Chinese Chemical Letters 34 (2023) 107696

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Novel seven-membered ring-fused naphthalimide derivatives with potentials for cancer theranostics

Yonghui Yang[1], Xiayu Shi[1], Zhuo Chen*, Yufang Xu, Xuhong Qian, Weiping Zhu*

State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of Chemical Biology, Shanghai key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

ARTICLE INFO

Article history:
Received 14 April 2022
Revised 14 July 2022
Accepted 19 July 2022
Available online 21 July 2022

Keywords:
Seven-membered ring
Antitumor activity
High fluorescent quantum yield
Theranostics

ABSTRACT

Naphthalimide derivatives have good planarity and large conjugated structure and therefore possess photophysical properties and biological activities. Previously, our group discovered seven-membered heterocyclic derivatives *via* modifying 4- and 5-positions of naphthalimide skeleton and found the derivatives had good water solubility and showed large stokes shift and strong fluorescence in water. In this article, we designed and synthesized more seven-membered ring-fused naphthalimide derivatives (**Y1**-**Y16**) by introducing different substitutions on the imide group. Among them, **Y1**, **Y5**, **Y9** were found to show similar cytotoxic activities with Amonafide against A549 and HL60 cells, with IC_{50} values at 10^{-6} mol/L. What is more, the asymmetry derivatives (**Y1** and **Y5**) showed high fluorescent quantum yields in the aqueous phase ($\Phi = 0.47$). Considering the great fluorescence quantum yields in water and the potent anti-tumor activities of the representative seven-membered ring-fused naphthalimides, they have potentials to be used as agents for cancer theranostics.

Cancer is still an important factor endangering the safety of people all over the world, and there were nearly 20 million new cases in 2020 [1–4]. Early detection and treatment of cancer are important for improving the survival rate and the quality of life of tumor patients [5–8]. Fluorescent probes have prospects of being applied in early diagnosis because of their advantages in sensitivity and *in situ* detection [9–15]. Therefore, it is worthwhile to develop fluorescent reagents that can diagnose and treat tumor lesions at the same time [16–24].

On one hand, the antitumor activities of naphthalimide derivatives was firstly discovered by Braña and his colleagues [25–28]. The representative compound Amonafide exhibited great antitumor activities both *in vitro* and *in vivo* (Fig. 1). However, due to the *N*-acetylation metabolism of the side chain in humans, Amonafide would cause high variable and unpredictable toxicity, which leads to great obstacles in phase III clinical trials [29]. Cyclization was an important structural modification strategy to avoid *N*-acetylation [30–36]. For example, **R16** could inhibit the proliferation of a panel of human cancer cell lines and suppress tumor growth in mice implanted with S-180 sarcoma and H22 hepatoma. Though the antitumor activities *in vitro* were improved significantly, the solubility of these derivatives were reduced greatly, which would induce low yield of the target compounds and affect the antitumor efficacy *in vivo* [37].

On the other hand, naphthalimide and its analogues are conventional fluorescent dyes for fibers and wildly used as fluorescent probes [38–40]. The conjugated polycyclic systems of naphthalimide derivatives are the structural basis of chromophores and fluorophores, which have many applications in fluorescence sensing. As reported, rigidifying the structure of fluorophore can reduce the energy consumption from rotation and vibration, which can efficiently improve the fluorescence properties [41]. Many similar modified strategies are applied in BODIPY fluorescent dyes. For example, Mei *et al.* reported a selective and sensitive chemosensor (**1**) for Cu^{2+}, which has longer wavelength through extending the rigidity of the BODIPY core [42]. Zhao and Carreira also reported a highly fluorescent ($\Phi = 0.28$), photostable aza-dipyrromethene dye (**2**) with very sharp and intense absorption (full width at half maximum height, fwhm = 30.4 nm; $\varepsilon = 159\ 000$) in the NIR region ($\lambda_{max} = 740$ nm) [43].

Recently, our research group developed a class of naphthalimide derivatives modified with seven-membered heterocycles at the 4- and 5- positions [44]. These compounds exhibited excellent optical properties. Besides, we observed that these compounds have good solubility, which may overcome the solubility problems of cy-

* Corresponding authors.
E-mail addresses: chenzhuo@ecust.edu.cn (Z. Chen), wpzhu@ecust.edu.cn (W. Zhu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cclet.2022.07.039
1001-8417/

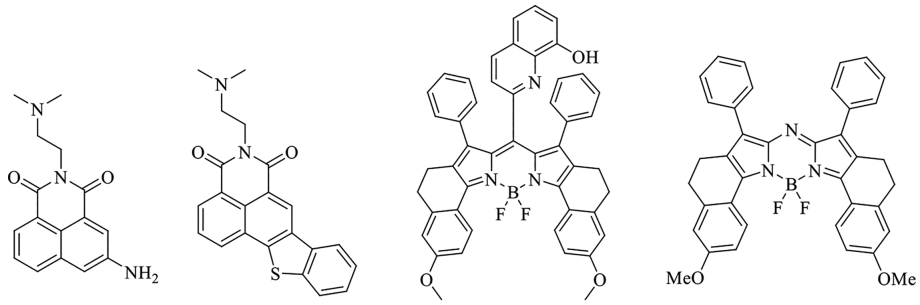
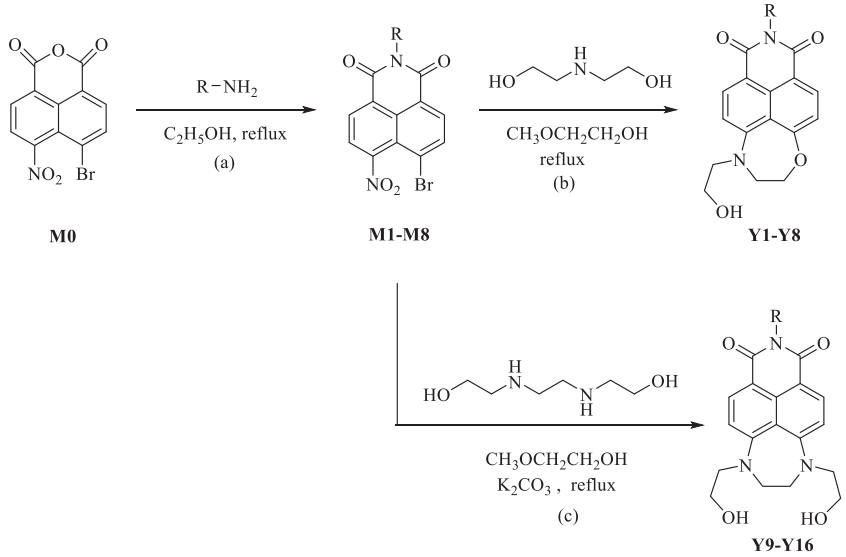


Fig. 1. Structures of Amonafide, **R16** and BODIPY derivatives **1** and **2**.



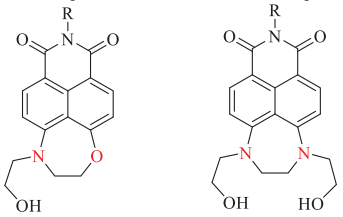
Scheme 1. Syntheses of target compounds **Y1**-**Y16**. (a) C_2H_5OH, reflux, 6 h; (b) $CH_3OCH_2CH_2OH$, reflux, 8 h; (c) K_2CO_3, $CH_3OCH_2CH_2OH$, reflux, 8 h.

clization. Therefore, we designed and synthesized more derivatives with different nitrogen-containing substituent groups. The newly-synthesized compounds were evaluated of their antitumor activities against leukemia and solid tumor cell lines compared with Amonafide. Besides, their potentials as theranostic agents were primarily explored.

As shown in Scheme 1, the synthesis of the target molecule was similar to the scheme previously reported by our group. The intermediate 4-bromo-5-nitro-1,8-naphthalic anhydride was synthesized as we described before [44]. It was then substituted with different nitrogen-containing substituents and subjected to a ring-forming reaction at the 4,5-position with diethanolamine to yield **Y1**-**Y8** and *N,N'*-bis(2-hydroxyethyl)ethylenediamine to yield **Y9**-**Y16**.

The antiproliferative activity of compounds **Y1**-**Y16** were evaluated against human cancer cell lines A549 (human non-small cell lung tumor cell), HL60 (Human promyelocytic leukemia cells). Amonafide were tested as a reference compound. In HL60 cells, *N',N'*-dimethylethane-1,2-diamine derivatives **Y1** and **Y9** exhibited great antitumor activities, which was consistent with previous reported naphthalimide derivatives (3.1 and 1.4 μmol/L, Table 1). The *N',N'*-diethyl analogues of **Y1** and **Y9** (**Y2**, **Y10**) exhibited relatively weaker antiproliferative activities (6.2 and 2.2 μmol/L). However, extention of the linker between the imide and the amino group to three carbon chain (**Y3**, **Y4**, **Y11** and **Y12**) greatly decreased their antiproliferative activities. No obvious antitumor activities were observed for the four compounds in HL60 cells. Compounds increasing the rigidity of the amino group (**Y5**, **Y6**, **Y13** and **Y14**) also exhibited potent antiproliferative activities. For example, the

Table 1
The antiproliferative activities of compounds **Y1-Y16**.



Compd.	R	Cell lines IC_{50} (μmol/L)	
		A549	HL60
Y1	$-CH_2CH_2N(CH_3)_2$	7.1	3.1
Y2	$-CH_2CH_2N(C_2H_5)_2$	17.2	6.2
Y3	$-CH_2CH_2CH_2N(CH_3)_2$	27.2	>100
Y4	$-CH_2CH_2CH_2N(C_2H_5)_2$	52.6	>100
Y5	$-CH_2CH_2N(CH_2)_4$	2.5	1.9
Y6	$-CH_2CH_2N(CH_2)_5$	8.0	3.4
Y7	$-CH_2CH_2OH$	>100	>100
Y8	$-CH_2CH_2N(CH_2)_4NCH_3$	67.2	>100
Y9	$-CH_2CH_2N(CH_3)_2$	3.6	1.4
Y10	$-CH_2CH_2N(C_2H_5)_2$	16.6	2.2
Y11	$-CH_2CH_2CH_2N(CH_3)_2$	>100	>100
Y12	$-CH_2CH_2CH_2N(C_2H_5)_2$	>100	>100
Y13	$-CH_2CH_2N(CH_2)_4$	21.2	6.6
Y14	$-CH_2CH_2N(CH_2)_5$	7.0	1.7
Y15	$-CH_2CH_2OH$	>100	>100
Y16	$-CH_2CH_2N(CH_2)_4NCH_3$	>100	>100
Amonafide		1.6	0.7

Table 2
The antiproliferative activities of compounds **Y1**, **Y5** and **Y9**.

Compd.	Cell lines (IC_{50}, μmol/L)			
	Eca-109	HCT116	HT29	MDA-MB-231
Y1	4.9	2.1	4.5	1.7
Y5	4.6	1.6	>10	1.2
Y9	5.5	1.2	3.3	1.2
Amonafide	2.4	0.9	2.7	1.2

Table 3
The fluorescence properties of compounds **Y1**, **Y5** and **Y9**.[a]

Compd.	Solvent	λ_{abs} (nm)	λ_{em}[b] (nm)	ε (L mol^{-1}cm^{-1})	Φ[c]
Y1	CH_3CN	442	504	6480	0.67
	DMSO	454	504	6450	0.76
	H_2O	466	535	7070	0.47
Y5	CH_3CN	444	497	14100	0.35
	DMSO	448	505	13800	0.47
	H_2O	448	533	15600	0.41
Y9	CH_3CN	464	502	11200	0.69
	DMSO	474	502	11940	0.64
	H_2O	480	533	14400	0.12

ε: Molar extinction coefficient at longest wavelength transition; Φ: fluorescence quantum yields.
[a] Control compound: *N*-butyl-4-butylamine-1,8-naphthalimide, usually used as reference ($\Phi_s = 0.81$ in alcohol).
[b] Excited at maximum absorption wavelength.
[c] Determined relative to fluorescein ($\Phi_f = 0.79$ in 0.1 mol/L sodium hydroxide aqueous solution).

pyrrolidine and piperidine derivatives (**Y5** and **Y6**) showed potent antitumor activities with IC_{50} values at 1.9 and 3.4 μmol/L. Furthermore, compounds without amino side chain (**Y7** and **Y15**) or with the methylpiperazine side chain (**Y8** and **Y16**) did not display obvious antitumor activities. Similar structure-activity relationship was observed in A549 cells.

Compounds **Y1**, **Y5** and **Y9** were then chosen and evaluated against more solid tumor cell lines, including Eca-109 (human esophageal cancer cell), HCT116 (human colon cancer cell), HT29 (human colon cancer cell), MDA-MB-231 (triple negative breast cancer cell). Amonafide was also tested as reference compounds. As shown in Table 2, compound **Y9** was the most potent one of these compounds. It showed similar antiproliferative activities with Amonafide in HCT116, HT29 and MDA-MB-231 cells. Compounds **Y1** and **Y5** also displayed great antiproliferative activity with IC_{50} at single digital micromolars in these tested cells except for **Y5** in HT29 cells.

In our previous report, we found that the water solubility of the seven-membered ring-fused naphthalimide derivatives was greatly improved compared to previous heterocycle-fused naphthalimide derivatives [44]. Besides, according to the spectroscopic tests, the fluorescence quantum yields and molar extinction coefficients of these derivatives in aqueous solution were found to be much higher. As shown in Table 3 and Fig. S49 (Supporting information), it was found that the absorption wavelength of the asymmetric derivatives (**Y1** and **Y5**) in organic phases was around 450 nm and the emission wavelength was around 500 nm. Therefore, the Stokes shift was around 50 nm. Besides, the fluorescence quantum yield was between 0.4 and 0.89. While in water, the absorption wavelength shifted to about 470 nm and the emission wavelength shifted to about 530 nm. What is more, the fluorescence quantum yield in water was still about 0.4–0.5, which was a very rare spectral property.

The absorption wavelength of the symmetric compounds (**Y9**) in organic phases was around 470 nm, and the emission length had no obvious changes with its asymmetric analogues. The Stokes shift decreased to around 30 nm. The fluorescence quantum yield

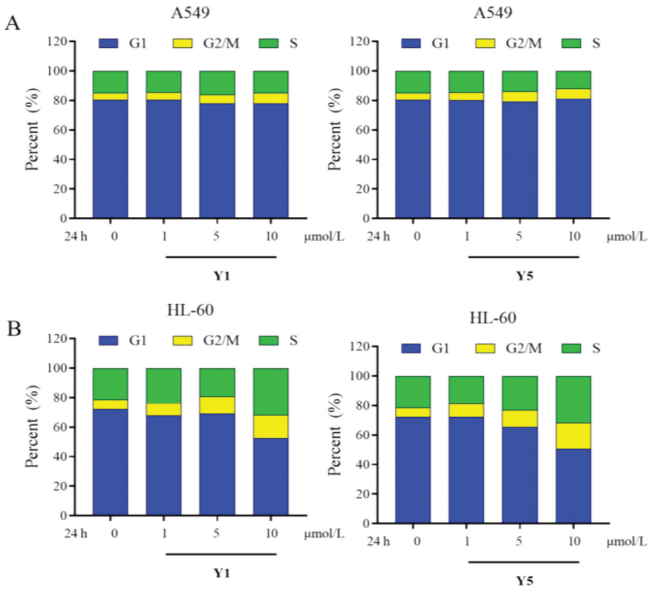


Fig. 2. Compounds **Y1** and **Y5** induced G2/M arrest in A549 and HL-60 cells. A549 cells (A) and HL60 cells (B) were treated with 0, 1, 5, 10 μmol/L **Y1** and **Y5** for 24 h and analyzed by DNA flow cytometry.

in organic solvents of **Y9** was close to that of its asymmetric analogues. However, the fluorescence quantum yield of **Y9** in the aqueous phase decreased to 0.12. Therefore, the representative compounds of the asymmetric series could exert green fluorescence in aqueous medium, which have advantages for cell staining and labelling in biological system. We hypothesize symmetric structures tend to form aggregates in aqueous solution more easily, so the fluorescence quantum yield of **Y9** were much lower than that of **Y1** and **Y5** in aqueous solution.

The representative compounds of the asymmetric derivatives were selected for further study. Based on the growth inhibitory activities of **Y1** and **Y5** in A549 and HL-60 cells, the effects of compounds on cell cycle progression were then investigated by analyzing cellular DNA content by flow cytometric.

As shown in Fig. 2, cell cycle arrest in G2/M phase was found after treated with compounds at different concentrations (0, 1, 5, 10 μmol/L). Compared with the control group (4.57%), the proportion of A549 cells arrested at the G2/M phase increased to 7.21% and 6.81% with 10 μmol/L **Y1** and **Y5**, respectively. Similarly, the percentage of HL-60 cells in the G2/M phase for control group is 6.45%, while treated with 10 μmol/L **Y1** and **Y5**, the percentage of cells arrested at the G2/M phase increased to 15.4% and 17.56%.

To further validate the antitumor mechanism of action of compounds **Y1** and **Y5**, the apoptosis rates were assessed. After incubated with different concentrations of compounds, cells were stained with Annexin V-Alexa Fluor 647 and PI which were used to assess the early and late apoptotic cell population, respectively. Apoptotic cells were observed in a dose-dependent manner when treated with increasing concentrations of **Y1** and **Y5** compared with the control group (Fig. 3). As illustrated in Fig. 3A, 20 μmol/L **Y1** led to 32.18% of apoptotic A549 cells while 20 μmol/L **Y5** induced 30.77% apoptosis cells, in comparison with 15.78% apoptosis rate for control group. Likewise, as shown in Fig. 3B, 20 μmol/L **Y1** and **Y5** induced the apoptosis of 78.11% and 81.98% HL-60 cells, respectively.

In this paper, a series of seven-membered ring-fused naphthalimide derivatives were designed and synthesized. We evaluated their effects to inhibit A549 and HL60 tumor cell growth and summarized their structure-activity relationship. The *N*′,*N*′-dimethylethane-1,2-diamine derivatives **Y1** and **Y9** and their *N*-(2-

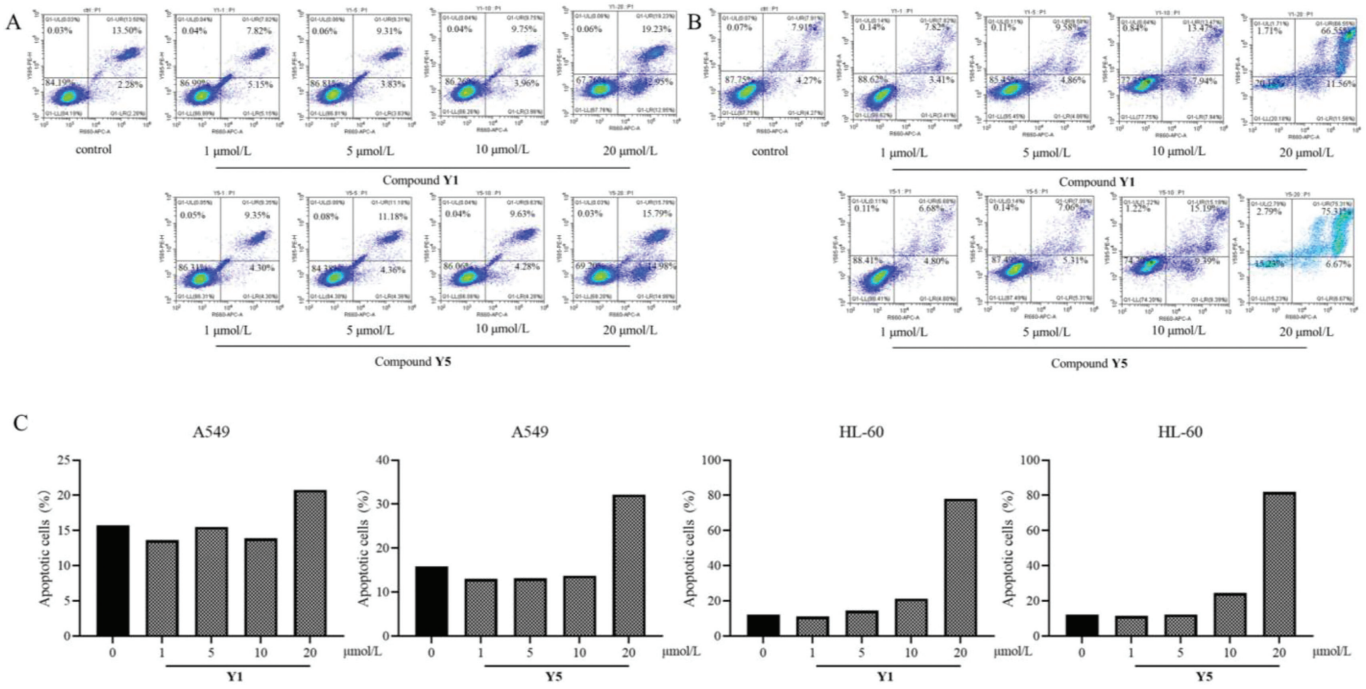


Fig. 3. Apoptosis ratio of the A549 and HL-60 cells. (A) A549 cells were treated with **Y1** and **Y5** at the indicated concentrations for 24 h. (B) HL-60 cells were treated with **Y1** and **Y5** at different concentrations. (C) Representative flow cytometric quantification of apoptotic cells.

aminoethyl)pyrrolidine analogue **Y5** were proved to exhibit great antiproliferative activities in A549 and HL60 cells. They could also effectively inhibit growth of human esophageal, colon and breast cancer cells at single digital micromolar, which was similar with Amonafide. Besides, **Y1**, **Y5** and **Y9** were tested of their photophysical properties. The asymmetric derivatives (**Y1** and **Y5**) have high fluorescence quantum yields in water, which is a rare property among naphthalimide derivatives. Furthermore, compounds **Y1** and **Y5** exerted their antitumor effects *via* G2/M phase cell cycle arrest and apoptosis induction in HL60 and A549 in dose dependent manner. Since seven-membered ring-fused naphthalimide derivatives possess great fluorescence quantum yields in water and potent anti-tumor activites at the same time, they have great potential to be used as theranostic agents for cancer.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank the National Natural Science Foundation of China (Nos. 21878088, 21476077), and Key Project of the Shanghai Science and Technology Committee (No. 18DZ1112703) for financial support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.07.039.

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, CA Cancer J. Clin. 70 (2020) 7–30.
[2] R.L. Siegel, K.D. Miller, A. Jemal, CA Cancer J. Clin. 71 (2021) 7–33.
[3] R.L. Siegel, K.D. Miller, A. Jemal, CA Cancer J. Clin. 68 (2018) 329–339.
[4] B. Zhou, J. Xu, Y.G. Cheng, et al., Int. J. Cancer 141 (2017) 231–241.
[5] D.R. Elias, L.J. Daniel, A.K. Chen, J.L. Czupryna, A. Tsourkas, Cancer Biomark. 4 (2008) 287–305.
[6] T. Hussain, Q.T. Nguyen, Adv. Drug Deliv. Rev. 66 (2014) 90–100.
[7] N. Jiang, J.L. Fan, F. Xu, et al., Angew. Chem. 127 (2015) 2540–2544.
[8] D.A. Diamantis, A. Agalou, M.V. Chatziathanasiadou, et al., Sens. Actuators B 337 (2021) 129807.
[9] Z.P. She, Y. Tian, Y.S. Xia, et al., Dyes Pigments 179 (2020) 108402–108404.
[10] N. Cheng, D. Du, X. Wang, et al., Trends Biotechnol. 37 (2019) 1236–1254.
[11] S.G. Kandlikar, I.P. Raya, P.A. Raghupathi, et al., Int. J. Heat Mass Transf. 108 (2017) 2303–2320.
[12] Z. Luo, Z. Huang, K. Li, et al., Anal. Chem. 90 (2018) 2875–2883.
[13] J. Massin, D.A. Charaf Ed, F. Appaix, et al., Chem. Sci. 4 (2013) 2833–2843.
[14] M. Qian, L. Zhang, Z. Pu, et al., Sens. Actuators B 344 (2021) 130261.
[15] R. Shi, L. Huang, X. Duan, et al., Anal. Chim. Acta 988 (2017) 66–73.
[16] O.M. Kolawole, W.M. Lau, H. Mostafid, V.V Khutoryanskiy, Int. J. Pharm. X 532 (2017) 105–117.
[17] B.N. Ames, L.S. Gold, W.C. Willett, Environ. Health Perspect. 4 (1995) 865–873.
[18] E. Raschi, V. Vasina, M.G. Ursino, et al., Pharmacol. Ther. 125 (2010) 196–218.
[19] M.E. Gellina, Y. ZHOU, T. Cass, et al., Mater. Sci. Eng. C 59 (2016) 324–332.
[20] S. Zhu, R. Tian, A.L. Antaris, X.Y. Chen, H.J. Dai, Adv. Mater. 31 (2019) 1900321.
[21] A. Nbm, B. Map, A. Adp, et al., J. Photochem. Photobiol. B 223 (2021) 112294.
[22] J. Xia, L. Zhang, M. Qian, et al., J. Colloid Interface Sci. 498 (2017) 170–181.
[23] Z. Yuan, M. Xu, T. Wu, et al., Talanta 198 (2019) 323–329.
[24] J. Zhang, L.L. Ning, J. Huang, C. Zhang, K. Pu, Chem. Sci. 11 (2019) 618–630.
[25] M.F. Braña, J.M. Castellano, C.M. Roldan, et al., Cancer Chemother. 4 (1980) 61–66.
[26] M.F. Braña, M. Cacho, A. Ramos, A. Gradillas, B.P. Teresa, Curr. Pharm. Des. 7 (2001) 1745–1780.
[27] M.F. Braña, M. Cacho, A. Ramos, et al., Org. Biomol. Chem. 1 (2003) 648–654.
[28] J. Kelly, S. Banerjee, T. Gunnlaugsson, et al., Chem. Soc. Rev. 42 (2014) 1601–1618.
[29] M.F. Braña, A. Ramos, Curr. Med. Chem. Anti Cancer Agents 1 (2001) 237–255.
[30] Z.G. Li, Q. Yang, X.H. Qian, Bioorg. Med. Chem. Lett. 15 (2005) 1769–1772.
[31] Z. Chen, Y.F. Xu, X.H. Qian, Chin. Chem. Lett. 29 (2018) 1741–1756.
[32] D. Gudeika, Synth. Met. 262 (2020) 116328.
[33] Q. Yang, Y. Peng, X.H. Qian, L.P. Tong, Bioorg. Med. Chem. Lett. 18 (2008) 6210–6213.
[34] X.L. Li, Y.J. Lin, Y.K. Yuan, K. L, X.H. Qian, Tetrahedron 67 (2011) 2299–2304.
[35] X.L. Li, Y.J. Lin, X.H. Qian, et al., Eur. J. Med. Chem. 46 (2011) 1274–1279.
[36] M.F. Braña, M. Cacho, M.A. Ramos, et al., J. Med. Chem. 45 (2002) 5813–5816.
[37] M. Lv, H. Xu, Curr. Med. Chem. 16 (2009) 4797–4813.
[38] X.T. Jia, Y. Yang, Y.F. Xu, X.H. Qian, Pure Appl. Chem. 86 (2014) 1237–1246.
[39] F.B. Yu, X.Y. Han, L.X. Chen, Chem. Commun. 50 (2014) 12234–12249.
[40] L. Zhou, L.J. Xie, C.H. Liu, Y. Xiao, Chin. Chem. Lett. 30 (2019) 1799–1808.
[41] J.B. Grimm, B.P. English, J. Chen, et al., Nat. Methods 12 (2015) 244–250.
[42] Y.J. Mei, P.A. Bentley, W. Wang, Tetrahedron Lett. 47 (2006) 2447–2249.
[43] W. Zhao, E.M. Carreira, Angew. Chem. Int. Ed. 44 (2005) 1677–1679.
[44] J.W. Zhang, C. Wang, L. Zhang, et al., RSC Adv. 6 (2016) 71624–71627.