European Journal of Medicinal Chemistry 54 (2012) 232–238

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

A novel class of small-molecule caspase-3 inhibitors prepared by multicomponent reactions

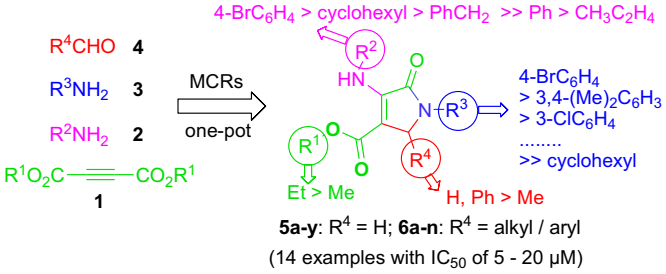
Qiuhua Zhu [a,b], Lixin Gao [c], Zhipeng Chen [a], Sichao Zheng [a], Huafei Shu [a], Jia Li [c,***], Huanfeng Jiang [b,**], Shuwen Liu [a,*]

[a] School of Pharmaceutical Sciences, Southern Medical University, 1838 Northern Guangzhou Road, Guangzhou, Guangdong 510515, China
[b] School of Chemistry and Chemical Engineering, South China University of Technology, 381 Wushan Road, Guangzhou 510640, China
[c] Chinese National Center for Drug Screening, Shanghai Institute of Materia Medica, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, 189 Guo Shou Jing Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China

HIGHLIGHTS

- The MCR scope for the synthesis of pentasubstituted polyfunctional dihydropyrroles **6** were expanded.
- Tetra- and pentasubstituted dihydropyrroles **5** and **6** are a novel series of caspase-3 inhibitors.
- The inhibitory activity of **5** and **6** depend on the nature of substituents on different positions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 January 2012
Received in revised form
30 April 2012
Accepted 1 May 2012
Available online 11 May 2012

Keywords:
Caspase-3 inhibitor
Dihydropyrrole
Multicomponent reaction
Synthesis

ABSTRACT

A series of tetra- and pentasubstituted polyfunctional dihydropyrroles **5** and **6** were synthesized via practical multicomponent reactions (MCRs) for research on their structure–activity relationship as caspase-3 inhibitors. Among 39 compounds evaluated, 14 of them exhibited inhibition against caspase-3 with IC_{50} ranging from 5 to 20 μM. The inhibitory activities of **5** and **6** depend on the nature of substituents on different positions. **5** and **6** possess a different scaffold from those previously reported and are the first caspase-3 inhibitors prepared via MCRs. The most active compounds **5k** ($IC_{50} = 5.27$ μM) could therefore be used as a lead for the development of highly potent caspase-3 inhibitors as drug candidates for therapeutic agents by taking advantage of MCRs.

1. Introduction

Apoptosis, or programmed cell death, is an essential physiological process of tissue development and homeostasis. The pathogenesis of many diseases is closely connected with aberrantly regulated apoptosis [1–6]. For example, cancer and Alzheimer's disease are characterized by insufficient and excessive apoptosis, respectively [4,5]. Caspases, a family of cysteinyl aspartate-specific proteases, have been proved to be critical in mediating the signal transduction and execution of apoptosis [7]. Evidence from caspase-deficient cells and cell lines, as well as immunodepleted cell-free extracts, have indicated that caspase-3 is a key executioner caspase and the inhibition of caspase-3 activity can significantly prevent apoptosis *in vitro* and *in vivo* [8–10]. Consequently, great

* Corresponding author. Tel.: +86 20 61648538; fax: +86 20 61648655.
** Corresponding author. Tel.: +86 20 22236518; fax: +86 20 87112906.
*** Corresponding author. Tel./fax: +86 21 50801552.
E-mail addresses: jli@mail.shcnc.ac.cn (J. Li), jianghf@scut.edu.cn (H. Jiang), liusw@smu.edu.cn (S. Liu).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2012.05.001

interest has emerged in developing caspase-3 inhibitors for use as therapeutic drugs in the treatment of excessive apoptosis-related diseases such as Alzheimer's and Parkinson's diseases.

Until now, two kinds of inhibitors of caspase-3, peptidic and nonpeptidic inhibitors, have been reported. Most of peptidic inhibitors exhibit high inhibitory activities against caspase-3 with IC_{50} in the nanomolar range [11] and even with *Ki* in the picomolar range [12]. However, they have limited clinical utility due to poor cell permeability and metabolic stability. Thus, considerable efforts have been devoted to the development of nonpeptidic caspase-3 inhibitors.

High throughput and virtual screening of libraries of small molecules already on hand have helped identify novel lead compounds, such as lead compounds **I**–**III** (Fig. 1) [13–15], which have been used as starting points for further developments in the field of caspase-3 inhibitors. New inhibitors with higher potency can often be developed from lead compounds by further structure-based design and optimization. For example, lead compound isatin (**I**, Fig. 1) has led to a series of more potent analogs, such as **IV** in Fig. 1 [16], with IC_{50} in the nanomolar range. Although rapid advances on nonpeptidic caspase-3 inhibitors have been made, none of these compounds have been developed as a drug for treatment of caspase-3-related diseases. Therefore, diverse potent and specific caspase-3 inhibitors are needed.

Multicomponent reaction (MCRs) is an ideal reaction mode which is consistent with the concept of green chemistry, such as atom economy, high efficiency. In addition, MCRs could be used to build structural diversity and complexity of compound libraries [17], which let them have played important role in modern drug discovery [18,19]. In order to discover new lead compounds, we developed practical MCRs for the synthesis of heterocyclic compounds with biological activity [20–25]. It was found that the MCR products, tetra- and pentasubstituted polyfunctional dihydropyrroles **5** and **6**, reported in Ref. [24] also acted as caspase-3 inhibitors [26]. To our knowledge, **5** and **6** possess a different molecular scaffold from existing nonpeptidic cascpase-3 inhibitors and are the first inhibitors against caspase-3 prepared by MCRs. Therefore, new dihydropyrroles **5** and **6** were synthesized for research on their structure–activity relationship as caspase-3 inhibitors.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, the reaction conditions for the synthesis of **5** and **6** correlate with the nature of substituents. Tetrasubstituted polyfunctional dihydropyrroles **5** with R^2 = alkyl and R^3 = aryl or alkyl (all of **5** with the exception of **5g**, **5i**–**5k**) as well as **5** with $R^2 = R^3$ = aryl (**5g**, **5i**–**5k**) were synthesized by the MCRs of but-2-ynedioates **1**, amines **2** and **3**, formaldehyde **4a** under conditions A and B, respectively [24]. Pentasubstituted polyfunctional dihydropyrroles **6** with R^2 = aryl or alkyl and R^3 = alkyl (all of **6** with the exception of **6f**, **6g** and **6n**) and **6n** with $R^2 = R^3$ = aryl (Ph) were prepared by the MCRs under conditions C and D, respectively [24]. When **6f** and **6g** (R^2 = 4-$HOOCC_6H_4$) were prepared under condition C or D, complex products were obtained. We found that triethylamine ($N(Et)_3$, 0.5 equiv) was needed for the synthesis of **6f** as well as **6g** and that the ratios of starting materials were related to the nature of groups R^4. Conditions E and F were suitable for the synthesis of **6f** with R^4 = aryl and **6g** with R^4 = alkyl, respectively.

A total of 39 tetra- and pentasubstituted polyfunctional dihydropyrroles **5** and **6** were synthesized in 60–98% yields (19 compounds of which were not previously reported). All the new compounds were characterized by ^1H and ^{13}C NMR, MS (GC–MS or ESI–MS) and elemental analysis. Satisfactory analytical data consistent with the shown molecular structures and a purity of at least 95% (determined by elemental analysis) were obtained for all compounds.

2.2. Biological activity

Compounds **5** and **6** were evaluated for their inhibitory activities against caspase-3 by the method as described in our previous work [14]. For all the compounds that exhibited more than 50% inhibition at the concentration of 20 μg/mL, the concentration-dependent caspase-3 inhibition curves were further conducted and the IC_{50} values were calculated by using PRISM 4 (GraphPad) software. Final IC_{50} values were the average of three independent experimental results. Peptidic caspase-3 inhibitor Ac-DEVD-CHO was used as positive control compound. In the same experimental conditions, Ac-DEVD-CHO inhibited the caspase-3 activity with IC_{50} value of 8.75 ± 0.63 nM. The IC_{50} values against caspase-3 for **5** and **6** are summarized in Table 1.

The activity results indicate that most of **5** displayed inhibitory activities against caspase-3 (Table 1). Replacement of the methyl group by the ethyl group (R^1) caused a modest activity increase up to 2-fold (**5b** > **5a**, **5d** > **5c**). Both of R^2 and R^3 could significantly influence the inhibitory activities of **5**. The orders of inhibitory activities of **5** with different R^2 and R^3 against caspase-3 was 4-BrC_6H_4 > cyclohexyl > $PhCH_2$ >> Ph > $CH_3C_2H_4$; and 4-BrC_6H_4 > 3,4-$(Me)_2C_6H_3$ > 3-ClC_6H_4 ≈ 3,4-$(Cl)_2C_6H_3$ > 4-MeC_6H_4 > Ph > 4-$NO_2C_6H_4$ > 4-$MeOC_6H_4$ ≈ 4-ClC_6H_4 > Naphthalen-1-yl >> cyclohexyl > $CH_3C_2H_4$, allyl and Pyridin-2-yl, respectively. Generally, 4-BrC_6H_4 group was an optimized group for both of R^2 and R^3 (**5k**, 5.27 μM) in this *in vitro* caspase-3 inhibition assay. Cyclohexyl as R^2 increased compounds activity, but as R^3 afforded inactive compounds. In addition, **5y** showed no inhibition, which may indicate that the hydrogen on the nitrogen in **5** is needed for their inhibitory activity against caspase-3.

As shown in Table 1, R^1 showed similar influence on the inhibitory activity of **6** to that on **5** with Et > Me. All of R^2, R^3 and R^4 could significantly influence the inhibitory activity of **6**. The activity order

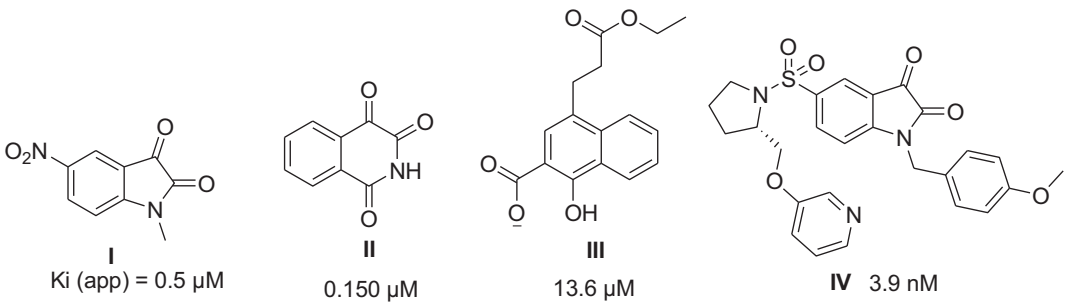
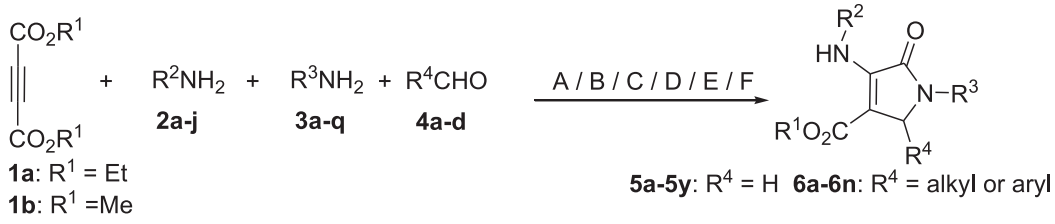
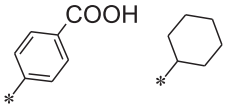


Fig. 1. Structures and IC_{50} values of several lead compounds with different scaffolds.



	1	2 /equive	3	4	Solvent	Additive /equiv	T /°C	t /h	R^2	R^3	5/6 yields / %
A	1	1	2	1.5	EtOH	HOAc/2	70	0.5-8	alkyl	alkyl/aryl	**5a-f**, **5h**, **5l-y**, 85-93
B	1	1	4	1.5	EtOH	HOAc/2	70	0.5-8	aryl	aryl	**5g**, **5i-k**, 85-89
C	1	1	2	3	EtOH		rt/70	2-16	alkyl/aryl	alkyl	**6a-e**, **6h-m**, 60-98
D	1	1	2	3	EtOH	HOAc/2	70	6	Ph	Ph	**6n**, 92
E	1.5	1	2	3	MeOH-EtOH (1:10)	$N(Et)_3$/0.5	rt	24			**6f** (R^4 = Ph), 76
F	1	1.5	2	3	MeOH-EtOH (1:10)	$N(Et)_3$/0.5	rt	24			**6g** (R^4 = Me) , 79

Scheme 1. Synthesis of tetra- and pentasubstituted polyfunctional dihydropyrroles **5** and **6**.[a]

of **6** with different R^2 was Naphthalen-1-yl > 4-BrC_6H_4 > Ph > 4-$NO_2C_6H_4$ >> 4-$HOOCC_6H_4$. The most potent caspase-3 inhibitor among this series was **6d** with an IC_{50} of 7.92 μM. **6** with carboxyl or hydroxyl groups show much lower inhibitory activity (**6f**, **6g**, **6i**–**6k**), which indicates active hydrogen on these substituted positions could significantly decrease the activity of **6**.

Compared with the structures of most of existing peptide and nonpeptide caspase-3 inhibitors, **5** and **6** have not high electrophilic groups that react with the active site cysteine residue of caspase-3, such as high electrophilic carbonyl groups in **I** and **II** (Fig. 1) [11–14] as well as high electrophilic Michael addition acceptors [27,28]. Because different binding modes are observed with different kinds of inhibitors bound to the same enzyme frequently [11,29], a different inhibitory mechanism might be expected for the novel class of caspase-3 inhibitors **5** and **6**.

In view of the influences of the logarithm of the partition coefficient (log *P*) on hydrophile–lipophile balance of drugs [30] as well as their release [31] and transport [32] *in vivo*, the log *P* values in octanol–water of compounds **5** and **6** were calculated (ClogP in Table 1). The ClogP error codes indicate that the ClogP values of only 14 compounds are based on trusted fragment values, while the other 25 compounds are based on approximated, priori fragment values or even very high log *P* unrealistic in nature (note d in Table 1). Therefore, experimental log *P* values are needed to evaluate the calculated ones. Considering structure, biological activity and solubility, the log *P* values of **5k**, **5w**, **6c** and **6d** were determined by shake-flask method [30,33]. To our surprise, the experimental log *P* values for these compounds are much lower than the calculated ones (Table 1). Since other compounds in Table 1 are the derivatives of these compounds, it is expected that the experimental log *P* values of **5** and **6** may be lower than 3. According to Lipinski's Five of Rules (H-bond donors < 5, molecular weight < 500, Log *P* < 5 and H-bond acceptors < 10) [34], both of **5** and **6** are drug-like compounds and hence could be used as new drug leads for the development of more potent analogs by taking advantages of MCRs.

3. Conclusions

In conclusion, we have expanded the scope of the MCRs for the synthesis of pentasubstituted dihydropyrroles **6** to include aromatic amines with carboxyl by developing new reaction conditions. 39 tetra- and pentasubstituted polyfunctional dihydropyrroles **5** and **6** were synthesized (19 compounds of which were not previously reported) and evaluated for their inhibitory activities against caspase-3. The activity results show that **5** and **6** are a series of caspase-3 inhibitors as well as that the activities of these compounds depend on the nature of substituents on different positions. These caspase-3 inhibitors possess a different scaffold from those previously reported and are the first caspase-3 inhibitors prepared via MCRs. The most active compounds **5k** (IC_{50} = 5.27 μM) could therefore be used as a lead for the development of highly potent caspase-3 inhibitors as drug candidates for therapeutic agents by taking advantage of MCRs. The scope of the MCRs for the synthesis of **5** and **6** as well as their inhibitory mechanism, permeability and further structure–activity relationship as caspase-3 inhibitors are under investigation in our groups.

4. Experimental protocols

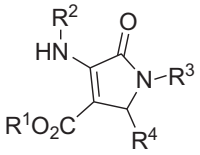
4.1. Chemistry

All melting points were taken on an XT-4 micro melting point apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded using a Bruker Avance 400 MHz NMR spectrometer and respectively referenced to 7.24 and 77.0 ppm for chloroform-*d* with TMS as internal standard. Mass spectra were recorded on an API 4000QTRAP. TLC was performed using commercially prepared 100–400 mesh silica gel plates (GF254), and visualization was effected at 254 and 365 nm. All the other chemicals were purchased from Aldrich Chemicals.

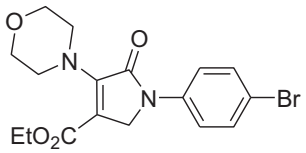
4.1.1. General procedure for the preparation of tetrasubstituted polyfunctional dihydropyrroles *5* with alkyl R^2 and alky/aryl R^3 (condition A) [24]

Primary amines **3** (2 mmol), 38% formaldehyde **4a** (120 mg, 1.5 mmol) and HOAc (120 mg, 2 mmol) were dropwise added into the mixture of EtOH (4 mL), but-2-ynedioates **1** (1 mmol) and primary or secondary aliphatic amines **2** (1 mmol) kept at room temperature for 10–30 min in sequence, followed with stirring at 70 °C for desired time (monitored by TLC). After completion of the reactions, the product mixture was purified by preparative TLC with *n*-hexane/ethyl acetate (10:1–1:1) as eluent to afford the desired products in 85–93% yields (Table 1, all of **5** with the exception of **5g**, **5i**–**k**). For the characterization data of **5a**–**5d**, **5f**, **5h** and **5x** see Ref. [24].

Table 1
Inhibitory activity of polyfunctional dihydropyrroles **5** and **6** against caspase-3.



5a-5x (R^4=H)
6a-6n (R^4=aryl, alkyl)



5y

.

Compound	R^1	R^2	R^3	R^4	Inhibition / %[a]	IC_{50} / μM[b]	ClogP[c]	Error[d]
5a	Me	Cyclohexyl	Ph	H	95.03 ± 3.25	28.18 ± 1.28	4.35	10
5b	Et	Cyclohexyl	Ph	H	100.5 ± 2.13	13.25 ± 0.46	4.88	10
5c	Me	$PhCH_2$	Ph	H	98.00 ± 2.97	19.67 ± 0.97	4.03	40
5d	Et	$PhCH_2$	Ph	H	98.36 ± 2.58	19.14 ± 1.05	4.56	40
5e	Me	$CH_3C_2H_4$	Ph	H	62.54 ± 15.86	>45	3.38	10
5f	Et	$CH_3C_2H_4$	Ph	H	28.21 ± 1.46		3.91	10
5g	Et	Ph	Ph	H	74.10 ± 10.94	>45	4.70	30
5h	Et	$PhCH_2$	$PhCH_2$	H	97.88 ± 2.95	16.58 ± 0.86	4.50	40
5i	Et	4-MeC_6H_4	4-MeC_6H_4	H	96.38 ± 1.17	11.33 ± 0.46	5.70	30
5j	Et	4-FC_6H_4	4-FC_6H_4	H	67.86 ± 6.83	>45	5.29	30
5k	Et	4-BrC_6H_4	4-BrC_6H_4	H	97.44 ± 2.52	5.27 ± 0.40	6.73/1.75[e]	30
5l	Et	Cyclohexyl	$CH_3C_2H_4$	H	17.57 ± 3.24		4.43	30
5m	Et	Cyclohexyl	Cyclohexyl	H	91.69 ± 7.97	>45	5.31	30
5n	Et	Cyclohexyl	allyl	H	12.75 ± 4.39		4.15	30
5o	Et	Cyclohexyl	4-MeC_6H_4	H	99.88 ± 3.85	11.32 ± 0.73	5.38	10
5p	Et	Cyclohexyl	3,4-MeC_6H_3	H	105.6 ± 2.92	9.76 ± 0.44	5.83	10
5q	Et	Cyclohexyl	4-$MeOC_6H_4$	H	97.04 ± 9.14	23.74 ± 2.18	4.80	10
5r	Et	Cyclohexyl	Naphthalen-1-yl	H	94.52 ± 1.59	25.23 ± 6.43	6.06	10
5s	Et	Cyclohexyl	Pyridin-2-yl	H	29.29 ± 10.27		3.38	10
5t	Et	Cyclohexyl	3-ClC_6H_4	H	99.25 ± 3.88	10.44 ± 1.02	5.59	10
5u	Et	Cyclohexyl	4-ClC_6H_4	H	97.14 ± 3.28	24.23 ± 2.66	5.59	10
5v	Et	Cyclohexyl	3,4-ClC_6H_3	H	97.72 ± 1.80	10.96 ± 1.06	6.19	10
5w	Et	Cyclohexyl	4-BrC_6H_4	H	100.4 ± 0.84	8.35 ± 1.07	5.74/1.80[e]	10
5x	Et	Cyclohexyl	4-$NO_2C_6H_4$	H	95.11 ± 2.26	15.99 ± 1.60	4.62	10
5y	Et	Morpholino	4-BrC_6H_4	H	48.71 ± 14.47		3.81	30
6a	Me	Ph	Cyclohexyl	Ph	69.29 ± 9.57	32.32 ± 1.19	5.76	40
6b	Et	Ph	Cyclohexyl	Ph	90.30 ± 1.19	27.66 ± 1.53	6.29	40
6c	Et	4-BrC_6H_4	Cyclohexyl	Ph	85.81 ± 14.88	9.98 ± 1.69	7.45/2.25[e]	51
6d	Et	Naphthalen-1-yl	Cyclohexyl	Ph	85.29 ± 2.78	7.92 ± 1.02	7.46/2.00[e]	51
6e	Et	4-$NO_2C_6H_4$	Cyclohexyl	Ph	98.72 ± 3.38	40.96 ± 6.15	6.68	40
6f	Et	4-$HOOCC_6H_4$	Cyclohexyl	Ph	38.37 ± 2.73		6.37	40
6g	Me	4-$HOOCC_6H_4$	Cyclohexyl	Me	24.21 ± 8.25		4.91	30
6h	Et	Ph	Cyclohexyl	Me	40.29 ± 8.99		5.35	30
6i	Et	Ph	HOC_2H_4	4-FC_6H_4	24.66 ± 8.95		4.07	40
6j	Et	Ph	HOC_2H_4	Ph	35.81 ± 2.89		3.93	40
6k	Et	4-MeC_6H_4	HOC_2H_4	Ph	33.27 ± 14.33		4.43	40
6l	Et	4-FC_6H_4	$PhCH_2$	Ph	36.31 ± 25.22		6.56	42
6m	Et	4-BrC_6H_4	$CH_3C_2H_4$	Ph	89.66 ± 5.40	>45	6.57	40
6n	Et	4-MeC_6H_4	4-MeC_6H_4	Ph	91.70 ± 4.18	26.80 ± 2.66	7.41	51

[a] Inhibition in a initial screening at the concentration of 20 μg/mL.
[b] Mean value ± standard deviation (at least three independent assays).
[c] Calculated log *P* value using ClogP program in Sybyl 7.3.
[d] ClogP error codes: 10 (trusted fragment value used), 30 (approximated fragment value used), 40 (a priori fragment value used), 42 (vinyl approximation based on priori value) and 51 (very high log *P* unrealistic in nature).
[e] Experimental log *P*.

*4.1.1.1. Methyl 2,5-dihydro-5-oxo-1-phenyl-4-(propylamino)-1H-pyrrole-3-carboxylate (**5e**).* 91% yield, White solid, mp = 87.0–88.0 °C; ^{1}H NMR (400 MHz, DMSO): δ = 7.80–7.77 (m, 2H), 7.47–7.38 (m, 2H), 7.26–7.17 (m, 1H), 4.44 (s, 2H), 3.91–3.80 (m, 5H), 1.70–1.64 (m, 2H), 1.01 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 164.80, 164.77, 139.06, 129.35, 125.27, 119.71, 96.33, 51.14, 48.31, 29.92, 24.72, 11.31 ppm; GC–MS: m/z = 274 (M^+); Anal. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21; O, 17.50; Found: C, 65.71; H, 6.53; N, 10.02.

*4.1.1.2. Ethyl 4-(cyclohexylamino)-2,5-dihydro-5-oxo-1-propyl-1H-pyrrole-3-carboxylate (**5l**).* 86% yield, yellow solid, mp = 64–67 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.75 (b, 1H), 4.595–4.591 (m, 1H), 4.20 (q, J = 7.2 Hz, 2H), 3.93 (s, 2H), 3.41 (t, J = 7.2 Hz, 2H), 1.98–1.96 (m, 2H), 1.73–1.67 (m, 11H), 0.92 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.4, 100.0, 59.4, 44.5, 34.9, 25.5, 24.7, 21.5, 14.6, 11.3 ppm; GC–MS: m/z = 294 (M^+); Anal. Calcd for $C_{16}H_{26}N_2O_3$; C, 65.28; H, 8.90; N, 9.52; O, 16.30; Found: C, 65.41; H, 8.79; N, 9.38.

*4.1.1.3. Ethyl 1-cyclohexyl-4-(cyclohexylamino)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate (**5m**).* 80% yield, White solid, mp = 101.5–104.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.62 (b, 1H), 4.48 (b, 1H), 4.09–4.08 (m, 2H), 3.89 (b, 1H), 3.80 (b, 2H), 1.87–1.28 (m, 10H), 1.19–1.05 (m, 13H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.6, 164.7, 96.4, 59.2, 51.1, 50.3, 43.4, 34.8, 31.0, 25.5, 25.4, 24.7, 14.5 ppm; GC–MS: m/z = 334 (M^+); Anal. Calcd for $C_{19}H_{30}N_2O_3$: C, 68.23; H, 9.04; N, 8.38; O, 14.35; Found: C, 68.10; H, 9.31; N, 8.17.

*4.1.1.4. Ethyl 1-allyl-4-(cyclohexylamino)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate (**5n**).* 54% yield, yellow solid, mp = 94.5–97.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.80–5.71 (m, 1H), 5.20–5.16 (m, 2H), 4.60–4.54 (m, 1H), 4.18 (q, J = 7.2 Hz, 2H), 4.04 (d, J = 6.0 Hz, 2H), 3.89 (s, 2H), 1.97–1.93 (m, 2H), 1.72–1.67 (m, 2H), 1.38–1.35 (m, 1H), 1.38–1.14 (m, 8H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.67, 165.30, 132.46, 120.05, 118.14, 96.75, 59.42, 50.40, 47.17, 45.45, 34.88, 25.55, 24.69, 14.55 ppm; GC–MS: m/z = 292 (M^+); Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58; O, 16.42; Found: C, 65.61; H, 8.12; N, 9.37.

*4.1.1.5. Ethyl 4-(cyclohexylamino)-2,5-dihydro-5-oxo-1-p-tolyl-1H-pyrrole-3-carboxylate (**5o**).* 87% yield, White solid, mp = 106.4–107.4 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.63–7.18 (m, 4H), 4.63–4.62 (m, 1H), 4.38 (s, 2H), 4.25 (q, J = 7.2 Hz, 2H), 2.34 (s, 3H), 2.03–2.00 (m, 2H), 1.76–1.19 (m, 11H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.3, 136.4, 134.7, 129.6, 119.6, 59.6, 50.6, 48.1, 34.8, 25.6, 24.7, 20.9, 14.6 ppm; GC–MS: m/z = 342 (M^+); Anal. Calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18; O, 14.02; Found: C, 70.01; H, 7.49; N, 8.32.

*4.1.1.6. Ethyl 4-(cyclohexylamino)-2,5-dihydro-1-(3,4-dimethylphenyl)-5-oxo-1H-pyrrole-3-carboxylate (**5p**).* 91% yield, White solid, mp = 113.9–114.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53–7.10 (m, 3H), 4.63–4.58 (m, 1H), 4.35 (s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 2.26 (s, 3H), 2.22 (s, 3H), 2.01–1.97 (m, 2H), 1.74–1.58 (m, 3H), 1.41–1.15 (m, 8H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.4, 164.2, 137.3, 136.5, 133.4, 130.0, 120.8, 119.5, 117.0, 94.3, 59.5, 50.5, 48.1, 34.8, 25.5, 24.6, 20.0, 19.2, 14.5 ppm; GC–MS: m/z = 356 (M^+); Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86; O, 13.47; Found: C, 70.93; H, 7.71; N, 7.69.

*4.1.1.7. Ethyl 4-(cyclohexylamino)-2,5-dihydro-1-(4-methoxyphenyl)-5-oxo-1H-pyrrole-3-carboxylate (**5q**).* 90% yield, White solid, mp = 101.8–102.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.64–6.91 (m, 4H), 4.62 (b, 1H), 4.37 (s, 2H), 4.25 (q, J = 7.2 Hz, 2H), 3.82 (s, 3H), 2.03–2.00 (m, 2H), 1.76–1.61 (m, 3H), 1.45–1.19 (m, 8H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.2, 156.9, 132.0, 121.5, 114.2, 59.6, 55.5, 50.5, 48.4, 34.8, 25.5, 24.7, 14.6 ppm; GC–MS: m/z = 358 (M^+); Anal. Calcd for $C_{20}H_{26}N_2O_4$: C, 67.02; H, 7.31; N, 7.82; O, 17.85; Found: C, 66.89; H, 7.50; N, 7.69.

*4.1.1.8. Ethyl 4-(cyclohexylamino)-2,5-dihydro-1-(naphthalen-1-yl)-5-oxo-1H-pyrrole-3-carboxylate (**5r**).* 45% yield, White solid, mp = 152.5–153.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.92–7.40 (m, 7H), 4.66–4.62 (s, 1H), 4.44 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 2.08–1.25 (m, 13H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.6, 134.6, 134.5, 130.1, 128.9, 128.6, 127.1, 126.5, 125.5, 125.3, 122.7, 97.8, 59.6, 51.4, 50.6, 45.1, 35.0, 25.5, 24.8, 14.6 ppm; ESI-MS: m/z = 379 ($M + H^+$); Anal. Calcd for $C_{23}H_{26}N_2O_3$: C, 72.99; H, 6.92; N, 7.40; O, 12.68; Found: C, 72.71; H, 6.99; N, 7.35.

*4.1.1.9. Ethyl 4-(cyclohexylamino)-2,5-dihydro-5-oxo-1-(pyridin-2-yl)-1H-pyrrole-3-carboxylate (**5s**).* 84% yield, White solid, mp = 94.5–96.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.47–7.07 (m, 4H), 4.64 (s, 2H), 4.58 (b, 1H), 4.25 (q, J = 7.2 Hz, 2H), 2.03–2.00 (m, 2H), 1.77–1.74 (m, 3H), 1.44–1.22 (m, 8H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.9, 165.0, 151.2, 147.9, 137.9, 120.0, 114.2, 98.3, 59.7, 50.6, 46.7, 34.9, 31.1, 25.5, 24.7, 14.5 ppm; GC–MS: m/z = 329 (M^+); Anal. Calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76; O, 14.57; Found: C, 65.43; H, 7.21; N, 12.58.

*4.1.1.10. Ethyl 1-(3-chlorophenyl)-4-(cyclohexylamino)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate (**5t**).* 91% yield, White solid, mp = 102.5–103.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.86–7.14 (m, 4H), 4.59 (b, 1H), 4.38 (s, 2H), 4.26 (q, J = 7.2 Hz, 2H), 2.01–1.99 (m, 2H), 1.76–1.61 (m, 3H), 1.45–1.19 (m, 8H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.6, 140.0, 134.9, 130.1, 124,9, 119.3, 117.1, 59.8, 50.6, 47.9, 34.9, 34.8, 25.5, 24.7, 14.6 ppm; GC–MS: m/z = 362 (M^+); Anal. Calcd for $C_{19}H_{23}ClN_2O_3$: C, 62.89; H, 6.39; Cl, 9.77; N, 7.72; O, 13.23; Found: C, 62.91; H, 6.29; N, 7.51.

*4.1.1.11. Ethyl 1-(4-chlorophenyl)-4-(cyclohexylamino)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate (**5u**).* 94% yield, White solid, mp = 91.5–92.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.72–7.30 (m, 4H), 4.58–4.53 (m, 1H), 4.35 (s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 2.00–1.96 (m, 2H), 1.74–1.69 (m, 3H), 1.40–1.15 (m, 8H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.3, 164.4, 137.3, 129.9, 129.0, 120.2, 96.2, 59.7, 50.5, 47.8, 34.7, 25.4, 24.6, 14.5 ppm; GC–MS: m/z = 362 (M^+); Anal. Calcd for $C_{19}H_{23}ClN_2O_3$: C, 62.89; H, 6.39; Cl, 9.77; N, 7.72; O, 13.23; Found: C, 62.81; H, 6.32; N, 7.61.

*4.1.1.12. Ethyl 1-(3,4-dichlorophenyl)-4-(cyclohexylamino)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate (**5v**).* 91% yield, White solid, mp = 114.5–115.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.99–7.42 (m, 3H), 4.58–4.56 (m, 1H), 4.36 (s, 2H), 4.26 (q, J = 7.2 Hz, 2H), 2.01–1.99 (m, 2H), 1.76–1.19 (m, 11H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.6, 133.1, 130.6, 120.7, 118.1, 100.0, 59.9, 50.7, 47.8, 34.8, 25.6, 25.5, 24.7, 14.6 ppm; GC–MS: m/z = 397 (M^+); Anal. Calcd for $C_{19}H_{22}Cl_2N_2O_3$: C, 57.44; H, 5.58; Cl, 17.85; N, 7.05; O, 12.08; Found: C, 57.18; H, 5.69; N, 7.31.

*4.1.1.13. Ethyl 1-(4-bromophenyl)-4-(cyclohexylamino)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate (**5w**).* 91% yield, White solid, mp = 107.0–108.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.67–7.46 (m, 4H), 4.56–4.55 (m, 2H), 4.36 (s, 2H), 4.24–4.22 (m, 2H), 1.99–1.96 (m, 2H), 1.74–1.15 (m, 11H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.4, 164.4, 137.9, 132.0, 120.6, 119.5, 117.7, 96.2, 59.7, 50.5, 47.8, 34.7, 25.4, 24.6, 14.5 ppm; GC–MS: m/z = 407 (M^+); Anal. Calcd for $C_{19}H_{23}BrN_2O_3$: C, 56.03; H, 5.69; Br, 19.62; N, 6.88; O, 11.78; Found: C, 56.23; H, 5.48; N, 6.69.

*4.1.1.14. Ethyl 1-(4-bromophenyl)-2,5-dihydro-4-morpholino-5-oxo-1H-pyrrole-3-carboxylate (**5y**).* 85% yield, White solid, mp = 151.0–152.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65–7.47 (m, 4H), 4.39 (s, 2H), 4.22 (q, J = 7.2 Hz, 2H), 3.80–3.75 (m, 8H), 1.31 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.6, 162.7, 146.5, 137.7, 132.1, 120.8, 117.9, 104.1, 67.5, 60.3, 50.2, 48.7, 14.5 ppm; GC–MS: m/z = 394 (M^+); Anal. Calcd for $C_{17}H_{19}BrN_2O_4$: C, 51.66; H, 4.85; Br, 20.22; N, 7.09; O, 16.19; Found: C, 51.39; H, 4.94; N, 7.15.

*4.1.2. General procedure for the preparation of tetrasubstituted polyfunctional dihydropyrroles **5** with aryl R^2 and R^3 (condition B) [24]*

Primary aromatic amines **3** (4 mmol), 38% formaldehyde **4a** (120 mg, 1.5 mmol) and HOAc (120 mg, 2 mmol) were dropwise added into the mixture of EtOH (4 mL), but-2-ynedioates **1** (1 mmol) and primary aromatic amines **2** (1 mmol) kept at room temperature for 30–60 min in sequence, followed the same steps described in general procedure A to afford the desired products in 85–89% yields (Table 1, **5g**, **5i**–**k**). For the characterization data of **5g**, **5i**–**k** see Ref. [24].

*4.1.3. General procedure for the preparation of pentasubstituted polyfunctional dihydropyrroles **6** with aryl/alky R^2 and alkyl R^3 (condition C) [24]*

Aldehydes **4b**–**d** (3 mmol) and primary aliphatic amines **3** (2 mmol) were dropwise added into the mixture of EtOH (4 mL), but-2-ynedioates **1** (1 mmol) and primary amines **2** (1 mmol) kept at room temperature for 30–60 min in sequence, followed with

stirring at rt or 70 °C for desired time (monitored by TLC). After completion of the reactions, the product mixture was purified by preparative TLC with *n*-hexane/ethyl acetate (10:1–1:1) as eluent to afford the desired products in 60–98% yields (Table 1, all of **6** with the exception of **6f**, **6g** and **6n**). For the characterization data of **6a**, **6b**, **6e**, **6h**–**6l** see Ref. [24].

*4.1.3.1. Ethyl 4-(4-bromophenylamino)-1-cyclohexyl-2,5-dihydro-5-oxo-2-phenyl-1H-pyrrole-3-carboxylate (**6c**).* 83% yield, Yellow solid, mp = 161.5–163.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.14 (s, 1H), 7.43–7.04 (m, 9H), 5.21 (s, 1H), 3.97 (t, J = 7.1 Hz, 2H), 3.67 (m, 1H), 1.84–1.58 (m, 7H), 1.21–1.06 (m, 3H), 0.97 (d, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.6, 164.4, 142.7, 137.9, 137.6, 131.3, 128.3, 128.2, 128.0, 124.2, 117.3, 110.5, 61.8, 60.0, 54.4, 30.9, 30.6, 26.0, 25.8, 25.2, 13.8 ppm; MS (ESI): m/z = 484 ($M + H^+$); Anal. Calcd for $C_{25}H_{27}BrN_2O_3$: C, 62.12; H, 5.63; Br, 16.53; N, 5.80; O, 9.93; Found: C, 62.35; H, 5.25; N, 5.69.

*4.1.3.2. Ethyl 1-cyclohexyl-2,5-dihydro-4-(naphthalen-1-ylamino)-5-oxo-2-phenyl-1H-pyrrole-3-carboxylate (**6d**).* 83% yield, Yellow solid, mp = 190.5–191.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) : δ = 8.40 (s, 1H), 8.11 (d, J = 8.3 Hz, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.49 (m, 3H), 7.40–7.30 (m, 6H), 5.26 (s, 1H), 3.89 (t, J = 7.1 Hz, 2H), 3.75–3.66 (m, 1H), 1.84–0.84 (m, 13H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.7, 144.2, 138.7, 134.7, 134.1, 129.1, 128.4, 128.2, 128.1, 128.0, 126.2, 126.0, 125.7, 125.1, 121.4, 109.3, 100.0, 61.7, 59.8, 54.2, 31.0, 30.7, 16.0, 25.8, 25.2, 13.7 ppm; MS (ESI): m/z = 455 ($M + H^+$). Anal. Calcd for $C_{29}H_{30}N_2O_3$: C, 76.63; H, 6.65; N, 6.16; O, 10.56; Found: C, 76.32; H, 6.71; N, 6.02.

*4.1.3.3. Ethyl 4-(4-bromophenylamino)-2,5-dihydro-5-oxo-2-phenyl-1-propyl-1H-pyrrole-3-carboxylate (**6m**).* 89% yield, White solid, mp = 134.0–135.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.23 (s, 1H), 7.46–7.40 (m, 2H), 7.38–7.32 (m, 3H), 7.24–7.19 (m, 2H), 7.10–7.05 (m, 2H), 5.17 (s, 1H), 4.06–3.97 (m, 2H), 3.64 (s, 1H), 2.66 (s, 1H), 1.51 (m, 2H), 1.00 (t, J = 7.1 Hz, 3H), 0.85 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.5, 142.9, 137.7, 136.4, 131.4, 128.6, 128.4, 127.7, 124.2, 117.4, 110.1, 62.1, 60.1, 42.2, 21.5, 13.8, 11.3 ppm; MS (ESI): m/z = 444 ($M + H^+$); Anal. Calcd for $C_{22}H_{23}BrN_2O_3$: C, 59.60; H, 5.23; Br, 18.02; N, 6.32; O, 10.83; found: C, 59.79; H, 5.12; N, 6.51.

*4.1.4. Procedure for the preparation of pentasubstituted polyfunctional dihydropyrroles **6** with $R^2 = R^3$ = aryl (condition D) [24]*

Benzaldehyde **4b** (3 mmol), aniline **3b** (2 mmol) and HOAc (120 mg, 2 mmol) were dropwise added into the mixture of EtOH (4 mL), ethyl but-2-ynedioate **1a** (1 mmol) and aniline **2b** (1 mmol) kept at room temperature for 30 min in sequence, followed the same steps described in general procedure C to afford the desired product in 92% yield (Table 1, **6n**). For the characterization data of **6n** see Ref.[24].

*4.1.5. Procedure for the preparation of pentasubstituted polyfunctional dihydropyrroles **6f** (condition E)*

The reaction mixture of diethyl but-2-ynedioate **1a** (255 mg, 1.5 mmol), 4-aminobenzoic acid **2j** (137 mg, 1 mmol) and $N(Et)_3$ (55 mg, 0.5 mmol) in MeOH (0.5 mL) kept at room for 1 h was added dropwise to the mixture of EtOH (5 mL), benzaldehyde **4b** (318 mg, 3 mmol) and cyclohexanamine **3a** (198 mg, 2 mmol), followed with stirring at room temperature for desired time (about 24 h, monitored by TLC). After completion of the reactions, the product mixture was purified by preparative TLC with *n*-hexane/ethyl acetate/HOAc (48:12:1) as eluent to afford the desired products in 76% yield (Table 1, **6f**).

*4.1.5.1. 4-(4-(Ethoxycarbonyl)-1-cyclohexyl-2,5-dihydro-2-oxo-5-phenyl-1H-pyrrol-3-ylamino)benzoic acid (**6f**).* 76% yield, white solid, mp = 218–222 °C; ^{1}H NMR (400 MHz, MeOH): δ = 7.92 (d, J = 8.7 Hz, 2H), 7.41–7.26 (m, 5H), 7.12 (d, J = 8.7 Hz, 2H), 5.40 (s, 1H), 3.87 (q, J = 7.2 Hz, 2H), 3.62 (m, 1H), 1.91–1.46 (m, 6H), 1.25–1.11 (m, 3H), 0.96 (m, 1H), 0.86 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (101 MHz, MeOH): δ = 145.87, 138.70, 131.30, 129.53, 129.33, 121.11, 63.81, 61.26, 56.00, 49.63, 49.42, 31.87, 31.50, 27.06, 26.91, 26.36, 14.03 ppm; MS (ESI): m/z = 449 ($M + H^+$); Anal. Calcd for $C_{26}H_{28}N_2O_5$: C, 69.63; H, 6.29; N, 6.25; O, 17.84; Found: C, 69.83; H, 6.32; N, 6.18.

*4.1.6. Procedure for the preparation of pentasubstituted polyfunctional dihydropyrroles **6g** (condition F)*

The reaction mixture of diethyl but-2-ynedioate **1a** (142 mg, 1 mmol), 4-aminobenzoic acid **2r** (205 mg, 1.5 mmol) and $N(Et)_3$ (55 mg, 0.5 mmol) in MeOH (0.5 mL) kept at room for 1 h was added dropwise to the mixture of EtOH (5 mL), acetaldehyde **4c** (318 mg, 3 mmol) and cyclohexanamine **3a** (198 mg, 2 mmol), followed with stirring at room temperature for desired time (about 24 h, monitored by TLC). After completion of the reactions, the product mixture was purified by preparative TLC with *n*-hexane/ethyl acetate/HOAc (48:12:1) as eluent to afford the desired products in 79% yield (Table 1, **6g**).

*4.1.6.1. 4-(4-(Methoxycarbonyl)-1-cyclohexyl-2,5-dihydro-5-methyl-2-oxo-1H-pyrrol-3-ylamino)benzoic acid (**6g**).* 79% yield, white solid, mp = 223–225 °C; ^{1}H NMR (400 MHz, MeOH): δ = 7.90 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 8.4 Hz, 1H), 4.41 (q, J = 6.4 Hz, 0H), 3.71–3.60 (m, 2H), 2.06–1.62 (m, 8H), 1.51 (d, J = 6.4 Hz, 2H), 1.33 (ddd, J = 56.8, 28.5, 8.1 Hz, 6H) ppm; ^{13}C NMR (101 MHz, MeOH): δ = 165.99, 165.64, 145.82, 142.51, 131.23, 121.11, 120.99, 115.91, 56.41, 55.76, 51.69, 31.83, 31.29, 27.15, 27.03, 26.49, 19.90 ppm; MS (ESI): m/z = 373 ($M + H^+$); Anal. Calcd for $C_{20}H_{24}N_2O_5$: C, 64.50; H, 6.50; N, 7.52; O, 21.48; Found: C, 64.12; H, 6.30; N, 7.43.

4.2. Caspase-3 assays

Recombinant human caspase-3 catalytic domain was prepared according to our previous work [14] with minor modification. The typical assay of caspase-3 was carried out in a 100 μL system including 50 mM HEPES pH 7.5, 150 mM NaCl, 2 mM dithiothreitol, 1 mM EDTA, 100 μM Ac-DEVD-pNA (pNA, *p*-nitroaniline) and 20 nM caspase-3 in the presence or absence of 2 μL of inhibitor in DMSO. In screening, the enzyme was incubated with inhibitors for 30 min. The rate of hydrolysis product pNA was monitored continuously by change of absorbance at 405 nm for 3 min, and the initial rate of hydrolysis was determined using the early linear region of the enzymatic reaction curve. Compounds were tested in duplicate, and IC_{50} curves were calculated for all inhibitors assayed. Final IC_{50} values were the average of three independent experiments.

4.3. Shake-flask determination of partition coefficients

The octanol–water partition coefficients (log *P*) were measured using the shake-flask technique as described in references [30,33] with minor difference. Briefly, 0.10 M phosphate buffer (pH = 7) and sample in buffer-saturated octanol (1 mM) were prepared. Then, 0.5 mL of sample in buffer-saturated octanol was added to 20 mL octanol-saturated buffer. The partitioning was carried out in 50 ml centrifuge tubes. The two phases were mutually saturated by 100 inversions in roughly 5 min at room temperature [30]. Then, the phases were allowed to separate after centrifugation at 3000 rpm for 5 min (Eppendorf 5424 centrifuge). Sample concentrations in octanol phase were determined.

Thirty μL of samples in octanol phase were diluted to 3 mL with buffer-saturated octanol. The absorption spectra of samples were determined by TU-1901 UV–vis spectrophotometer. The concentration measurements were performed by HPLC using a liquid chromatograph Waters 2695 separation module equipped with a Waters 2996 photodiode array detector. The column was a Phenomenex P/NO.006-4337-E0 (4 μm, 4.60 × 250 mm). The mobile phase was the mixture of 90% methanol and 10% water, and four samples had retention times of 6.45–11.88 min.

Different concentrations of sample (10, 20, 40, 60, 80, 100 and 120 μM) were prepared by diluting 1 mM of sample solutions with buffer-saturated octanol and determined by HPLC under the above mentioned conditions. The correlation coefficients R^2 of linear equations for the stand curves of four samples are 0.9997–0.9999. The concentration of sample in octanol phase was calculated using the linear equation.

Acknowledgments

We thank the National Natural Foundation of China (U0832001), National Basic Research Program of China (973 Program 2011CB808603), Guangdong Natural Science Foundation (10351086000000) and Guangdong Science and Technology Project (2008B060600066) for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2012.05.001. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] C.B. Thompson, Science 267 (1995) 1456–1462.
[2] D.W. Nicholson, Nature 407 (2000) 810–816.
[3] H.U. Simon, Apoptosis 14 (2009) 439–446.
[4] G.I. Evan, K.H. Vousden, Proliferation, Nature 411 (2001) 342–348.
[5] N. Bulata, C. Widmanna, Brain Res. Bull. 80 (2009) 251–267.
[6] B. Geering, H.-U. Simon, Cell Death Differ. 18 (2011) 1457–1469.
[7] W.C. Earnshaw, L.M. Martins, S.H. Kaufmann, Rev. Biochem. 68 (1999) 383–424.
[8] R.M. Siegel, Nat. Rev. Immunol. 6 (2006) 308–317.
[9] C.H. Yi, J. Yuan, Dev. Cell 16 (2009) 21–34.
[10] S. Kumar, Cell Death Differ. 14 (2007) 32–43.
[11] J.W. Becker, J. Rotonda, S.M. Soisson, R. Aspiotis, C. Bayly, S. Francoeur, M. Gallant, M. Garcia-Calvo, A. Giroux, E. Grimm, Y. Han, D. McKay, D.W. Nicholson, E. Peterson, J. Renaud, S. Roy, N. Thornberry, R. Zamboni, J. Med. Chem. 47 (2004) 2466–2474.
[12] M.F. Schmidt, A. El-Dahshan, S. Keller, J. Rademann, Angew. Chem. Int. Ed. 48 (2009) 6346–6349.
[13] D. Lee, S.A. Long, J.L. Adams, G. Chan, K.S. Vaidya, T.A. Francis, K. Kikly, J.D. Winkler, C.-M. Sung, C. Debouck, S. Richardson, M.A. Levy, W.E. DeWolf Jr., P.M. Keller, T. Tomaszek, M.S. Head, M.D. Ryan, R.C. Haltiwanger, P.-H. Liang, C.A. Janson, P.J. McDevitt, K. Johanson, N.O. Concha, W. Chan, S.S. Abdel-Meguid, A.M. Badger, M.W. Lark, D.P. Nadeau, L.J. Suva, M. Gowen, M.E. Nuttall, J. Biol. Chem. 275 (2000) 16007–16014.
[14] Y.H. Chen, Y.H. Zhang, H.J. Zhang, D.Z. Liu, M. Gu, J.Y. Li, F. Wu, X.Z. Zhu, J. Li, F.J. Nan, J. Med. Chem. 49 (2006) 1613–1623.
[15] J. Sakai, A. Yoshimori, Y. Nose, A. Mizoroki, N. Okita, R. Takasawaa, S. Tanuma, Bioorg. Med. Chem. 16 (2008) 4854–4859.
[16] W. Chu, J. Zhang, C. Zeng, J. Rothfuss, Z. Tu, Y. Chu, D.E. Reichert, M.J. Welch, R.H. Mach, J. Med. Chem. 48 (2005) 7637–7647.
[17] J.E. Biggs-Houck, A. Younai, J.T. Shaw, Curr. Opin. Chem. Biol. 14 (2010) 371–382.
[18] L.A. Marcaurelle, M.A. Foley, Curr. Opin. Chem. Biol. 14 (2010) 285–288.
[19] N. Dahan-Farkas, C. Langley, A.L. Rousseau, D.B. Yadav, H. Davids, C.B. de Koning, Eur. J. Med. Chem. 46 (2011) 4573–4583.
[20] M. Zhang, H.F. Jiang, H. Liu, Q.H. Zhu, Org. Lett. 9 (2007) 4111–4113.
[21] H. Cao, X.J. Wang, H.F. Jiang, Q.H. Zhu, M. Zhang, X.H. Liu, Chem. Eur. J. 14 (2008) 11623–11633.
[22] H.F. Jiang, R.H. Mai, H. Cao, Q.H. Zhu, X.H. Liu, Org. Biomol. Chem. 7 (2009) 4943–4953.
[23] H.F. Jiang, Q.H. Zhu, S.W. Liu, M. Zhang, (2009) CN 101497580.
[24] Q.H. Zhu, H.F. Jiang, J.H. Li, S.W. Liu, C.L. Xia, M. Zhang, J. Comb. Chem. 11 (2009) 685–696.
[25] Q.H. Zhu, H.F. Jiang, J.H. Li, S.W. Liu, M. Zhang, X.J. Wang, C.R. Qi, Tetrahedron 65 (2009) 4604–4613.
[26] H. Jiang, Q. Zhu, J. Li, L. Gao. (2010) CN 101838260A.
[27] W. Chu, J. Rothfuss, A. d'Avignon, C. Zeng, D. Zhou, R.S. Hotchkiss, R.H. Mach, J. Med. Chem. 50 (2007) 3751–3755.
[28] P.M.C. Glória, I. Coutinho, L.M. Gonçalves, C. Baptista, J. Soares, A.S. Newton, R. Moreira, L. Saraiva, M.M.M. Santos, Eur. J. Med. Chem. 46 (2011) 2141–2146.
[29] J.C. Powers, J.L. Asgian, O.D. Ekici, K.E. James, Chem. Rev. 102 (2002) 4639–4750.
[30] A. Leo, C. Hansch, D. Elkins, Chem. Rev. 71 (1971) 525–616.
[31] D.B. Larsen, H. Parshad, K. Fredholt, C. Larsen, Int. J. Pharm. 232 (2002) 107–117.
[32] S. Mitragotri, J. Controll. Release 71 (2001) 23–29.
[33] X. Liu, G. Bouchard, H.H. Girault, B. Testa, P.-A. Carrupt, Anal. Chem. 75 (2003) 7036–7039.
[34] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliver. Rev. 46 (2001) 3–26.