Asian Journal of Chemistry; Vol. 33, No. 6 (2021), 1420-1424

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23199

Sildenafil (VIAGRA™): A Promising Anticancer Drug Against Certain Human Cancer Cell Lines

MOHAMMED A. HUSSEIN[1,*], MOHAMED M. SALAH EL-DIN[2], ESRAA M. SALEH[2], AHMED T. MOSTAFA[2], MAHMOUD T. ABD-ELAZZIZ[2], OMNIA M. ABDELRAHMAN[2], AHMED S. MAHMOUD[3], AHMED M. MORO[4], EBTSAM A. ABDEL-WAHAB[4] and ALI A. ALI[5]

[1]Department of Biochemistry, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, Egypt
[2]Department of Medical Laboratories, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, Egypt
[3]Department of Biophysics, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, Egypt
[4]Department of Physics (experimental physics), Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, Egypt
[5]Vice President of Post Graduate Studies, October 6 University, Sixth of October City, Egypt

*Corresponding author: E-mail: prof.husseinma@o6u.edu.eg

Received: 28 March 2021; *Accepted*: 28 April 2021; *Published online*: 5 June 2021; AJC-20378

Sildenafil has been identified as the first agent for treating male erectile dysfunction and is a selective inhibitor of phosphodiesterase 5 (PDE5). Its chemical structure consists of three moieties named; 1-methyl-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one, 5-(2-ethoxy-1-ylsulfonyl)phenyl and 4-methylpiperazine. Many articles are reported the cytotoxic activity of each moiety individually. The combination into a single molecule (sildenafil) of these three structural features could have promising anti-cancer and cytotoxic effects. The study evaluated sildenafil cytototoxic activity *in vitro* against mammalian cell lines: MCF-7, HCT-116, HeLa cells and A-549 cells with their IC_{50} values. Sildenafil showed considerable cytotoxic activity (IC_{50} = 28.2 ± 0.92, 45.2 ± 1.5, 30.5 ± 0.87 and 60.5 ± 3.2 µg/mL) against HCT-116, MCF-7, A-549 and HeLa cells, respectively. HCT-116 was the most sensitive cell line towards sildenafil followed by A-549, A375, MCF-7 and HeLa cells. These findings shed light on the antitumor activity of sildenafil and its possible impact on potentiating of cytokines, antitumor and anti-inflammatory markers in tumour cells. These effects might be related to the structure feature of sildenafil.

Keywords: Sildenafil, Cytotoxicity activity, Structure activity relationship, MCF-7, HCT-116, HeLa, A-549.

INTRODUCTION

There have been reports of various biological activities in quinazolines and 1,2,4-triazoles [1-5]. The combination of these two structural characteristics in one molecule may lead to a potential biologically promising compound [6]. Quinazolines have a well-known potential activity as antitumor [7], anticancer [8] and antimicrobial activity [9]. A major drug-like scaffold [10] has been shown to be pyrazolopyrimidine′s moiety, including the inhibitor of bruton tyrosine kinase ibrutinib [11], tumour necrosis factor-associated protein1 (TRAP1) inhibitor [12], cycline-dependant kinase (CDK) inhibitors [13,14], anti-inflammation [15] and bumped kinase inhibitors; and a variety of clinical applications have been identified [16].

On the other hand, sulfa drugs are a very important class of compounds in the pharmaceutical industry and a key pharmacophore in many marketed drugs. In addition, sulfonamide derivatives possess very interesting diversified pharmacological and biological properties, like antifungal [17], antiviral [18], antitumor [19], anti-inflammatory [20] and as a carbonic anhydrase inhibitor [21].

Chegwidden & Spencer [22] also reported the inhibition growth of human cancer cells in the culture by the direct action of specific sulfonamide carbonic anhydrase (CA) inhibitors. They showed that potent clinically used inhibitors CA-sulfonamide, such as acetazolamide, methazolamide and ethoxzolamide, inhibited human lymphoma cell growth, with GI_{50} values ranging from 0.5 mM for acetazolamide to 0.25 mM for ethoxzolamide [22].

In addition, piperazine and its analogues are the important pharmacophores, showing multiple bioactivities including antifungal activity [23-25]. The use of piperazine as oral anticancer drug in Japan has been approved as a clinical use for

oral cancer in Japan [26], including 4,4,1,2-(ethanediyl)*bis*(1-isobutoxycarbonyloxy-methyl-2,6-piperazinedione (MST-16). The MST-16 compound showed strong anti-proliferative activity for tumors such as colon, prostate, breast, lung and leukemia [27]. In another study a multiple protein kinase inhibitor, a compound of piperazine-pyrimidine, 1-aryl-2-(*N*-methylpiperazinomethyl)-2-propen-1-one dihydrochloride [26,28-30].

Sildenafil, was initially developed as an antihypertensive agent. Due to the unexpected side effect of improvement of penile erection. Additionally, other PDE-5 inhibitors were shown to induce apoptosis in different human tumors [31,32]. Hussein *et al*. [33] explained that sildenafil structure consists of three moieties; pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one, 5-(2-ethoxy-1-ylsulfonyl)phenyl and 4-methylpiperazine (Fig. 1).

Sulfonamide derivatives also play an important role in their anticancer activity because of their good protein tyrosine kinases (PTKs) inhibitory activity [34-37]. Aryl sulfonyl piperazine in sildenafil is an important class of therapeutical agents that provide good models for many biological objectives. A great deal of research has been conducted during recent years to develop new piperazine derivatives to improve their biological activity. Anticancer [38,39] anti-allergic [40], neuronal nicotinic acetyl cholin receptor [41], antibacterial, anti-acetyl cholinesterase [42] and transglutaminase 2 selective covalent inhibitors were reported to carry benzene sulfonyl group [43] for the activities of Huntington's disease. Compound 1 inhibitor (11β-HSD1 inhibitors) was also found to have efficacy in a cynomolgus monkey *ex vivo* enzymes inhibition model [44], as a selective and orally bioavailable inhibitor.

Combining the three structural characteristics of a single molecule could produce compounds with promising anticancer effects. Sildenafil's structure prompted us to assess its activity in anticancer. Moreover, there is no report about the sildenafil human cancer cell lines which have anticancer effect. The aim of this study was to conduct the effect of sildenafil on human cancer cell lines (HCT-116, MCF-7, A-549 and HeLa) as a promising target drug.

EXPERIMENTAL

Mammalian cell lines: Human breast carcinoma (MCF-7), human colon carcinoma (HCT-116), human cervical carcinoma (HeLa) and human lung carcinoma (A-549) cell lines were purchased from the VACSERA Tissue Culture Unit in Egypt.

Chemicals: Dimethyl sulfoxide (DMSO, Sigma, USA), violet crystal and black trypan (St. Louis, USA), fetal bovine serum, DMEM, RPMI-1640 and 0.25% of trypsin-EDTA were purchased from Lonza Chemicals, Switzerland with buffer solution from HEPES, L-glutamine and gentamycin. Crystal violet stain (1%) was prepared by 0.5% (w/v) crystal violet and 50% methanol then finally made up the volume by using double distilled water and then filtered with Whatman No.1 paper.

Propagation of cell lines: Cells were propagated with 10% heat-inactivated foetal foetal bovine serum, 1% L-glutamine, HEPES and 50 µg/mL gentamycin in Dulbecco's modified Eagle medium (DMEM). All cells were retained at 37 ºC and sub-cultivated at 5% CO_2 humidified atmosphere twice weekly.

Cytototoxicity assessment using a viability test: The cells were seeded in a 96-well plate for cytotoxicity test at 1×10^4 cells per pot in 100 µL of cellular concentrations of the growth medium. After 24 h of seeding, the fresh medium was added containing different sample concentration. A multi-channel pipette was used to add standard twice dilutions of the test chemical compound to cell monolayers confluents dispensed into 96-well, flat-bottomed microtiter plates (Falcon, USA). In a humidified incubator with 5% CO_2, the microtiter plates have been incubated at 37 ºC for 24 h.

Each concentration of the test sample was used with three wells. Without a test sample and with or without DMSO, the control cells were incubated. There was no impact on the experiment on the small percentage of DMSO present in the wells (maximum 0.1%). The viable cell yield was determined by colorimetric method after incubation of the cells at 37 ºC for 24 h. In brief, the media were sucked in and the crystal-violet solution (1%) was added to each well for at least 0.5 h following the end of the incubation period. Take the stain away and rinse the sheets with tap water until excess stain is removed. Glacial acetic acid (30%) was then added to all wells and thoroughly mixed and measured the plaque absorption at the wavelength of 490 nm, after being shaken gently in the microplate reader (TECAN, Inc.) [45]. For background absorption in wells without any additional blemish all results were corrected. In the

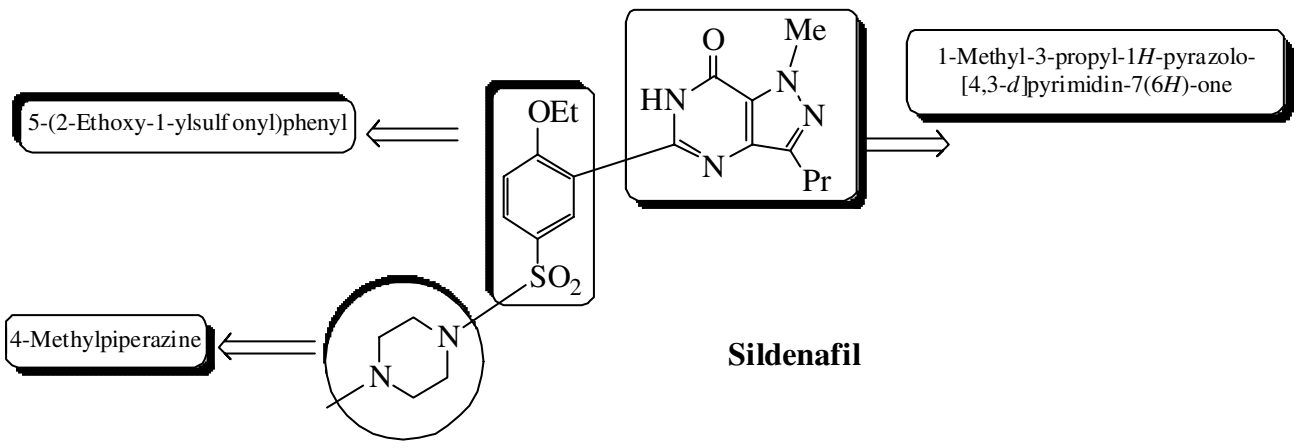

Fig. 1. Structure feature of VIAGRA™

absence of the tested compounds, the treated samples were compared with the cell control. All experiments were carried out in triplicate.

Each compound tested was calculated for its cell cytotoxic effect. In order to determine the number of sustainable cells and the percentage of viability, the optical density was measured using a microplate reader, (SunRise, TECAN, Inc., USA).

$$\frac{OD_t}{OD_c} \times 100\%$$

where OD_t is the mean optical density of wells treated with the tested sample; OD_c is the mean optical density of untreated cells.

After treatment with the compound specified for surviving cells the relation between drug concentration and survival curve was drawn. A graphical dose response curve for each concentration was estimated using the Graphpad Prism software (San Diego, USA) to provide the 50% inhibitory concentration (IC_{50}, the required concentration for cause of toxicity in 50% of intact cells) [46].

RESULTS AND DISCUSSION

Mammalian cell lines: MCF-7 cells (human breast carcinoma), HCT-116 cells (human colon carcinoma), HeLa cells (human cervical carcinoma) and A-549 cells (human lung carcinoma) were used as tumor cell lines. Table-1 shows that the IC_{50} value of sildenafil against HCT-116 cell line is 28.2 ± 0.92 µg/mL (Fig. 2), while Table-1 shows that the IC_{50} value of sildenafil (the concentration of the compounds which kills 50% of the cells) against MCF-7 cell line is 45.2 ± 1.5 µg/mL (Fig. 3). In addition, Table-1 show that the IC_{50} value of sildenafil against A-549 and HeLa cell lines are 30.5 ± 0.87 and 60.5 ± 3.2 µg/mL, respectively (Figs. 4 and 5).

Sildenafil was shown to be a substantial cytotoxic agent in mammalian cells. The study suggested that sildenafil cytotoxicity is associated with its structural characteristics. Sildenafil consists of three moieties *viz*. pyrazol[4,3-*d*]pyrimidine, 5-(2-ethoxy-1-ylsulfonyl)phenyl and 4-methylpiperazine in its structure. Shao *et al*. [47] synthesized as potential anti-tumor agents a derivative from pyrazolo pyrimidine CDK inhibitors. A panel of cell-cancer cells, including the colorectal, breast, pulmonary, ovarian, cervical and pancreatic was evaluated for synthesized substitute pyrimidine products in relation to

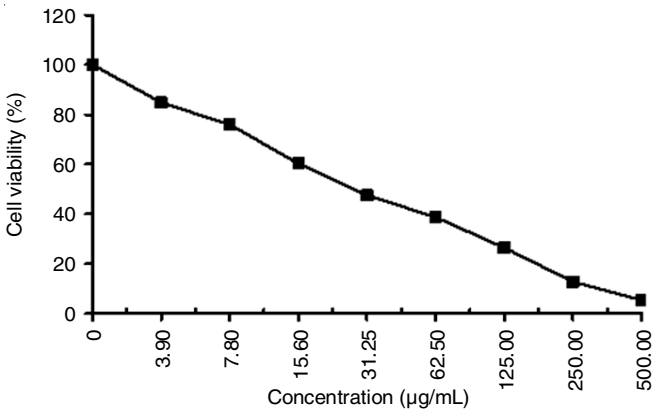

Fig. 2. % of HCT-116 cell viability against concentration of sildenafil

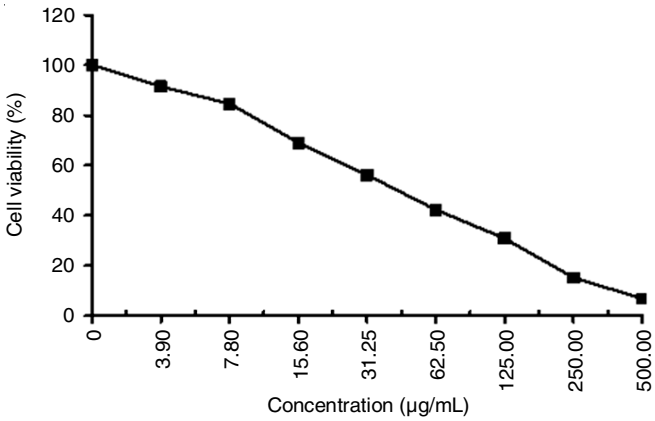

Fig. 3. % of MCF-7 cell viability against concentration of sildenafil

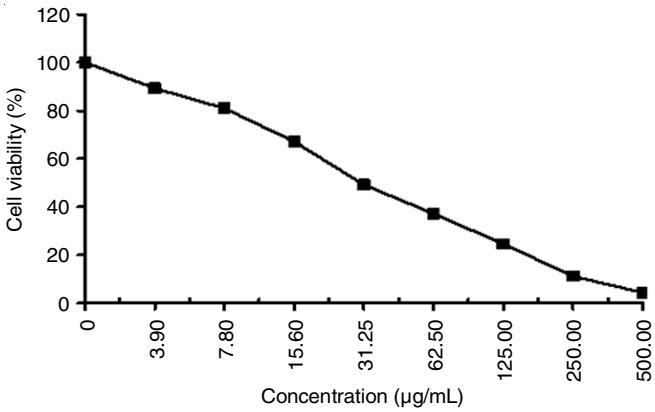

Fig. 4. % of A-549 cell viability against concentration of sildenafil

TABLE-1
IC_{50} VALUE OF SILDENAFIL AGAINST HCT-116, MCF-7, A-549 AND HeLa CELLS LINE

Sample conc. (µg/mL)	HCT-116			MCF-7			A-549			HeLa		
	Viability (%)	Inhibitory (%)	S.D. (±)	Viability (%)	Inhibitory (%)	S.D. (±)	Viability (%)	Inhibitory (%)	S.D. (±)	Viability (%)	Inhibitory (%)	S.D. (±)
500	5.44	94.56	0.28	6.93	93.07	0.75	6.93	93.07	0.75	8.79	91.21	1.35
250	12.78	87.22	0.94	15.28	84.72	1.34	15.28	84.72	1.34	19.40	80.6	0.64
125	26.50	73.50	1.73	30.94	69.06	2.42	30.94	69.06	2.42	35.27	64.73	2.31
62.5	38.72	61.28	2.86	42.31	57.69	1.77	42.31	57.69	1.77	48.02	51.98	2.94
31.25	47.58	52.42	2.43	56.17	43.83	2.95	56.17	43.83	2.95	78.56	21.44	2.88
15.6	60.34	39.66	3.19	69.02	30.98	2.84	69.02	30.98	2.84	94.43	5.57	0.95
7.8	75.92	24.08	1.64	84.59	15.41	1.73	84.59	15.41	1.73	99.52	0.48	0.46
3.9	84.83	15.17	0.95	91.46	8.54	1.32	91.46	8.54	1.32	100	0	–
0	100	0	0	100	0	0	100	0	0	100	0	0
IC_{50}	28.2 ± 0.92 µg/mL			45.2 ± 1.5 µg/mL			30.5 ± 0.87 µg/mL			60.5 ± 3.2 µg/mL		

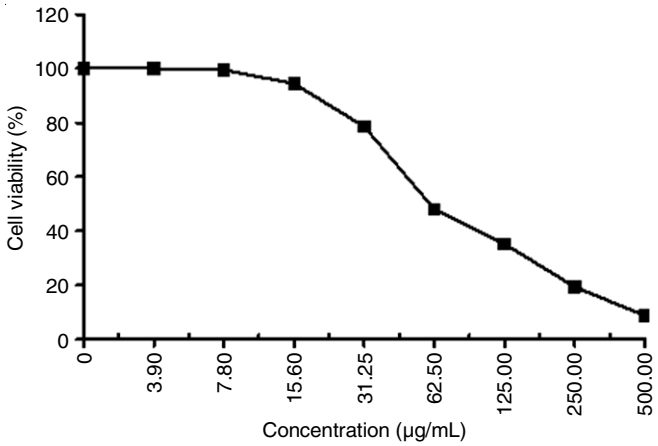

Fig. 5. % of HELA cell viability against concentration of sildenafil

their antitumor activity. CDKs can trigger caspase 3, decrease Mcl-1 levels of anti-apoptotic protein and cause apoptosis of the cancer cell [47]. Some pyrazolo pyrimidines have also been taken as standard medication as a highest affinity with DNA, and the highest percentage increase in lifespan of Ehrlich ascites cells injected into the mouse was 5-furouracil [48].

In addition, some derivative of pyrazolo-pyrimidine of the tested compounds exhibited high growth inhibitory potential against PC-3 cell [49]. However, 2,4-diaminofuro[2,3-*d*]pyrimidine has been developed by Hu *et al*. [50], who reported the *in vitro* anticancer activity against A459 and SPC-A-1 cell lines. Song *et al*. [51] synthesized and evaluated for anti-tumor potential for human leukemia (HL-60), a new library with microwave irradiation pyrazolo[3,4-*d*]pyrimidine derivatives. The new pyrrole[2,3-*d*] molecules were developed and evaluated for anticancer activities against the HCT-116 [52].

In sildenafil, the electron retraction substitute for the sulfur atom present in 5-(2-ethoxy-1-ylsulfonyl)phenyl moiety, because the S-O bonds have high internal rotation barriers and are increased by their pre-encryption effect, different conformers may be considered rather rigid molecules [52].

Conclusion

Sildenafil has been investigated for cytotoxicity in mammalian cell lines: cells MCF-7 (human breast carcinoma), HCT-116 (human colon carcinoma), HeLa cells (human cervical carcinoma) and A-549 cells (humann lung carcinoma) *in vitro*. Present results showed that sildenafil can be used as a traditional chemotherapy agent as antitumor and inflammatory markers in tumour cells. More trials were needed to demonstrate *in-vitro* and *in-vivo* sildenafil proliferation, apoptosis and angiogenesis and antitumor activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. R. Sompalle, S.M. Roopan, N.A. Al-Dhabi, K. Suthindhiran, G. Sarkar and M.V. Arasu, *J. Photochem. Photobiol. B*, **162**, 232 (2016); https://doi.org/10.1016/j.jphotobiol.2016.06.051
2. P.S. Auti, G. George and A.T. Paul, *RSC Adv.*, **10**, 41353 (2020); https://doi.org/10.1039/D0RA06642G
3. M.A. Hussein, *Med. Chem. Res.*, **21**, 1876 (2012); https://doi.org/10.1007/s00044-011-9707-0
4. M.M. Ghorab, Z.H. Ismail, M. Abdalla and A.A. Radwan, *Arch. Pharm. Res.*, **36**, 660 (2013); https://doi.org/10.1007/s12272-013-0094-6
5. N. Garg, T. Chandra, S. Lata and K.K. Saxana A. Kumar, *Pak. J. Sci. Ind. Res.*, **8**, 52 (2009).
6. M.M. Ghorab, Z.H. Ismail, A.A. Radwan and M. Abdalla, *Acta Pharm.*, **63**, 1 (2013); https://doi.org/10.2478/acph-2013-0006
7. M.A. Hussein, H.A. El-Gizawy, N.A. Gobba and Y.O. Mosaad, *Curr. Pharm. Biotechnol.*, **18**, 677 (2017); https://doi.org/10.2174/1389201018666171004144615
8. H.A. El-Gizawy, F. Omar and M.A. Hussein, *Int. J. Drug Deliv.*, **10**, 25 (2018).
9. E. Jafari, M.R. Khajouei, F. Hassanzadeh, G.H. Hakimelahi and G.A. Khodarahmi, *Res. Pharm. Sci.*, **11**, 1 (2016).
10. S. Schenone, M. Radi, F. Musumeci, C. Brullo and M. Botta, *Chem. Rev.*, **114**, 7189 (2014); https://doi.org/10.1021/cr400270z
11. R.H. Advani, J.J. Buggy, J.P. Sharman, S.M. Smith, T.E. Boyd, B. Grant, K.S. Kolibaba, R.R. Furman, S. Rodriguez, B.Y. Chang, J. Sukbuntherng, R. Izumi, A. Hamdy, E. Hedrick and N.H. Fowler, *J. Clin. Oncol.*, **31**, 88 (2013); https://doi.org/10.1200/JCO.2012.42.7906
12. H.-K. Park, H. Jeong, E. Ko, G. Lee, J.-E. Lee, S.K. Lee, A.-J. Lee, J.Y. Im, S. Hu, S.H. Kim, J.H. Lee, C. Lee, S. Kang and B.H. Kang, *J. Med. Chem.*, **60**, 7569 (2017); https://doi.org/10.1021/acs.jmedchem.7b00978
13. S. Wyllie, M. Thomas, S. Patterson, S. Crouch, M. De Rycker, R. Lowe, S. Gresham, M.D. Urbaniak, T.D. Otto, L. Stojanovski, F.R.C. Simeons, S. Manthri, L.M. MacLean, F. Zuccotto, N. Homeyer, H. Pflaumer, M. Boesche, L. Sastry, P. Connolly, S. Albrecht, M. Berriman, G. Drewes, D.W. Gray, S. Ghidelli-Disse, S. Dixon, J.M. Fiandor, P.G. Wyatt, M.A.J. Ferguson, A.H. Fairlamb, T.J. Miles, K.D. Read and I.H. Gilbert, *Nature*, **560**, 192 (2018); https://doi.org/10.1038/s41586-018-0356-z
14. J.A. Markwalder, M.R. Arnone, P.A. Benfield, M. Boisclair, C.R. Burton, C.-H. Chang, S.S. Cox, P.M. Czerniak, C.L. Dean, D. Doleniak, R. Grafstrom, B.A. Harrison, R.F. Kaltenbach, D.A. Nugiel, K.A. Rossi, S.R. Sherk, L.M. Sisk, P. Stouten, G.L. Trainor, P. Worland and S.P. Seitz, *J. Med. Chem.*, **47**, 5894 (2004); https://doi.org/10.1021/jm020455u
15. A.H. Abdelazeem, S.A. Abdelatef, M.T. El-Saadi, H.A. Omar, S.I. Khan, C.R. McCurdy and S.M. El-Moghazy, *Eur. J. Pharm. Sci.*, **62**, 197 (2014); https://doi.org/10.1016/j.ejps.2014.05.025
16. M.A. Hulverson, I. Bruzual, E.V. McConnell, W. Huang, R.S.R. Vidadala, R. Choi, S.L.M. Arnold, G.R. Whitman, M.C. McCloskey, L.K. Barrett, K.L. Rivas, S. Scheele, A.E. DeRocher, M. Parsons, K.K. Ojo, D.J. Maly, E. Fan, W.C. Van Voorhis and J.S. Doggett, *J. Infect. Dis.*, **219**, 1464 (2019); https://doi.org/10.1093/infdis/jiy664
17. M. Feng, B. Tang, S. H. Liang and X. Jiang, *Curr. Top. Med. Chem.*, **16**, 1200 (2016); https://doi.org/10.2174/1568026615666150915111741
18. P. Zoumpoulakis, Ch. Camoutsis, G. Pairas, M. Sokovic, J. Glamoclija, C. Potamitis and A. Pitsas, *Bioorg. Med. Chem.*, **20**, 1569 (2012); https://doi.org/10.1016/j.bmc.2011.12.031
19. G.H. Al-Ansary, M.A. Ismail, D.A. Abou El Ella, S. Eid and K.A. Abouzid, *Eur. J. Med. Chem.*, **68**, 19 (2013); https://doi.org/10.1016/j.ejmech.2013.07.006
20. M.S. Al-Dosari, M.M. Ghorab, M.S. Alsaid, Y.M. Nissan and A.B. Ahmed, *Eur. J. Med. Chem.*, **69**, 373 (2013); https://doi.org/10.1016/j.ejmech.2013.08.048
21. A. Weber, A. Casini, A. Heine, D. Kuhn, C.T. Supuran, A. Scozzafava and G. Klebe, *J. Med. Chem.*, **47**, 550 (2004); https://doi.org/10.1021/jm030912m

22. W.R. Chegwidden and I.M. Spencer, *Inflammopharmacol.*, **3**, 231 (1995); https://doi.org/10.1007/BF02659120
23. C.T. Supuran, *Nat. Rev. Drug Discov.*, **7**, 168 (2008); https://doi.org/10.1038/nrd2467
24. R. Gillet, P. Jeannesson, M.-L. Arnould-Guérin, S. Kirkiacharian, H. Sefraoui, J.-C. Jardillier and F. Pieri, *Cancer Chemother. Pharmacol.*, **41**, 252 (1997); https://doi.org/10.1007/s002800050737
25. L.M. Slater, G.F. Eilon, J. Gu, J.W. Jacobs and K. Hara, *Cancer Chemother. Pharmacol.*, **45**, 183 (2000); https://doi.org/10.1007/s002800050028
26. M. Yoshida, Y. Maehara and K. Sugimachi, *Clin. Cancer Res.*, **5**, 4295 (1999).
27. C. Hulme and M.-P. Cherrier, *Tetrahedron Lett.*, **40**, 5295 (1999); https://doi.org/10.1016/S0040-4039(99)00960-0
28. M. Font, A. Gonzalez, J.A. Palop and C. Sanmartin, *Eur. J. Med. Chem.*, **46**, 3887 (2011); https://doi.org/10.1016/j.ejmech.2011.05.060
29. G.V. Scagliotti and S. Novello, *Cancer Treat. Rev.*, **38**, 292 (2012); https://doi.org/10.1016/j.ctrv.2011.07.008
30. S. Schenone, C. Brullo, F. Musumeci and M. Botta, *Expert Opin. Investig. Drugs*, **19**, 931 (2010); https://doi.org/10.1517/13543784.2010.499898
31. X.L. Mei, Y. Yang, Y.J. Zhang, Y. Li, J.-M. Zhao, J.-G. Qiu, W.-J. Zhang, Q.-W. Jiang, Y.-Q. Xue, D.-W. Zheng, Y. Chen, W.-M. Qin, M.-N. Wei and Z. Shi, *Am. J. Cancer Res.*, **5**, 3311 (2015).
32. M. Sarfati, V. Mateo, S. Baudet, M. Rubio, C. Fernandez, F. Davi, J.-L. Binet, J. Delic and H. Merle-Béral, *Blood*, **101**, 265 (2003); https://doi.org/10.1182/blood-2002-01-0075
33. M.A. Hussein, Y.O. Mosaad and N.A.E.K. Gobba, *Med. Chem.*, **8**, 136 (2018); https://doi.org/10.4172/2161-0444.1000505
34. J. Li, J. Tan, L. Chen, J. Zhang, X. Shen, C. Mei, L. Fu, L. Lin, J. Ding, B. Xiong, X. Xiong, H. Liu, X. Luo and H. Jiang, *Acta Pharmacol. Sin.*, **27**, 1259 (2006); https://doi.org/10.1111/j.1745-7254.2006.00437.x
35. X. Xiong, H. Liu, L. Fu, L. Li, J. Li, X. Luo and C. Mei, *Chemotherapy*, **54**, 463 (2008); https://doi.org/10.1159/000159272
36. H.-Q. Li, T. Yan, Y. Yang, L. Shi, C.-F. Zhou and H.-L. Zhu, *Bioorg. Med. Chem.*, **18**, 305 (2010); https://doi.org/10.1016/j.bmc.2009.10.054
37. P.-C. Lv, H.-Q. Li, J. Sun, Y. Zhou and H. Zhu, *Bioorg. Med. Chem.*, **18**, 4606 (2010); https://doi.org/10.1016/j.bmc.2010.05.034
38. A.W. Shaw, Y. Chang, Y. Hsu, P.-J. Lu, C.-N. Yang, H.-L. Chen, C.-W. Lo, C.-W. Shiau and M.-K. Chern, *Eur. J. Med. Chem.*, **45**, 2860 (2010); https://doi.org/10.1016/j.ejmech.2010.03.008
39. D. Kommula, S. Polepalli, N. Jain and M.S. Murty, *Indian J. Pharm. Sci.*, **80**, 930 (2018); https://doi.org/10.4172/pharmaceutical-sciences.1000440
40. L. Wang, T. Wang, B. Yang, Z. Chen and H. Yang, *Med. Chem. Res.*, **21**, 124 (2012); https://doi.org/10.1007/s00044-010-9512-1
41. B.J. Henderson, D.J. Carper, T.F. González-Cestari, B. Yi, K. Mahasenan, R.E. Pavlovicz, M.L. Dalefield, R.S. Coleman, C. Li and D.B. McKay, *J. Med. Chem.*, **54**, 8681 (2011); https://doi.org/10.1021/jm201294r
42. M.E. Prime, O.A. Andersen, J.J. Barker, M.A. Brooks, R.K.Y. Cheng, I. Toogood-Johnson, S.M. Courtney, F.A. Brookfield, C.J. Yarnold, R.W. Marston, P.D. Johnson, S.F. Johnsen, J.J. Palfrey, D. Vaidya, S. Erfan, O. Ichihara, B. Felicetti, S. Palan, A. Pedret-Dunn, S. Schaertl, I. Sternberger, A. Ebneth, A. Scheel, D. Winkler, L. Toledo-Sherman, M. Beconi, D. Macdonald, I. Muñoz-Sanjuan, C. Dominguez and J. Wityak, *J. Med. Chem.*, **55**, 1021 (2012); https://doi.org/10.1021/jm201310y
43. B.R. Rao, R. Katiki, K. Dileep, C.G. Kumar, G.N. Reddy, J.B. Nanubolu and M.S.R. Murty, *Lett. Org. Chem.*, **16**, 723 (2019); https://doi.org/10.2174/1570178615666181113094539
44. D. Sun, Z. Wang, M. Cardozo, R. Choi, M. DeGraffenreid, Y. Di, X. He, J.C. Jaen, M. Labelle, J. Liu, J. Ma, S. Miao, A. Sudom, L. Tang, H. Tu, S. Ursu, N. Walker, X. Yan, Q. Ye and J.P. Powers, *Bioorg. Med. Chem. Lett.*, **19**, 1522 (2009); https://doi.org/10.1016/j.bmcl.2008.12.114
45. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983); https://doi.org/10.1016/0022-1759(83)90303-4
46. S.M. Gomha, S.M. Riyadh, E.A. Mahmmoud and M.M. Elaasser, *Heterocycles*, **91**, 1227 (2015); https://doi.org/10.3987/COM-15-13210
47. H. Shao, S. Shi, D.W. Foley, F. Lam, A.Y. Abbas, X. Liu, S. Huang, X. Jiang, N. Baharin, P.M. Fischer and S. Wang, *Eur. J. Med. Chem.*, **70**, 447 (2013); https://doi.org/10.1016/j.ejmech.2013.08.052
48. N.S. El-Sayed, E.R. El-Bendary, S.M. El-Ashry and M.M. El-Kerdawy, *Eur. J. Med. Chem.*, **46**, 3714 (2011); https://doi.org/10.1016/j.ejmech.2011.05.037
49. M. Fares, S.M. Abou-Seri, H.A. Abdel-Aziz, S.E.S. Abbas, M.M. Youssef and R.A. Eladwy, *Eur. J. Med. Chem.*, **83**, 155 (2014); https://doi.org/10.1016/j.ejmech.2014.06.027
50. Y.-G. Hu, Y. Wang, S.-M. Du, X.-B. Chen and M.-W. Ding, *Bioorg. Med. Chem. Lett.*, **20**, 6188 (2010); https://doi.org/10.1016/j.bmcl.2010.08.122
51. X.J. Song, Y. Shao and X.G. Dong, *Chin. Chem. Lett.*, **22**, 1036 (2011); https://doi.org/10.1016/j.cclet.2011.05.012
52. S.J. Tangeda and A. Garlapati, *Eur. J. Med. Chem.*, **45**, 1453 (2010); https://doi.org/10.1016/j.ejmech.2009.12.050