Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

In vitro cytotoxicity evaluation of thiourea derivatives bearing *Salix sp.* constituent against HK-1 cell lines

Norsyafikah Asyilla Nordin, Vannessa Lawai, Zainab Ngaini, Ainaa Nadiah Abd Halim, Siaw San Hwang, Reagan Entigu Linton, Boon Kiat Lee & Paul Matthew Neilsen

To cite this article: Norsyafikah Asyilla Nordin, Vannessa Lawai, Zainab Ngaini, Ainaa Nadiah Abd Halim, Siaw San Hwang, Reagan Entigu Linton, Boon Kiat Lee & Paul Matthew Neilsen (2018): *In vitro* cytotoxicity evaluation of thiourea derivatives bearing *Salix sp.* constituent against HK-1 cell lines, Natural Product Research, DOI: 10.1080/14786419.2018.1517120

To link to this article: https://doi.org/10.1080/14786419.2018.1517120

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2018.1517120

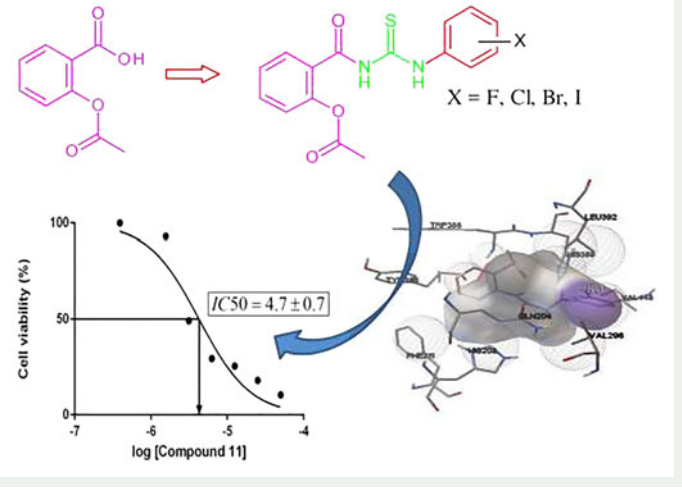
In vitro cytotoxicity evaluation of thiourea derivatives bearing *Salix sp.* constituent against HK-1 cell lines

Norsyafikah Asyilla Nordin[a,b], Vannessa Lawai[b], Zainab Ngaini[b], Ainaa Nadiah Abd Halim[b], Siaw San Hwang[c], Reagan Entigu Linton[c], Boon Kiat Lee[c] and Paul Matthew Neilsen[d]

[a]Faculty of Pharmacy, Universiti Sultan Zainal Abidin, Besut Campus, Terengganu, Malaysia; [b]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Sarawak, Malaysia; [c]Faculty of Engineering, Computing and Science, Swinburne University of Technology Sarawak Campus, Sarawak, Malaysia; [d]School of Health Medical and Applied Sciences, Central Queensland University, Norman Gardens, Australia

ABSTRACT

In searching for drugs from natural product scaffolds has gained interest among researchers. In this study, a series of twelve halogenated thiourea (**ATX 1-12)** *via* chemical modification of aspirin (a natural product derivative) and evaluated for cytotoxic activity against nasopharyngeal carcinoma (NPC) cell lines, HK-1 via MTS-based colorimetric assay. The cytotoxicity studies demonstrated that halogens at *meta* position of **ATX** showed promising activity against HK-1 cells (IC_{50} value $\leq$15 μM) in comparison to cisplatin, a positive cytotoxic drug (IC_{50} value $=8.9 \pm 1.9$ μM). **ATX 11**, bearing iodine at *meta* position, showed robust cytotoxicity against HK-1 cells with an IC_{50} value of 4.7 ± 0.7 μM. Molecular docking interactions between **ATX 11** and cyclooxygenase-2 demonstrated a robust binding affinity value of −8.1 kcal/mol as compared to aspirin's binding affinity value of −6.4 kcal/mol. The findings represent a promising lead molecule from natural product with excellent cytotoxic activity against NPC cell lines.



ARTICLE HISTORY
Received 7 May 2018
Accepted 24 August 2018

KEYWORDS
Aspirin; thiourea; cytotoxicity; molecular docking; cyclooxygenase-2

CONTACT Zainab Ngaini nzainab@unimas.my

Supplemental data for this article can be accessed https://doi.org/10.1080/14786419.2018.1517120.

1. Introduction

Willow tree (*Salix sp.*) has been reported as an effective traditional remedy to treat fever and pain (Desborough and Keeling 2017). Willow tree is a Salicaceae family which comprised of several species including *Salix alba* L., *Salix pentandra* L. and *Salix purpurea* L (Mahdi 2010). Salicylic acid is an active metabolite extracted from willow bark. Chemical modification of salicylic acid into acetylsalicylic acid or aspirin has been reported for gastrointestinal study (Mahdi et al. 2006).

Aspirin is a type of non-steroidal anti-inflammatory drug which commonly used to relieve pain, reduce fever and prevent diseases related to aging (Vane and Botting 2003). Prolonged use of aspirin, however, has caused gastrointestinal bleeding and ulceration. Several efforts in modifying aspirin has been reported to increase its pharmacological properties (Turnbull et al. 2008; Ngaini and Mortadza 2018). Phosphoaspirin is an example of structural modification of aspirin that exhibits less gastrointestinal toxicity and enhanced anti-inflammatory effect compared to the conventional aspirin (Huang et al. 2011). Other aspirin modifications were also reported to augment its antifungal (Chavan et al. 2012) and anticancer activities (Huang et al. 2014).

Synthesis of new drugs and studies on their efficacy requires higher cost and longer times. Many new drugs fail in the clinical trials due to poor bioavailability or pharmacokinetic properties. Medicinal chemistry approaches to modify known drugs seems to be more viable and practical option. Our preliminary natural product modification of aspirin *via* thiourea moieties bearing amino acids showed excellent antibacterial activities (Ngaini et al. 2012, Wan Zullkiplee et al. 2014). The potential of thiourea moieties as an effective drug has been widely reported *via* production of several commercial drug-based thioureas such as carbimazole, propylthiouracil and methylthiouracil (Ansari et al. 2014). Thiourea is an organic compound which consist of thiocarbonyl and amine groups and known to display broad range of biological properties such as antibacterial (Abd Halim and Ngaini 2017; Nordin et al. 2017), larvicidal (Kaymakcioglu et al. 2013) and anticancer (Yahyazadeh and Ghasemi 2013). The presence of substituents such as halogens has been reported for excellent antibacterial (Zhou et al. 2006; Prabhashankar et al. 2012), antifungal (Feng et al. 2014), cytotoxic (El-Gohary and Shaaban 2013) and antitumor properties (Jain et al. 2012).

Herein, we report the synthesis of halogenated thiourea moieties (**ATX 1-12**) bearing aspirin, a natural product-derived molecule, by reacting acetoxybenzoyl thiocyanate with a series of halogenated anilines. All 12 compounds were evaluated for potential cytotoxic agent against NPC cell line, HK-1. The interaction of the compounds with the targeted enzyme was evaluated *via* molecular docking analysis against COX-2 protein.

2. Results and discussion

2.1. Chemistry

Series of novel aspirin analogues (**ATX 1-12**) bearing halogenated thiourea moieties were prepared from the reaction of aspirin, an active scaffold of natural product derivative, and oxalyl chloride in the presence of potassium thiocyanate (Ngaini et al.

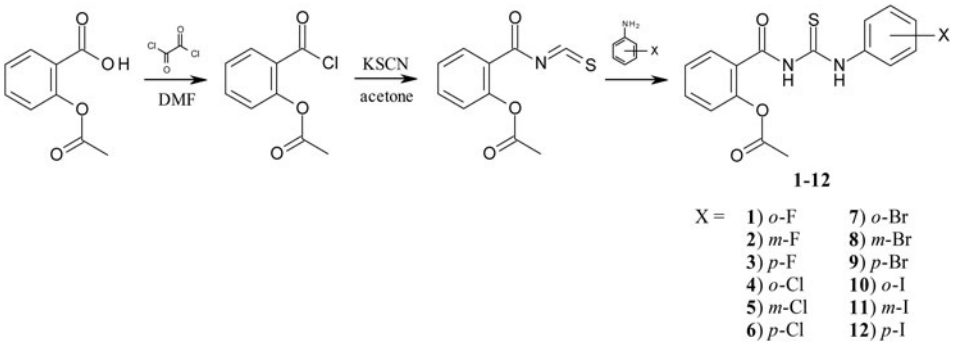
2012) to form acetoxybenzoyl isothiocyanate as intermediates. Further synthesis of the intermediates with a series of halogenated anilines afforded **ATX 1-12** in 30.7-42.0% yield (Scheme 1). The synthesis of thiourea also form urethane as side product which contributed to the low product yield (Katritzky et al. 2004).

The structural elucidation of **ATX 1-12** was performed using FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy. The FTIR spectra showed sharp peaks corresponded to *v*(NH) group at 3356–3382 cm^{-1}. The disappearance of peak at 2000–2500 cm^{-1} for –NCS indicated the successful conversion of intermediates into thioureas (Fathalla et al. 2001). The absorption peaks at 1774–1785 cm^{-1} and 1663–1675 cm^{-1} were assigned to *v*(C=O) ester and amide, respectively. The absorption peak attributed to aryl groups was observed at 1524–1561 cm^{-1}. The presence of *v*(C–N) was attributed to the peaks at 1142–1169 cm^{-1} (Saeed et al. 2009) while peaks at 749–863 cm^{-1} were assigned to *v*(C=S) (Bielenica et al. 2015).

^{1}H NMR spectra of **ATX 1-12** indicated the presence of CH_3 at δ_H 2.31–2.32 ppm and aromatic groups at δ_H 7.13–8.16 ppm. The resonance at δ_H 11.65–11.86 ppm and δ_H 12.27–60 ppm were attributed to CSNH and CONH, respectively. The deshielding effect of carbonyl and thiocarbonyl groups has shifted the signal to downfield region (Saeed et al. 2010). ^{13}C NMR spectra also gave resonance peaks corresponded to the proposed structures. The peaks at δ_C 20.7–21.4 ppm were attributed to the methyl group in aspirin. The resonance at δ_C 91.7–163.0 ppm were attributed to aromatic carbons, while signals at δ_C 166.3–169.4 ppm and δ_C 178.8–179.9 ppm were corresponded to C=O and C=S, respectively (Rauf et al. 2009).

2.2. Anticancer assay

The application of chemically modified natural product compounds of aspirin bearing thioureas moieties were evaluated for *in vitro* cytotoxic activity against NPC cell line, HK1 at different concentrations ranging from 0.39 to 50 μM using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. The relative influence of the aspirin-thiourea derivatives on the viability of the NPC cell line was determined *via* concentration-response curves using regression analysis to determine the IC_{50} (concentration causing 50% cell growth inhibition) (Figure 1). The IC_{50} of **ATX 1-12** is summarized in Table S1 with values ranging from 4.7 to 14.6 μM.



Scheme 1. Synthesis of **ATX 1-12**.

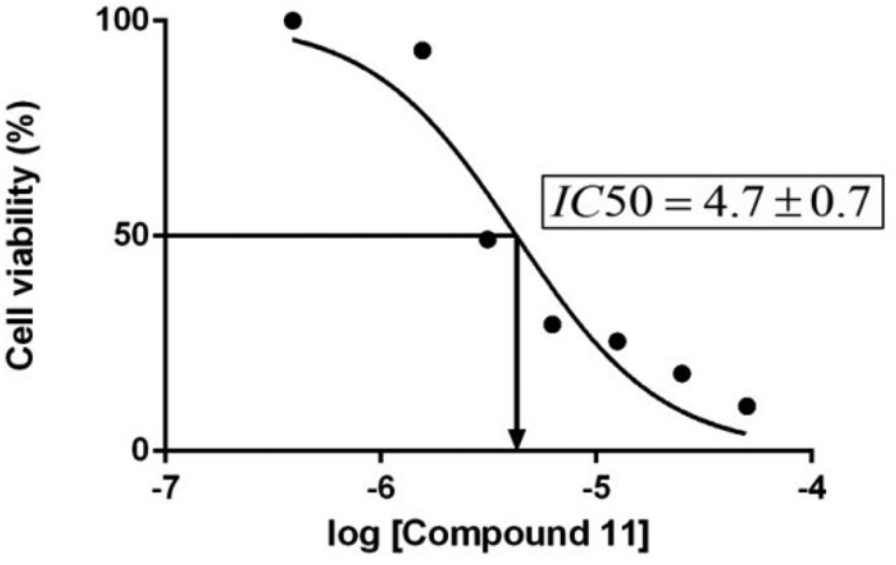


Figure 1. Cell viability of NPC cells, HK1 treated with various concentrations of **ATX 11** for 7 h. The cell viability was measured by the MTS assay.

All compounds with the exception of **ATX 1**, **3**, **4**, **7**, **8** and **10** showed promising activities against HK1 with IC_{50} values $\leq$10 μM. This is encouraging compared to the parental aspirin, which showed no anticancer activity at the same concentrations on HK1 cells ($IC_{50} > 50$ μM) and a reference drug cisplatin ($IC_{50} \leq 8.9$ μM). Cisplatin is the chemotherapeutic drug used as a standard for treating NPC (Al-Sarraf et al. 1998).

ATX with halogens (except bromine) introduced at the *meta* position in thiourea moieties displayed excellent activities ($IC_{50} \leq 8.0$ μM) as compared to *ortho* and *para* positions. Compound with substituents at *meta* position was reported to be more selective to bind on specific protein (Vaz and Klabunde 2008), thus giving excellent activities by affecting the cell's survival functions.

ATX 11, with iodine at *meta* position, showed the most potent cytotoxic activity against HK1 amongst the tested aspirin derivatives, with an IC_{50} of 4.7 μM. Importantly, neither the halogenated aniline used to synthesise **ATX 11** (with the iodine at the *meta* position) nor aspirin itself, showed activity against HK1 cells ($IC_{50} > 50$ μM). In other words, the cytotoxic activity of **ATX 11** is likely to be a result of the modification to aspirin, rather than an independent cytotoxicity associated with the halogenated aniline. The presence of iodine has increased lipophilicity of the aspirin-thiourea compared to the other halogens (Shirasaka et al. 1990) and more lipophilic character resulted in greater cytotoxicity (Modi et al. 2011). The presence of halogens at *ortho* position in thiourea moieties showed a noticeably reduced cytotoxicity (IC_{50} 10.8 to 13.9 μM) ($P < 0.05$) as compared to *meta* and *para* positions. While in **ATX 9**, the introduction of bromine at *para* position has displayed good cytotoxicity with an IC_{50} value of 5.7 μM. Based on the IC_{50} values in this study, it can be concluded that the presence of iodine and chlorine at *meta* position as well as bromine at *para* position of the phenyl ring has improved the cytotoxicity profiles against NPC cells, HK1 ($P < 0.05$).

In comparison to aspirin, the presence of thiourea moiety in the molecular structure of **ATX 1-12** provides important functional groups for a promising anticancer activity. High electronegative sulfur atom presence could incorporate with protein through hydrogen bonding in cancer cell and subsequently inhibited the cells' growth (Zhao et al. 2015).

2.3. Molecular docking

Cyclooxygenase-2 (COX-2) enzyme is an inducible protein which expressed abnormally in many types of cancers (Chen et al 2010). The suppression of viability of the NPC cell line, HK1 could be due to the inhibition of COX-2 (Chen et al 2010). In this regard, the binding interaction between **ATX 11** (the most potent cytotoxic activity against HK1) to the active site of COX-2 was performed *via* molecular docking for further estimation of the binding energy in comparison to aspirin. The aspirin-thiourea derivative of **ATX 11** demonstrated robust molecular docking in the binding pockets of COX-2 and displayed strong electrostatic interaction with Trp388, Leu392, His389, His208, Tyr 386, Phe211, Gln204, Val296 and Val448 (Adinarayana et al. 2012) (Figure 2). **ATX 11** showed good binding affinity value of -8.1 Kcal/mol. In contrast, aspirin showed weak interaction with the residue Trp388, Leu391, Leu392 and Gln204 and a binding energy value of -6.4 Kcal/mol (Khan et al. 2015) (Figure 3). These interaction further explained and supported the anticancer activity of **ATX 11** against COX-2 by significant

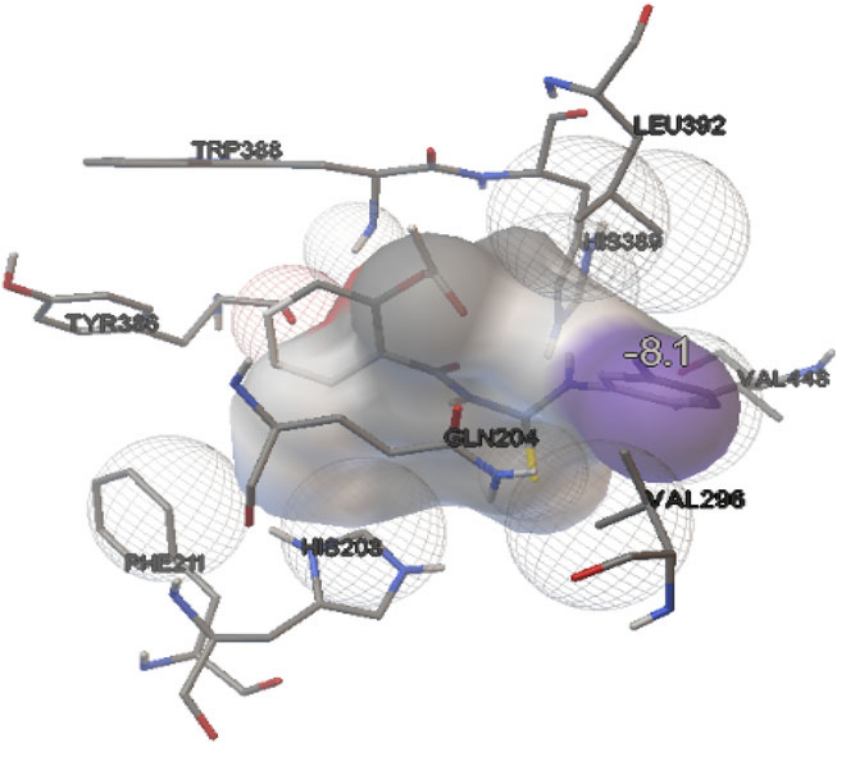


Figure 2. Binding interaction of **ATX 11** at the active site of COX-2.

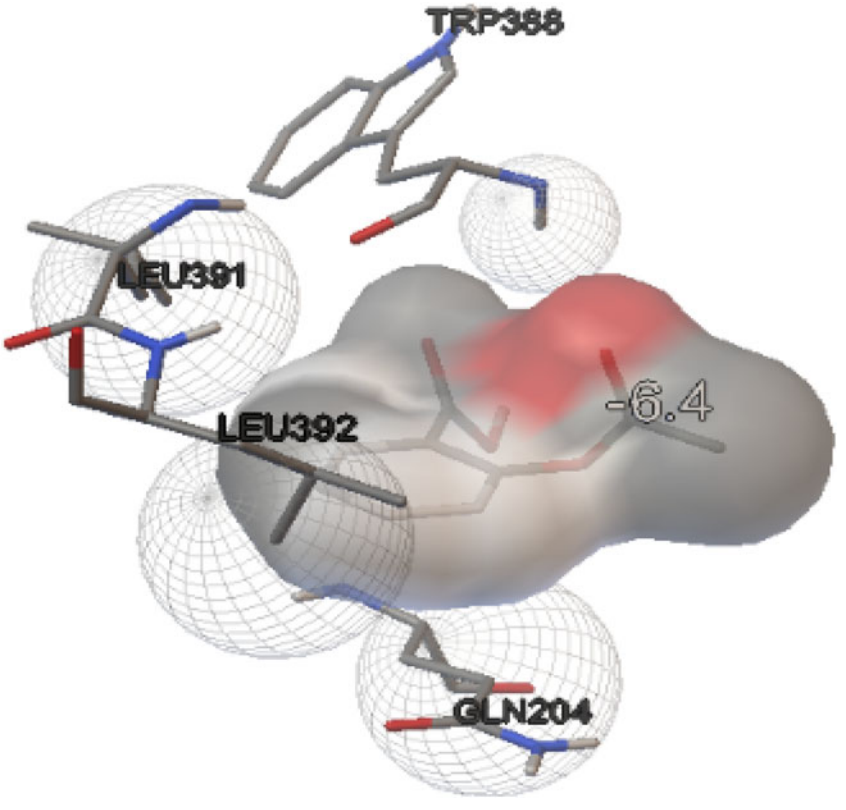


Figure 3. Binding interaction of aspirin at the active site of COX-2.

interaction with specific amino acid residues with higher binding affinity compared to aspirin.

3. Experimental

3.1. General

Aspirin, potassium thiocyanate and halogenated anilines series were obtained from Merck. All other reagents were used without further purification nevertheless the acetone was distilled before used. Melting points were determined on Stuart SMP3 using open tube capillary method. FTIR spectra (ν/cm^{-1}) were recorded as KBr pellets on Perkin Elmer 1605 FTIR Spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL ECA 500 at 500 MHz (^{1}H) and 125 MHz (^{13}C) with the chemical shift reported relative to DMSO-d_6 as the standard reference and chemical shift values were expressed in δ ppm.

3.2. General synthesis of ATX 1-12

Aspirin (0.1802 g, 1.0 mmol) was reacted with oxalyl chloride and DMF was added to initiate the reaction. Acetylsalicyloyl chloride formed was added drop wise into a solution of KSCN (0.0972 g, 1.0 mmol) in dry acetone (10 mL). The mixture was stirred for

30 min at room temperature and white precipitate (KCl) was filtered off from the mixture. A series of halogenated aniline (1.0 mmol) in 10 mL dry acetone was added into the filtrate and the mixture was stirred at room temperature for 24 h to form precipitate. Purification by column chromatography (silica gel, 1:6 ethyl acetate/hexane) afforded **ATX 1-12** as shown in Supplementary Materials (Data S1).

3.3. Cell culture and cell viability assay

MTS based colorimetric assay was used to evaluate the cytotoxic activity of **ATX 1-12** on NPC cell line, HK1 (Huang et al. 1980). The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in RPMI 1640 (GIBCO) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and 1% penicillin-streptomycin (GIBCO). The cells were seeded onto a 96 well plate at $5x10^3$ cells/100 μL. **ATX 1-12** were dissolved in DMSO at different concentrations of which 100 μL of the diluted mixtures were added onto designated wells of the 96-well plate; with final concentration ranging from 0.39 to 50 μM. Cells added with the equivalent concentration of DMSO vehicle were analysed as controls. The 96-well plate was incubated for 72 hours at 37 °C and culture media was carefully removed after the incubation. Subsequently, 10 μL of MTS (Cell Titer 96@ Aqueous Non-Rad Cell Proliferation Assay Kit, Promega) and 50 μL of fresh media were added to each well, followed by an incubation of 90 minutes at 37 °C. The absorbance was read at 490 nm using microplate reader (Synergy HT, BioTek, USA).

3.4. Molecular docking

Binding interaction of the most active compound **ATX 11** and binding site of cyclooxygenase-2 (COX-2) was performed using AutodockTools 1.5.6 and the binding energy was further estimated using Autodock Vina programme (Nguyen et al. 2016). The crystal structure of COX-2 was obtained from RCSB Protein Data Bank with PDB ID: 5IKR (Orlando and Malkowski, 2016). A grid box with $60 \times 60 \times 60$ Å dimension with a spacing of 0.375 Å was centered at active site of the protein. Aspirin was also docked into active site of COX-2 as reference.

3.5. Statistical analysis

Statistical analysis was performed using *t*-test (SPSS version 21). A value of $P < 0.05$ was considered as significant.

4. Conclusion

Twelve aspirin-thiourea derivatives (**ATX 1-12)** have been successfully prepared and demonstrated as potential anticancer agents. Among the 12 compounds in this study, **ATX 11** showed excellent effect towards cell viabilities of nasopharyngeal cancer cell line compared to aspirin alone. **ATX 11** (with iodine at *meta* position) showed the most potent cytotoxic/inhibitory effect on NPC cell viability with IC_{50} of 4.7 μM. Molecular docking demonstrated good binding interaction of **ATX 11** against COX-2

compared to aspirin, in correlation to the IC_{50} values. This study offers significant breakthrough in producing a potential chemotherapeutic agent in NPC treatment using natural product derivatives as a reactive scaffold in the presence of thiourea moieties. *In vitro* study of **ATX 11** as enzyme inhibitor can be further investigated for potential pharmaceutical applications.

Acknowledgements

We acknowledge support from Universiti Malaysia Sarawak and Malaysia Ministry of Science, Technology and Innovation for the financial support through FRGS/ST02(01)/1115/2014(01) and FRGS/ST01(01)/1298/2015(15). We also acknowledge Universiti Sultan Zainal Abidin for grant support through UniSZA/2017/DPU/02. We thank Universiti Teknologi MARA for providing CHN elemental analysis services and Corine Neilsen for outstanding editing and proof reading of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

We acknowledge support from Universiti Malaysia Sarawak and Malaysia Ministry of Science, Technology and Innovation for the financial support through FRGS/ST02(01)/1115/2014(01) and FRGS/ST01(01)/1298/2015(15). We also acknowledge Universiti Sultan Zainal Abidin for grant support through UniSZA/2017/DPU/02. We thank Universiti Teknologi MARA for providing CHN elemental analysis services and Corine Neilsen for outstanding editing and proof reading of the manuscript.

References

Abd Halim AN, Ngaini Z. 2017. Synthesis and characterization of halogenated bis(acylthiourea) derivatives and their antibacterial activities. Phosphorus, Sulfur, Silicon Relat Elem. 192(9): 1012–1017.

Adinarayana KPS, Reddy PA, Babu PA. 2012. Structural studies on docking selective COX-2 inhibitors. J Bioinform Res. 1:21–26.

Al-Sarraf M, LeBlanc M, Giri PG, Fu KK, Cooper J, Vuong T, Forastiere AA, Adams G, Sakr WA, Schuller DE, Ensley JF. 1998. Chemoradiotherapy versus radiotherapy in patients with advanced nasopharyngeal cancer: phase III randomized intergroup study 0099. J Clin Oncol. 16(4):1310–1317.

Ansari MM, Deshmukh SP, Khan R, Musaddiq M. 2014. Synthesis antimicrobial and anticancer evaluation of 1-Aryl-5-(o-methoxyphenyl)-2-S-benzyl isothiobiurets. Int J Med Chem. 2014:1–6.

Bielenica A, Stefańska J, Stępień K, Napiórkowska A, Augustynowicz-Kopeć E, Sanna G, Madeddu S, Boi S, Giliberti G, Wrzosek M, Struga M. 2015. Synthesis, cytotoxicity and antimicrobial activity of thiourea derivatives incorporating 3-(trifluoromethyl)phenyl moiety. Eur J Med Chem. 101:111–125.

Chavan BB, Chitte PD, Choudhary NP, Albhar KG, Hukkeri VI. 2012. Synthesis and biological evaluation of novel benzimidazole derivative with aspirin as potent antimicrobial & antifungal agents. Int J Sci Res Rev. 1:22–30.

Chen J, Ran Y, Hong C, Chen Z, You Y. 2010. Anticancer effects of celecoxib on nasopharyngeal carcinoma HNE-1 cells expressing COX-2 oncoprotein. Cytotechnology. 62(5):431–438.

Desborough MJR, Keeling DM. 2017. The aspirin story – from willow to wonder drug. Br J Haematol. 177(5):674–683.
El-Gohary NS, Shaaban MI. 2013. Synthesis, antimicrobial, antiquorum-sensing, antitumor and cytotoxic activities of new series of fused [1,3,4]thiadiazoles. Eur J Med Chem. 63:185–195.
Fathalla WM, Čajan M, Marek J, Pazdera P. 2001. One-pot quinazolin-4-ylidenethiourea synthesis via N-(2-cyanophenyl)benzimidoyl isothiocyanate. Molecules. 6(7):574–587.
Feng ZQ, Yang XL, Ye YF, Hao LY. 2014. The effect of electron-withdrawing group functionalization on antibacterial and catalytic activity of palladium (II) complexes. Bull Korean Chem Soc. 35(4):1121–1127.
Huang DP, Ho JHC, Poon YF, Chew EC, Saw D, Lui M, Li CL, Mak LS, Lai SH, Lau WH. 1980. Establishment of a cell line (NPC/HK1) from a differentiated squamous carcinoma of the nasopharynx. Int J Cancer. 26(2):127–132.
Huang L, Mackenzie GG, Ouyang N, Sun Y, Xie G, Johnson F, Komninou D, Rigas B. 2011. The Novel phospho-non-steroidal anti-inflammatory drugs, OXT-328, MDC-22 and MDC-917, inhibit adjuvant-induced arthritis in rats. Br J Pharmacol. 162(7):1521–1533.
Huang L, Wong CC, Mackenzie GG, Sun Y, Cheng KW, Vrankova K, Alston N, Ouyang N, Rigas B. 2014. Phospho-aspirin (MDC-22) inhibits breast cancer in preclinical animal models: an effect mediated by EGFR inhibition, p53 acetylation and oxidative stress. BMC Cancer. 14(1):1–14.
Jain AK, Vaidya A, Ravichandran V, Kashaw SK, Agrawal RM. 2012. Recent developments and biological activities of thiazolidinone derivatives: a review. Bioorg Med Chem. 20(11):3378–3395.
Katritzky AR, Ledoux S, Witek RM, Nair SK. 2004. 1-(Alkyl/arylthiocarbamoyl)benzotriazoles as stable isothiocyanate equivalents: synthesis of di- and trisubstituted thioureas. J Org Chem. 69(9):2976–2982.
Kaymakcioglu BK, Celen AO, Tabanca N, Ali A, Khan SI, Khan IA, Wedge DE. 2013. Synthesis and biological activity of substituted urea and thiourea derivatives containing 1,2,4-triazole moieties. Molecules. 18(3):3562–3576.
Khan MF, Rashid R, Rashid MA. 2015. Computational study of geometry, molecular properties and docking study of aspirin. World J Pharm Res. 4:2702–2714.
Mahdi JG. 2010. Medicinal potential of willow: a chemical perspective of aspirin discovery. J Saudi Chem Soc. 14(3):317–322.
Mahdi JG, Mahdi AJ, Mahdi AJ, Bowen ID. 2006. The historical analysis of aspirin discovery, its relation to the willow tree and antiproliferative and anticancer potential. Cell Prolif. 39(2): 147–155.
Modi NR, Shah RJ, Patel MJ, Suthar M, Chauhan BF, Patel LJ. 2011. Design, synthesis, and QSAR study of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-phenyl acetamide derivatives as cytotoxic agents. Med Chem Res. 20(5):615–625.
Ngaini Z, Arif MAM, Hussain H, Er SM, Tang D, Kamaluddin DHA. 2012. Synthesis and antibacterial activity of acetoxybenzoyl thioureas with aryl and amino acid side chains. Phosphorus, Sulfur, Silicon Relat Elem. 187(1):1–7.
Ngaini Z, Mortadza NA. 2018. Synthesis of halogenated azo-aspirin analogues from natural product derivatives as the potential antibacterial agents. Nat Prod Res. 1. DOI: 10.1080/14786419.2018.1486310
Nguyen TT, Tran DP, Huy PDQ, Hoang Z, Carloni P, Pham PV, Nguyen C, Li MS. 2016. Ligand binding to anti-cancer target CD44 investigated by molecular simulations. J Mol Model. 22: 165–179.
Nordin NA, Chai TW, Tan BL, Choi CL, Halim AN, Hussain H, Ngaini N. 2017. Novel synthetic monothiourea aspirin derivatives bearing alkylated amines as potential antimicrobial agents. J Chem. 2017:1–7.
Orlando BJ, Malkowski MG. 2016. Substrate-selective Inhibition of cyclooxygeanse-2 by fenamic acid derivatives is dependent on peroxide tone. J Biol Chem. 291(29):15069–15081.
Prabhashankar J, Govindappa VK, Renuka N, Akshatha KN, Mahadevamurthy S, Kariyappa AK. 2012. Evaluation and Studies on the Structural Impact of 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles on their Biological Activities. Pharm Lett. 4:1685–1691.

Rauf MK, Imtiaz-Ud-Din BA, Gielen M, Ebihara M, de Vos D, Ahmed S. 2009. Synthesis, structural characterization and in vitro cytotoxicity and anti-bacterial activity of some copper (I) complexes with N,N′-disubstituted thioureas. J Inorg Biochem. 103:1135–1144.

Saeed A, Shaheen U, Hameed A, Naqvi SZH. 2009. Synthesis, characterization and antimicrobial studies of some new 1-(fluorobenzoyl)-3-(fluorophenyl) thioureas. J Fluorine Chem. 130(11): 1028–1034.

Saeed S, Rashid N, Ali M, Hussain R, Jones P. 2010. Synthesis, spectroscopic characterization, crystal structure and pharmacological properties of some novel thiophene-thiourea core derivatives. Eur J Chem. 1(3):221–227.

Shirasaka T, Murakami K, Ford H, Kelley JA, Yoshioka H, Kojima E, Aoki S, Broder S, Mitsuy H. 1990. Lipophilic Halogenated Congeners of 2′,3′-dideoxypurine Nucleosides Active Against Human Immunodeficiency Virus In Vitro. Proc Natl Acad Sci USA. 87(23):9426–9430.

Turnbull CM, Marcarino P, Sheldrake TA, Lazzarato L, Cena C, Fruttero R, Gasco A, Fox S, Megson IL, Rossi AG. 2008. A novel hybrid aspirin-NO-releasing compound inhibits TNF alpha release from LPS-activated human monocytes and macrophages. J Inflamm. 5(1):10–12.

Vane JR, Botting RM. 2003. The mechanism of action of aspirin. Thromb Res. 110(5-6):255–258.

Vaz RJ, Klabunde T. 2008. Antitargets: prediction and prevention of drug side effects. Germany: Wiley-VCH.

Wan Zullkiplee WSH, Abd Halim AN, Ngaini Z, Mohd Ariff MA, Hussain H. 2014. Bis-thiourea bearing aryl and amino acids side chains and their antibacterial activities. Phosphorus, Sulfur Silicon Relat. Elem. 189(6):832–838.

Yahyazadeh A, Ghasemi Z. 2013. Synthesis of unsymmetrical thiourea derivatives. Eur Chem Bull. 2:573–575.

Zhao W, Bai JK, Li HM, Chen T, Tang YJ. 2015. Tubulin structure-based drug design for the development of novel 4β-sulfur-substituted podophyllum tubulin inhibitors with anti-tumor activity. Sci Rep. 5:10172–10183.

Zhou Y, Sun Z, Froelich JM, Hermann T, Wall D. 2006. Structure-activity relationships of novel antibacterial translation inhibitors: 3,5-diamino-piperidinyl triazines. Bioorg Med Chem Lett. 16(20):5451–5456.