mL Emrys reaction vial, a 4-arylmethylene-2-phenyloxazol-5(4*H*)-one (**1**, 1 mmol), amine (**2**{*1*−*5*}, 1 mmol), and ethylene glycol (2 mL) were mixed, and then the vial was capped. The mixture was heated for a given time at 120 °C under microwave irradiation (initial power 150 W and maximum power 350 W). Upon completion, monitored by TLC, the reaction mixture was cooled to room temperature and poured into cold water. The solid product was collected by Büchner filtration and purified by flash column chromatography (silica gel, mixtures of petroleum ether/acetic ester, 5:1, v/v) to afford the desired pure dipeptide mimetic compounds **3**.

Selected Examples . *N-(1-(1H-indazol-5-ylcarbamoyl)-2-(3,4-dimethoxyphenyl)vinyl)benzamide* (**3**{*7,1*}). White solid, mp: 225−229 °C. IR (KBr, ν, cm^{-1}): 3165, 3047, 3012, 2945, 2877, 1687, 1636, 1620, 1516, 1399, 1270, 1217, 1135, 1033, 844, 817, 767, 745, 697. ^{1}H NMR (400 MHz, DMSO-d_6): 12.99 (1H, s, NH), 10.06 (2H, s, 2NH),

dx.doi.org/10.1021/co1000458 |*ACS Comb. Sci.* 2011, 13, 147–153

8.18 (1H, s, -N=CH), 8.09 (2H, d, J = 7.6 Hz, ArH), 8.04 (1H, s, ArH), 7.60 (2H, d, J = 8.0 Hz, ArH), 7.55−7.47 (3H, m, ArH), 7.31 (1H, s, =CH), 7.24−7.20 (2H, m, ArH), 6.99 (1H, d, J = 8.4 Hz, ArH), 3.76 (3H, s, -OCH_3), 3.55 (3H, s, -OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 165.9, 164.3, 149.4, 148.3, 146.4, 137.0, 133.6, 133.4, 132.2, 131.7, 129.3, 128.7, 128.3, 127.9, 126.9 123.5, 122.7, 121.5, 112.5, 111.6, 111.0, 109.8, 55.6, 55.1. HRMS (ESI): m/z calcd for $C_{25}H_{23}N_4O_4$: 443.1714 $[M+H]^+$; found: 443.1731 $[M+H]^+$.

*N-(1-(2-methylbenzo[d]thiazol-5-ylcarbamoyl)-2-p-tolylvinyl)-benzamide (**3**{6,2}).* Pale yellow solid, mp: 255−258 °C. IR (KBr, ν, cm^{-1}): 3230, 3067, 1646, 1576, 1520, 1466, 1323, 1294, 1256, 1183, 804, 696. ^{1}H NMR (400 MHz, DMSO-d_6): 10.38 (1H, s, NH), 10.13 (1H, s, NH), 8.39 (1H, s, ArH), 8.04 (2H, d, J = 7.6 Hz, ArH), 7.95 (1H, d, J = 8.4 Hz, ArH), 7.73 (1H, d, J = 8.4 Hz, ArH), 7.62−7.51 (5H, m, ArH), 7.21 (2H, d, J = 7.6 Hz, ArH), 7.17 (1H, s, =CH), 2.79 (3H, s, $-CH_3$), 2.31 (3H, s, $-CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 167.7, 164.6. 153.4, 138.4, 137.8, 136.4, 133.6, 133.5, 133.1, 131.7, 131.4, 130.1, 129.6, 129.5, 129.1, 128.7, 128.3, 127.9, 121.5, 118.1, 112.9, 112.7, 20.9, 19.8. HRMS (ESI): m/z calcd for $C_{25}H_{22}N_3O_2S$: 428.1428 $[M+H]^+$; found: 428.1442 $[M+H]^+$.

*N-(1-(9H-fluoren-7-ylcarbamoyl)-2-(4-nitrophenyl)vinyl)benzamide (**3**{3,3}).* Yellow solid, mp: 285−287 °C. IR (KBr, ν, cm^{-1}): 3257, 3107, 2944, 1637, 1616, 1547, 1519, 1466, 1343, 1317, 1256, 865, 802, 768, 736, 712. ^{1}H NMR (400 MHz, DMSO-d_6): 10.47 (1H, s, NH), 10.34 (1H, s, NH), 8.27 (2H, d, J = 8.8 Hz, ArH), 8.07 (1H, s, ArH), 8.03 (2H, d, J = 7.6 Hz, ArH), 7.89 (2H, d, J = 8.8 Hz, ArH), 7.85 (2H, t, J = 7.6 Hz, ArH), 7.73 (1H, d, J = 7.6 Hz, ArH), 7.65−7.52 (4H, m, ArH), 7.37 (1H, t, J = 7.6 Hz, ArH), 7.28 (1H, t, J = 7.6 Hz, ArH), 7.18 (1H, s, =CH), 3.93 (2H, s, $-CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6): 166.0, 163.9, 146.5, 143.6, 142.9, 141.6, 141.0, 138.2, 136.6, 134.6, 133.2, 132.0, 130.3, 128.4, 128.0, 126.7, 126.2, 125.0, 124.5, 123.7, 120.0, 119.5, 118.8, 116.7, 36.5. HRMS (ESI): m/z calcd for $C_{29}H_{22}N_3O_4$: 476.1605 $[M+H]^+$; found: 476.1606 $[M+H]^+$.

*N-(1-(methylcarbamoyl)-2-(2,4-dichlorophenyl)vinyl)benzamide (**3**{12,4}).* Pale yellow solid, mp: 218−219 °C. IR (KBr, ν, cm^{-1}): 3313, 3238, 3067, 1637, 1551, 1483, 1279, 868, 788, 693. ^{1}H NMR (400 MHz, DMSO-d_6): 9.91 (1H, s, NH), 8.24 (1H, d, J = 4.4 Hz, NH), 7.91 (2H, d, J = 7.6 Hz, ArH), 7.70 (1H, s, ArH), 7.57−7.47 (4H, m, ArH), 7.37 (1H, d, J = 8.4 Hz, ArH), 7.16 (1H, s, =CH), 2.70 (3H, d, J = 4.4 Hz, $-CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 164.8, 134.0, 133.5, 133.3, 132.1, 131.7, 130.8, 130.6, 128.9, 128.2, 127.8, 127.3, 112.7, 26.3. HRMS (ESI): m/z calcd for $C_{17}H_{14}Cl_2N_2O_2Na$: 371.0325 $[M+Na]^+$; found: 371.0326 $[M+Na]^+$.

*N-(1-(cyclopropylcarbamoyl)-2-(4-chlorophenyl)vinyl)benzamide (**3**{2,5}).* White solid, mp: 211−212 °C. IR (KBr, ν, cm^{-1}): 3231, 2998, 1669, 1644, 1524, 1486, 1311, 887, 810, 714. ^{1}H NMR (400 MHz, DMSO-d_6): 9.87 (1H, s, NH), 8.23 (1H, d, J = 4.0 Hz, NH), 7.97 (2H, d, J = 7.6 Hz, ArH), 7.59−7.48 (5H, m, ArH), 7.41 (2H, d, J = 8.8 Hz, ArH), 7.03 (1H, s, =CH), 2.78−2.73 (1H, m, −CH), 0.68−0.63 (2H, m, $-CH_2$), 0.54−0.51 (2H, m, $-CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6): 166.3, 133.6, 133.4, 132.7, 131.6, 131.1, 130.8, 128.4, 128.3, 127.9, 126.4, 112.7, 23.0, 5.7. HRMS (ESI): m/z calcd for $C_{19}H_{17}ClN_2O_2Na$: 363.0871 $[M+Na]^+$; found: 363.0880 $[M+Na]^+$.

Cytotoxic Assay to Colon Carcinoma Cell Line SW1116. SW1116 cell was maintained in RMPI 1640 (HyClone) with 10% fetal bovine serum (FBS), 50 μg/mL streptomycin, and 50 μg/mL of penicillin in a humidified atmosphere of 5% CO_2 at 37 °C. The cytotoxicity of the tested compounds **3** to SW1116 cells was assayed using the MTT (methyl thiazolyl tetrazolium) method. Briefly, SW1116 cells were collected and seeded in 96-well plates at a density of 4.5 × 10^5 cells/mL. After incubation in a humidified atmosphere of 5% CO_2 at 37 °C for 24 h, cells were exposed to fresh 1640 medium containing tested compounds in the concentration of 10 μg/mL or three different concentrations of 0.1, 1, and 10 μg/mL at 37 °C and allowed to culture for another 24 h. The fresh 1640 medium containing no tested compounds was used as the blank control. An aliquot of 20 μL of MTT tetrazolium salt dissolved in Hank's balanced salt solution at a final concentration of 5 mg/mL was added to each well and incubated in the CO_2 incubator for 4 h. Finally, the medium was aspirated from each well and 150 μL of DMSO was added to dissolve the formazan crystals. The absorbance of each well was obtained using a Dynatech MR5000 plate counter at a wavelength of 570 nm. The inhibition rate was calculated according to the following formula: Inhibition rate = $(OD_{570\ \text{blank control}} - OD_{570\ \text{tested compounds}}) / OD_{570\ \text{blank control}} \times 100\%$, where OD stands for optical density at 570 nm.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, characterization data, and ^{1}H and ^{13}C NMR spectra of compounds **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 0086-516-83500065. Fax: 0086-516-83500065. E-mail: laotu@xznu.edu.cn.

Funding Sources

We are grateful for the financial support from the Natural Science Foundation of China (No. 21002083 and 21072163), the Natural Science Foundation (09KJB150011) and Qing Lan Project (08QL001) of Jiangsu Education Committee, and the Interdisciplinary Key Item of Xuzhou Normal University (09XKXK01).

■ REFERENCES

(1) (a) Tian, F.-F.; Zhou, P.; Li, Z.-L. T-scale as A Novel Vector of Topological Descriptors for Amino Acids and Its Application in QSARs of Peptides. *J. Mol. Struct.* **2007,** *830*, 106–115. (b) Shu, M.; Mei, H.; Yang, S.; Liao, L.; Li, Z. Structural Parameter Characterization and Bioactivity Simulation Based on Peptide Sequence. *QSAR Comb. Sci.* **2009,** *28*, 27–35. (c) Day, T.; Greenfield, S. A. Bioactivity of A Peptide Derived from Acetylcholinesterase in Hippocampal Organotypic Cultures. *Exp. Brain Res.* **2004,** *155*, 500–508.

(2) Strom, K.; Sjogren, J.; Broberg, A.; Schnurer, J. Lactobacillus Plantarum MiLAB 393 Produces the Antifungal Cyclic Dipeptides Cyclo (L-Phe-L-Pro) and Cyclo (L-Phe-trans-4-OH-L-Pro) and 3-Phenyllactic acid. *Appl. Environ. Microbiol.* **2002,** *68*, 4322–4327.

(3) Tan, C.; Zhang, X.; Jiang, Y.; Zhang, D.; Chen, J. Antitumor Activity of Vilon Dipeptide Lys2Glu. *Chin. Pharmacol. Bull.* **2007,** *23*, 233–236.

(4) (a) Cui, C. B.; Kakeya, H.; Okada, G.; Onose, R.; Ubukata, M.; Takahashi, I.; Isono, K.; Osada, H. Tryprostatins A and B, Novel Mammalian Cell Cycle Inhibitors Produced by Aspergillus Fumigatus. *Antibiotics* **1995,** *48*, 1382–1384. (b) Cui, C. B.; Kakeya, H.; Osada, H. Novel Mammalian Cell Cycle Inhibitors, Cyclotroprostatins A-D, Produced by Aspergillus Fumigatus, Which Inhibit Mammalian Cell cCycle at G2/ M Phase. *Tetrahedron* **1997,** *53*, 59–72.

(5) (a) Faden, A. I.; Movsesyan, V. A.; Knoblach, S. M.; Ahmed, F.; Cernak, I. Neuroprotective Effects of Novel Small Peptides in vitro and after Brain Injury. *Neuropharmacology* **2005,** *49*, 410–424. (b) Hlinak, Z.; Vinsova, J.; Kasafirek, E. Effect of Alaptide, Its An Alogues and Oxiracetam on Memory for An Elevated Plusmaze in Mice. *Eur. J. Pharmacol.* **1996,** *314*, 1–7.

(6) Tang, X.; Fan, L.; Yu, H.; Liao, Y.; Yang, D. Facile Synthesis of Dipeptidomimetics of *p*-Aminobenzoic Acid and Their Antidiabetic Activity. *Chin. J. Org. Chem.* **2009,** *29*, 595–600.

(7) Zhou, Z.-J.; Ye, H.-Y.; Wu, S.-Z.; Meng, S.-R. Experimental Study of L2Arg2Glups Activity on Antiplatelet and Anticoagulation in Rats and Its Mechanism. *Chin. Pharmacol. Bull.* **2000,** *16*, 556–559.

(8) (a) Barros, T. G.; Pinheiro, S.; Williamson, J. S.; Tanuri, A.; Pereira, H. S.; Brindeiro, R. M.; Neto, J. B. A.; Antunes, O. A. C.; Muri, E. M. F. Novel Peptide Mimetic Inhibitors of Hepatitis C Serine Protease Derived from Isomannide. *Synthesis* **2009,** 620–626. (b) Girgis, A. S.; Ellithey, M. Facile Synthesis of Non-steroidal Anti-inflammatory Active Bisbenzamide-containing Compounds. *Bioorg. Med. Chem.* **2006,** *14*, 8527–8532.

(9) Cerecetto, H.; Gerpe, A.; Gonzalez, M.; Aran, V. J.; Ochoa de Ocariz, C. Pharmacological Properties of Indazole Derivatives: Recent Developments. *Mini-Rev. Med. Chem.* **2005,** *5*, 869–878.

(10) (a) Gerpe, A.; Aguirre, G.; Boiani, L.; Cerecetto, H.; Gonzalez, M.; Olea-Azar, C.; Rigol, C.; Maya, J. D.; Morello, A.; Piro, O. E.; Aran, V. J.; Azqueta, A.; Lopez de Cerain, A.; Monge, A.; Rojas, M. A.; Yaluff, G. Indazole N-oxide Derivatives as Antiprotozoal Agents: Synthesis, Biological Evaluation and Mechanism of Action Studies. *Bioorg. Med. Chem.* **2006,** *14*, 3467–3480. (b) Li, X.; Chu, S.; Feher, V. A.; Khalili, M.; Nie, Z.; Margosiak, S.; Nikulin, V.; Levin, J.; Sprankle, K. G.; Tedder, M. E.; Almassy, R.; Appelt, K.; Yager, K. M. Structure-based Design, Synthesis, and Antimicrobial Activity of Indazole-derived SAH/MTA Nucleosidase Inhibitors. *J. Med. Chem.* **2003,** *46*, 5663–5673.

(11) Abouzid, K. A. M.; El-Abhar, H. S. Synthesis and Antiinflammatory Activity of Novel Indazolones. *Arch. Pharm. Res.* **2003,** *26*, 1–8.

(12) Aran, V. J.; Flores, M.; Munoz, P.; Ruiz, J. R.; Sanchez-Verdu, P.; Stud, M. Cytostatic Activity Against HeLa Cells of A Series of Indazole and Indole Derivatives; Synthesis and Evaluation of Some Analogs. *Liebigs Ann.* **1995,** 817–824.

(13) Dutta Gupta, S.; Hari Narayana Moorthy, N. S.; Sanyal, U. Synthesis, Cytotoxic Evaluation, in Silico Pharmacokinetic and QSAR Study of Some Benzothiazole Derivatives. *Int. J. Pharm. Pharm. Sci.* **2010,** *2*, 57–62.

(14) Wells, G.; Bradshaw, T. D.; Diana, P.; Seaton, A.; Shi, D.-F.; Westwell, A. D.; Stevens, M. F. G. Antitumour Benzothiazoles. Part 10. The Synthesis and Antitumour Activity of Benzothiazole-substituted Quinol Derivatives. *Bioorg. Med. Chem. Lett.* **2000,** *10*, 513–515.

(15) Kamal, A.; Reddy, K. S.; Khan, M. N. A.; Shetti, R. V. C. R. N. C.; Ramaiah, M. J.; Pushpavalli, S. N. C. V. L.; Srinivas, C.; Pal-Bhadra, M.; Chourasia, M.; Sastry, G. N.; Juvekar, A.; Zingde, S.; Barkume, M. Synthesis, DNA-binding Ability and Anticancer Activity of Benzothiazole/benzoxazole-pyrrolo[2,1-c][1,4]benzodiazepine Conjugates. *Bioorg. Med. Chem.* **2010,** *18*, 4747–4761.

(16) (a) Saeed, S.; Rashid, N.; Jones, P. G.; Ali, M.; Hussain, R. Synthesis, Characterization and Biological Evaluation of Some Thiourea Derivatives Bearing Benzothiazole Moiety as Potential Antimicrobial and Anticancer Agents. *Eur. J. Med. Chem.* **2010,** *45*, 1323–1331. (b) Shaikh Kabeer, A.; Baseer, M. A.; Mote, N. A. Synthesis and Antimicrobial Activity of Some Schiff Bases from Benzothiazoles. *Asian J. Chem.* **2001,** *13*, 496–500.

(17) Paget, C. J.; Kisner, K.; Stone, R. L.; DeLong, D. C. Heterocyclic Substituted Ureas. II. Immunosuppressive and Antiviral Activity of Benzothiazole- and Benzoxazoleureas. *J. Med. Chem.* **1969,** *12*, 1016–1018.

(18) (a) Agrawal, K. C. Fluorene Derivatives for Antitumor Activity. *J. Med. Chem.* **1967,** *10*, 99–101. (b) Pan, H.-L.; Fletcher, T. L. Derivatives of Fluorene. XXI. New Halofluorenes. 2. Further Potential Antitumor Agents. *J. Med. Chem.* **1965,** *8*, 491–497.

(19) Zeng, W.; Ballard, T. E.; Tkachenko, A. G.; Burns, V. A.; Feldheim, D. L.; Melander, C. Mimicking the Biological Activity of Diazobenzo[b]fluorene Natural Products with Electronically Tuned Diazofluorene Analogs. *Bioorg. Med. Chem. Lett.* **2006,** *16*, 5148–5151.

(20) Albrecht, W. L.; Fleming, R. W.; Horgan, S. W.; Kihm, J. C.; Mayer, G. D. Bisbasic-substituted Polycyclic Aromatic Compounds. New Class of Antiviral Agents. 3. 2,7-Bis(aminoacyl)fluorenes and -Fluorenones. *J. Med. Chem.* **1974,** *17*, 886–889.

(21) Kemnitzer, W.; Sirisoma, N.; Jiang, S.; Kasibhatla, S.; Crogan-Grundy, C.; Tseng, B.; Drewe, J.; Cai, S. X. Discovery of N-Aryl-9-oxo-9H-fluorene-1-carboxamides as A New Series of Apoptosis Inducers Using A Cell- and Caspase-based High-throughput Screening Assay. 2. Structure-activity Relationships of the 9-Oxo-9H-fluorene Ring. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 1288–1292.

(22) Bahekar, R. H.; Jain, M. R.; Jadav, P. A.; Goel, A.; Patel, D. N.; Prajapati, V. M.; Gupta, A. A.; Modi, H.; Patel, P. R. Synthesis of 3,8,9-Trisubstituted-1,7,9-triaza-fluorene-6-carboxylic Acid Derivatives as A New Class of Insulin Scretagogues. *Bioorg. Med. Chem.* **2007,** *15*, 5950–5964.

(23) (a) Ushenko, I. K.; Chovnik, L. I. Chemistry of Cyanine Dyes. XVI. Biscyanines. *Zh. Obshch. Khim.* **1960,** *30*, 2665–2669 CAN 55:62085. (b) Block, M. H.; Donald, S. C.; Foote, K.; Schofield, P.; Marsham, P. R. Preparation of Carbazoles as Neuropeptide Y5 Receptor Ligands. *PCT Int. Appl.* 2001, WO 2001007409 CAN 134:147496.

(24) For reviews, see: (a) Schreiber, S. L. Target-oriented and Diversity-oriented Organic Synthesis in Drug Discovery. *Science* **2000,** *287*, 1964–1969. (b) Biggs-Houck, J. E.; Younai, A.; Shaw, J. T. Recent Advances in Multicomponent Reactions for Diversity-oriented Synthesis. *Curr. Opin. Chem. Biol.* **2010,** *14*, 371–382.

(25) For reviews, see: (a) Kappe, C. O. Microwave Dielectric Heating in Synthetic Organic Chemistry. *Chem. Soc. Rev.* **2008,** *37*, 1127–1139. (b) Polshettiwar, V.; Varma, R. S. Aqueous Microwave Chemistry: A Clean and Green Synthetic Tool for Rapid Drug Discovery. *Chem. Soc. Rev.* **2008,** *37*, 1546–1557.

(26) (a) Jiang, B.; Tu, S.-J.; Kaur, P.; Wever, W.; Li, G. Four-Component Domino Reaction Leading to Multifunctionalized Quinazolines. *J. Am. Chem. Soc.* **2009,** *131*, 11660–11661. (b) Jiang, B.; Li, C.; Shi, F.; Tu, S.-J.; Kaur, P.; Wever, W.; Li, G. Four-Component Domino Reaction Providing an Easy Access to Multifunctionalized Tricyclo-$[6.2.2.0^{1,6}]$dodecane Derivatives. *J. Org. Chem.* **2010,** *75*, 2962–2965. (c) Han, Z.-G.; Miao, C.-B.; Shi, F.; Ma, N.; Zhang, G.; Tu, S.-J. Diversity Synthesis of N-Substituted 2-Amino-1,6-naphthyridine Derivatives under Microwave Irradiation. *J. Comb. Chem.* **2010,** *12*, 16–19.

(27) (a) Tu, S.; Zhang, J.; Jia, R.; Zhang, Y.; Jiang, B.; Shi, F. A novel Reaction of 4-(Arylmethylene)-2-phenyloxazol-5(4H)-ones with Pyridin-2-amine: Formation of 3-(Arylmethyl)-3-(benzoylamino)imidazo-[1,2-a]pyridin-2(3H)-ones. *Synthesis* **2007,** 558–564. (b) Maekawa, K.; Kubo, K.; Igarashi, T.; Sakurai, T. Electron transfer-initiated Asymmetric Photocyclization of Chiral Auxiliary-substituted N-Acyl-dehydro(1-naphthyl)-alaninamides to the Corresponding 3,4-Dihydrobenzo[f]quinolinone derivatives. *Tetrahedron* **2005,** *61*, 11211–11224. (c) Bailey, K. L.; Molinski, T. F. Entropically Favorable Macrolactamization. Synthesis of Isodityrosine Peptide Analogs by Tandem Erlenmeyer Condensation-Macrolactamization. *J. Org. Chem.* **1999,** *64*, 2500–2504. (d) Tripathy, P. K.; Mukerjee, A. K. A Facile Synthesis of N-Substituted 2-Acylamino-2-alkenamides. *Synthesis* **1985,** 285–288. (e) Cativiela, C.; Garcia, J. I.; Melendez, E. Stereospecific Synthesis of the (Z/E)-Isomers of N-Aryl-(alkyl)-2-benzoylamino-3-aryl-2-butenecarboxamides. *Synthesis* **1982,** 763–765.