Bioorganic & Medicinal Chemistry xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Small molecular inhibitors of miR-1 identified from photocycloadducts of acetylenes with 2-methoxy-1,4-naphthalenequinone

Su-Bee Tan [a], Chengmei Huang [b], Xuejiao Chen [b], Yihan Wu [b], Mi Zhou [b], Chenyu Zhang [a,*], Yan Zhang [b,*]

[a] School of Life Science, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, PR China
[b] School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Inhibitor of miR-1
Photocycloaddition
2-Methoxy-1,4-naphthalenequinone
Acetylene
Cyclobutene

ABSTRACT

Small molecules which can modulate endogenous microRNAs are important chemical tools to study microRNA regulational network. In this Letter we screened the [2+2] photocycloadducts of 2-methoxy-1,4-naphthalenequinone with a series of aryl acetylenes on their activity to modulate endogenous microRNAs. A potent inhibitor of the muscle-specific miR-1 which is closely related with cardiac development and disease was identified. The small molecular inhibitor was the cyclobutene type product derived from the photocycloaddition of 2-methoxy-1,4-naphthalenequinone with *tert*-butyl (5-(phenylethynyl)quinolin-8-yl) carbonate. Analogues of the small molecular inhibitor were then prepared using similar photocycloaddition reactions for evaluation on inhibition activity on miR-1 to provide structure–activity relationship of the miR-1 inhibitor.

1. Introduction

MicroRNAs (miRNAs) are a class of endogeneous noncoding small RNA which have emerged recently as a new class of modulators of gene expression at the post-transcriptional level.[1,2] The biogenesis pathway of mature miRNA from primary miRNAs has been elucidated.[2] Mature miRNAs can silence their target mRNA through binding to its 3′ untranslated region (3′-UTR) upon loading into the RNA-induced silencing complex (RISC) to repress mRNA transcription or to trigger mRNA degradation.[3] Over the past several years, more and more evidences have indicated that dysregulation in the expression of miRNAs contributes to the pathogenesis of many human diseases.[4,5] For example, miRNAs such as miR-21,[6–8] miR-25, miR-214, miR-221,[9,10] miR-17-92 cluster,[11,12] were found to overexpress in various tumor tissue, while miRNAs such as miR-34,[13] miR-15, miR-16,[14,15] are down-regulated in tumorgenesis.

The regulation of gene expression by miRNAs which are abundant in specific tissues also plays fundamental roles in diverse biological processes including cell proliferation and differentiation, apoptosis, immune response, etc.[2] For example, miR-1 is enriched in skeletal muscle and is believed to mediate myogenesis, muscle development and heart development.[16–19] Due to the wide regulatory functions of miRNAs on gene expression, they have become attractive biomarkers and therapeutic targets for various diseases.[20–22]

Small molecules which can regulate the expression or function of miRNAs are important tools for elucidating the regulatory mechanism of miRNAs and hold the promise to develop novel therapeutic agents. By far only a few type of small molecules of miRNAs have been identified.[23] Dieters et al. identified small molecules that can specifically regulate the expression of miR-21 or miR-122 from established chemical libraries.[24,25] Maiti and co-workers, discovered a tuberculosis drug, streptomycin from 15 aminoglycosides that can down-regulates miR-21 by binding to pre-miRNA (precursor miRNA) and blocking the miRNA maturation process.[26] Other molecules such as enoxacin[27] and our previously established photoreaction product[28] have been proved to activate the function of miRNAs by enhancing TRBP activity or upregulating TRBP (TAR RNA binding protein) expression.

Photocycloaddition reactions of carbonyl compounds with alkenes or acetylenes have been used by us as efficient method to generate structurally diversified compounds.[29–35] In the photoreactions of naphthalene-1,4-dione and acetylenes, both the carbonyl group and the C=C bond between the two carbonyl groups could react with the acetylene. Therefore both the benzoanthracenone product and the cyclobutene type product were generated.[28] Successful identification of a universal activator of miRNAs from the photoreaction products of naphthalene-1,4-dione and acetylenes encouraged us to evaluate systematically on the modulation activity of different photocycloadducts on various endogeneous miRNAs. In this Letter, we synthesized a series of substituted 2*a*-methoxy-cyclobuta[*b*]naphthalene-3,8(2*aH*,8*aH*)-diones via chemo- and regio-selectively [2+2] photocycloadditions of 2-methoxy-1,4-naphthalenequinone with various aryl acetyelens and

* Corresponding authors. Tel.: +86 25 83686234; fax: +86 25 83686234 (C.Z); tel.: +86 25 83593072; fax: +86 25 83685976 (Y.Z.).
E-mail addresses: cyzhang@nju.edu.cn (C. Zhang), njuzy@nju.edu.cn (Y. Zhang).

0968-0896/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmc.2013.04.058

evaluated the activity of these compounds to modulate endogenous miRNAs.

2. Results and discussion

2.1. Photocycloaddition of 2-methoxy-1,4-naphthalenequinone with aryl acetylenes

With the methoxy group substituted on the 2-position of 1,4-naphthalenequinone, photoreactions of 2-methoxy-1,4-naphthalenequinone **1** with biphenylacetylene **2a** happened exclusively on the C=C bond to give cyclobutene type product. We therefore synthesized a series of acetylenes including 2-phenyl or 2-azaaryl phenylacetylenes **2a**–**2c**, aza-aryl cyclopropyl acetylenes **3a**–**3f** and 5-ethynylquinolin-8-ol derivatives **4a**–**4c** (Chart 1) to react with 2-methoxy-1,4-naphthalenequinone to get compounds with the same fused cyclobutene framework but different substitution groups on the cyclobutene ring.

Irradiation of **1** with 2-phenyl or 2-azaaryl phenylacetylenes **2a**–**2c** in benzene gave cyclobutenes **5**–**7** with yields ranging from 60% to 80% (Scheme 1). The photocycloaddition proceeded with high chemo- and regio-selectivity. The photoreaction proceeded via the $\pi\pi^*$ excited state and the C=C bond conjugated with two carbonyl groups reacted with the C≡C bond in the acetylenes to form the cyclobutene ring. For the acetylenes substituted with different aryl groups, only one regio-isomer was obtained.

We used NOESY spectra to determine the relative position of the aryl rings to the methoxy group (Supplementary data). In the NOESY spectrum of **6**, the relevance between the methoxy proton and the proton on the phenyl group was observed. In the NOESY of compound **7**, the proton on the pyrazine ring showed relevance with the proton on the cyclobutene ring. The regio-selectivity of the photocycloaddition can be rationalized by the fact that phenyl ring is more electron-sufficient than the aza-aryl ring.

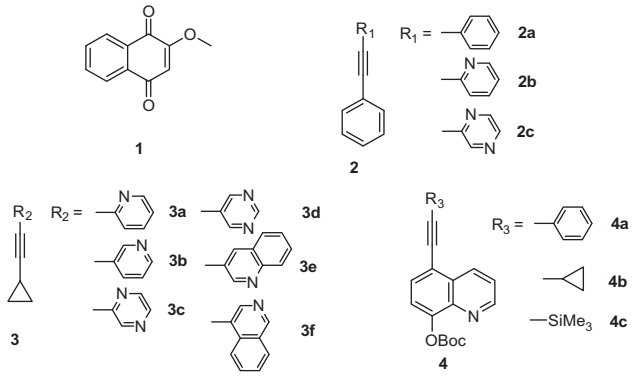
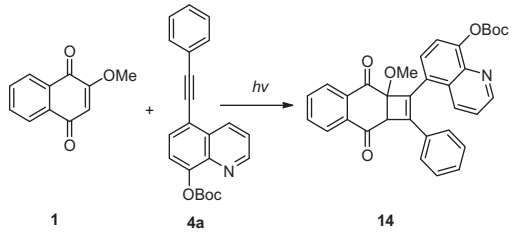


Chart 1.



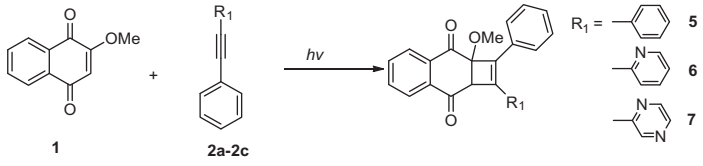
Scheme 3. Photoreaction of **1** with **4a**.

The photoreaction of 2-methoxy-1,4-naphthalenequinone **1** with cyclopropylethynyl aza-aryls **3a**–**3f** also proceeded with high regio-selectivity to give one regio-isomer predominantly (Scheme 2). We also used NOESY spectra to determine the relative position of the cyclopropyl ring to the methoxy group (Supplementary data). In the NOESY spectra of the products, relevance between the cyclopropyl proton and the proton on the cyclobutene ring was also detected. The regio-selectivity in the photocycloaddition may be rationalized by the ability of the aryl ring to stabilize the conjugated radical center in the diradical intermediates.

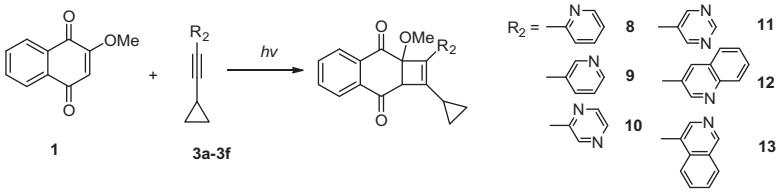
To get the acetylene substituted with 8-hydroxyquinoneline ring, we used 5-bromoquinolin-8-yl *tert*-butyl carbonate to couple with phenylacetylene catalyzed by $Pd(PPh_3)_4$. Then we tested the photoreaction of **4a** with 2-methoxy-1,4-naphthalenequinone **1**. Irradiation of **1** and **4a** in benzene gave **14** as the only product with a yield of 80% (Scheme 3). According to the NOESY spectrum of **14**, the quinoline ring resides on the carbon adjacent to the methoxy substituted carbon. The regio-selectivity can also be rationalized by the most stable diradical intermediates.

2.2. Preliminary assay of compounds 5–14 on different endogenous miRNAs

With compounds **5**–**14** obtained from the photocycloaddition of 2-methoxy-1,4-naphthalenequinone with different aryl substituted acetylenes, we were able to perform preliminary evaluation on their effects on endogenous miRNAs. The cell-based evaluation system was similar to that reported previously by us and oth-



Scheme 1. Photoreactions of 2-methoxynaphthalene-1,4-dione **1** with 1-phenyl-2-arylethynes **2a**–**2c**.



Scheme 2. Photoreactions of **1** with cyclopropyl aza-arylethynes **3a**–**3f**.

ers.[24,28] In general, luciferase reporter gene with the complementary sequence of the miRNAs of interest was tranfected into the cells. β-Gal was used as internal reference for transfection efficacy and cell viability. The disturbed expression or function of the endogeneous miRNAs upon incubation with the compounds could therein be read out from the increased or decreased luciferase signal from the cells relative to cells treated by DMSO as control.

We selected miR-1, miR-214, miR-25 and miR-150 as miRNAs of our interest and constructed the corresponding cellular reporter systems. MiR-1 is expressed specifically in muscle tissue and its misregulation has been reported in coronary artery diseases and heart failing.[36,37] MiR-25 is highly conserved and is one of the key modulator of TGFB signaling in many tumors, interfering with cell cycle arrest and apoptosis.[38] Expression of miR-214 was significantly deregulated in various types of cancer and miR-214 has been reported to play distinct roles in different tumors.[39,40] MiR-150 is stably expressed in most immune cells, including macrophage, monocyte, mature T and B cell.[41–43] It has been reported closely related to inflammation, immune-response and autoimmune diseases.[44,45]

According to the abundance of the four miRNAs of our interests, we selected three different types of cell-lines to construct the cellular assay systems. Mouse myoblast cell-line C2C12 was transfected with luciferase reporter for miR-1. Mouse leukaemic mococyte macrophage cell-line RAW267.4 was transfected with luciferase reporter for miR-150. Human lung adenocarcinoma cell-line A549 was transfected with luciferase reporter for miR-25 and miR-214 respectively. According to reports concerning the identification of small molecules for miRNA regulator, 10 μM of compounds were normally used for screening. Therefore, activity assay of our photocycloadducts were carried out under this concentration as well.

Modulation of compounds **5**–**14** on the endogenous miR-1, miR-150, miR-25 and miR-214 was then evaluated. The relative luciferase signal from the reporter cells upon treatment of compounds **5**–**14** at 10 μM relative to that from the same cells treated with DMSO control were summarized in Table 1. Firstly, we investigated the regulatory effect of the compounds on miR-1 which is abundant in differentiated C2C12 cells. Since miR-1 is expressed mainly in myotube, we incubated our reporter C2C12 cell-lines in culture medium containing 2% horse serum after transfection of reporter plasmid to induce differentiation. In the meanwhile, 10 μM of compounds were added and the cells were further incubated for 48 h. Luciferase assay results indicated that compound **14** had potent inhibition on miR-1 since an increase of luciferase signal in C2C12 cells by ~7 fold was observed in reporter cell treated by **14** compared with that of DMSO control. Compared with compound **14**, the effect of other photocycloadducts **6**–**13** showed much lower modulation activity on miR-1. Compound **5** showed activation activity on miR-1 since the cellular luciferase signal was only 45% to DMSO control.

Then we measured the activity of compounds **5**–**14** in modulating miR-150 in mouse leukaemic mococyte macrophage RAW267.4 cells. The cells transfected with miR-150 reporter showed no distinct change on luciferase expression upon treatment with all these compounds compared with DMSO control (column 3 in Table 1). We further measured the activity of compounds **5**–**14** in the modulation of the oncogenic miR-25 and miR-214 in A549 cells. No significant change was observed on the luciferase signal of the reporter cells upon treatment of compounds **6**–**14** (column 4 and 5 in Table 1). The reporter cells for miR-25 and miR-214 upon treatment of compound **5** showed significantly decreased luciferase signal, which indicated that this compound could also activate miR-25 and miR-214.

Based on the preliminary screening on the 10 photocloadducts with respect to their modulation activity on the four types of miRNAs, we were able to identify compound **14** as a potent inhibitor of miR-1. This small molecule selectively inhibits the endogenous miR-1 in muscle cells but shows no influence on other miRNAs we tested. Due to the importance of miR-1 in coronary artery diseases, we explored further on this types of small molecular inhibitors of miR-1.

2.3. Inhibition of compound 14 on miR-1

Upon identification of compound **14** as selective inhibitor of miR-1, we investigated further the selectivity, dose-dependence and mechanism of action on its inhibition of miR-1. C2C12 cells transfected with control vector containing luciferase gene without the complimentary sequence of miRNA were constructed as negative control. Reporter systems for several other miRNAs were also constructed, including miR-21 in HeLa cells (cervical cancer cell-line) and miR-9 in MCF-7 cells (breast cancer cell-line). Figure 1 showed the relative luciferase signal from various reporter cells upon treatment with compound **14** at 10 μM for 48 h. In contrast to the potent inhibition on miR-1, the compound showed no significant alteration on the relative luciferase signal from the cells with control vector and reporters for miR-21, miR-9, miR-25, miR-150 and miR-214.

Table 1
Relative luciferase signals from reporter cells upon treatment with compounds **5**–**14**[a]

Compound	miR-1	miR-150	miR-25	miR-214
5	0.45 ± 0.01	0.91 ± 0.27	0.63 ± 0.01	0.58 ± 0.07
6	0.90 ± 0.04	1.16 ± 0.40	0.95 ± 0.05	0.95 ± 0.01
7	1.12 ± 0.09	1.32 ± 0.44	1.01 ± 0.05	0.93 ± 0.08
8	1.55 ± 0.17	1.20 ± 0.39	0.92 ± 0.05	0.82 ± 0.10
9	0.93 ± 0.16	1.04 ± 0.40	0.97 ± 0.09	0.91 ± 0.11
10	0.84 ± 0.25	1.63 ± 0.47	0.83 ± 0.08	0.74 ± 0.08
11	2.06 ± 0.61	1.02 ± 0.27	1.16 ± 0.11	1.28 ± 0.06
12	1.36 ± 0.02	0.83 ± 0.15	1.00 ± 0.04	0.93 ± 0.04
13	0.87 ± 0.23	1.75 ± 0.60	1.16 ± 0.25	1.10 ± 0.03
14	**7.11 ± 1.03**	1.35 ± 0.34	0.95 ± 0.01	1.01 ± 0.11

[a] All experiments were conducted in triplicates and normalized to DMSO control. The relative luciferase signals are presented as mean ± SE (n = 3).

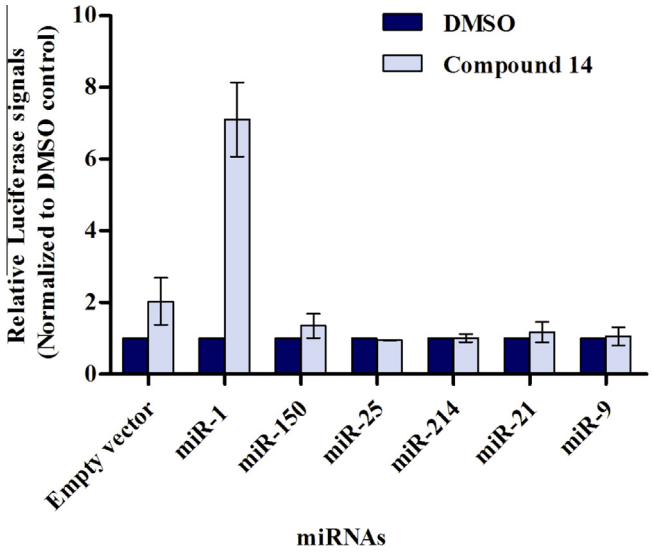


Figure 1. Selective inhibition of compound **14** on miR-1. The luciferase signal from various reporter cells was detected after 48 h incubation with compound **14**. All experiments were conducted in triplicates and normalized to DMSO control and the data are presented as mean ± SE (n = 3).

The dose-dependence of the compound on inhibition activity of miR-1 was then studied according to the relative luciferase signal from reporter cells treated by compound **14** at different concentrations. As shown in Figure 2, at concentrations between 0 and 5 μM, the inhibition activity of the compound on miR-1 increased rapidly with the increase of compound concentration in the cell culture media. While increase of concentration from 5 to 10 μM showed no further increase on the inhibition activity. The dose-dependent curve suggests that the inhibition of **14** on miR-1 was based on specific interaction between the compound and its target.

The selective inhibition of miR-1 by compound **14** is most likely due to the down-regulation of miR-1 expression in C2C12 cells. We then checked the expression level of mature miR-1 in differentiated C2C12 cells treated by the compound for 48 h using quantitative RT-PCR. As shown in Figure 3, the expression level of miR-1 in compound **14** treated cells decrease by ~4 fold compared with that in DMSO treated cells. As contrast, compound **14** did not significantly change the expression level of other miRNAs we tested. The results on the regulation of compound **14** on miRNA were consistent with the luciferase assay results. Therefore, we conclude that compound **14** decreases the cytoplasmic levels of mature miR-1.

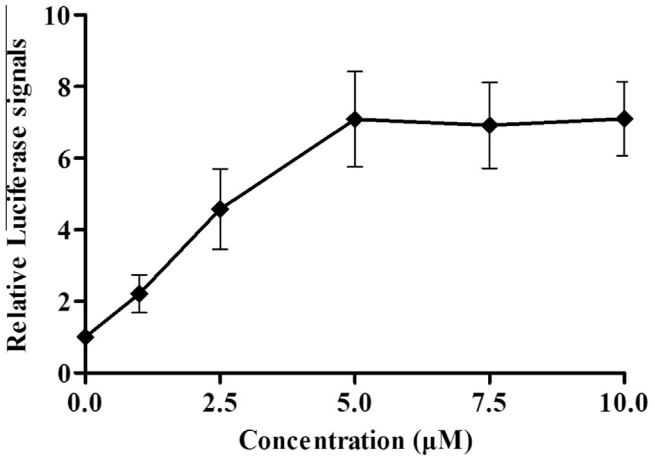


Figure 2. Dose-dependence curve of the inhibition activity of compound **14** on miR-1 in differentiated C2C12 cells. The values are presented as means ± SE (n = 3).

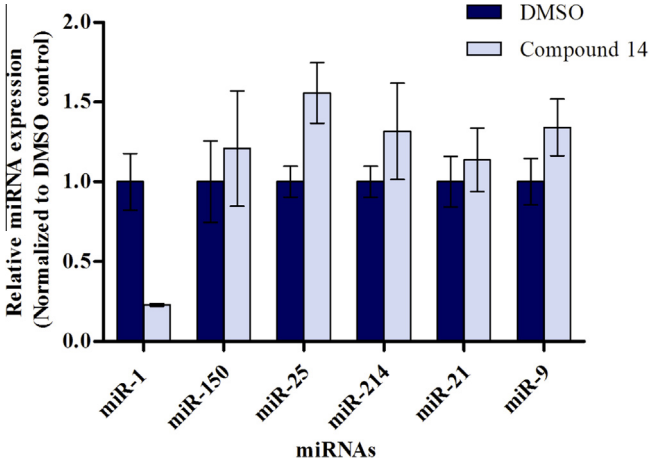


Figure 3. Influence of compound **14** on the expression level of different endogenous miRNAs. The expressions of mature miR-1 in differentiated C2C12 cells, miR-21 in HeLa cells, miR-150 in RAW267.4 cells, miR-9 in MCF-7 cells, and miR-25/miR-214 in A549 cells were determined by quantitative RT-PCR after treatment with compound **14** for 48 h. All experiments were conducted in triplicates and normalized to DMSO control and the data are presented as mean ± SE (n = 3).

2.4. Inhibition activity of compound 14 analogues

The distinct activity of compound **14** with other photocycloadducts **5**–**13** suggested that the 8-hydroxylquinoline ring substituted on the cyclobutene ring was crucial for its inhibition activity on miR-1. To get more information on the structure–activity relationship, we synthesized a series of **14** analogues with the same 8-hydroxylquinoline ring substituted cyclobutene framework (Chart 2). Change of the substitution group on the 8-hydroxyquinoline ring was straight-forward as described in the experimental part. The preparation of the cyclopropyl ring or trimethylsilyl group substituted cyclobutenes **18** and **22** was based on similar photocycloaddition reactions of 2-methoxy-1,4-naphthalenequinone with the 5-cyclopropylethynyl or 5-(trimethylsilyl)ethynyl 8-*tert*-butoxyquinolines, respectively. Removal of the trimethylsilyl group in **22**–**25** with TBAF gave **26**–**29**, respectively.

According to the dose-dependence curve of the inhibition activity of compound **14** on miR-1 in differentiated C2C12 cells, compound **14** reached its maxima effect at concentration of 5 μM. Therefore we evaluated the inhibition activity of compounds **15**–**29** on miR-1 in C2C12 cells at the concentration of 5 μM. Prior to activity evaluations, cytotoxicity of compounds were examined by MTT assay, and results showed that C2C12 cells were compatible with most of these compounds at the concentration of 5 μM. (Supplementary data) The relative luciferase signal from the reporter cells for miR-1 treated by these compounds was shown in Figure 4. It seemed that the majority of the analogues had comparable inhibition activity as compound **14** on miR-1.

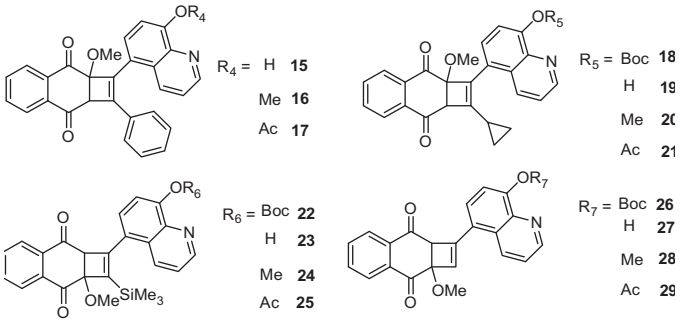


Chart 2.

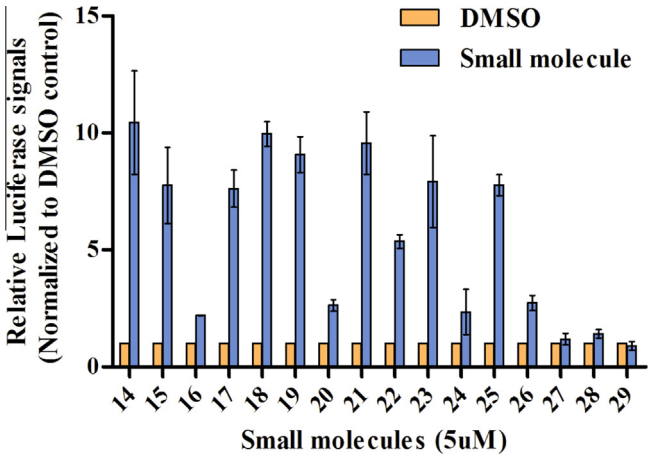


Figure 4. Inhibition activity of compounds **15**–**29** on miR-1. The luciferase signals from C2C12 transfected with Luc-miR-1 reporter were detected after 48 h incubation with compounds **15**–**29**. All experiments were conducted in triplicates and normalized to DMSO control. The relative luciferase signals are presented as mean ± SE (n = 3).

The structural features of the compounds which lost their activity provided us information on the structure–activity relationship on this type of miR-1 inhibitors. For the substitution groups on the hydroxyl group, it seemed that Ac or Boc substitution did not have significant influence on the inhibition activity. However, methylation of the hydroxyl group easily diminished the activity. Substitution group on the C=C of the cyclobutene seemed to be necessary since compounds **26**–**29** with H group at this position showed much lower activity compared with their counterparts with phenyl, cyclopropyl or trimethylsilyl substitution groups.

3. Conclusions

In summary, we have identified one type of miR-1 inhibitors from the cyclobutenes derived from the [2+2] photocycloaddition of aryl acetylenes with 2-methoxy-1,4-naphthalenequinone. This type of miR-1 inhibitors have the common framework as the photocycloadduct of 8-(*tert*-butoxy)-5-(phenylethynyl)quinoline with 2-methoxy-1,4-naphthalenequinone. The inhibitors were able to decrease the cytoplasmic levels of mature miR-1 inside C2C12 cells. Preparation of structural analogues and derivatives of the active compounds allowed us to analyze some structure–activity relationship. Due to the importance of miR-1 in the regulation of signal transduction pathway in muscle cells and its wide relevance with coronary artery diseases and heart failing, the small molecular inhibitor of miR-1 identified in this paper should provide useful information for the construction of miR-1 related small molecular probes. Exploration on the detailed mechanism for this type of compounds to inhibit miR-1 is underway in our group.

4. Experimental

4.1. Synthesis

4.1.1. General

Commercial reagents were used as supplied or purified by standard techniques where necessary. Benzene was refluxed with Na overnight before use. Dichloromethane (DCM) and triethylamine was freshly distillated over calcium hydride prior use. NMR spectra were recorded on Bruker 300 MHz spectrometers or Bruker 400 MHz spectrometers. Chemical shifts for ^{1}H NMR spectra are recorded in parts per million from tetramethylsilane with the solvent resonance as the internal standard (chloroform, 7.262 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad), integration and coupling constant in Hz. All the NOESY spectrum were measured on Bruker 400 MHz spectrometers. High resolution mass spectra (HRMS) were recorded on a Agilent 6540Q-TOF high resolution LC-MS spectrometer (ESI). Reagents:

4.1.2. Photocycloaddition reactions

General: the light source was a high-pressure mercury lamp (500 W) in a cooling water jacket which was further surrounded by a layer of filter solution (1 cm thick, 15% aqueous $NaNO_2$) to cut off light of wavelength shorter than 400 nm. The solution of 2-methoxynaphthalene-1,4-dione **1** (0.04 M) and corresponding alkynes (0.08 M) in anhydrous benzene was irradiated under continuous nitrogen purging. The reaction course was monitored by TLC. At the end of the reaction, the solvent was removed under reduced pressure and the crude products were further purified by flash column chromatography with petroleum ether/ethyl acetate to give the final products.

Photocycloaddition of **1** with **2a** gave **5** (81%), pale yellow crystal; MP: 124–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15–8.12 (m, 1H), 7.87–7.84 (m, 1H), 7.75–7.65 (m, 4H), 7.57–7.52 (m, 2H), 7.37–7.30 (m, 6H), 4.49 (s, 1H), 3.50 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.0, 195.1, 143.9, 141.2, 134.7, 134.4, 133.7, 133.6, 132.2, 131.6, 129.7, 129.2, 128.7 (4C), 128.2, 127.4 (2C), 127.0 (3C), 84.1, 56.9, 53.4; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{18}NaO_3$ $[M+Na]^+$ 389.1148, found 389.1150.

Photocycloaddition of **1** with **2b** gave **6** (58%), pale yellow crystal; MP: 144–145 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.7–8.68 (m, 1H), 8.39 (d, 1H, *J* = 1.8 Hz), 8.37 (d, 1H, *J* = 1.2 Hz), 8.06–8.03 (m, 1H), 7.86–7.83 (m, 1H), 7.77 (td, 1H, *J* = 7.8, 1.8 Hz), 7.70–7.63 (m, 3H), 7.43–7.34 (m, 3H), 7.26–7.22 (m, 1H), 4.56 (s, 1H), 3.53 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.1, 194.5, 151.1, 149.7, 145.2, 141.4, 136.7, 134.6, 134.4, 133.7, 133.6, 131.3, 130.0, 129.1 (2C), 128.4 (2C), 128.1, 126.9, 123.6, 123.5, 84.2, 55.9, 53.3; HRMS (ESI): *m*/*z* calcd for $C_{24}H_{18}NO_3$ $[M+H]^+$ 368.1281, found 368.1286.

Photocycloaddition of **1** with **2c** gave **7** (74%), white crystal; MP: 142–143 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.93 (s, 1H), 8.62 (s, 1H), 8.48 (d, 1H, *J* = 2.1 Hz), 8.31–8.28 (m, 2H), 8.04–7.99 (m, 1H), 7.88–7.85 (m, 1H), 7.67–7.64 (m, 2H), 7.42–7.37 (m, 3H), 4.56 (s, 1H), 3.52 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.4, 194.0, 148.2, 146.9, 144.5, 144.2, 143.9, 138.3, 134.9, 134.6, 133.6, 133.5, 130.9, 130.6, 129.2 (2C), 128.5 (2C), 128.2, 127.1, 84.6, 55.6, 53.5; HRMS (ESI): *m*/*z* calcd for $C_{23}H_{16}N_2NaO_3$ $[M+Na]^+$ 391.1053, found 391.1062.

Photocycloaddition of **1** with **3a** gave **8** (46%), white crystal; MP: 167–168 °C; ^{1}H NMR(300 MHz, $CDCl_3$): δ 8.51 (d, 1H, *J* = 4.2 Hz), 8.04 (dd, 1H, *J* = 7.5, 1.2 Hz), 7.92 (d, 1H, *J* = 7.2 Hz), 7.76–7.60 (m, 4H), 7.09 (t, 1H, *J* = 5.7 Hz), 3.94 (s, 1H), 3.42 (s, 3H), 2.78–2.73 (m, 1H), 1.13–1.02 (m, 2H), 0.87–0.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.0, 194.5, 155.5, 151.2, 149.6, 139.7, 136.6, 134.8, 134.4, 133.9, 133.8, 128.2, 126.8, 122.4, 122.2, 82.9, 56.1, 53.4, 13.4, 7.4, 6.8; HRMS (ESI): *m*/*z* calcd for $C_{21}H_{18}NO_3$ $[M+H]^+$ 332.1281, found 332.1282.

Photocycloaddition of **1** with **3b** gave **9** (86%), yellow crystal; MP: 173–174 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.88 (s, 1H), 8.48 (s, 1H), 8.08 (dd, 1H *J* = 7.5, 1.5 Hz), 8.00–7.96 (m, 2H), 7.82–7.71 (m, 2H), 7.32–7.28 (m, 1H), 3.95 (s, 1H), 3.42 (s, 3H), 1.96–1.89 (m, 1H), 1.08–1.00 (m, 2H), 0.86–0.71 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.6, 194.5, 151.5, 148.7, 147.8, 138.4, 134.9, 134.8, 134.5, 133.9, 133.7, 133.6, 128.2, 126.9, 123.7, 83.2, 56.5, 53.5, 12.8, 6.9, 6.3; HRMS (ESI): *m*/*z* calcd for $C_{21}H_{18}NO_3$ $[M+H]^+$ 332.1281, found 332.1283.

Photocycloaddition of **1** with **3c** gave **10** (70%), white crystal; MP: 156–157 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.89 (s, 1H), 8.49–8.47 (m, 1H), 8.40 (d, 1H, *J* = 2.1 Hz), 8.11–8.08 (m, 1H), 7.79–7.96(m, 1H), 7.81–7.71 (m, 2H), 3.98 (s, 1H), 3.44 (s, 3H), 2.67–2.61 (m, 1H), 1.26–1.04 (m, 2H), 0.87–0.78 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.3, 193.9, 158.4, 146.9, 144.2, 143.5, 143.3, 142.9, 137.3, 134.9, 134.6, 133.7, 128.3, 126.9, 82.5, 56.6, 53.6, 13.6, 7.9, 7.1; HRMS (ESI): *m*/*z* calcd for $C_{20}H_{16}N_2NaO_3$ $[M+Na]^+$355.1053, found 355.1051.

Photocycloaddition of **1** with **3d** gave **11** (68%), white crystal; MP: 158–159 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.08 (s, 1H), 9.01 (s, 2H), 8.12–7.98 (m, 1H), 8.01–7.98 (m, 1H), 7.84–7.73 (m, 2H), 3.96 (s, 1H), 3.41 (s, 3H), 1.93–1.85 (m, 1H), 1.13–1.05 (m, 2H), 0.91–0.74 (m, 2H); ^{13}C NMR (75 MHz $CDCl_3$): δ 195.9, 194.0, 157.3, 154.4 (3C), 153.9, 135.1, 134.7, 133.5, 133.4, 128.3, 127.1, 126.2, 82.7, 56.8, 53.8, 13.1, 7.3, 6.7; **HRMS** (ESI): *m*/*z* calcd for $C_{20}H_{17}N_2O_3$ $[M+H]^+$ 333.1234, found 333.1240.

Photocycloaddition of **1** with **3e** gave **12** (85%), pale yellow crystal; MP: 172–173 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.19 (d, 1H, *J* = 2.1 Hz), 8.50 (d, 1H, *J* = 1.5 Hz), 8.10 (d, 1H, *J* = 8.4 Hz), 8.07 (dd, 1H, *J* = *J* = 7.8, 1.2 Hz), 8.00 (dd, 1H, *J* = 7.8, 1.2 Hz), 7.94 (d, 1H, *J* = 8.1 Hz), 7.80 (td, 1H, *J* = 7.2, 1.5 Hz), 7.76–7.70 (m, 2H), 7.62–7.56 (m, 1H), 4.02 (s, 1H), 3.47 (s, 3H), 2.06–1.99 (m, 1H), 1.13–1.06 (m, 2H), 0.93–0.76 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.6, 194.4, 151.3, 148.6, 147.1, 138.6, 134.9, 134.5, 133.6,

133.3, 129.9, 129.2, 128.5, 128.2, 127.7, 127.0, 126.9, 125.3, 83.3, 56.5, 53.5, 12.9, 7.0, 6.5; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{20}NO_3[M+H]^+$ 382.1438, found 382.1440.

Photocycloaddition of **1** with **3f** gave **13** (84%), pale yellow crystal; MP: 125–126 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.19 (s, 1H), 8.45 (s, 1H), 8.08–8.04 (m, 2H), 8.00–7.96 (m, 2H), 7.84 (td, 1H, *J* = 7.5, 1.5 Hz), 7.77(td, 1H, *J* = 7.5, 1.5 Hz), 7.68–7.57 (m, 2H), 4.06 (s, 1H), 3.47 (s, 3H), 1.56–1.54 (m, 1H), 1.0–0.97 (m, 2H), 0.91–0.85 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.8, 194.4, 155.6, 152.9, 142.7, 138.5,134.8, 134.6, 133.9, 133.6, 130.8, 128.3, 128.0 (3C), 127.4, 127.1, 124.8, 123.1, 84.9, 56.8, 54.3, 12.7, 6.6, 5.9; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{20}NO_3$ $[M+H]^+$ 382.1438, found 382.1437.

Photocycloaddition of **1** with **4a** gave **14** (80%), white powder from acetone/petroleum ether; 175–177 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.88 (dd, 1H, *J* = 4.0, 1.4 Hz), 8.11–8.08 (m, 1H), 8.01–7.98 (m, 1H), 7.90 (dd, 1H, *J* = 8.6, 1.4 Hz), 7.78–7.75 (m, 2H), 7.57 (d, 1H, *J* = 7.9 Hz), 7.51 (d, 1H, *J* = 7.9 Hz), 7.25–7.14 (m, 6H), 4.59 (s, 1H), 3.50 (s, 3H), 1.61 (s, 9H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 196.4, 194.1, 151.8, 150.5, 148.2, 148.1, 141.5, 138.6, 134.9, 134.6, 133.9, 133.8, 133.6, 131.7, 130.1, 128.7 (2C), 127.8, 127.7, 127.5(3C), 126.7, 126.6, 121.9, 120.8, 85.6, 83.9, 56.7, 54.3, 27.7 (3C); HRMS (ESI): *m*/*z* calcd for $C_{33}H_{28}NO_6$ $[M+H]^+$ 534.1911, found 534.1919.

4.1.3. Preparation of analogues of compound 14

Procedure A: to a solution of compound **14** in DCM (0.1 M) was added 20 equiv of TFA. The mixture was stirred at room temperature and monitored by TLC. Upon complete conversion, the reaction mixture was poured into saturated $NaHCO_3$ solution. The mixture was extracted with DCM. The organic layer was dried over Na_2SO_4. The solvent was removed under vacum, and the crude products were separated by flash column chromatography with petroleum ether/ethyl acetate to give **15** (91%).

Compound **15**, yellow powder;176–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (dd, 1H, *J* = 4.0, 1.6 Hz), 8.11–8.07 (m, 1H), 8.00–7.96 (m, 1H), 7.89 (dd, 1H, *J* = 8.6, 1.4 Hz), 7.77–7.72 (m, 2H), 7.48 (d, 1H, *J* = 8.0 Hz), 7.24–7.14 (m, 7H), 4.57 (s, 1H), 3.50 (s, 3H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 196.6, 194.3, 153.3, 147.9, 146.9, 139.5, 138.3, 134.8, 134.5 (2C), 133.8, 133.7, 132.1, 129.7, 128.5 (3C), 127.8, 127.4 (3C), 125.6, 122.1, 120.0, 110.1, 85.7, 56.5, 54.2**; HRMS** (ESI): *m*/*z* calcd for $C_{28}H_{20}NO_4$ $[M+H]^+$ 434.1387, found 434.1388.

Procedure B: to the solution of **15** in acetone(0.1 M) was added 10 equiv of CH_3I and 3 eq. of K_2CO_3. The mixture was stirred at reflux temperature and monitored by TLC until complete conversion. After cooling to the room temperature, the mixture was poured into water and extracted with DCM. The organic layer was dried over Na_2SO_4. Then, the solvent was removed and the residue was separated by flash column chromatography with petroleum ether/ethyl acetate to give the final products **16** (84%).

Compound **16**, pale yellow powder; 179–181 °C; ^{1}H NMR(400 MHz, $CDCl_3$) δ 8.87 (dd, 1H, *J* = 4.0, 1.6 Hz), 8.08–8.06 (m, 1H), 8.00–7.98 (m, 1H), 7.82 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.78–7.71 (m, 2H), 7.51 (d, 1H, *J* = 8.0 Hz), 7.24–7.12 (m, 6H), 7.09 (d, 1H, *J* =8.0 Hz), 4.57 (s, 1H), 4.12 (s, 3H), 3.50 (s, 3H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 196.5, 194.2, 156.2, 149.2, 147.1, 140.2, 139.3, 134.8, 134.5, 133.9, 133.8, 133.6, 132.0, 129.8, 128.5 (2C), 127.7, 127.5, 127.4 (3C), 126.3, 121.8, 121.3, 107.5, 85.7, 56.6, 56.1, 54.2; HRMS (ESI): *m*/*z* calcd for $C_{29}H_{22}NO_4$ $[M+H]^+$ 448.1543, found 448.1550.

Procedure C: to a solution of **15** in anhydrous DCM (0.1 M) was added 1.2 equiv of anhydrous TEA and 1.1 eq. of acetyl chloride drop wise at 0 °C. The mixture solution was left to be stirrers at rt for 5 min. The solvent was removed and the crude product was separated by flash column chromatography with petroleum ether/ethyl acetate to give the final products to give **17** (96%).

Compound **17**, pale yellow powder; 181–183 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (dd, 1H, *J* = 4.0, 1.2 Hz), 8.11–8.09 (m, 1H), 8.01–7.98 (m, 1H), 7.93 (d, 1H, *J* = 8.4 Hz), 7.79–7.74 (m, 2H), 7.53 ((d, 1H, *J* = 7.6 Hz), 7.49 (d, 1H, *J* = 8.0 Hz), 7.25–7.15 (m, 6H), 4.59 (s, 1H), 3.50 (s, 3H), 2.52 (s, 3H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 196.2, 194.1, 169.4, 150.5, 148.2, 148.1, 141.4, 138.6, 134.9, 134.6, 134.1, 133.8, 133.6, 131.7, 130.1, 128.7 (2C), 127.8, 127.5 (3C), 126.7, 126.6, 121.9, 121.4, 85.6, 56.7, 54.3, 21.0; **HRMS** (ESI): *m*/*z* calcd for $C_{30}H_{22}NO_5$ $[M+H]^+$ 476.1492, found 476.1495.

Photocycloaddition of **1** with **4b** gave **18** (90%), white powder;161–163 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.87 (d, 1H, *J* = 3.0 Hz), 8.27 (d, 1H, *J* = 8.4 Hz), 8.03 (d, 2H, *J* = 7.5 Hz), 7.84–7.72 (m, 2H), 7.56 (d, 1H, *J* = 7.8 Hz), 7.49 (d, 1H, *J* = 7.8 Hz), 7.33 (dd, 1H, *J* = 8.4, 3.9 Hz), 4.01 (s, 1H), 3.45 (s, 3H), 1.58 (s, 9H), 1.52–1.44 (m, 1H), 1.01–0.94 (m, 1H), 0.90–0.82 (m, 1H), 0.75–0.61 (m, 2H);^{13}C NMR(75 MHz, $CDCl_3$) δ 196.8, 194.4, 154.1, 151.8, 150.2, 147.7, 141.3, 139.5, 134.9, 134.6, 134.0, 133.8, 133.6, 128.0, 127.4, 127.3, 127.0, 126.7, 121.6, 120.7, 84.9, 83.8, 56.5, 54.2, 27.6 (3C), 12.8, 6.5, 5.8; HRMS (ESI): *m*/*z* calcd for $C_{30}H_{28}NO_6$ $[M+H]^+$ 498.1911, found 498.1912.

Treatment of **18** according to procedure A gave **19** (86%), pale yellow powder; 158–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (dd, 1H, *J* = 4.2, 1.4 Hz), 8.33 (dd, 1H, *J* = 8.4, 1.2 Hz), 8.04 (s, 1H), 8.02 (s,1H), 7.83–7.79 (m, 1H), 7.76–7.72 (m, 1H), 7.51 (d, 1H, *J* = 8.0 Hz), 7.37 (dd, 1H, *J* = 8.6, 4.2 Hz), 7.14 (d, 1H, *J* = 8.0 Hz), 4.02 (s, 1H), 3.46 (s, 3H); 1.56–1.49 (m, 1H), 1.00–0.93 (m, 1H), 0.89–0.82 (m, 1H), 0.75–0.67 (m, 1H), 0.66–0.59 (m, 1H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 197.1, 194.6, 152.8, 152.3, 147.7, 140.2, 138.2, 134.7, 134.5(2C), 133.9, 133.6, 128.5, 127.9, 127.0, 126.4, 121.8, 119.8, 109.7, 85.0, 56.4, 54.1, 12.7, 6.3, 5.6; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{20}NO_4$ $[M+H]^+$ 398.1387, found 398.1392.

Treatment of **19** according to procedure B gave **20** (78%), yellow powder;164–166 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, 1H, *J* = 2.8 Hz), 8.24 (d, 1H, *J* = 8.8 Hz), 8.04 (s, 1H), 8.02 (s,1H), 7.82 (t, 1H, *J* = 7.6 Hz), 7.74 (t, 1H, *J* = 7.6 Hz), 7.54 (d, 1H, *J* = 8.0 Hz), 7.33 (dd, 1H, *J* = 8.4, 4.0 Hz), 7.04 (d, 1H, *J* = 8.0 Hz), 4.08 (s, 3H), 4.02 (s, 1H), 3.46 (s, 3H), 1.53–1.45 (m, 1H), 0.99–0.92 (m, 1H), 0.87–0.82 (m, 1H), 0.74–0.69 (m, 1H), 0.65–0.60 (m, 1H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 197.0, 194.6, 155.8, 152.7, 149.0, 140.1, 139.9, 134.8, 134.4, 133.9, 133.6, 127.9, 127.4, 127.1, 127.0, 121.6, 121.1, 107.3, 85.0, 56.4, 56.0, 54.1, 12.7, 6.3, 5.6; HRMS (ESI): *m*/*z* calcd for $C_{26}H_{22}NO_4$ $[M+H]^+$ 412.1543, found 412.1546.

Treatment of **19** according to procedure C gave **21** (93%), white powder; 163–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (dd, 1H, *J* = 4.0, 1.6 Hz), 8.28(dd, 1H, *J* = 8.6, 1.4 Hz), 8.06–8.02 (m, 2H), 7.82 (td, 1H, *J* = 7.6, 1.2 Hz), 7.76 (td, 1H, *J* = 7.6, 1.2 Hz), 7.59 (d, 1H, *J* = 7.6 Hz), 7.42 (d, 1H, *J* = 7.6 Hz), 7.33 (dd, 1H, *J* = 8.6, 4.2 Hz), 4.02 (s, 1H), 3.46 (s, 3H), 2.49 (s, 3H). 1.54–1.47 (m, 1H), 1.04–0.98 (m, 1H), 0.92–0.85 (m, 1H), 0.76–0.70 (m, 1H), 0.69–0.62 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8, 194.4, 169.6, 154.1, 150.2, 147.7, 141.2, 139.4, 134.9, 134.6, 134.1, 133.8, 133.6, 128.0, 127.6, 127.3, 127.0, 126.7, 121.6, 121.2, 84.9, 56.5, 54.2, 21.0, 12.8, 6.5, 5.8; HRMS (ESI): *m*/*z* calcd for $C_{27}H_{22}NO_5$ $[M+H]^+$ 440.1492, found 440.1490.

Photocycloaddition of **1** with **4c** gave **22** (75%), pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (dd, 1H, *J* = 4.4, 1.6 Hz), 8.20 (dd, 1H, *J* = 8.8, 1.6 Hz), 8.08–8.04 (m, 2H), 7.85 (dt, 1H, *J* = 7.6, 1.6 Hz), 7.79 (dt, 1H, *J* = 7.6, 1.2 Hz), 7.45 (d, 1H, *J* = 8.0 Hz), 7.33 (dd, 1H, *J* = 8.6, 4.2 Hz), 7.23 (d, 1H, *J* = 8.0 Hz), 4.17 (s, 1H), 3.44 (s, 3H), 1.57 (s, 9H), −0.13 (s, 9H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 196.4, 194.5, 160.8, 159.1, 151.5, 150.3, 147.9, 141.0, 135.0, 134.5, 134.1, 133.7, 133.3, 129.3, 127.7, 127.1, 126.4, 121.7, 120.1, 87.8, 83.6, 58.0, 54.1, 27.6 (3C), −2.2 (3C); HRMS (ESI): *m*/*z* calcd for $C_{30}H_{22}NO_6Si$ $[M+H]^+$ 530.1993, found 530.1995.

Treatment of **22** according to procedure A gave **23** (88%), pale yellow powder; 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73

(dd, 1H, J = 4.0, 1.6 Hz), 8.27 (dd, 1H, J = 8.6, 1.4 Hz), 8.05 (dd, 1H, J = 7.6, 1.2 Hz), 8.01 (dd, 1H, J = 7.6, 0.8 Hz), 7.81 (td, 1H, J = 7.2, 1.2 Hz), 7.74 (td, 1H, J = 7.6, 1.6 Hz), 7.34 (dd, 1H, J = 8.4, 4.4 Hz), 7.18 (d, 1H, J = 8.0 Hz), 7.08 (d, 1H, J = 8.0 Hz), 4.16 (s, 1H), 3.44 (s, 3H), –0.11 (s, 9H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 198.9, 197.0, 162.0, 161.5, 155.1, 150.0, 140.0, 137.0, 136.9, 136.5, 135.9, 135.4, 130.5, 129.8, 129.2, 129.0, 124.1, 124.0, 111.6, 59.9, 56.2, –0.01 (3C); HRMS (ESI): m/z calcd for $C_{25}H_{24}NO_4Si$ $[M+H]^+$ 430.1469, found 430.1469.

Treatment of **23** according to procedure B gave **24** (80%), yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (dd, 1H, J = 4.2, 1.4 Hz), 8.16 (d, 1H, J = 8.4 Hz), 8.07 (dd, 1H, J = 7.8, 1.0 Hz), 8.03 (dd, 1H, J = 7.8, 1.0 Hz), 7.84 (td, 1H, J = 7.6, 1.6 Hz), 7.77 (td, 1H, J = 7.6, 1.2 Hz), 7.32 (dd, 1H, J = 8.6, 4.2 Hz), 7.25 (d, 1H, J = 8.0 Hz), 6.99 (d, 1H, J = 8.0 Hz), 4.17 (s, 1H), 4.08 (s, 3H), 3.44 (s, 3H), –0.12 (s, 9H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 196.7, 194.8, 159.7, 159.5, 155.8, 149.1, 139.8, 134.9, 134.4, 133.7, 133.3, 127.7, 127.6, 127.2, 127.1, 123.1, 121.7, 106.9, 87.9, 57.9, 56.0, 54.1, –2.26 (3C); HRMS (ESI): m/z calcd for $C_{26}H_{26}NO_4Si$ $[M+H]^+$ 444.1626, found 444.1630.

Treatment of **23** according to procedure C gave **25** (94%), colorless foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (dd, 1H, J = 4.0, 1.6 Hz), 8.21 (dd, 1H, J = 8.6, 1.4 Hz), 8.08 – 8.05 (m, 2H), 7.85 (td, 1H, J = 7.6, 1.2 Hz), 7.79 (td, 1H, J = 7.6, 1.2 Hz), 7.38 (d, 1H, J = 8.0 Hz), 7.33 (dd, 1H, J = 8.6, 4.2 Hz), 7.25 (d, 1H, J = 8.0 Hz), 4.18 (s, 1H), 3.44 (s, 3H), 2.48 (s, 3H), –0.13 (s, 9H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 196.4, 194.6, 169.2, 160.8, 159.0, 150.3, 147.9, 141.0, 135.0, 134.5, 134.2, 133.7, 133.3, 129.4, 127.7, 127.1, 126.4, 121.7, 120.8, 87.8, 58.0, 54.1, 20.9, –2.2 (3C); HRMS (ESI): m/z calcd for $C_{25}H_{20}NO_4$ $[M+H]^+$ 398.1387, found 398.1392. HRMS (ESI): m/z calcd for $C_{27}H_{26}NO_5Si$ $[M+H]^+$ 472.1575, found 472.1570.

Procedure D: To a solution of **22**–**25** in DCM (0.1 M) was added 10 eq. of TBAF. The solution was stirred at rt for 3 h. When **22**–**25** was completely conversed, the solvent was removed and the residue was septerated by flash column chromatography with petroleum ether/ethyl acetate to give **26**–**29**, respectively.

Compound **26** (95%), pale yellow powder; 73–75 °C; ^{1}H NMR(400 MHz, $CDCl_3$) δ 8.87 (d, 1H, J = 4.0 Hz), 8.46 (d, 1H, J = 8.8 Hz), 8.09 (d, 1H, J = 8.0 Hz), 8.03–8.00 (m, 2H), 7.75 (t, 1H, J = 7.6 Hz), 7.69 (t, 1H, J = 7.6 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.39 (dd, 1H, J = 8.0, 4.0 Hz), 6.98 (s, 1H), 4.26 (s, 1H), 3.50 (s, 3H), 1.56 (s, 9H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 195.0, 194.3, 151.6, 150.0, 148.4, 148.2, 141.3, 135.6, 134.9, 134.6, 133.3, 133.2, 132.4, 128.4, 128.3, 127.3, 127.1, 125.5, 122.2, 120.7, 87.0, 83.9, 56.3, 53.3, 27.6 (3C); HRMS (ESI): m/z calcd for $C_{27}H_{23}NNaO_6$ $[M+Na]^+$ 480.1418, found 480.1417.

Compound **27** (87%), pale yellow powder; 178–180 °C; ^{1}H NMR(400 MHz, $CDCl_3$) δ 8.76 (d, 1H, J = 3.2 Hz), 8.49 (d, 1H, J = 8.4 Hz), 8.07–8.02 (m, 3H), 7.77 (td, 1H, J = 7.6, 1.2 Hz), 7.70 (td, 1H, J = 7.6, 1.2 Hz), 7.45 (dd, 1H, J = 8.4, 4.0 Hz), 7.19 (d, 1H, J = 8.0 Hz), 6.84 (d, 1H, J = 1.6 Hz), 4.26 (d, 1H, J = 1.2 Hz), 3.52 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 195.5, 194.7, 153.5, 148.7, 147.6, 138.2, 134.8, 134.5, 133.3, 133.2, 133.0, 131.9, 130.5, 128.2, 127.1, 126.5, 122.5, 118.4, 109.7, 87.0, 56.2, 53.2; HRMS (ESI): m/z calcd for $C_{22}H_{16}NO_4$ $[M+H]^+$ 358.1074, found 358.1073.

Compound **28** (83%) yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.48 (d, 1H, J = 8.4 Hz), 8.12(d, 1H, J = 8.0 Hz),8.05 (s, 1H), 8.03(s, 1H), 7.77 (t, 1H, J = 7.6 Hz), 7.71(t, 1H, J = 7.6 Hz), 7.45 (dd, 1H, J = 8.0, 3.2 Hz), 7.10 (d, 1H, J = 8.0 Hz), 6.88 (s, 1H), 4.28 (d, 1H, J = 1.2 Hz), 4.12 (s, 3H), 3.53 (s, 3H);^{13}C NMR(100 MHz, $CDCl_3$) δ 195.5, 194.7, 156.3, 148.8, 148.4, 134.8, 134.5, 133.3, 133.2, 132.7,129.7, 128.2, 127.3, 127.1, 122.2, 119.6, 107.4, 87.0, 56.3, 56.2, 53.2; HRMS (ESI): m/z calcd for $C_{23}H_{18}NO_4$ $[M+H]^+$ 372.1230, found 372.1226.

Compound **29** (96%), white powder; 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (d, 1H, J = 2.8 Hz), 8.52 (d, 1H, J = 8.8 Hz), 8.12 (d, 1H, J = 8.0 Hz), 8.06–8.03(m, 2H), 7.78 (t, 1H, J = 7.6 Hz), 7.73 (t, 1H, J = 7.6 Hz), 7.49 (d, 1H, J = 8.0 Hz), 7.43 (dd, 1H, J =8.4, 4.0 Hz), 7.00 (s, 1H), 4.29 (s, 1H), 3.53 (s, 3H), 2.51 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 195.0, 194.3, 169.5, 149.9, 148.2, 135.7, 134.9, 134.7, 133.3, 133.2, 128.5, 128.4, 127.4, 127.1, 125.7, 122.2, 121.5, 87.0, 56.4, 53.4, 21.0; HRMS (ESI): m/z calcd for $C_{24}H_{18}NO_5$ $[M+H]^+$ 400.1179, found 400.1176.

4.2. Biological assay

4.2.1. Cell culture and reagents

HeLa, C2C12, MCF-7, RAW267.4 and A549 cells were cultured in high glucose DMEM (Dulbecco's Modified Eagle's Medium, Invitrogen) containing 10% fetal bovine serum (FBS) and 1% penincilin/streptomycin at 37 °C and 5% CO_2. C2C12 cells were induced to differentiate in DMEM containing 2% horse serum (Invitrogen). Compounds were pre-dissolved in DMSO; the final concentration of DMSO in culture medium was 1:1000.

4.2.2. Toxicity test of compounds

The cultured cells were seeded on 96 wells plates with around 10,000 cells/well. The next day, they were incubated with 10uM compounds in high glucose DMEM containing 2% FBS. MTT assay was performed after 48 h of incubation. Briefly, cells were incubated with 0.5 mg/mL of MTT (Sigma–Aldrich) for 4 h in a CO_2 incubator at 37 °C. After that, the medium was removed completely and the purple precipitates were dissolved in DMSO for 10 min at room temperature. The absorbance of solution was measured at 490 nm. Each experiment was performed in triplicates.

4.2.3. Reporter plasmids construction and isolation

The luciferase reporter plasmid was sequentially digested with HindIII and SpeI restriction endonuclease (Takara) and was gel purified. The complementary DNA sequences of mature miR-1, miR-150, miR-25 and miR-214 containing HindIII and SpeI 'sticky' end were individually cloned into the purified luciferase reporter by using T4 ligase (Takara) at 16 °C overnight. The plasmids were transformed into chemically competent *Escherichia coli* by incubating the plasmid and competent *E. coli* on ice for 30 min, followed by a pulse of heat shock (42 °C, 90 s). The *E. coli* was concentrated and plate on agar plate containing 100 ug/ml Ampicillin. The agar plate was incubated at 37 °C for 16 h. Positive colonies were selected by PCR colony screens, and the construction of Luciferase miRNA reporters were confirmed by sequencing (Invitrogen). All mature miRNAs complementary sequences were purchased from Invitrogen Company and were 3′ phosphorylated. The followings were their corresponding sequences:

miR-1: 5′-ATACATACTTCTTTACATACCA-3′
miR-25: 5′-TCAGACCGAGACAAGTGCAATG-3′
miR-214: 5′-ACTGCCTGTCTGTGCCTGCTGT-3′
miR-150: 5′-CACTGGTACAAGGGTTGGGAGA-3′

Positive plasmids were isolated from *E. coli* by using plasmid isolation kit (Invitrogen).

4.2.4. Screening of photocycloadducts for miRNAs function modifier

Luciferase assays were performed to identify effect of compounds on function of endogeneous miRNAs: For miR- miR-1, C2C12 cells at 70% confluence in 48-well plates were co-transfected with miR-1 luciferase reporter (0.25 μg) or respective luciferase reporters and β-galactosidase expressing plasmid (0.25 μg) by using lipo-fectamine 2000 (Invitrogen) according to manufacturer's protocol. The β-galactosidase was used as transfection control. After 6 h of transfection, the medium was changed to high glucose DMEM containing 2% horse serum, 1% penincilin/streptomycin and 10 μM of compounds. The cells were further incubated for 48 h at 37 °C, 5% CO_2, and then assayed using luciferase assay

kits (Promega). DMSO was used as negative control. For miR-150, RAW267.4 cells were transfected with miR-150 luciferase reporter. For miR-214 and miR-25, A549 cells were transfected with miR-214 or miR-25 luciferase reporters. High glucose DMEM containing 2% FBS was used in the case of RAW267.4 and A549 cells.

4.2.5. Quantitative RT-PCR

Total RNA from cells treated with compound **14** and DMSO was isolated using TRIZOL reagent (Invitrogen) according to manufacturer's protocol. Mature mmu-miR-1 in differentiated C2C12 cells, hsa-miR-21 in HeLa cells, hsa-miR-25 and hsa-miR-214 in A549 cells, miR-9 in MCF-7 cells and miR-150 in RAW267.4 cells were quantified using Taqman miRNA detection assay (Applied biosystems). In general, 2 μg of RNA extracted from cells was used for each reaction. cDNA of interested miRNA was synthesized from total RNA using specific mature miRNA primers (Applied Biosystems) using high capacity cDNA Reverse Transcription Kit (Takara). The reactions were carried out in a thermal cycler for 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C and then held at 4 °C. Real-time PCR was performed on Applied Biosystems 7300 Fast Real Time PCR system using rTaq polymerase (Takara). The reaction was carried out at 95 °C for 10 min followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The relative expression level was calculated using the comparative C1 method.

Acknowledgments

Financial support the National Basic Research Program of China (No. 2011CB935800), National Science Foundation of China (81250044) and the Natural Science Foundation of Jiangsu Province (BK2012012) was acknowledged.

Supplementary data

Supplementary data (Viability tests on C2C12 cells. Copies of NMR spectra of all the photocycloadducts and their derivatives.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.04.058.

References and notes

1. Bartel, D. P. *Cell* **2004**, *116*, 281.
2. He, L.; Hannon, G. J. *Nat. Rev. Genet.* **2004**, *5*, 522.
3. Winter, J.; Jung, S.; Keller, S.; Gregory, R. I.; Diederichs, S. *Nat. Cell Biol.* **2009**, *11*, 228.
4. Croce, C. M. *Nat. Rev. Genet.* **2009**, *10*, 704.
5. Esquela-Kerscher, A.; Slack, F. J. *Nat. Rev. Cancer* **2006**, *6*, 259.
6. Asangani, I. A.; Rasheed, S. A. K.; Nikolova, D. A.; Leupold, J. H.; Colburn, N. H.; Post, S.; Allgayer, H. *Oncogene* **2008**, *27*, 2128.
7. Kulda, V.; Pesta, M.; Topolcan, O.; Liska, V.; Treska, V.; Sutnar, A.; Rupert, K.; Ludvikova, M.; Babuska, V.; Holubec, L.; Cerny, R. *Cancer Genet. Cytogen.* **2010**, *200*, 154.
8. Meng, F.; Henson, R.; Wehbe-Janek, H.; Ghoshal, K.; Jacob, S. T.; Patel, T. *Gastroenterology* **2007**, *133*, 647.
9. Galardi, S.; Mercatelli, N.; Giorda, E.; Massalini, S.; Frajese, G. V.; Ciafre, S. A.; Farace, M. G. *J. Biol. Chem.* **2007**, *282*, 23716.
10. Pineau, P.; Volinia, S.; McJunkin, K.; Marchio, A.; Battiston, C.; Terris, B.; Mazzaferro, V.; Lowe, S. W.; Croce, C. M.; Dejean, A. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *107*, 264.
11. Connolly, E.; Melegari, M.; Landgraf, P.; Tchaikovskaya, T.; Tennant, B. C.; Slagle, B. L.; Rogler, L. E.; Zavolan, M.; Tuschl, T.; Rogler, C. E. *Am. J. Pathol.* **2008**, *173*, 856.
12. Tsuchida, A.; Ohno, S.; Wu, W.; Borjigin, N.; Fujita, K.; Aoki, T.; Ueda, S.; Takanashi, M.; Kuroda, M. *Cancer Sci.* **2011**, *102*, 2264.
13. Ji, Q.; Hao, X. B.; Zhang, M.; Tang, W. H.; Meng, Y.; Li, L.; Xiang, D. B.; DeSano, J. T.; Bommer, G. T.; Fan, D. M.; Fearon, E. R.; Lawrence, T. S.; Xu, L. *Plos One* **2009**, *4*, e6816.
14. Aqeilan, R. I.; Calin, G. A.; Croce, C. M. *Cell Death Differ.* **2009**, *17*, 215.
15. Cimmino, A.; Calin, G. A.; Fabbri, M.; Iorio, M. V.; Ferracin, M.; Shimizu, M.; Wojcik, S. E.; Aqeilan, R. I.; Zupo, S.; Dono, M.; Rassenti, L.; Alder, H.; Volinia, S.; Liu, C. G.; Kipps, T. J.; Negrini, M.; Croce, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 2464.
16. Chen, J. F.; Mandel, E. M.; Thomson, J. M.; Wu, Q. L.; Callis, T. E.; Hammond, S. M.; Conlon, F. L.; Wang, D. Z. *Nat. Genet.* **2006**, *38*, 228.
17. Kwon, C.; Han, Z.; Olson, E. N.; Srivastava, D. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 18986.
18. Thum, T.; Catalucci, D.; Bauersachs, J. *Cardiovasc. Res.* **2008**, *79*, 562.
19. Zhao, Y.; Samal, E.; Srivastava, D. *Nature* **2005**, *436*, 214.
20. Liu, R.; Chen, X.; Du, Y. Q.; Yao, W. Y.; Shen, L.; Wang, C.; Hu, Z. B.; Zhuang, R.; Ning, G.; Zhang, C. N.; Yuan, Y. Z.; Li, Z. S.; Zen, K.; Ba, Y.; Zhang, C. Y. *Clin. Chem.* **2012**, *58*, 610.
21. Yang, C. H.; Wang, C.; Chen, X.; Chen, S. D.; Zhang, Y. N.; Zhi, F.; Wang, J. J.; Li, L. M.; Zhou, X. J.; Li, N. Y.; Pan, H.; Zhang, J. F.; Zen, K.; Zhang, C. Y.; Zhang, C. N. *Int. J. Cancer* **2013**, *132*, 116.
22. Zen, K.; Zhang, C. Y. *Med. Res. Rev.* **2012**, *32*, 326.
23. Deiters, A. *AAPS J.* **2010**, *12*, 51.
24. Gumireddy, K.; Young, D. D.; Xiong, X.; Hogenesch, J. B.; Huang, Q.; Deiters, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 7482.
25. Young, D. D.; Connelly, C. M.; Grohmann, C.; Deiters, A. *J. Am. Chem. Soc.* **2010**, *132*, 7976.
26. Bose, D.; Jayaraj, G.; Suryawanshi, H.; Agarwala, P.; Pore, S. K.; Banerjee, R.; Maiti, S. *Angew. Chem., Int. Ed.* **2012**, *51*, 1019.
27. Melo, S.; Villanueva, A.; Moutinho, C.; Davalos, V.; Spizzo, R.; Ivan, C.; Rossi, S.; Setien, F.; Casanovas, O.; Simo-Riudalbas, L.; Carmona, J.; Carrere, J.; Vidal, A.; Aytes, A.; Puertas, S.; Ropero, S.; Kalluri, R.; Croce, C. M.; Calin, G. A.; Esteller, M. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 4394.
28. Chen, X.; Huang, C.; Zhang, W.; Wu, Y.; Zhang, C. Y.; Zhang, Y. *Chem. Commun.* **2012**, *48*, 6432.
29. Huang, C. M.; Yu, H. T.; Miao, Z. R.; Zhou, J.; Wang, S. A.; Fun, H. K.; Xu, J. H.; Zhang, Y. *Org. Biomol. Chem.* **2011**, *9*, 3629.
30. Wang, L.; Huang, Y. C.; Liu, Y.; Fun, H. K.; Zhang, Y.; Xu, J. H. *J. Org. Chem.* **2010**, *75*, 7757.
31. Jiang, H.; Huang, C. M.; Guo, J. J.; Zeng, C. Q.; Zhang, Y.; Yu, S. Y. *Chem. Eur. J.* **2012**, *18*, 15158.
32. Wu, D. D.; He, M. T.; Liu, Q. D.; Wang, W.; Zhou, J.; Wang, L.; Fun, H. K.; Xu, J. H.; Zhang, Y. *Org. Biomol. Chem.* **2012**, *10*, 3626.
33. Wu, D. D.; Wang, L.; Xu, K.; Song, J.; Fun, H. K.; Xu, J. H.; Zhang, Y. *Chem. Commun.* **2012**, *48*, 1168.
34. Jiang, H.; Chen, X. J.; Zhang, Y.; Yu, S. Y. *Adv. Syn. Catal.* **2013**, *355*, 809–813.
35. Yu, H. T.; Li, J. B.; Kou, Z. F.; Du, X. W.; Wei, Y.; Fun, H. K.; Xu, J. H.; Zhang, Y. *J. Org. Chem.* **2010**, *75*, 2989.
36. Ai, J.; Zhang, R.; Gao, X.; Niu, H. F.; Wang, N.; Xu, Y.; Li, Y.; Ma, N.; Sun, L. H.; Pan, Z. W.; Li, W. M.; Yang, B. F. *Cardiovasc. Res.* **2012**, *95*, 385.
37. Lu, Y.; Zhang, Y.; Shan, H.; Pan, Z.; Li, X.; Li, B.; Xu, C.; Zhang, B.; Zhang, F.; Dong, D.; Song, W.; Qiao, G.; Yang, B. *Cardiovasc. Res.* **2009**, *84*, 434.
38. Petrocca, F.; Vecchione, A.; Croce, C. M. *Cancer Res.* **2008**, *68*, 8191.
39. Yang, H.; Kong, W.; He, L.; Zhao, J. J.; O'Donnell, J. D.; Wang, J.; Wenham, R. M.; Coppola, D.; Kruk, P. A.; Nicosia, S. V.; Cheng, J. Q. *Cancer Res.* **2008**, *68*, 425.
40. Yang, Z.; Chen, S.; Luan, X.; Li, Y.; Liu, M.; Li, X.; Liu, T.; Tang, H. *IUBMB Life* **2009**, *61*, 1075.
41. Zhang, Y. J.; Liu, D. Q.; Chen, X.; Li, J.; Li, L. M.; Bian, Z.; Sun, F.; Lu, J. W.; Yin, Y. A.; Cai, X.; Sun, Q.; Wang, K. H.; Ba, Y.; Wang, Q. A.; Wang, D. J.; Yang, J. W.; Liu, P. S.; Xu, T.; Yan, Q. A.; Zhang, J. F.; Zen, K.; Zhang, C. Y. *Mol. Cell.* **2010**, *39*, 133.
42. Zheng, Q.; Zhou, L.; Mi, Q. S. *J. Immunol.* **2012**, *188*, 2118.
43. Zhou, B.; Wang, S.; Mayr, C.; Bartel, D. P.; Lodish, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7080.
44. Contreras, J.; Rao, D. S. *Leukemia* **2012**, *26*, 404.
45. Bian, Z.; Li, L. M.; Cui, J. L.; Zhang, H. J.; Liu, Y.; Zhang, C. Y.; Zen, K. *J. Pathol.* **2011**, *225*, 544.