Silica Gel-Mediated Amide Bond Formation: An Environmentally Benign Method for Liquid-Phase Synthesis and Cytotoxic Activities of Amides

Xiao-Dong Yang, Xiang-Hui Zeng, Yuan-Hong Zhao, Xue-Quan Wang, Zhi-Qiang Pan, Liang Li, and Hong-Bin Zhang*

Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming, 650091, P. R. China

The amide bond plays a major role in the elaboration and composition of biological systems. It is one of the most important linkages in organic chemistry and constitutes the key functional group in peptides, polymers, and many natural products and pharmaceuticals.[1] An in-depth analysis of the comprehensive medicinal chemistry database revealed that the carboxamide group appears in more than 25% of known drugs.[2] For example, atorvastatin (**1** in Figure 1, blocker of cholesterol production), the top selling drug worldwide since 2003, contains an amide bond,[1a,3] as do Reyataz (**2**, a protease inhibitor used to treat HIV),[4] Gleevec (**3**, a protein-tyrosine kinase inhibitor used to treat chronic myeloid leukemia),[5] and Altace (**4**, an ACE inhibitor used to treat hypertension and heart disease).[6]

Because of its importance in biological systems, formation of the amide bond has been one of the most widely studied reactions in organic chemistry.[7] Numerous methods have been developed toward the synthesis of amides; contemporary trends in amide formation, however, have been directed toward the design and synthesis of efficient coupling reagents.[8] It has been taken for granted that amide bond forming is realized by reacting an activated carboxylic acid with an amine. Utilization of an activating strategy unavoidably brings about problems associated with harmful waste materials, of which dicyclohexyl urea arising from the commonly used coupling reagent dicyclohexylcarbodiimide (DCC) is a particularly troublesome example. The situation is even worse in combinatorial liquid-phase synthesis of amide libraries for drug discovery where there are the difficulties of removing byproducts produced during coupling reactions, as well as excessive activating agents. In other words, the advantages of solution-phase synthesis over solid-phase approaches, such as unlimited scale, easy manipulation, easy analysis of the reaction progress, efficiency, ready optimization of reaction conditions and cost effectiveness, are compromised by the time-consuming purification process.[9] On the basis of the current criteria of green chemistry,
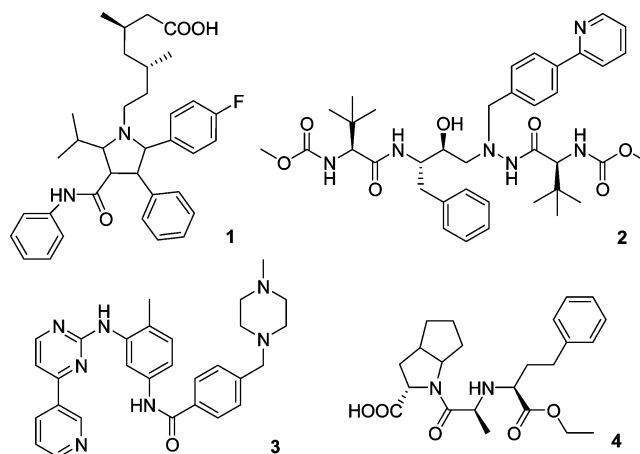

Figure 1. Chemical structures of marketed drugs containing an amide bond.
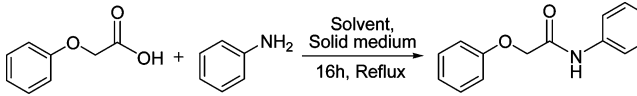
amide libraries syntheses should be as efficient as possible while being environmentally benign. Development of a new method that could be used for the generation of amide libraries in the solution phase is one of our interests. In this paper, we report a convenient approach toward the synthesis of amides, as well as amide libraries based on pyrolyzation mediated by silica gel in the solution phase (Scheme 1).

Although amide bond formation by direct pyrolyzation of salts generated by mixing an acid with an amine appears an attractive method, little attention has been paid seriously to this approach. Only a few attempts dealing with the synthesis of amides under microwave irradiation conditions have been discussed in the recent literature. High temperatures are generally associated with pyrolyzation and thus preclude its further application. In typical recent research, the synthesis of amides by directly combining amines and carboxylic acids under solvent-free microwave conditions has been studied;[10] however, it is less likely that the extremely high temperature (250−300 °C) employed would be appropriate for a liquid-phase generation of amide libraries. Besides, this microwave-mediated method provided amides in only 10−25% yields when secondary amines were used. Because solid medium, such as silica gel, had been used previously as a solid support for acylthiourea formation under solvent-free microwave condition,[11] we decided to try pyrolazation that was mediated by silica gel in solution phase, aiming to develop a new method for the synthesis of amides (Table 1). By screening of solvents, we found that toluene was a good solvent for amide formation. To our delight, treatment of phenoxyacetic acid with phenylamine in toluene in the presence of silica gel afforded the desired amide in 90% yield. Without addition of silica gel, only a 28% yield was obtained after reflux for 16 h with azotropic removal of water. When silica gel was

Scheme 1. Preparation of Amides by Silica Gel-Mediated Reaction
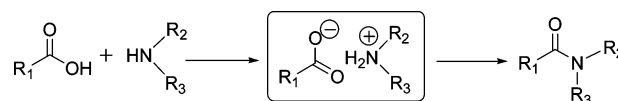
* To whom correspondence should be addressed. Tel.: +86-871-5031119. Fax: +86-871-5035538. E-mail: zhanghbyd@gmail.com or zhang_hongbin@hotmail.com.

Table 1. Attempts Towards the Synthesis of Amides[a]

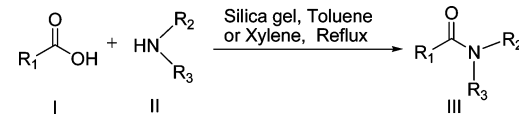| entry	solvent	solid medium	yield (%)
1	benzene		0
2	1,2-dichloroethane		0
3	1,4-dioxane		trace
4	toluene		28
5	xylene		31
6	toluene	molecular sieves (4 Å)	20
7	toluene	anhydrous magnesium sulfate	15
8	toluene	aluminum oxide	12
9	1,4-dioxane	silica gel	56
10	toluene	silica gel	90

[a] A mixture of acid (1.2 mmol), amine (1.0 mmol) and solid medium (500 mg) was stirred in solvent (10 mL) at reflux for 16 h.

changed to molecular sieves, only a 20% yield of the amide was obtained under identical conditions. For some acids and amines, elevation of the temperature by using xylene as solvent provided better yields (Table 2, compounds **III-7−III-18** and **III-25−III-30**). A number of amides were synthesized and the results are summarized in Table 2.[12] It is noteworthy that purification of the amide products was easy to handle and pure samples could be obtained by filtration and washing with aqueous sodium bicarbonate to remove excess acid. The silica gel can be reused several times

without any significant loss of activity after being washed with acetone and ethanol and dried at 120 °C in an oven overnight.

The cytotoxic potential of all synthesized amides was evaluated in vitro against AGZY, U937, and PC12 tumor cell lines according to procedures described in the literature.[13] The results are summarized in Table 2 (IC$_{50}$ value is defined as the concentration corresponding to 50% growth inhibition). From Table 2, it has been found that cinnamic amides **III-15**, **III-16**, **III-17**, **III-18**, and **III-28** possess moderate cytotoxic activities, although cinnamic amides **III-13** and **III-14** were inactive. Among them, compound **III-17**, prepared from an electron-rich cinnamic acid and an electron-rich arylamine, was the most active. These results suggested that cinnamic amides with an electron-rich substitution on both of the aromatic rings may play a vital role in the modulation of the cytotoxic activities.

With the optimized reaction conditions in hand, we turned our attention next to the synthesis of amides with the aim of finding more potent compounds. On the basis of the above cytotoxic activities, we selected 3,4,5-trimethoxycinnamic acid (**I-17**) as the starting acid. We synthesized cinnamic amides **IV-1** to **IV-10**, respectively, by using 3,4,5-trimethoxycinnamic acid and ten amines (including five arylamines and five alkylamines) via silica gel-mediated reaction (Table 3). The cytotoxic potential

Table 2. Synthesis of Amides via Silica Gel-Mediated Reaction[a] and Cytotoxic Activities in Vitro[c] (IC$_{50}$, μM)

compound	acid (I)	amine (II)	yield (%)	IC$_{50}$ for AGZY	IC$_{50}$ for U937	IC$_{50}$ for PC12
III-1	phenylpropanoic acid	*o*-anisidine	92	>100	>100	>100
III-2	phenoxy acetic acid	2-amino-4,6-dimethylpyrimidine	94	>100	>100	>100
III-3	phenoxy acetic acid	diethylamine	77	>100	>100	>100
III-4	phenoxy acetic acid	2-aminopyridine	84	>100	>100	>100
III-5	1-cyclohexenecarboxylic acid	3,4-dimethoxyphenylethanamine	83	>100	>100	>100
III-6	sorbic acid	3,4-dimethoxyphenylethanamine	88	>100	>100	>100
III-7	sorbic acid	morpholine	55[b]	>100	63.3	>100
III-8	3,4,5-trimethoxybenzoic acid	morpholine	74[b]	>100	>100	>100
III-9	3,4,5-trimethoxybenzoic acid	*o*-anisidine	62[b]	87.4	>100	>100
III-10	nicotinic acid	morpholine	78[b]	>100	>100	>100
III-11	isobutyric acid	3,4,5-trimethylaniline	75[b]	>100	>100	>100
III-12	3-iodobenzoic acid	3,5-dimethylaniline	61[b]	44.6	>100	32.6
III-13	3-chlorocinnamic acid	2-aminopyridine	90[b]	>100	>100	>100
III-14	3,4-dimethoxycinnamic acid	morpholine	91[b]	>100	>100	>100
III-15	3,4-dimethoxycinnamic acid	1-adamantylamine	81[b]	39.1	39.1	72.8
III-16	3,4,5-trimethoxycinnamic acid	2-amino-4,6-dimethylpyrimidine	55[b]	76.5	82.6	88.5
III-17	3,4,5-trimethoxycinnamic acid	*m*-anisidine	75[b]	18.8	25.3	29.9
III-18	3,4-dimethoxycinnamic acid	2-aminopyridine	86[b]	55.7	41.5	19.8
III-19	phenoxy acetic acid	1-adamantylamine	70	>100	>100	>100
III-20	phenoxy acetic acid	2,4,6-trimethylaniline	88	>100	>100	>100
III-21	phenoxy acetic acid	benzylamine	92	>100	>100	>100
III-22	4-methoxylphenylacateic acid	2-aminoethanol	78	>100	>100	>100
III-23	phenoxy acetic acid	8-aminoquinoline	80	>100	>100	>100
III-24	phenoxy acetic acid	2,4-dimethoxybenzenamine	88	>100	>100	>100
III-25	4-nitrobenzoic acid	*p*-anisidine	75[b]	>100	>100	>100
III-26	3,5-dinitrobenzoic acid	*p*-anisidine	51[b]	>100	>100	>100
III-27	4-methoxylphenylacateic acid	*p*-nitroaniline	88[b]	>100	>100	>100
III-28	3,4,5-trimethoxycinnamic acid	*p*-nitroaniline	70[b]	82.1	78.3	66.2
III-29	4-nitrobenzoic acid	*p*-nitroaniline	38[b]	>100	>100	>100
III-30	3,5-dinitrobenzoic acid	*p*-nitroaniline	31[b]	>100	>100	>100

[a] Yields represent isolated yield. [b] Xylene as solvent. [c] Cytotoxicity, as IC$_{50}$ for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.

Table 3. Synthesis of Cinnamic Amides via Silica Gel-Mediated Reaction[a] and Cytotoxic Activities in Vitro[b] (IC$_{50}$, μM)
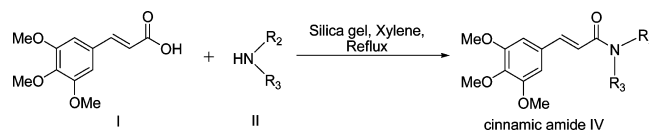
entry	amine (II)	yield (%)	HepG2	Hep-2	EJ	Raji
IV-1	*m*-anisidine	75	27.9	36.3	26.8	54.5
IV-2	*m*-toluidine	69	47.6	46.1	84.7	>100
IV-3	*o*-anisidine	72	69.5	>100	53.8	>100
IV-4	aniline	76	9.0	11.2	12.0	16.9
IV-5	2-aminopyridine	67	>100	>100	>100	>100
IV-6	1-adamantylamine	78	>100	>100	>100	>100
IV-7	4-methoxyphenylethanamine	66	>100	>100	>100	>100
IV-8	cyclohexylamine	85	>100	>100	>100	>100
IV-9	morpholine	90	>100	>100	>100	>100
IV-10	diethylamine	72	>100	>100	>100	>100
DDP			13.6	18	7.9	4.1

[a] Yields represent isolated yield. [b] Cytotoxicity, as IC$_{50}$ for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.

of all synthesized cinnamic amides was evaluated in vitro against liver carcinoma (HepG2), laryngeal carcinoma (Hep-2), bladder carcinoma (EJ), and lymphoma (Raji). Cisplatin (DDP) was used as the reference drug. The results of the cytotoxicity studies are summarized in Table 3. The results of the cytotoxicity studies showed that arylamines (**IV-1**−**IV-5**) exhibited higher cytotoxic activities than alkylamines (**IV-6**−**IV-10**). From Table 3, it has been found that cinnamic amide **IV-4** displayed significant potency with the in vitro cytotoxic activities comparable to DDP. Additionally, cinnamic amide **IV-1** (**III-17**) exhibited moderate cytotoxic activities.

In conclusion, an efficient, functional group tolerant, and environmentally benign process for the synthesis of amides was developed. Our protocol provides an alternative for the combinatorial liquid-phase synthesis of amide libraries for drug discovery. No activation reagents or scavengers are required in this process. Purification of desired compounds is easy, and cost-effective. By this method, a number of amides were prepared and evaluated in vitro against a panel of human tumor cell lines. Cinnamic amide **IV-4** was found to be the most potent compound synthesized against four human tumor cell lines. Further utilization of this method for the synthesis of complex and active amides is under investigation.

Supporting Information Available. General procedures for the preparation of amides and spectroscopic data for all products. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Valeur, E.; Bradley, M. *Chem. Soc. Rev.* **2009**, *38*, 606–631. (b) Greenberg, A.; Breneman, C. M.; Liebman, J. F. *the Amide Linkage: Structural Significance in Chemistry, Biochemistry, And Materials Science*; John Wiley & Sons: New York, 1999. (c) Rebek, J. *Acc. Chem. Res.* **1999**, *32*, 278–286. (d) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232. (e) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4011.

(2) (a) Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827–10852. (b) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Comb. Chem.* **1999**, *1*, 55–68.

(3) Graul, A.; Castaner, J. *Drugs Future* **1997**, *22*, 956–968.

(4) Jr. Roskoski, R. *Biochem. Biophys. Res. Commun.* **2003**, *309*, 709–717.

(5) Patel, R. N. *Coord. Chem. Rev.* **2008**, *252*, 659–701.

(6) Hogan, B. L.; Williams, M.; Idiculla, A.; Veysoglu, T.; Parente, E. *J. Pharm. Biomed. Anal.* **2000**, *23*, 637–651.

(7) (a) Sheehan, J. C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067–1068. (b) Jursic, B. S.; Zdravkovski, Z. *Synth. Commun.* **1993**, *23*, 2761–2770. (c) Katritzky, A. R.; Rogovoy, B. V.; Kirichenko, N.; Vvedensky, V. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1809–1811. (d) Poondra, R. R.; Turner, N. J. *Org. Lett.* **2005**, *7*, 863–866. (e) Valeur, E.; Bradley, M. *Tetrahedron* **2007**, *63*, 8855–8871. (f) Nordstrøm, L. U.; Vogt, H.; Madsen, R. *J. Am. Chem. Soc.* **2008**, *130*, 17672–17673. (g) Gustafsson, T.; Ponténb, F.; Seeberger, P. H. *Chem. Commun.* **2008**, 1100–1102. (h) Pu, Y. J.; Vaid, R. K.; Boini, S. K.; Towsley, R. W.; Doecke, C. W.; Mitchell, D. *Org. Process Res. Dev.* **2009**, *13*, 310–314. (i) Colombo, M.; Bossolo, S.; Aramini, A. *J. Comb. Chem.* **2009**, *11*, 335–337.

(8) (a) Hoeg-Jensen, T.; Olsen, C. E.; Holm, A. *J. Org. Chem.* **1994**, *59*, 1257–1263. (b) Li, P.; Xu, J. C. *Tetrahedron* **2000**, *56*, 4437–4445. (c) Carpino, L. A.; Ferrer, F. J. *Org. Lett.* **2001**, *3*, 2793–2795. (d) Gomez, L.; Ngouela, S.; Gellibert, F.; Wagner, A.; Mioskowski, C. *Tetrahedron Lett.* **2002**, *43*, 7597–7599. (e) Wischnat, R.; Rudolph, J.; Hanke, R.; Kaese, R.; May, A.; Theisc, H.; Zuther, U. *Tetrahedron Lett.* **2003**, *44*, 4393–4394. (f) Han, S.-Y.; Kim, Y.-A. *Tetrahedron* **2004**, *60*, 2447–2467. (g) Ye, Y.; Li, H.; Jiang, X. *Pept. Sci.* **2005**, *80*, 172–178. (h) Kaminski, Z. J.; Kolesinska, B.; Kolesinska, J.; Sabatino, G.; Chelli, M.; Rovero, P.; Blaszcyk, M.; Glowka, M. L.; Papini, A. M. *J. Am. Chem. Soc.* **2005**, *127*, 16912–16920. (i) Valeur, E.; Bradley, M. *Tetrahedron* **2007**, *63*, 8855–8871. (j) El-faham, A.; Albericio, F. *J. Org. Chem.* **2008**, *73*, 2731–2737.

(9) (a) Cheng, S.; Comer, D. D.; Williams, J. P.; Myers, P. L.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567–2573. (b) Boger, D. L.; Chai, W.; Ozer, R. S.; Andersson, C.-M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 463–468. (c) Flynn, D. L.; Crich, J. Z.; Devraj, R. V.; Hockerman, S. L.; Parlow, J. J.; South, M. S.; Woodard, S. *J. Am. Chem. Soc.* **1997**, *119*, 4874–4881. (d) Kaldor, S. W.; Siegel, M. G. *Curr. Opin. Chem. Biol.* **1997**,

1, 101–106. (e) Falorni, M.; Giacomelli, G.; Porcheddu, A.; Taddei, M. *Eur. J. Org. Chem.* **2000**, 1669–1675.

(10) Gelens, E.; Smeets, L.; Sliedregt, L. A. J. M.; van Steen, B. J.; Kruse, C. G.; Leurs, R.; Orru, R. V. A. *Tetrahedron Lett.* **2005**, *46*, 3751–3754.

(11) Márquez, H.; Plutín, A.; Rodríguez, Y.; Perez, E.; Loupy, A. *Synth. Commun.* **2000**, *30*, 1067–1073.

(12) The general procedure for the preparation of amides **III-1** to **III-30** and **IV-1** to **IV-10** via silica gel mediated reaction is as follows: A mixture of acid (1.2 mmol), amine (1.0 mmol) and silica gel (450−600 mg, 300−400 mesh) was stirred in toluene or xylene (10 mL) at reflux for 12−48 h. After completion of the reaction as indicated by TLC, the solution was cooled to room temperature. Silica gel was filtered through a small pad of Celite and washed with toluene or ethyl acetate (3 × 10 mL). The filtrate washed with saturated aqueous $NaHCO_3$ (3 × 15 mL) to remove excess acid, then washed with water and brine, and dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the residue was chromatographed on silica gel (petroleum ether/ethyl acetate) to afford the products.

(13) (a) Kim, D. K.; Ryu, D. H.; Lee, J. Y.; Lee, N.; Kim, Y. W.; Kim, J. S.; Chang, K.; Im, G. J.; Kim, T. K.; Choi, W. S. *J. Med. Chem.* **2001**, *44*, 1594–1602. (b) Cao, R.; Chen, Q.; Hou, X.; Chen, H.; Guan, H.; Ma, Y.; Peng, W.; Xu, A. *Bioorg. Med. Chem.* **2004**, *12*, 4613–4623.