JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY, 2017
VOL. 33, NO. 1, 147–150
https://doi.org/10.1080/14756366.2017.1404593

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Symmetric molecules with 1,4-triazole moieties as potent inhibitors of tumour-associated lactate dehydrogenase-A

Abdul-Malek S. Altamimi[a], Ahmed M. Alafeefy[b], Agnese Balode[c,d], Igor Vozny[c], Aleksandrs Pustenko[c,d], Mohey Eldin El Shikh[e], Fatmah A. S. Alasmary[f], Sherif A. Abdel-Gawad[a,g] and Raivis Žalubovskis[c]

[a]Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Alkharj, Saudi Arabia; [b]Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia; [c]Latvian Institute of Organic Synthesis, Riga, Latvia; [d]Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia; [e]Experimental Medicine and Rheumatology, William Harvey Research Institute, Queen Mary University of London, London, UK; [f]Chemistry Department, College of Science, King Saud University, Saudi Arabia, Riyadh; [g]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ABSTRACT

A series of symmetric molecules incorporating aryl or pyridyl moieties as central core and 1,4-substituted triazoles as a side bridge was synthesised. The new compounds were investigated as lactate dehydrogenase (LDH, EC 1.1.1.27) inhibitors. The cancer associated LDHA isoform was inhibited with $IC_{50} = 117–174$ μM. Seven compounds exhibited better LDHA inhibition (IC_{50} 117–136 μM) compared to known LDH inhibitor – galloflavin (IC_{50} 157 μM).

ARTICLE HISTORY
Received 2 September 2017
Revised 7 November 2017
Accepted 7 November 2017

KEYWORDS
Lactate dehydrogenase; triazole; inhibitors

Introduction

The lactate dehydrogenase (LDH, EC 1.1.1.27) is one of the most abundant proteins and it is expressed in all tissues[1]. The main function of LDH is interconversion of lactate and pyruvate with accompanying interconversion of NAD^+ and NADH.

Three LDH isoforms are present in humans – LDHA, LDHB and LDHC. LDHA and LDHB are expressed in all cells, whereas LDHC is produced only in testis[1]. In the active form, LDH is a tetramer formed of LDHA and LDHB in various ratios making five tetramers: LDH1 ($4 \times$ LDHB), LDH2 ($1 \times$ LDHA/$3 \times$ LDHB), LDH3 ($2 \times$ LDHA/$2 \times$ LDHB), LDH4 ($1 \times$ LDHA/$3 \times$ LDHB) and LDH5 ($4 \times$ LDHA)[2].

In normal cells, predominant is LDHB where it converts lactate to pyruvate with interconversion of NAD^+ into NADH, which allows cells to use lactate as a nutrient source for oxidative metabolism, and/or for gluconeogenesis[2]. LDHA is the predominant isoform found in skeletal muscle and other highly glycolytic tissues. In contrast to LDHB, LDHA has a higher affinity for pyruvate, that is, LDHA and LDH5 tetramer in particular predominantly converts pyruvate to lactate with consumption of one NADH molecule to produce NAD^+ which in turn is essential in glycolysis[3]. Cancer cells mainly generate energy through glycolysis even in the presence of normal oxygen pressure[4]. Since the LDHA is the final enzyme in glycolysis pathway where generated NAD^+ is necessary for continued high glycolysis rate in cancer cells (Warburg effect)[4], LDHA is an important supporter of glucose metabolism in cancer cells and can affect tumourigenesis and metastasis[5]. Additionally, elevated levels of LDHA are markers of many tumours, the majority of them are highly glycolytic, and high LDHA levels are related with poor prognosis, for instance in several human malignancies[6]. Therefore, LDHA is defined as anticancer drug target[6,7]. Notably, a limited number of LDHA inhibitors is reported in the literature so far[8]. In several papers, very promising LDHA inhibition results have been reported. As a one of the first inhibitors with low-micromolar inhibition activity ($K_i = 13.5$ μM) compound **FX11** (Figure 1) along with two similar compounds was reported in 2010[9]. Utilising NMR and SPR (surface plasmon resonance) fragment based hit identification technique and further optimisation of structure a series with low- and submicromolar LDHA inhibition was obtained with best lead **A** ($IC_{50} = 0.27$ μM)[10]. In the other study, also using fragment-based hit identification, series of new LDHA inhibitors was obtained. In this series, IC_{50} values ranged from 59 to 0.12 μM, where the best inhibition was observed for compound **B**.[11] It was noted that in this series carbohydrate moiety in the middle of the molecule and its stereochemistry had significant influence on the inhibition activity. In very recent work through docking-based virtual screening, several potential LDHA inhibitors were identified. One of the compounds identified (**C**) showed very good LDHA inhibition potency *in vitro* with an IC_{50} value of 0.33 μM.[12]

Results and discussions

Here, we report the synthesis of symmetric molecules incorporating aryl or pyridyl moieties as central core and 1,4-substituted triazoles as a side bridge and they evaluation as LDHA inhibitors.

At the beginning of our study based on literature data[11], we assumed that V-shape structures are beneficiary for good LDHA inhibition. Our assumption was based on published X-ray structures, for instance for **B**–LDHA complex (PDB code 4I9H). It was also obvious that besides V-shape of the molecule and appropriate length of V-"arms" the terminal groups have to be able to make

CONTACT Raivis Žalubovskis raivis@osi.lv Latvian Institute of Organic Synthesis, Aizkraukles 21, LV-1006, Riga, Latvia; Ahmed M. Alafeefy alafeefy@hotmail.com Department of Chemistry, Kulliyyah of Science, International Islamic University, P.O. Box 141, 25710 Kuantan, Malyasia

Supplemental data for this article can be accessed here.

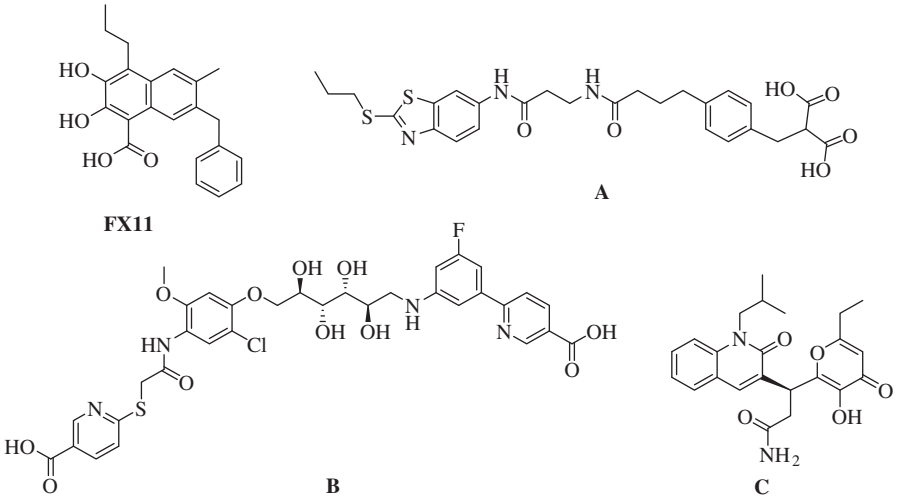
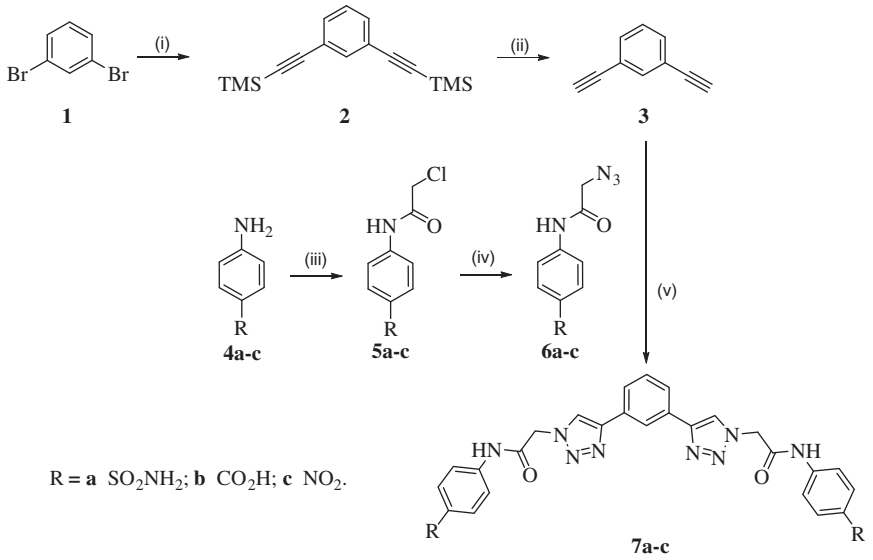


Figure 1. Examples of chemical structures of known LDHA inhibitors.



Scheme 1. Reagents and conditions: (i) trimethylsilylacetylene, $Pd_2(PPh_3)_2$, CuI, *i*-Pr_2NH, THF, 70 °C; (ii) KF, MeOH/THF, rt, 69% for two steps; (iii) chloroacetyl chloride, K_2CO_3, THF, 0 °C, **5a** (98%), **5b** (84%), **5c** (89%); (iv) NaN_3, DMF, rt, **6a** (97%), **6b** (68%), **6c** (95%); (v) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, AcOH, DMF/H_2O, rt, **7a** (32%), **7b** (71%), **7c** (41%). All detailed experimental procedures are provided in the Supplemental data.

hydrogen bonding, therefore we chose carboxylic, sulphonamide and nitro groups as terminal ones for this study.

Chemistry

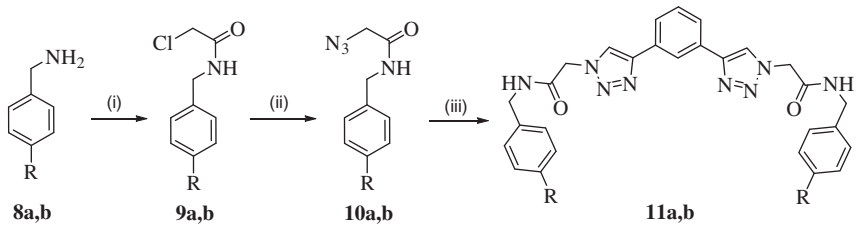
The synthesis of desired inhibitors **7a-c** was started from commercially available 1,3-dibromobenzene (**1**) which was reacted with trimethylsilylacetylene in Sonogashira reaction to provide bis-TMS protected derivative **2** (Scheme 1). Following deprotection with KF afforded building block **3** in good yield over two steps[13]. Azides **6a-c** necessary for Cu-mediated click reaction were prepared in two steps from commercially available anilines **4a-c**. Acylation of anilines **4a-c** with chloroacetyl chloride afforded chlorides **5a-c**[14–16] in good yields and following treatment with NaN_3 provided azide building blocks **6a-c** also in good yields. Reaction of building block **3** with **6a-c** under acidic click reaction condition[17] provided inhibitors **7a-c**.

The same strategy as described earlier was utilised for the synthesis of inhibitors **11a** and **11b**. First, aminomethylphenyl derivatives **8a,b** were acylated with chloroacelyl chloride to obtain intermediates **9a** and **9b**, which were converted into corresponding azides **10a,b** by treatment with NaN_3 (Scheme 2). Following reaction of azides **10a,b** with building block **3** under acidic click reaction conditions provided desired inhibitors **11a** and **11b**.

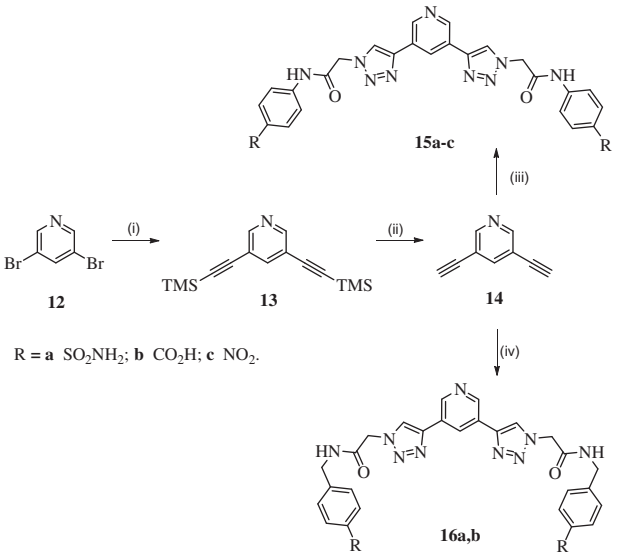
Bis-acetylenylpyridine building block **14** was prepared in similar way to compound **3**, where 3,5-dibromopyridine **12** was first reacted with TMS-acetylene under Sonogashira reaction conditions and then TMS were eliminated in obtained intermediate **13** by treatment with K_2CO_3 proving building block **14** in 67% yield over two steps (Scheme 3)[18].

The reaction between building blocks **13** and **6a-c** under acidic click reaction conditions provided inhibitors **15a-c** (Scheme 3). In turn, treatment of **13** by azides **10a,b** under the same condition provided inhibitors **16a,b** with extended bridge.

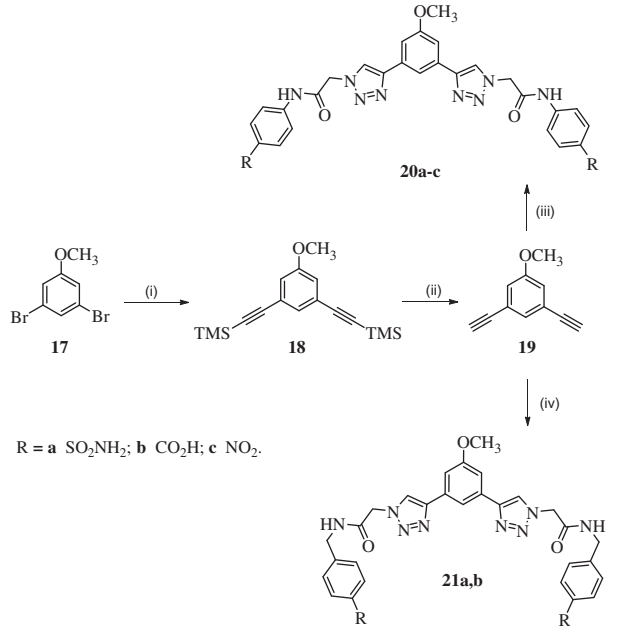
And finally, for the synthesis of inhibitors **20a-c** and **21a,b,** anisole building block **19** was synthesised in analogy to **3** and **14** as above, starting synthesis from dibromoanisole **17**. Bis-TMS protected intermediate **18** was obtained under Sonogashira reaction conditions and following deprotection by potassium hydroxide provided building block **19** in 61% yield over two steps (Scheme 4)[19].



Scheme 2. Reagents and conditions: (i) chloroacetyl chloride, K_2CO_3, THF, 0 °C to rt, **9a** (33%), **9b** (69%); (ii) NaN_3, DMF, rt, **10a** (78%), **10b** (77); (iii) **3**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, AcOH, DMF/H_2O, rt, **11a** (17%), **11b** (59%).



Scheme 3. Reagents and conditions: (i) trimethylsilylacetylene, $Pd_2(PPh_3)_2$, CuI, Et_3N, 75 °C; (ii) K_2CO_3, MeOH/THF, rt, 67% for two steps; (iii) **6a-c**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, AcOH, DMF/H_2O, rt, **15a** (85%), **15b** (39%), **15c** (73%); (iv) **10a,b**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, AcOH, DMF/H_2O, rt, **16a** (63%), **16b** (46%).



Scheme 4. Reagents and conditions: (i) trimethylsilylacetylene, $Pd_2(PPh_3)_2$, CuI, Et_3N, THF 65 °C; (ii) KOH, MeOH/THF, rt, 61% for two steps; (iii) **6a-c**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, AcOH, DMF/H_2O, rt, **20a** (64%), **20b** (56%), **20c** (47%); (iv) **10a,b**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, AcOH, DMF/H_2O, rt, **21a** (31%), **21b** (85%).

Table 1. Inhibition data of human LDHA.

Compound	Inhibition, IC_{50} (μM)*
7a	128
7b	120
7c	172
11a	158
11b	156
15a	136
15b	125
15c	156
16a	174
16b	117
20a	165
20b	127
20c	163
21a	173
21b	128
Galloflavin	157
Blank	0

*Mean from three different assays, by colorimetric assay measuring the absorbance at 450 nm (errors were in the range of ±5–10% of the reported values).

Treatment of **19** with azides **6a-c** under the same condition as described above provided inhibitors **20a-c** (Scheme 4). In turn, treatment of **19** with azides **10a,b** afforded desired inhibitors **21a,b**.

The structures of all new compounds synthesised were fully approved by ^1H and ^{13}C NMR, IR and HRMS data (see Supplemental data).

Inhibition studies

All 15 symmetrical compounds were evaluated for their ability to inhibit LDHA, for the comparison galloflavin as a known isoform nonselective LDHA inhibitor[20] was used. Our choice of galloflavin was based on the fact that it is extensively studied as potent anti-cancer agents in recent years[21–25]; additionally, galloflavin is already commercially available.

Even though all compounds exhibited similar LDHA inhibition and it is difficult to perform structure-activity-relationship (SAR) analysis, we can divide this compound into two groups. First one, the most active compounds (**7a**, **7b**, **15a**, **15b**, **16b**, **20b** and **21b**) showed better LDHA inhibition compared to galloflalvin, ranging IC_{50} values from 117 to 136 μM for compounds in first group, whereas galloflavin has $IC_{50} = 157$ μM (Table 1). The most active compound in this group was **16b** ($IC_{50} = 117$ μM), which incorporated pyridyl moiety as a central core and carboxylic groups as a terminal ones. Second group of compounds (**7c**, **11a**, **11b**, **15c**, **16a**, **20a**, **20c** and **21a**) exhibited equal or weaker inhibition compared to galloflavin, ranging IC_{50} values from 156 to 174 μM. The most active compounds in this group were **11a**, **11b** and **15c** (IC_{50} 158, 156 and 156 μM, respectively). Similar activity of this

three compounds is hard to explain, where compounds **11a** and **11b** have phenyl ring as a central core and sulphonamide and carboxyl groups as terminal ones on aminomethylphenyl moieties, but compounds **15c** has pyridyl central moiety and nitro groups as terminal one on shorter, aniline containing, bridge.

We hypothesise that the putative interaction of the inhibitors described in this work with LDHA is similar to the published one, that is, inhibitors bound in the active centre close to conserved residues involved in the catalytic processing of LDHA substrates[26]. We also assume that at the same time the NADH cofactor is bound in the active centre near the inhibitor molecule. This binding region of the protein is known to undergo considerable conformational changes during the catalytic cycle, that is, it has rather high flexibility. Therefore, inhibitor binding might be unstable and this reflects in IC_{50} values obtained.

Conclusions

In conclusion, a series of new symmetric molecules have been designed and synthesises as potent LDHA inhibitors. The compounds synthesised exhibited promising *in vitro* LDHA inhibition activity, where seven compounds were better inhibitors (IC_{50} 117–136 μM) as known LDH inhibitor – galloflavin (IC_{50} 157 μM), and other eight showed equal or slightly lower inhibitory activity (IC_{50} 156–174 μM) as galloflavin. The results obtained are promising base for further development of novel LDH inhibitors.

Disclosure statement

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

Funding

This project was supported by the National Plan of Science, Technology and Innovation [Grant No. 12-MED2980–54], Prince Sattam bin Abdulaziz University, Alkharj, PO Box 173, 11942.

References

1. Di Stefano G, Manerba M, Di Ianni L, et al. Lactate dehydrogenase inhibition: exploring possible applications beyond cancer treatment. Future Med Chem 2016;8:713–25.
2. Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. J Clin Invest 2013;123:3685–92.
3. Dawson DM, Goodfriend TL, Kaplan NO. Lactic dehydrogenases: functions of the two types rates of synthesis of the two major forms can be correlated with metabolic differentiation. Science 1964;143:929–33.
4. Warburg O. On the origin of cancer cells. Science 1956;123:309–14.
5. Talaiezadeh A, Shahriari A, Tabandeh MR, et al. Kinetic characterization of lactate dehydrogenase in normal and malignant human breast tissues. Cancer Cell Int 2015;15:19.
6. Fiume L, Manerba M, Vettraino M, et al. Inhibition of lactate dehydrogenase activity as an approach to cancer therapy. Future Med Chem 2014;6:429–45.
7. Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. Cell Death Dis 2013;4:e532.
8. Rani R, Kumar V. Recent update on human lactate dehydrogenase enzyme 5 (hLDH5) inhibitors: a promising approach for cancer chemotherapy. J Med Chem 2016;59:487–96.
9. Le A, Cooper CR, Gouw AM, et al. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. PNAS 2010;107:2037–42.
10. Ward RA, Brassington C, Breeze AL, et al. Design and synthesis of novel lactate dehydrogenase A inhibitors by fragment-based lead generation. J Med Chem 2012; 55:3285–306.
11. Kohlmann A, Zech SG, Li F, et al. Fragment growing and linking lead to novel nanomolar lactate dehydrogenase inhibitors. J Med Chem 2013;56:1023–40.
12. Fang A, Zhang Q, Fan H, et al. Discovery of human lactate dehydrogenase A (LDHA) inhibitors as anticancer agents to inhibit the proliferation of MG-63 osteosarcoma cells. Med Chem Commun 2017;8:1720–6.
13. Neenan TX, Whitesides GM. Synthesis of high carbon materials from acetylenic precursors. Preparation of aromatic monomers bearing multiple ethynyl groups. J Org Chem 1988;53:2489–96.
14. Addy PS, Saha B, Singh PND, et al. 1,3,5-Trisubstituted benzenes as fluorescent photoaffinity probes for human carbonic anhydrase II capture. Chem Commun 2013;49:1930–2.
15. Harte AJ, Gunnlaugsson T. Synthesis of α-chloroamides in water. Tetrahedron Lett 2006;47:6321–4.
16. Deepkumar J, Kalpesh P. Synthesis and evaluation of novel benzimidazole derivatives as antimicrobial agents. Med Chem Res 2014;23:1290–9.
17. Shao C, Wang X, Xu J, et al. Carboxylic acid-promoted copper(I)-catalyzed azide-alkyne cycloaddition. J Org Chem. 2010;75:7002–5.
18. Goto H, Heemstra JM, Hill DJ, et al. Single-site modifcation and their effect on the folding stability of *m*-phenylene ethynylene oligomers. Org Lett 2004;6:889–92.
19. Beves JE, Blanco V, Blight BA, et al. Towards metal complexes that can directionally walk along tracks: controlled stepping of a molecular biped with a palladium(II) foot. J Am Chem Soc 2014;136:2094–100.
20. Manerba M, Vettraino M, Fiume L, et al. Galloflavin (CAS 568-80-9): a novel inhibitor of lactate dehydrogenase. ChemMedChem 2012;7:311–17.
21. Farabegoli F, Vettraino M, Manerba M, et al. Galloflavin, a new lactate dehydrogenase inhibitor, induces the death of human breast cancer cells with different glycolytic attitude by affecting distinct signaling pathways. Eur J Pharm Sci 2012;47:729–38.
22. Vettraino M, Manerba M, Govoni M, et al. Galloflavin suppresses lactate dehydrogenase activity and causes MYC downregulation in Burkitt lymphoma cells through NAD/NADH-dependent inhibition of sirtuin-1. Anticancer Drugs 2013;24:862–70.
23. Manerba M, Di Ianni L, Fiume L, et al. Lactate dehydrogenase inhibitors sensitize lymphoma cells to cisplatin without enhancing the drug effects on immortalized normal lymphocytes. Eur J Pharm Sci 2015;74:95–102.
24. Han X, Sheng X, Jones HM, et al. Evaluation of the anti-tumor effects of lactate dehydrogenase inhibitor galloflavin in endometrial cancer cells. J Hematol Oncol 2015;8:2.
25. Manerba M, Di Ianni L, Govoni M, et al. Lactate dehydrogenase inhibitors can reverse inflammation induced hanges in colon cancer cells. Eur J Pharm Sci 2017;96:37–44.
26. Dragovich PS, Fauber BP, Corson LB, et al. Identification of substituted 2-thio-6-oxo-1,6-dihydropyrimidines as inhibitors of human lactate dehydrogenase. Bioorg Med Chem Lett 2013;23:3186–94.