Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2023, **33**, 73–76

Mendeleev Communications

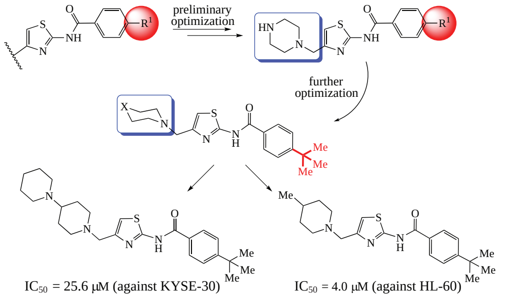
2-Amino-4-(aminomethyl)thiazole-based derivatives as potential antitumor agents: design, synthesis, cytotoxicity and apoptosis inducing activities

Min Liao,[a] Danling Huang*[a,b] and Yong-Xian Cheng*[a,b]

[a] *School of Pharmaceutical Sciences, Health Science Center, Shenzhen University, 518060 Shenzhen, China.*
E-mail: leonchemistry@szu.edu.cn, yxcheng@szu.edu.cn
[b] *Institute for Inheritance-Based Innovation of Chinese Medicine, Marshall Laboratory of Biomedical Engineering, School of Pharmaceutical Sciences, Health Science Center, Shenzhen University, 518060 Shenzhen, China*

DOI: 10.1016/j.mencom.2023.01.023

Novel 2-amino-4-(aminomethyl)thiazole derivatives were designed and synthesized by a facile method including the Hantzsch construction of thiazole core followed by amidation and nucleophilic substitution steps. Bioassay results showed that 4-(*tert*-butyl)-*N*-[4-(piperazin-1-ylmethyl)thiazol-2-yl]-benzamide and 4-(*tert*-butyl)-*N*-{4-[(4-piperidinopiperidin-1-yl)methyl]thiazol-2-yl}benzamide possessed similar activities compared with 5-fluorouracil. The 4-piperidino-piperidin-1-yl-containing derivative also suppressed proliferation of cultured tumor cells by inducing apoptosis.



Keywords: thiazole derivatives, antitumor, drug design, Hantzsch reaction, amidation, structure–activity relationship, apoptosis.

Thiazole scaffolds are potential in development of derivatives with diverse matrices due to high flexible introduction of substituents, *i.e.*, at C-2, C-4 and C-5 positions. By dint of the biological importance of multitudinous thiazole derivatives such as antibacterial,[1–3] fungicidal,[4,5] antiviral,[6,7] anti-inflammatory,[8–10] antioxidative,[11] and antitumor activities,[12–14] thiazole fragment has drawn attention extensively in medicinal chemistry. In particular, thiazoles are widely utilized in drug design of antitumor agents [Figure 1(*a*)]. Some of them are developed as Raf kinase inhibitor (dabrafenib[15]) while several 2-aminothiazoles are considered as phosphatidylinositol 3-kinase (PI3K) inhibitor (alpelisib[16]). Also, microtubule inhibitor ixabepilone[17] and tyrosine kinase inhibitors (TKI) dasatinib[18] contain thiazole rings. *N*-(Thiazol-2-yl)benzamide derivatives were considered to be active core of antitumor molecules. In 2002, Bischof[19] described two compounds **A** and **B** inhibiting proliferation of tumor cell lines in specific manner. A few years ago, Lee[20] synthesized a series of 4-aryl-*N*-arylcarbonyl-2-aminothiazoles while one of them (**C**) showed antiproliferative activity with IC_{50} values of 16.3–42.7 nM against different tumor cells.

Inspired by the above discussion, we designed herein a series of *N*-(thiazol-2-yl)benzamide derivatives aminomethylated at position 4 [see Figure 1(*b*)]. Piperazine, a hydrophilic group, was common in antitumor drug molecules such as flumatinib,[21] imatinib[22] and dasatinib,[18] hence it was considered as introducing an amino group into the molecule. Then evaluation of substitution in benzene ring affecting the antitumor activity was carried out by keeping piperazine ring constant and varying the R^1 group. When determined the optimal group R^1, conversion of 4-positioned substituents in thiazole was implemented in order to ascertain the optimal group R^2_2N. The target *N*-(4-aminomethylthiazol-2-yl)benzamide analogues **3a–g** and **4a–j** were synthesized as shown in Scheme 1.

Initially, thiazole frame was built employing the Hantzsch reaction (see Scheme 1).[23] Amides **2a–g** were then synthesized by the reaction of compound **1** with various acyl chloride in the presence of $NaHCO_3$. Stronger base such as Na_2CO_3, NaOH, pyridine, triethylamine and tetramethylethylenediamine should be avoided because intermolecular self-coupling between amino and chloromethyl groups in compound **1** would occur. Further nucleophilic reaction between compounds **2a–g** and piperazine in refluxing anhydrous acetonitrile afforded derivatives **3a–g**, excess piperazine having been used to avoid disubstitution in piperazine. Subsequently, compound **3b** was reacted with acetyl and acryloyl chlorides in the presence of triethylamine to get products **4a** and **4b**, respectively. Compound **2b** was transformed into derivatives **4c–o** *via* the method similar to that for preparation of **3a–g** when it was reacted with various amines including 1-methylpiperazine, morpholine, thiomorpholine, dipropylamine, dibutylamine and piperidine analogue instead of piperazine.

Initially, we assessed the influence of substituents R^1 attached to benzene ring on the proliferation inhibition activity against the tested cell lines at 40 μM. Compound **3b** with a *tert*-butyl group compared with the analogue having no substituent gives significant contribution to anti-proliferative activity (see Online Supplementary Materials, Table S1) and possesses highest inhibition rates (IRs) against all tested cell lines. It seems that *tert*-butyl attached to benzene ring enhances cytotoxicity of these compounds. Further structure optimization was carried out through the substituents on position 4 of thiazole keeping R^1 as Bu^t constant. As a result (Tables 1 and S2), most of optimized compounds displayed different levels of anticancer activity

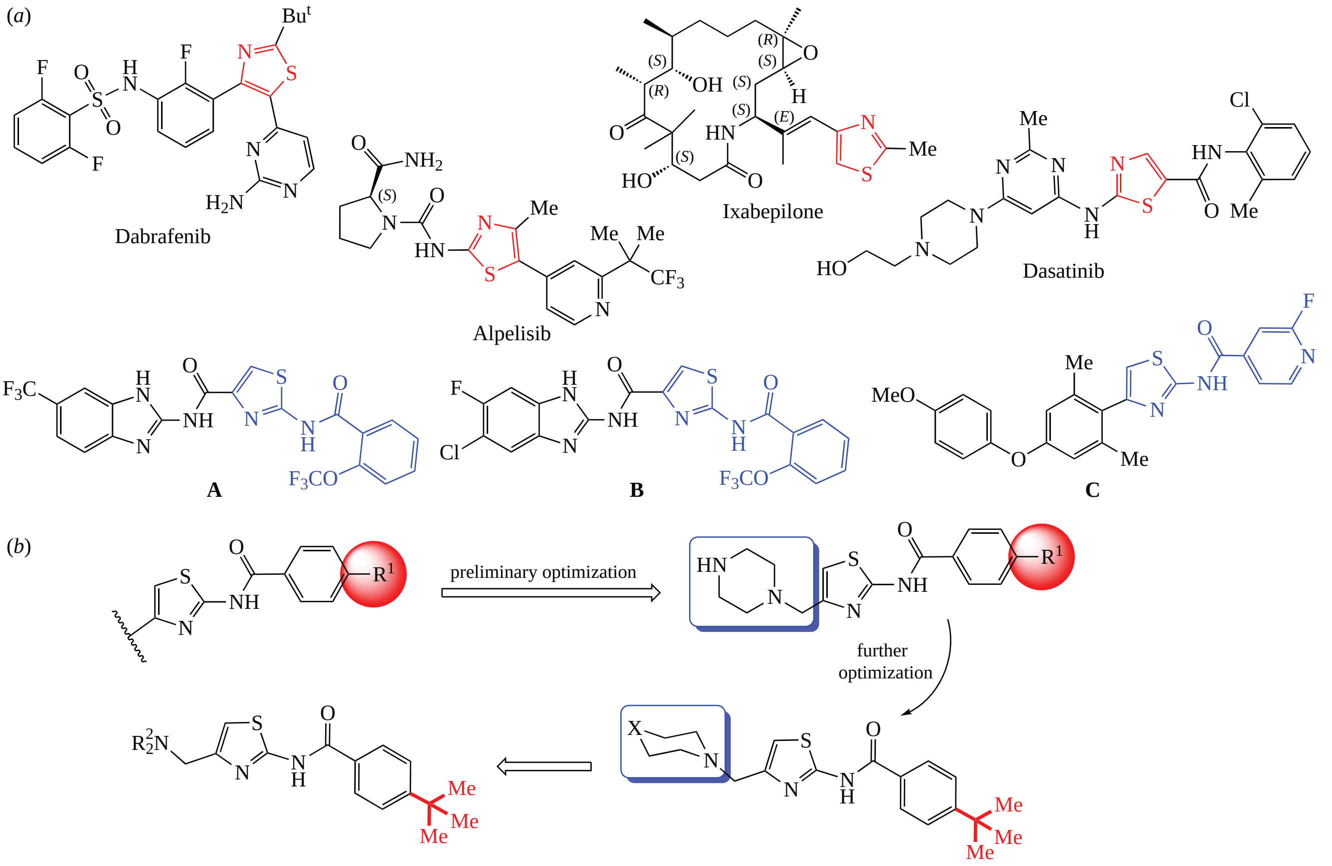
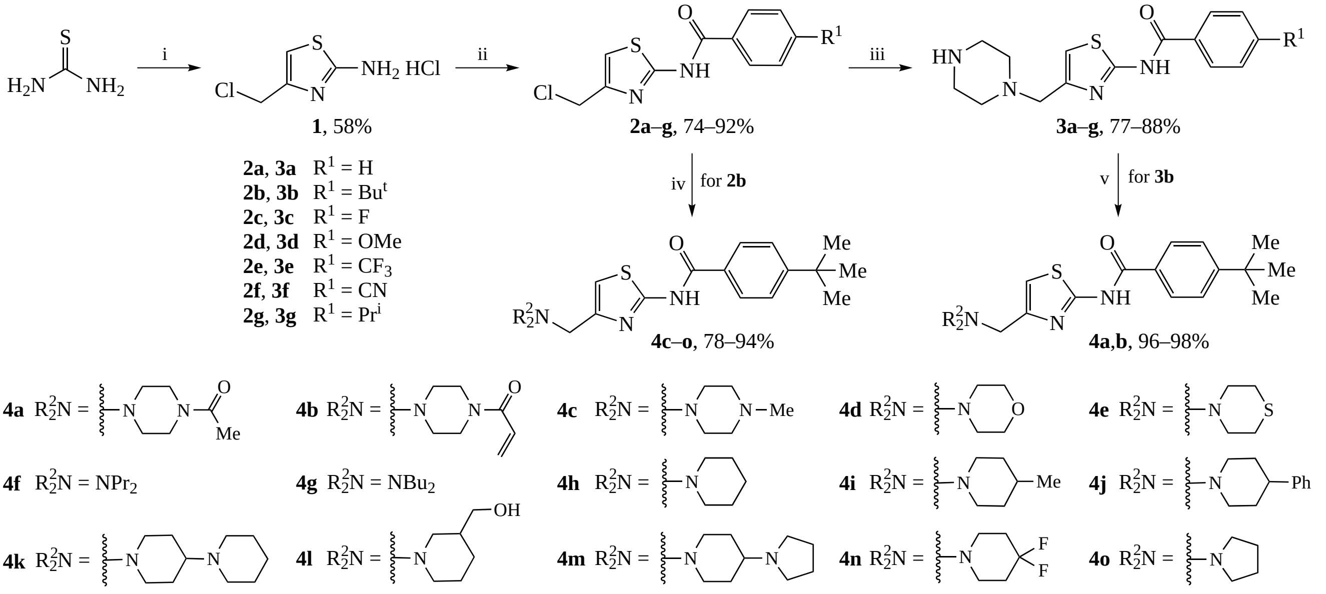


Figure 1 (*a*) Thiazole-containing antitumor active molecules in recent drug discovery and development. (*b*) Design and optimization of the target compounds.

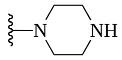
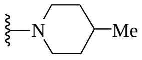
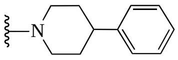
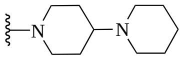
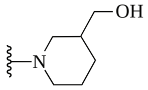
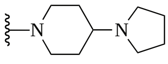
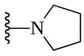


Scheme 1 *Reagents and conditions*: i, $ClCH_2C(O)CH_2Cl$, EtOH, room temperature, 4 h; ii, 4-$R^1C_6H_4C(O)Cl$, $NaHCO_3$, CH_2Cl_2, 0 °C, 8 h; ii, piperazine, acetonitrile, KI, reflux; iv, amine R^2_2NH, MeCN, reflux, 2 h; v, acyl chloride, Et_3N, CH_2Cl_2, 0 °C, 4 h.

against different tumour cells while some compounds exhibited better antitumor activity than the positive control drug 5-fluorouracil. Compound **4i** showed strongest growth inhibitory activity against HL-60 cells with an IC_{50} value of 4.0 μM among all the title compounds. Derivative **4k** showed best antitumor activity against KYSE-30 cells and its cytotoxicity was closest to that of 5-fluorouracil (IC_{50} = 16.8 μM). In HePG2 cells, compounds **3b**, **4j**, **4k**, **4l** and **4m** displayed satisfactory anti-proliferative activity with IC_{50} values below 50 μM. The antitumor activities of derivatives **4l** (IC_{50} = 25.5 μM) and **4o** (IC_{50} = 29.7 μM) against HePG2 cells were superior to that of 5-fluorouracil (IC_{50} = 62.9 μM). In addition, most of compounds exhibited moderate cytotoxicity against A-549 and BGC-823. It is worth mentioning that these compounds showed lower cytotoxicity against normal human breast cells (MCF-10A) than that of human cancer cells. However, although **3b** and **4k** showed slightly lower antitumor activities than cisplatin, they reveal much lower cytotoxicity than cisplatin. In balance, compounds **3b** and **4k** were the optimized ones and may be regarded as potential compounds with a broad spectrum of antitumor activity.

Apparently, according to the analysis of biological data, the change of substituent R^1 influences antitumor activities to some

Table 1 IC_{50} (μM) for some of the title compounds against human tumor cells.

Compound	R^2_2N	IC_{50}/μM					
		A549	BGC-823	HePG2	KYSE-30	HL-60	MCF-10A
3b		**38.1 ± 2.**1	**38.8 ± 0.4**	**44.4 ± 1.6**	**34.6 ± 0.8**	**39.0 ± 1.3**	>100
4i		47.3 ± 0.8	83.3 ± 0.3	103.6 ± 2.1	65.5 ± 1.6	**4.0 ± 0.3**	>100
4j		83.3 ± 0.4	84.9 ± 3.2	47.0 ± 1.3	46.0 ± 0.5	92.2 ± 2.3	>100
4k		**55.0 ± 2.6**	**37.0 ± 0.0**	**43.7 ± 2.2**	**25.6 ± 1.1**	**39.9 ± 3.1**	51.7 ± 1.0
4l		84.9 ± 0.6	>100	25.5 ± 1.8	77.4 ± 2.4	66.7 ± 1.0	>100
4m		80.1 ± 0.3	73.3 ± 1.84	46.9 ± 1.0	50.8 ± 2.1	67.7 ± 0.8	>100
4o		90.3 ± 1.3	>100	29.7 ± 1.7	66.5 ± 1.8	63.90 ± 2.7	90.7 ± 3.7
5-Fluorouracil		62.4 ± 4.1	43.8 ± 0.7	62.9 ± 3.3	16.8 ± 1.1	13.7 ± 0.6	39.7 ± 1.1
Cisplatin		14.3 ± 2.1	4.1 ± 3.7	11.6 ± 1.6	6.6 ± 0.7	14.1 ± 0.7	5.1 ± 1.4

extent. Roughly, for A549, KSYE-30 and HL-60, the contribution of R^1 substituted benzene moiety to activity is $Bu^t > Pr^i >$ $>$ OMe > other electron-withdrawing substituent. For BGC-823, the order of the R^1 moiety affecting the cytotoxicity of compounds is $Bu^t >$ OMe > CN ≈ $CF_3 > Pr^i >$ F > H. It is obvious that the growing cytotoxicity was observed due to introduction Bu^t as the R^1substituent. On the other hand, nature of R^2_2N plays key role in the cytotoxicity activities of drugs as well. When keeping R^1 as Bu^t, the antitumor activities of compounds were floated along with the conversion of R^2_2N regularly. Superficially, the influence of R^2_2N moiety on improving activity may be summarized as piperazine > Bu_2N > 4-substituted piperidine > piperidine ≈ ≈ morpholine ≈ thiomorpholine, while the cytotoxicity is correlated as follows: **3a** > **4g** > **4**i ≫ **4h**, **4d**, and **4e**. Deeply, it is obvious that electron density in position 4 of six-membered heterocycles influenced biological activities of compounds. A complex relationship and significant regularity indicated that the electron enrichment in position 4 of six-membered heterocycles of R^2_2N gave more contribution to the growth suppression of tumour cells than electron deficiency. For instance, with N atom modified in position 4 of six-membered heterocycles of R^2_2N, acetyl-substituted derivative **4a** ($IC_{50} > 100$ μM) and acryloyl-substituted one **4b** ($IC_{50} > 100$ μM) exerted feeble antitumour activities compared with those of methyl-substituted **4c** (IC_{50} values ranging from 43.0 to 65.3 μM against different tumour cells) and hydrogen-substituted **3b** (IC_{50} values ranging from 34.6 to 44.4 μM against different tumour cells). Similarly, when keeping R^2_2N as 4-substituted piperazine group, the antitumor activities are correlated as follows: 4-piperidine-substituted (strong electron donor) **4k** > 4-methyl-substituted (moderate electron donor) **4i** > 4,4-difluoro-substituted (electron withdraw) **4n**. Although the regularity mentioned above is shallow, the analysis of these results does provide guiding significance for optimizing this kind of derivatives.

Representative compounds **3b** and **4k** with best bioactivities were selected to evaluate their influence on expression of apoptosis associated protein. The poly (ADP-ribose) polymerase (PARP) represents a protein family that acts a key part in multifarious cellular procedure including programmed cell death. Cleavage of PARP (c-PARP) is often considered to be a biomarker for the detection of apoptosis. Besides, protein kinase B (AKT) also plays a significant role in cell survival and apoptosis. Thus, the PARP, c-PARP, AKT, phosphorylated AKT (p-AKT) in KYSE-30 cell were measured after treatment with **3b** and **4k**, using Western blot analysis. Compounds **3b** and **4k** induced KYSE-30 cell apoptosis in a dose-dependent manner, associated with c-PARP and p-AKT [Figure 2(*a*),(*b*)]. To further

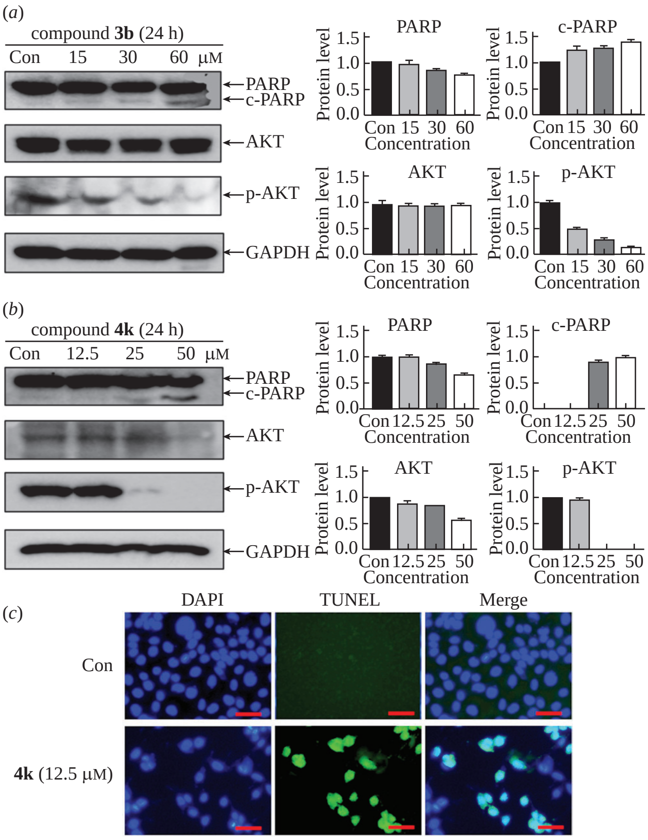


Figure 2 Induction of apoptosis by **4k** of KYSE30. Effects of (*a*) compound **3b** and (*b*) compound **4k** on the protein expression of PARP, c-PARP, AKT, and p-AKT. KYSE-30 cells were untreated or treated with different concentration of the test material for 24 h, following which western blot using GAPDH as the loading control. (*c*) DAPI/TUNEL double staining experiment of **4k** on KYSE-30 cells.

prove that **4k** could induce cell apoptosis, **4k**-treated cells were analyzed for apoptosis by DAPI/TUNEL double staining and TUNEL labeling [see Figure 2(*c*)]. Clearly, apoptotic changes, chromatin condensation and fragmentation were detected especially after approximately 48 h of **4k** treatment. The fraction of apoptotic cells, as determined by DAPI/TUNEL double staining, truly enhanced. Thus, based on the above evidence of cells undergoing apoptosis, we suppose that compounds **3b** and **4k** can inhibit tumour cell proliferation *via* inducing apoptosis.

In summary, a class of 2-amino-4-(aminomethyl)thiazole analogues was synthesized *via* a facile pathway. These compounds possess a broad spectrum of antitumor activities, especially **3b** and **4k**, which exhibited an excellent inhibitory activity against the proliferation of A549, BGC-823, HePG2, KYSE-30, and HL-60 cells. In addition, compounds **3b** and **4k** effectively suppressed the apoptosis-associated inhibition of KYSE-30 cell growth, while both of them significantly inhibited p-AKT and activated c-PARP in KYSE-30 cell. Further development of the new class of 2-amino-4-(aminomethyl)-thiazole derivatives as antitumor agent is prospective.

This work was financially supported by Shenzhen University Top Ranking Project no. 86000000210.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.01.023.

References

1 M. P. Christy, T. Johnson, C. D. McNerlin, J. Woodard, A. T. Nelson, B. Lim, T. L. Hamilton, K. M. Freiberg and D. Siegel, *Org. Lett.*, 2020, **22**, 2365.
2 H. S. Lalithamba, K. Uma, T. S. Gowthami and G. Nagendra, *Org. Prep. Proced. Int.*, 2020, **52**, 181.
3 E. Abdel-Galil, M. M. Girges and G. E. Said, *ChemistrySelect*, 2020, **5**, 3075.
4 Q. Wu, B. Zhao, Z. Fan, X. Guo, D. Yang, N. Zhang, B. Yu, S. Zhou, J. Zhao and F. Chen, *J. Agric. Food Chem.*, 2019, **67**, 1360.
5 Z. Yan, A. Liu, Y. Ou, J. Li, H. Yi, N. Zhang, M. Liu, L. Huang, J. Ren, W. Liu and A. Hu, *Bioorg. Med. Chem.*, 2019, **27**, 3218.
6 I. Conti, R. Morigi, A. Locatelli, M. Rambaldi, G. Bua, G. Gallinella and A. Leoni, *Molecules*, 2019, **24**, 1420.
7 T. K. Venkatachalam, E. A. Sudbeck, C. Mao and F. M. Uckun, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 523.
8 S. Sinha, M. Doble and S. L. Manju, *Eur. J. Med. Chem.*, 2018, **158**, 34.
9 M. T. E. Maghraby, O. M. F. Abou-Ghadir, S. G. Abdel-Moty, A. Y. Ali and O. I. A. Salem, *Bioorg. Med. Chem.*, 2020, **28**, 968.
10 M. M. El-Kerdawy, M. A. Ghaly, S. A. Darwish, H. A. Abdel-Aziz, A. R. Elsheakh, R. S. Abdelrahman and G. S. Hassan, *Bioorg. Chem.*, 2019, **83**, 250.
11 I. Stankova, K. Chuchkov, S. Shishkov, K. Kostova, L. Mukova and A. S. Galabov, *Amino Acids*, 2009, **37**, 383.
12 A. Andreani, S. Burnelli, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, L. Varoli, N. Calonghi, C. Cappadone, G. Farruggia, M. Zini, C. Stefanelli, L. Masotti, N. S. Radin and R. H. Shoemaker, *J. Med. Chem.*, 2008, **51**, 809.
13 R. M. Mohareb and B. M. Mostafa, *J. Heterocycl. Chem.*, 2020, **57**, 1275.
14 N.-Y. Chen, Y.-L. Xie, G.-D. Lu, F. Ye, X.-Y. Li, Y.-W. Huang, M.-L. Huang, T.-Y. Chen and C.-P. Li, *Mol. Diversity*, 2021, **25**, 967.
15 G. S. Falchook, G. V. Long, R. Kurzrock, K. B. Kim, T. H. Arkenau, M. P. Brown, O. Hamid, J. R. Infante, M. Millward, A. C. Pavlick, S. J. O'Day, S. C. Blackman, C. M. Curtis, P. Lebowitz, B. Ma, D. Ouellet and R. F. Kefford, *Lancet*, 2012, **379**, 1893.
16 I. A. Mayer, V. Abramson, J. M. Balko, M. Sanders, D. Juric, D. Solit, Y. Li, L. Cantley, E. Winer and C. Arteaga, *Cancer Res.*, 2015, **75** (15 Supplement), CT232.
17 M. Steinberg, *Clin. Ther.*, 2008, **30**, 1590.
18 M. Steinberg, *Clin. Ther.*, 2007, **29**, 2289.
19 J. Bischof, J. Leban, M. Zaja, A. Grothey, B. Radunsky, O. Othersen, S. Strobl, D. Vitt and U. Knippschild, *Amino Acids*, 2012, **43**, 1577.
20 Y.-S. E. Lee, S.-H. Chuang, L. Y. L. Huang, C.-L. Lai, Y.-H. Lin, J.-Y. Yang, C.-W. Liu, S.-C. Yang, H.-S. Lin, C.-C. Chang, J.-Y. Lai, P.-S. Jian, K. Lam, J.-M. Chang, J. Y. N. Lau and J.-J. Huang, *J. Med. Chem.*, 2014, **57**, 4098.
21 J. Zhao, H. Quan, Y. Xu, X. Kong, L. Jin and L. Lou, *Cancer Sci.*, 2014, **105**, 117.
22 D. G. Savage and K. H. Antman, *N. Engl. J. Med.*, 2002, **346**, 683.
23 A. Hantzsch and J. H. Weber, *Ber. Dtsch. Chem. Ges.*, 1887, **20**, 3118.

Received: 19th May 2022; Com. 22/6905