Bioorganic & Medicinal Chemistry Letters 20 (2010) 7540–7543

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

N-(8-(3-Ethynylphenoxy)octyl-1-deoxynojirimycin suppresses growth and migration of human lung cancer cells

Yunxue Zhao [a,*], Wenjia Liu [a], Ying Zhou [b], Xiumei Zhang [a], Paul V. Murphy [b,*]

[a] Department of Pharmacology, School of Medicine, Shandong University, 44 West Wenhua Road, Jinan 250012, PR China
[b] School of Chemistry, National University of Ireland, Galway, University Road, Galway, Ireland

ARTICLE INFO

Article history:
Received 4 August 2010
Revised 8 September 2010
Accepted 10 September 2010
Available online 28 October 2010

Keywords:
N-(8-(3-Ethynylphenoxy)octyl-1-deoxynojirimycin
Lung cancer
Cell cycle
Apoptosis
Migration
Stress fibre

ABSTRACT

Previously *N*-(8-(3-ethynylphenoxy)octyl-1-deoxynojirimycin 1 has been shown to display properties associated with inhibition of angiogenesis. Here we examined the anti-tumourigenic role of **1** in a lung cancer cell line. This agent altered cell surface oligosaccharide expression and inhibited the growth of A549 cells by inducing G1 phase cell cycle arrest and apoptosis. Furthermore, stress fibre assembly and cell migration in A549 cells was markedly suppressed by **1**.

Lung cancer is one of the most common and life-threatening malignancies worldwide. The poor prognosis of lung cancer is largely attributed to the frequent occurrence of metastasis, since most metastatic tumours are unresectable at the time of presentation. The severe morbidity and poor prognosis of lung cancer highlight the importance of searching for new and effective agents against lung cancer.[1,2] Malignant transformation is associated with changes in the glycosylation of cell surface proteins and lipids. In tumour cells, alterations in cellular glycosylation may play a key role in their growth and metastatic behaviour.[3,4] Our previously study showed that *N*-(8-(3-ethynylphenoxy)octyl-1-deoxynojirimycin **1** (Fig. 1) inhibited an α-glucosidase, altered cell surface oligosaccharide expression and inhibited BAEC growth and migration. Its activity was superior to other analogues, which had shorter alkyl groups linking DNJ with the aryl alkyne and thus **1** was chosen for this study with tumour cells.[5] Hence the aim was to investigate the anticancer effects of **1** on human lung cancer cell A549, in an attempt to define the multifaceted role of **1** in cancer cell proliferation, apoptosis and migration.

Previous work has shown that **1** inhibited an α-glucosidase at the enzyme level and in a separate assay altered the expression of oligosaccharides on the surface of bovine aortic endothelial cells (BAECs).[6] The effect on the bioprocessing of A549 lung cancer cell surface oligosaccharides was thus investigated as described previously.[6] Accordingly, A549 cells were treated with 1-deoxynojirimycin (DNJ, positive control) and **1** (50 μM) for 24 h and then the binding of fluorescein labelled phytohemagglutinin-L (L-PHA-FITC) to the cells was monitored by fluorescence activated cell sorting (FACS) analysis in a flow cytometer. L-PHA recognises branched oligosaccharides that contain β-linked *N*-acetyl-lactosamine. Results of the analyses are shown in Figure 2. As expected, A549 cells that had been treated with DNJ and **1** for 24 h showed decreased L-PHA binding compared to the control. Although **1** is less potent as an α-glucosidase inhibitors than DNJ at the enzyme level,[6] it was found to alter surface oligosaccharide expression on A549 cells to the same level as DNJ. This is explained by the possibility that **1** has increased cellular permeability compared to DNJ, thereby facilitating diffusion to the ER and enhancing access to the glycoprocessing enzymes therein.

The alkyne **1** inhibited the growth of BAECs during the previous study.[6] The inhibition of A549 cell growth by **1** was therefore also determined using the MTT assay[7] and the results are summarised in Table 1. The IC_{50} of DNJ was greater than 1 mM. The aromatic acetylene derivative **2** (Fig. 1) was likewise inactive. However, **1**, which is a hybrid of DNJ and **2**, inhibited the growth of A549 cells with an IC_{50} of 51.2 μM.

N-(8-(3-Ethynylphenoxy)octyl-1-deoxynojirimycin **1** has previously been shown to inhibit the proliferation of BAECs by arresting these cells at the G1 phase of the cell cycle.[6] We further examined how *N*-(8-(3-ethynylphenoxy)octyl-1-deoxynojirimycin suppressed

* Corresponding authors. Tel.: +353 91 492465; fax: +353 91 525700 (P.V.M.).
E-mail addresses: zhaoyunxue@sdu.edu.cn (Y. Zhao), paul.v.murphy@nuigalway.ie (P.V. Murphy).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.065

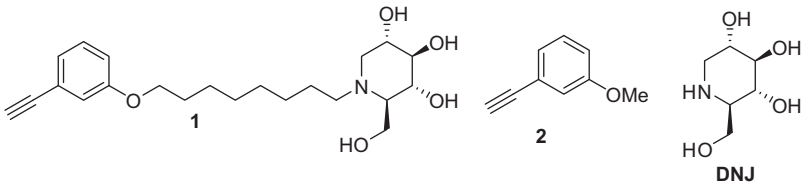

Figure 1. Structures of compounds.

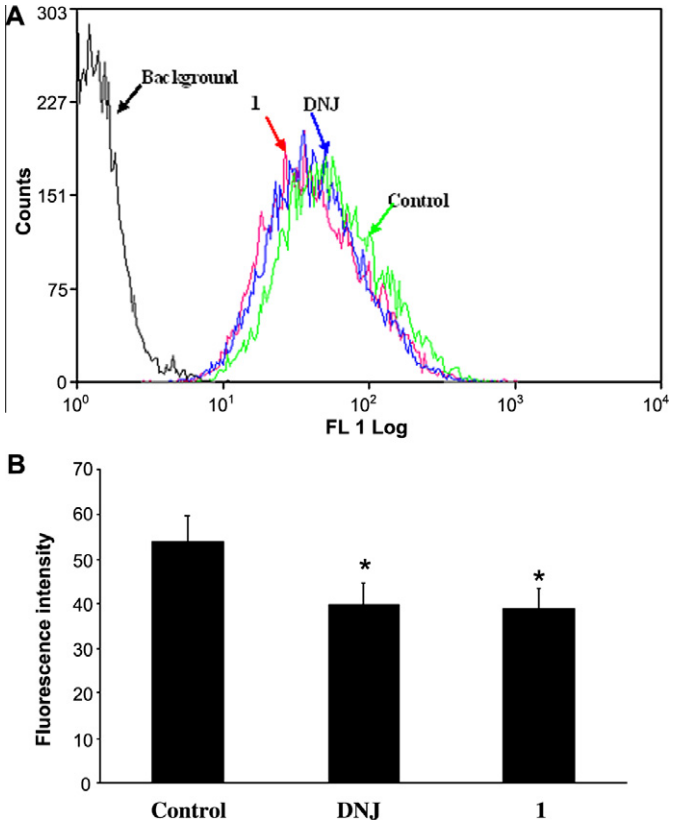

Figure 2. Effect of glycosidase inhibitors on cell surface oligosaccharides. (A) Lung cancer cells A549 were cultured in the presence of DNJ and **1** (5×10^{-5} M) for 24 h. Cells were harvested with trypsin/EDTA, treated with L-PHA-FITC and analysed by flow cytometry. Plots show the relationship between fluorescence intensity (FL1Log, *x*-axis) and cell number (counts, *y*-axis) for populations of cells treated with compounds. (B) Cell associated mean relative fluorescence intensities. Each bar represents the mean (±SD) of fluorescence intensity from triplicate determinations. *P <0.05 comparing with control.

Table 1
Inhibition of A549 cells growth by **DNJ**, **1** and **2**

Compd	A549 cell growth inhibition IC_{50}[a] (μM)
DNJ	>1000
1	51.2
2	>1000

[a] Values are means of three experiments.

the growth of lung cancer cells.[8] Its effect on cell cycle distribution was analysed and the result of a typical experiment is shown in Figure 3. As determined by flow cytometry, exposure of A549 cells to **1** (50 and 100 μM doses) after 24 h, resulted in a clear increase of the percentage of cells in the G1 phase with a concomitant decrease in the percentage of cells in the S and G2/M phase when compared with the control.

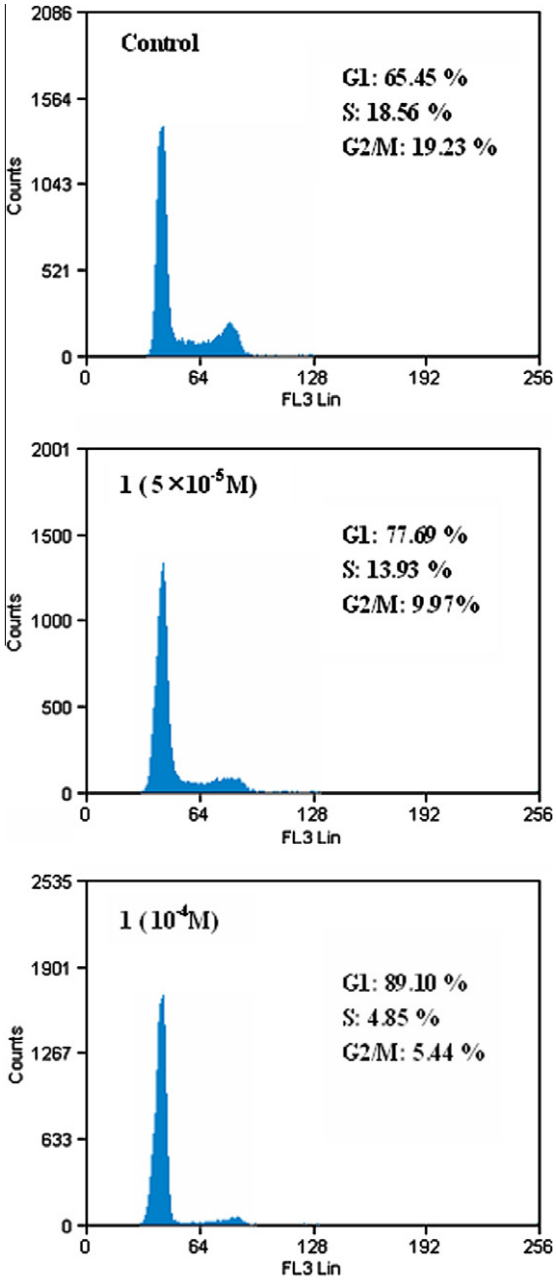

Figure 3. Effect of **1** on A549 cell cycle progression. A549 cells were treated with or without **1** for 24 h, respectively. Cells were then stained with propidium iodide, and the nuclei were analysed for DNA content by flow cytometry. A total of 10,000 nuclei were analysed from each sample, and the percentages of cells within G1, S and G2/M were determined. Representative profiles are shown, and the % of cells in G1, S and G2/M are shown in the upper right corner of the profiles.

The hybrid compound **1** did not induce apoptosis in BAECs (50 and 100 μM doses)[6] and it was of interest to establish whether it

would act similarly on the lung cancer cells. The A549 cells were thus treated with **1** (50 and 100 μM doses) for 24, 48 and 72 h, and subsequently subjected to staining with annexin V and propidium iodide and flow cytometry analysis.[9] The treatment with **1** (50 and 100 μM) caused a considerable apoptotic effect in A549 cells in comparison with controls (Fig. 4A and B). To further determine the molecular events leading to apoptosis induced by **1**, we analysed the mitochondrial membrane potential in cancer cells which had been treated with **1**. The damage of mitochondrial integrity and the consequent loss of mitochondrial membrane potential ($\Delta\Psi m$) is an event in the initiation and activation of apoptotic cascades.[10] To determine whether **1** induced mitochondrial disruption in A549 cells, we examined the depolarisation of the mitochondrial membrane by measuring the fluorescence remission shift (red to green) of the $\Delta\Psi m$ sensitive cationic JC-1 (5,50,6,60-tetrachloro-1,10,3,30-tetraethylbenzamidazolocarbocyanin iodide) dye in A549 cells. Briefly these cells were treated with **1** for 48 h and subsequently processed and stained with the JC-1 dye and then analysed by flow cytometry. Cells which had been treated with **1** (100 μM) showed an increase in green/red fluorescence intensity indicating increased mitochondrial membrane depolarisation (Fig. 4C). Therefore, it was concluded that the induction of apoptosis by **1** in lung cancer cells is closely associated with mitochondrial membrane disruption.

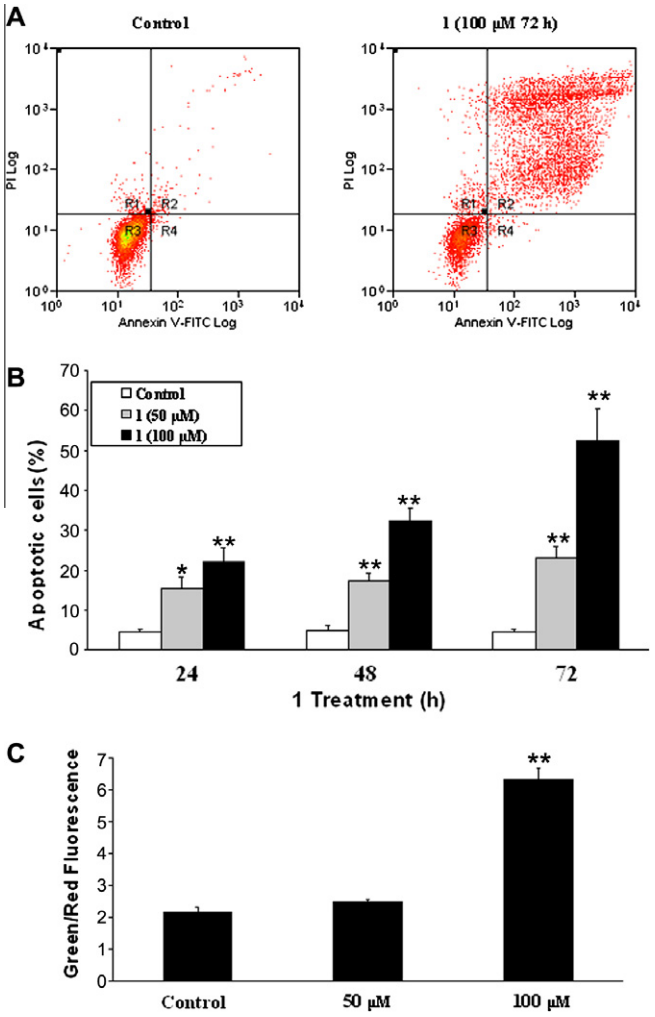

Figure 4. Apoptotic effect of **1** on human lung cancer A549 cells. (A) The fluorescence pattern of annexin V-FITC and propidium iodide-stained A549 cells after 72 h treatment with **1** (100 μM). (B) A549 cells were seeded and next day treated with different doses of **1** in complete medium as indicated. After 24, 48 and 72 h of treatments, cells were harvested and analysed for annexin V-positive apoptotic cell population as described in references and notes. (C) Effect of **1** on mitochondrial membrane potential in human lung cancer cell A549. Cells were treated with different doses of **1** for 48 h and then harvested and processed by JC-1 staining followed by flow cytometry analysis. Each bar represents the mean (±SD $n = 3$). $^{*}P < 0.01$, $^{**}P < 0.001$ comparing with control.

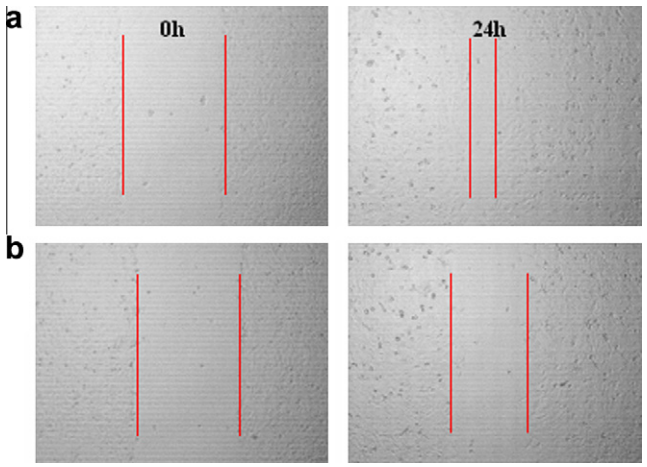

Figure 5. Effect of **1** on A549 cell migration. Migration of A549 cells was evaluated using the in vitro scratch-wound healing assay in the absence (a) and presence (b) of **1** (10^{-4} M).

Cancer patients die more often from metastasis rather than primary neoplastic lesions. Cell migration is a key feature of tumour progression and metastasis.[11,12] The effect of **1** on A549 cell migration was evaluated with the wound healing assay in vitro.[13] As shown in Figure 5, there is a significant drop in the ability of the **1** (100 μM) treated cells to migrate into the empty space. The results indicated that **1** can inhibit A549 cell migration (100 μM); this contrasts with observations for DNJ and **2**, which cannot inhibit cell migration at the same concentration (data not shown).

The DNJ derivative **1** inhibited the migration of A549 cells and it was thus of interest to explore the effect of **1** on actin polymerisation. It is well established that actin polymerisation, which leads to stress fibre assembly and depolymerisation, plays a crucial role in cell motility.[14,15] In an attempt to explore the molecular events in response to treatment with **1**, the formation of the actin cytoskeleton in A549 cells was assessed by staining with TRITC-phalloidin, which specifically binds F-actin.[16] The results showed that stress fibre assembly in A549 was abolished by **1** (100 μM) (Fig. 6). Therefore, **1** suppressed the migration of A549 at least in part through disrupting actin assembly and stress fibre formation.

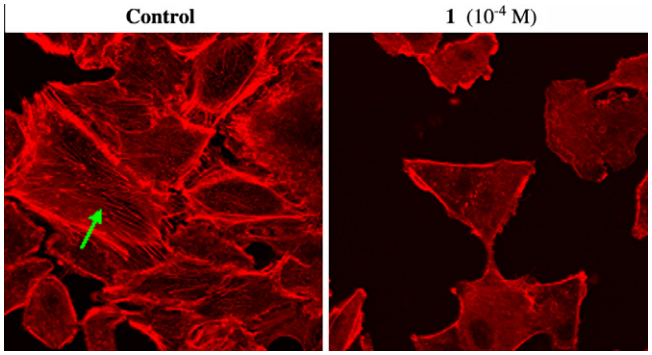

Figure 6. The effect of **1** on actin stress fibre polymerisation in A549 cells was assessed by fluorescence staining with TRITC-phalloidin, a substance which specifically detects F-actin. In the control, stress fibres are present (green arrow). Incubation of A549 cells with **1** (100 μM) led to marked disruption of stress fibre assembly.

In summary, we have demonstrated that the glycosidase inhibitor **1**, alters the cell surface oligosaccharide structure of A549. It displays remarkable anti-tumourigenic activities in lung cancer cells through multiple pathways, including cell cycle arrest, apoptosis induction and disruption of cell migration. In addition, we found that **1** disrupted cell migration at least in part through preventing stress fibre formation. The effects of **1** on A549 cell signal transduction and in an animal model for cancer are currently being evaluated.

Acknowledgments

The research was supported by Science Foundation Ireland (03/IBN/B352) and Shandong University (10000068961013).

References and notes

1. Spira, A.; Ettinger, D. S. *N. Eng. J. Med.* **2004**, *350*, 379.
2. Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J.; Murray, T.; Thun, MJ. *CA Cancer J. Clin.* **2008**, *58*, 71.
3. Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Disc.* **2005**, *4*, 477.
4. Partridge, EA.; Le Roy, C.; Di Guglielmo, GM.; Pawling, J.; Cheung, P.; Granovsky, M.; Nabi, I. R.; Wrana, J. L.; Dennis, J. W. *Science* **2004**, *306*, 120.
5. For the synthesis of **1** and analogues see: (a) Zhao, Y.; Zhou, Y.; O' Boyle, K. M.; Murphy, P. V. *Bioorg. Med. Chem.* **2008**, *16*, 6333; (b) Zhou, Y.; Zhao, Y.; O' Boyle, K. M.; Murphy, P. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 954–958.
6. Zhao, Y.; Zhou, Y.; O' Boyle, K. M.; Murphy, P. V. *Chem. Biol. Drug Des.* **2010**, *75*, 570. Lung cancer cells (A549 cells) were cultured in F12K medium supplemented with 10% heat inactivated FBS, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin and maintained at 37 °C in a humidified atmosphere of 5% CO_2. The cultured cells were treated with compounds (50 μM) for 24 h in 6 well plate. Cells were then harvested and washed with DPBS. Approximately 5×10^5 cells were re-suspended in test tubes and incubated in DPBS/1% BSA with 200 μl 2 μg/mL of the plant lectins L-PHA-FITC for 1 h at 4 °C. Cells were washed twice with DPBS, and fluorescence histogram profiles were determined using flow cytometric FACS analysis. Each compound was tested in triplicate and the experiments were repeated three times.
7. See Ref. 6. The A549 cells (2.5×10^3 cells) were seeded on 96-well microtitre plates in F12K medium with 10% FBS and incubated overnight. The cell culture medium was replaced by the different dose of a solution of **1** and the other compounds, and then the cells were cultured for another 72 h. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reagent was added to the cell supernatant for a final concentration of 0.5 mg/ml of MTT. After 3 h the cell culture medium was removed. Formazan crystals in adherent cells were dissolved in 200 μl DMSO and the absorbance of the formazan solution was measured. Each compound was tested in triplicate and the experiments were repeated three times.
8. See Ref. 6. Cells were seeded on 6 well plates in F12K medium with 10% FBS overnight. Then **1** was added to the cells, which were then cultured for another 24 h. Cells were collected with trypsin-EDTA and washed three times with PBS. The cells were re-suspended and fixed for at least 2 h at −20 °C with 70% ethanol. After washing twice with PBS, cells were incubated at 25 °C with 200 μg/ml RNase A for 30 min. The resulting cells were incubated with 50 μg/ml propidium iodide for 30 min at 4 °C. The treated cells were subjected to flow cytometry and the percentage of cells at each phase of the cell cycle was analysed. Compound **1** was tested in triplicate and the experiment was repeated three times.
9. Zhao, Y.; Yang, G.; Ren, D.; Zhang, X.; Yin, Q.; Sun, X. *Mol. Biol. Rep.* **2010** Jun 30. [Epub ahead of print] Briefly, after treatment with **1** (0, 50 and 100 μM for 24, 48 and 72 h), A549 cells were collected and washed with DPBS twice, and subjected to annexin V and propidium iodide staining using Annexin-V FITC apoptosis kit following the step-by-step protocol provided by the manufacturer (Biosource). After staining, flow cytometry was performed for the quantification of apoptotic cells. Compound **1** was tested in triplicate and the experiment was repeated three times in each case.
10. Tait, J. F. *J. Nucl. Med.* **2008**, *49*, 1573.
11. Christofori, G. *Nature* **2006**, *441*, 444.
12. Janik, ME.; Lityńska, A.; Vereecken, P. *Biochim. Biophys. Acta* **2010**, *1800*, 545.
13. See Ref. 9. Briefly A549 cells (3×10^4 cells) were seeded on 96-well microtitre plates in F12K with 10% FBS overnight. The confluent monolayers were wounded with a 200 μl plastic pipette tip. After washing, the chamber slide was incubated at 37 °C in 10% FBS F12K media in the presence or absence of compounds. Migration of cells into wounded areas were photographed at 0 and 24 h.
14. Palm, D.; Lang, K.; Brandt, B.; Zaenker, KS.; Entschladen, F. *Semin. Cancer Biol.* **2005**, *15*, 396.
15. Vignjevic, D.; Montagnac, G. *Semin. Cancer Biol.* **2008**, *18*, 12.
16. See Ref. 9. Cells were seeded to the glass coverslips in cell culture dishes (3.5 cm) overnight. Compound **1** was then added and cells were cultured for 24 h. After fixing with 4% paraformaldehyde, cells were treated with 0.1% Triton X-100 and blocked with 1% BSA. Cells were incubated with TRITC-conjugated phalloidin for 60 min and examined under Confocal Laser Scanning Microscope (100×, oil). The experiments were repeated at three times in each case.