Bioorganic & Medicinal Chemistry Letters 20 (2010) 4116–4119

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Recombination of diterpenoid structure units: Synthesis of antitumor amides bearing functionalized bicyclo[3.2.1]octane ring

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ARTICLE INFO

Article history:
Received 18 March 2010
Revised 14 May 2010
Accepted 18 May 2010
Available online 8 June 2010

Keywords:
Amide
Diterpenoid structure unit
Bicyclo[3.2.1]octane ring system
Antitumor activity

ABSTRACT

In this work, 23 new amides (**14**–**36**) bearing a representative diterpenoid structure unit, the functionalized bicyclo[3.2.1]octane ring, have been synthesized and its antitumor potential is studied. In vitro studies demonstrate that a number of amides with the bicyclo[3.2.1]oct-3-en-2-one subunit are active against HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 tumor cell lines. The hybrid derivative, compound **20**, was found to be the most potent compound (IC_{50} = 1.05 μM against HL-60) and more active than cisplatin (DDP), the positive control. Additionally, compound **20** exhibited broad spectrum in vitro anticancer activity with IC_{50} values of 1.1–4.3 μM against the five tested cancer cell lines.

Natural diterpenes constitute a large family of important bioactive compounds. Among them, the tetracyclic *ent*-kaurenoids are important source of antitumor agents and have attracted the attention of numerous research groups.[1] A number of antitumor *ent*-kaurenoid diterpenes (Fig. 1) have been reported in recent years, including eriocalyxin B (**1**),[2] oridonin (**2**),[3] and ludongnin J (**3**).[4] A representative structure unit presented in those diterpenoid compounds is the functionalized bridged C/D bicyclo[3.2.1]octane ring system. In the preceding Letter[5] we reported the synthesis and biological evaluation of a series of gibberellin derivatives and found that gibberellins bearing two α,β-unsaturated ketone units are potent antitumor agents. It is noteworthy that a functionalized bicyclo[3.2.1]octane carbon framework is also presented in our antitumor gibberellins. As an especially interesting example, the highly bioactive platensimycin (Fig. 1, compound **4**), an amide derived from bacterial oxidation of *ent*-kaurene,[6] is also endowed with the bicyclo[3.2.1]octane skeleton (Fig. 1). Because the structure of platensimycin can be viewed as a hybrid of diterpenoid structure unit with an amine by an amide linkage, we were inspired to initiate a research program towards the recombination of diterpenoid structure unit, with the aim of generation of antitumor amides on the basis of the functionalized bicyclo[3.2.1]octane ring system (Scheme 1). Herein we report the synthesis of bicyclo[3.2.1]oct-3-en-2-one ring containing amides and its in vitro cytotoxic properties against a number of human tumor cell lines.

The synthesis of amides containing the functionalized bicycle[3.2.1]octane framework commenced with protection of 1,3-cyclohexandione. Treatment of commercially available 1,3-cyclohexandione with isopropanol in reflux benzene in the presence of *p*-toluene sulfonic acid resulted in the 3-isoproxyl-2-cyclohexenone (**6**). Enolization of compound **6** with lithium diisopropyl amide (LDA) followed by carbonylation with methyl chloroformate provided intermediate **7** (82%). Allylation of compound **7** in the presence of sodium hydride afforded compound **8** (91%). After reduction with lith-

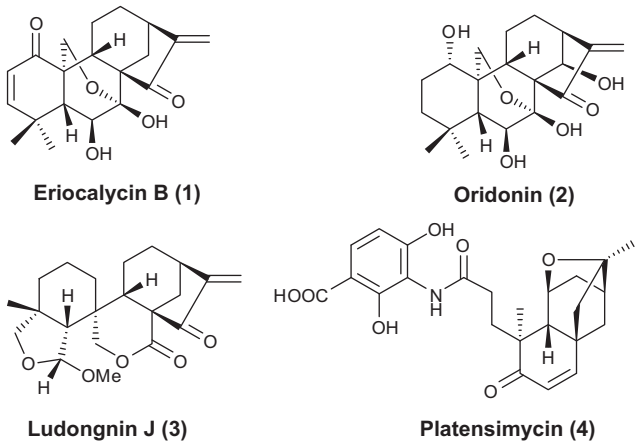

Figure 1. Representative molecules of tetracyclic diterpenes (**1–3**) and platensimycin (**4**).

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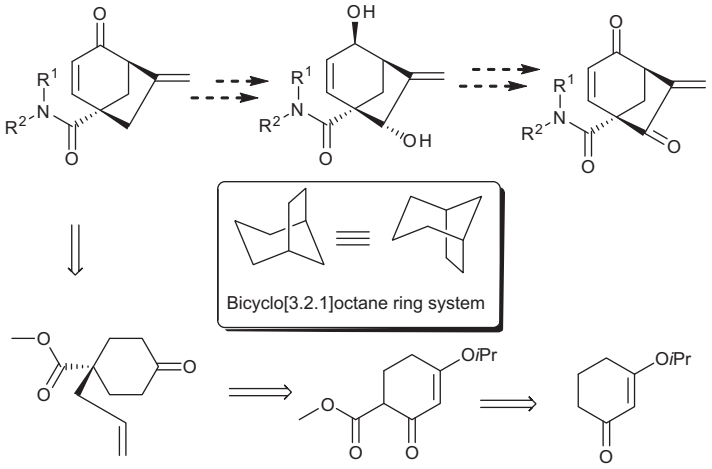
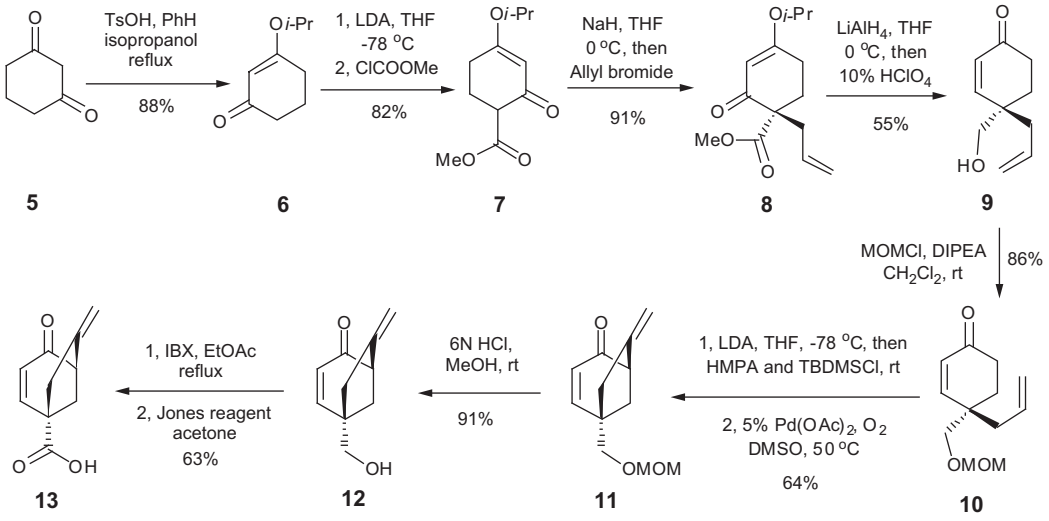
doi:10.1016/j.bmcl.2010.05.075

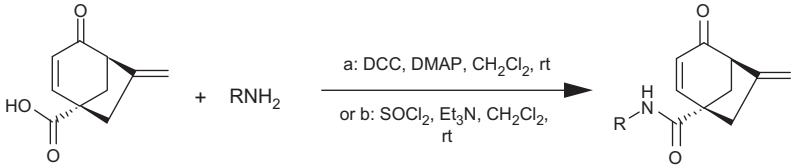


Scheme 1. Synthetic plan towards amides bearing a functionalized bicyclo[3.2.1]octane ring.



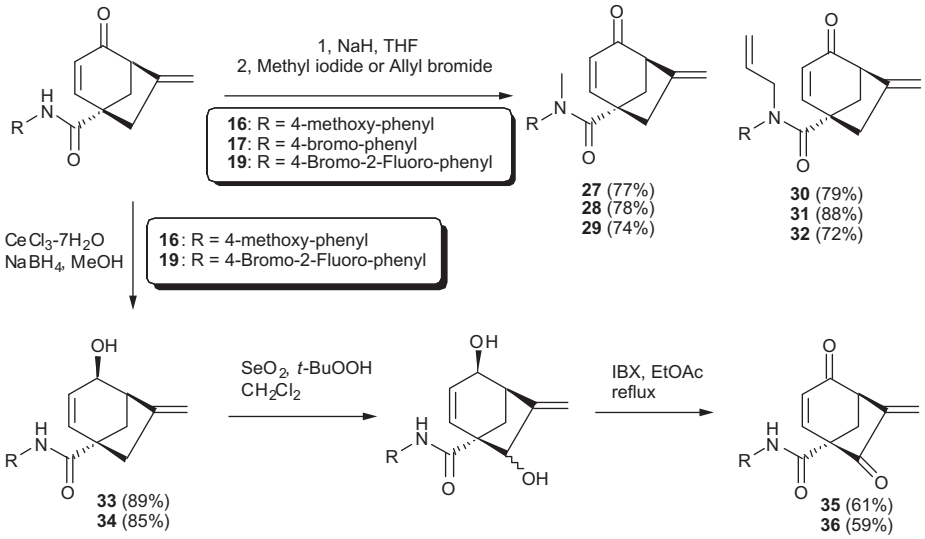
Scheme 2. Synthesis of 6-methylene-4-oxobicyclo[3.2.1]oct-2-ene-1-carboxylic acid **13**.

Table 1
Synthesis of primary amides bearing functionalized bicycle[3.2.1]octane ring[a,b]



Amide	R	Yield (%)
14	2-(4-Methoxy-phenyl)-ethyl	79[a]
15	Phenyl	72[a]
16	4-Methoxy-phenyl	81[a]
17	4-Bromo-phenyl	82[a]
18	2-Bromo-4-methyl-phenyl	71[a]
19	4-Bromo-2-fluoro-phenyl	69[a]
20	3,4-Dichloro-phenyl	67[b]
21	2-Chloro-phenyl	73[b]
22	3-Methoxy-phenyl	77[b]
23	2,4,6-Trimethyl-phenyl	81[b]
24	2,6-Dimethyl-4-bromo-phenyl	78[b]
25	4-Acetyl-phenyl	62[b]
26	*t*-Butyl	79[b]

[a] Method a: Treatment of acid with DCC in the presence of DMAP in dichloromethane for 30 min (rt) then addition of amine at room temperature; Method b: Treatment of acid with $SOCl_2$ in dichloromethane for 30 min then addition of amine and Et_3N at room temperature.
[b] Yields represent isolated yields.



Scheme 3. Synthesis of amides bearing functionalized bicycle[3.2.1]octane ring.

Table 2
In vitro cytotoxic activities of amides **17–41** (IC_{50}, μM)[a,b]

Amides	Cell lines				
	HL-60	SMMC-7721	A-549	SK-BR-3	PANC-1
14	>40	>40	>40	>40	>40
15	16.59 ± 0.43	>40	>40	>40	>40
16	13.09 ± 0.45	>40	>40	>40	>40
17	2.80 ± 0.42	3.78 ± 0.60	4.62 ± 1.79	10.03 ± 1.40	7.66 ± 0.05
18	4.40 ± 2.20	5.74 ± 0.91	15.49 ± 1.06	15.51 ± 0.24	15.14 ± 0.46
19	3.45 ± 0.10	5.41 ± 1.52	11.87 ± 5.10	11.27 ± 0.25	7.10 ± 2.12
20	1.05 ± 0.65	1.19 ± 0.19	3.16 ± 0.68	4.30 ± 0.26	3.14 ± 0.18
21	6.85 ± 0.35	>40	>40	>40	19.80 ± 0.35
22	>40	>40	>40	>40	>40
23	14.11 ± 0.35	>40	>40	>40	15.22 ± 1.25
24	8.44 ± 3.18	6.08 ± 1.03	16.64 ± 0.57	14.42 ± 3.79	16.63 ± 0.22
25	13.6 ± 0.25	>40	>40	>40	>40
26	>40	>40	>40	>40	>40
27	>40	>40	>40	>40	>40
28	>40	>40	>40	>40	>40
29	>40	>40	>40	>40	>40
30	20.51 ± 0.45	>40	>40	>40	>40
31	>40	>40	>40	>40	>40
32	15.20 ± 0.15	22.10 ± 1.35	>40	>40	>40
33	>40	>40	>40	>40	>40
34	>40	>40	>40	>40	>40
35	>40	>40	>40	>40	>40
36	>40	>40	>40	>40	>40
DDP	1.42 ± 0.35	14.31 ± 1.30	15.23 ± 0.35	14.77 ± 0.54	21.87 ± 6.62
1	0.66 ± 0.14	0.43 ± 0.27	1.40 ± 0.81	0.73 ± 0.21	0.74 ± 0.04

[a] Cytotoxicity as IC_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 570 nm relative to untreated cells using the MTT assay.
[b] Human promyelocytic leukemia (HL-60), human hepatocellular carcinoma (SMMC-7721), human lung adenocarcinoma (A-549), human breast adenocarcinoma (SK-BR-3), human pancreatic carcinoma (PANC-1).

ium aluminium hydride and subsequent acidification with perchloric acid, cyclohexenone **9** was obtained in 55% yield. Following the well established Ihara's protocol,[7] the key intermediate (**11**) was prepared in 64% yield over two steps (Scheme 2). Acid catalyzed hydrolysis of **11** resulted in alcohol **12** (91%), which provided acid **13** (63% in two steps) when exposed to oxidation first with IBX (*o*-iodoxybenzoic acid) then with John's reagent.[8]

With the desired acid (**13**) in hand, we began the synthesis of amides by treatment of acid **13** with a number of commercially available amines.[9] The synthesized amides are summarized in Table 1.

To get further insight toward the structure and activity relationship, a few tertiary amides (**27–32**) were synthesized (Scheme 3). Two amides (**33**, **34**), without the α,β-unsaturated ketone system, were also prepared by reduction of compound **16** and **19**, respectively under Luche reduction condition.[10] Two amides (**35** and **36**) containing two α,β-unsaturated ketone moiety were also prepared (Scheme 3) from the corresponding amide **33** and **34** by

allylic hydroxylation with selenium dioxide (SeO_2, CH_2Cl_2, *t*-BuOOH) and finally oxidation with IBX (IBX, EtOAc).

Next, the cytotoxic properties of all newly synthesized amides were evaluated in vitro against five human tumor cell lines (including HL-60, SMMC-7721, A-549, SK-BR-3, PANC-1) by MTT assay,[11] cisplatin (DDP) and eriocalycin B (**1**) were used as reference drugs. The results are summarized in Table 2 (IC_{50} value, defined as the concentrations corresponding to 50% growth inhibition). To our delight, compound **17** (IC_{50} = 2.80 ± 0.42 μM), **18** (IC_{50} = 4.41 ± 2.20 μM), **19** (IC_{50} = 3.45 ± 0.10 μM), **20** (IC_{50} = 1.05 ± 0.65 μM), **21** (IC_{50} = 6.85 ± 0.35 μM) and **24** (IC_{50} = 8.44 ± 3.18 μM) showed potential activities against myeloid leukaemia (HL-60); Compound **20** displayed potent or similar cytotoxic activity in vitro compared to DDP and eriocalycin B, and also possessed potent activities against SMMC-7721, A-549, SK-BR-3 and PANC-1 cells. Although secondary amides (compound **15–25**, except amide **22**) derived from aniline derivatives were shown to have certain level of activity against myeloid leukaemia (HL-60), secondary amide **14** and **26**, prepared from alkyl amines, were both inactive in this assay. In comparison with secondary amides, tertiary amides (**27–32**) were generally less potent than its secondary amide counterparts (**16**, **17** and **19**) against all tumor cell lines investigated. The loss of potency in tertiary amides is possibly due to the loss of a hydrogen bond donor (CONH bond). The fact that compound **33** and **34** were both inactive suggests the requirement of an α,β-unsaturated ketone moiety in the bicyclo[3.2.1]octane ring system (see Table 2). To our surprise, however, presence of two α,β-unsaturated ketone moiety did not appear to improve potency (Table 2, compound **35** and **36**). Even though the results are preliminary, we found a clear trend in the structure–activity relationship: the α,β-unsaturated ketone moiety is necessary for potency, with the best result being coupled with halogen containing anilines. Among the five tested cell lines, HL-60 exhibited more sensitivity to the rest of tumor cell lines. Particularly, HL-60 cells and SMMC-7721 cells were 3–4-fold more sensitive to amide **20** than the rest of tumor cell lines. Compound **20** was shown to have promising antiproliferative activities against a broad spectrum of human tumor cell lines from a diverse set of target organs, including leukemia and solid tumors (liver, lung, breast and pancreas cancer cell lines).

In conclusion, we have designed and synthesized a number of amides bearing bicycle[3.2.1]octane ring system. We also evaluated their antitumor activities by in vitro MTT assay. This study has revealed that the bicyclo[3.2.1]oct-3-en-2-one moiety is a potential cytotoxic pharmacophore, in particular, the amide **20** is a promising lead due to the cytotoxic potencies displayed. It is also of interests to note that amides bearing bicyclo[3.2.1]oct-3-en-2-one moiety are more selective towards HL-60 cell line. Further biological investigation is currently underway in our laboratory and the results will be reported in due course.

Acknowledgments

This work was supported by the Natural Science Foundation of China (20832005, 20925205), the National Basic Research Program of China (973 Program 2009CB522300) and the Natural Science Foundation of Yunnan Province (2007B0006Z).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.075.

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8. Acid **13**: colorless oil, ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.98 (s, 1H), 7.48–7.51 (dd, *J* = 1.5 Hz, 1.5 Hz, 1H), 5.90–5.93 (dd, *J* = 1.5 Hz, 1.5 Hz, 1H), 5.34 (s, 1H), 5.13 (s, 1H), 3.58 (d, *J* = 1.6 Hz, 1H), 2.97–3.03 (m, 1H), 2.60 (d, *J* = 15.8 Hz, 1H), 2.31–2.41 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 197.8, 178.6, 151.8, 142.7, 127.0, 113.7, 57.9, 51.6, 43.4, 41.3. EIMS *m*/*z* (%): 179 (M^++1, 21%), 178 (M^+, 68%), 160 (50), 150 (51), 133 (72), 132 (68), 105 (100), 91 (58), 79 (74), 77 (76). HRMS *m*/*z* Found: 178.0626, Calcd for $C_{10}H_{10}O_3$ (M^+: 178.0630.
9. Amide **17**: White powder, mp 133.1–134.2 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.01 (s, 1H), 7.38–7.45 (m, 5H), 5.88 (dd, *J* = 1.3, 9.9 Hz, 1H), 5.29 (s, 1H), 5.12 (s, 1H), 3.52 (d, *J* = 4.5 Hz, 1H), 3.00 (dt, *J* = 2.4, 16.0 Hz, 1H), 2.64 (d, *J* = 16.0 Hz, 1H), 2.36 (dd, *J* = 2.1, 10.8 Hz, 1H), 2.25–2.33 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 197.3, 170.7, 152.8, 142.6, 136.6, 132.0, 127.4, 122.2, 117.7, 113.8, 58.1, 53.8, 42.9, 40.4. EIMS *m*/*z* (%): 333 (M^++2, 74%), 331 (M^+, 74%), 305 (10), 303 (11), 288 (5), 280 (13), 278 (14), 250 (2), 224 (4), 199 (5), 198 (5), 172 (23), 170 (23), 160 (19), 145 (10), 133 (61), 119 (25), 105 (100), 91 (66), 79 (51), 77 (77). HRMS *m*/*z* Found: 331.0175, Calcd for $C_{16}H_{14}NO_2Br$ (M^+: 331.0208. Amide **18**: White plates, mp 146.2–146.9 °C ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.15 (d, *J* = 8.4 Hz, 1H), 7.82 (s, 1H), 7.46 (dd, *J* = 2.0, 9.9 Hz, 1H), 7.37 (d, *J* = 1.2 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 5.97 (dd, *J* = 1.3, 9.9 Hz, 1H), 5.36 (s, 1H), 5.17 (s, 1H), 3.61 (d, *J* = 4.8 Hz, 1H), 3.04 (dt, *J* = 2.4, 16.0 Hz, 1H), 2.71 (d, *J* = 16.0 Hz, 1H), 2.46 (dd, *J* = 2.1, 10.8 Hz, 1H), 2.30–2.40 (m, 1H), 2.31 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 196.7, 170.4, 152.0, 142.6, 136.1, 132.6, 132.4, 129.2, 127.7, 122.1, 114.0, 113.8, 58.0, 54.0, 43.0, 40.6, 20.6. EIMS *m*/*z* (%): 347 (M^++2, 84%), 345 (M^+, 84%), 332 (2), 330 (2), 319 (27), 317 (27), 303 (27), 294 (37), 292 (38), 267 (22), 266 (80), 238 (34), 212 (17), 210 (15), 187 (51), 185 (57), 184 (36), 161 (32), 160 (40), 149 (12), 134 (59), 133 (85), 132 (50), 119 (48), 105 (100), 104 (57), 91 (53), 78 (68), 77 (85). HRMS *m*/*z* Found: 345.0370, Calcd for $C_{17}H_{16}NBrO_2$ (M^+: 345.0364. Amide **19**: grey powder, mp 123.2 °C ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.18 (t, *J* = 8.4 Hz, 1H), 7.58 (s, 1H), 7.43 (dd, *J* = 2.1, 9.9 Hz, 1H), 7.27–7.33 (m, 2H), 5.98 (dd, *J* = 1.5, 9.9 Hz, 1H), 5.37 (s, 1H), 5.18 (s, 1H), 3.61 (d, *J* = 4.8 Hz, 1H), 3.04 (dt, *J* = 2.4, 16.0 Hz, 1H), 2.71 (dd, *J* = 1.2, 16.0 Hz, 1H), 2.44 (dd, *J* = 2.1, 10.8 Hz, 1H), 2.30–2.40 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 196.5, 170.6, 154.1 (150.8), 151.5, 142.3, 127.9, 125.1 (124.9), 123.3, 118.8, 118.5, 116.9 (116.8), 114.0, 57.9, 53.9, 42.9, 40.6. EIMS *m*/*z* (%): 351 (M^++2, 80%), 349 (M^+, 80%), 336 (10), 334 (11), 323 (32), 321 (32), 308 (17), 306 (18), 298 (36), 296 (37), 270 (12), 268 (9), 256 (2), 242 (8), 228 (3), 217 (11), 216 (11), 202 (7), 190 (43), 189 (46), 188 (45), 161 (93), 160 (50), 149 (27), 134 (51), 133 (85), 132 (51), 119 (51), 105 (100), 103 (56), 91 (54), 81 (39), 79 (72), 77 (80). HRMS *m*/*z* Found: 349.0109, Calcd for $C_{16}H_{13}NBrFO_2$ (M^+: 349.0114. Amide **20**: pale pink plates, mp 135.4–136.8 °C ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.97 (s, 1H), 7.76 (d, *J* = 2.1 Hz, 1H), 7.34–7.45 (m, 3H), 5.91 (dd, *J* = 1.5, 9.9 Hz, 1H), 5.31 (s, 1H), 5.15 (s, 1H), 3.55 (d, *J* = 4.6 Hz, 1H), 3.03 (dt, *J* = 2.4, 16.0 Hz, 1H), 2.67 (d, *J* = 16.0 Hz, 1H), 2.28–2.42 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 197.2, 170.8, 152.4, 142.3, 136.9, 132.8, 130.5, 128.2, 127.5, 122.2, 119.7, 114.0, 58.0, 53.8, 42.9, 40.4. EIMS *m*/*z* (%): 323 (M^++2, 78%), 322 (M^++1, 42%), 321 (M^+, 86%), 308 (6), 306 (9), 295 (29), 293 (40), 280 (13), 278 (22), 270 (30), 268 (40), 253 (5), 242 (8), 240 (11), 213 (11), 188 (10), 187 (9), 163 (30), 162 (32), 161 (91), 160 (79), 149 (23), 145 (21), 133 (100), 132 (55), 125 (18), 119 (50), 109 (17), 105 (94), 103 (49), 91 (52), 79 (67), 77 (75). HRMS *m*/*z* Found: 321.0329, Calcd for $C_{16}H_{13}NCl_2O_2$ (M^+: 321.0323.
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11. The cytotoxicity assay was in five kinds of cell lines (HL-60, SMMC-7721, A-549, SK-BR-3, PANC-1). Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal serum and dispersed in replicate 96-well plates. Compounds were then added. After 48-h exposure to the compounds, cells viability were determined by the [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) cytotoxicity assay by measuring the absorbance at 570 nm with a microplate spectrophotometer. Each test was performed in triplicate.