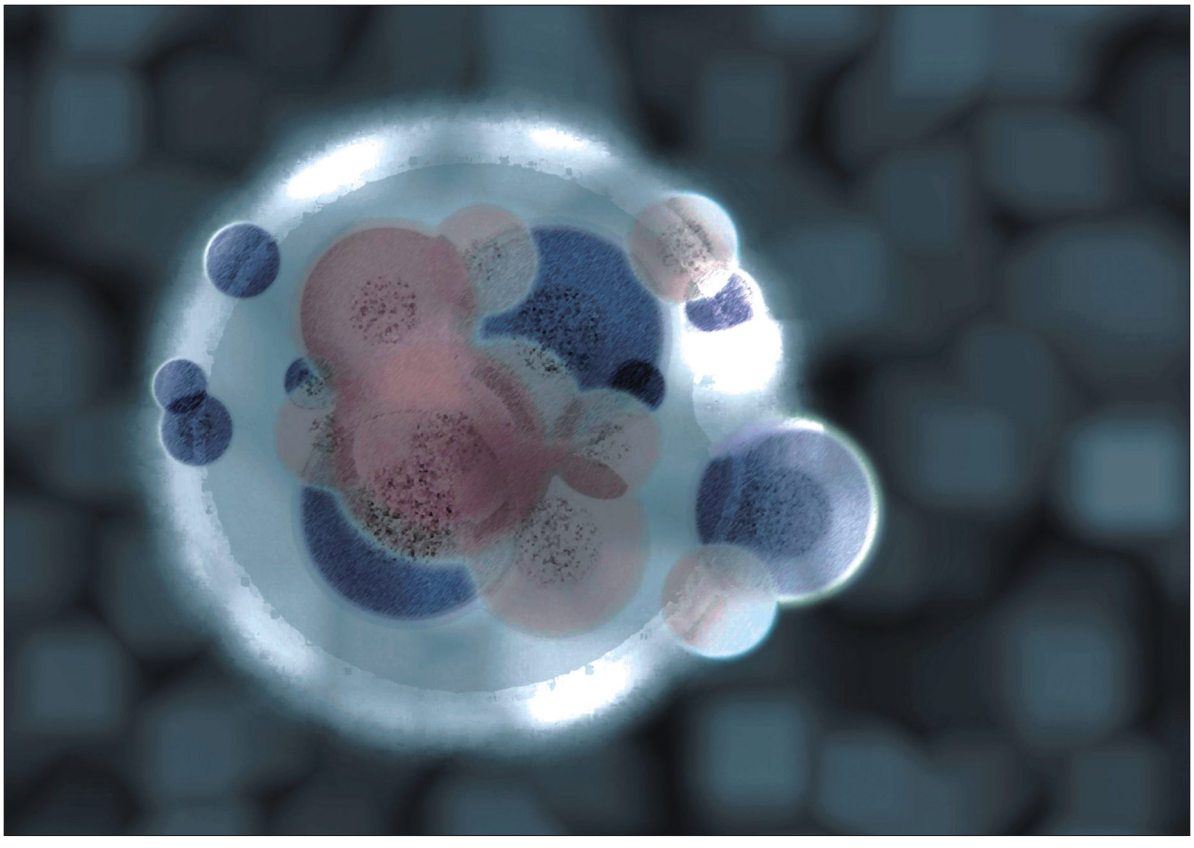

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Research Article
Effects of 6-Mercaptopurine and its Derivatives on Human Hepatocellular Carcinoma and Mammary Adenocarcinoma Cell Lines

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Abstract

Background and Objective: The 6-Mercaptopurine (6-MP) is used to treat autoimmune diseases and inflammation such as rheumatoid arthritis, Crohn's disease and ulcerative colitis. It is also very useful for acute leukemia in children. Therefore, the objective of this present study was to evaluate the cytotoxicity potential of 6-MP and its derivatives 6-Hydroxy-2-Mercaptopurine (6H2MP) and 2-amino-9-butyl-6-mercaptopurine (2A9B6-MP) on other cancer cells. **Materials and Methods:** The HepG2 cells, a cell line derived from human hepatocellular carcinoma and MCF-7 cells, a cell line derived from adenocarcinoma of mammary gland epithelial cells, were incubated *in vitro* with these three 6-MP derivatives and the cytotoxic potential was measured using 3-(4,5-Dimethylthiazole-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay. **Results:** The incubations revealed HepG2 cells were more susceptible to all derivatives when compared to MCF-7 cells. THe 6-MP was cytotoxic when compared to the other 2 related compounds. The HepG2 cells were only at 37.20 and 19.50% viable at 50 and 100 M concentration of 6-MP, respectively. Whereas, MCF-7 cells were at 60.31 and 55.41% viability at the same 6-MP concentration respectively. Viability of HepG2 cells were at 67.51 and 53.20% at the 2 highest concentration of 6H2-MP and MCF-7 showed better viability at 82.71 and 79.95% at 50 and 100 M concentration of 6H2-MP, respectively. The MCF-7 was also more resistant to 2A9B6MP where the viability of MCF-7 cells was approximately double to the viability of HepG2 at 100 M 2A9B6-MP. **Conclusion:** Analogues of 6-MP appeared to be less toxic to cancer cells when compared to 6-MP. The 6-MP is useful in the treatment of leukemia clinically and from this current study, the usefulness may extend to liver cancers. However, further investigations are needed to exactly evaluate the effectiveness of 6-MP in liver cancers.

Key words: 6-Mercaptopurine, 6-hydroxy-2-mercaptopurine, 2-amino-9-butyl-6-mercaptopurine, cytotoxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The 6-Mercaptopurine (6-MP) is a thiopurine drug which was widely used to treat various types of leukemia[1]. It was approved as an antitumor drug by Food and Drug Administration (FDA) USA back in 1953[2]. While 80% of children's leukemia diseases are treated with 6-MP concurrently with other anti-tumor drugs due to cancer resistance[3]. The incidence, mortality and survival rate are alarming[4]. The enhanced efficacy of the combined chemotherapy is very useful not only for leukemia but other cancers such as lungs, liver and breast[3,5] but the risk of adverse drug reactions (ADRs) was not reduced. Recently, 6-MP has been used also to treat arthritis, inflammatory diseases and ulcerative colitis[6]. Kantarjian *et al.*[7] reviewed the treatment and outlook.

Unfortunately, 6-MP induced a long list of ADRs which can be divided into three groups; bone marrow suppression, short- and long-term effects[8]. Short-term ADRs include hepatitis, pancreatitis, rash, fever, hypotension and nausea[6]. The long-term ADRsare the result of general immunosuppression such as cytomegalovirus infection, bacterial liver abscesses and even cancer risk[6]. One strategy for reducing ADRs induced by 6-MP was the introduction of azathioprine, a prodrug of 6-MP[6]. It was a good strategy; however, patients were still experiencing the 6-MP ADRs. Recently, concerns about the potential of azathioprine and 6-MP increased significantly the risk of non-Hodgkin's lymphoma by a factor of 1.6[9].

The next strategy for reducing ADRs induced by 6-MP is the synthesis of analogs of 6-MP. Analogues may possess similar/better efficacy to the parent compound with lower ADRs. In this present investigation, evaluate the cytotoxic potential of 6-MP and 2 analogs 6-Hydroxy-2-Mercaptopurine (6H2MP) and 2-amino-9-butyl-6-mercaptopurine (2A9B6-MP). All three compounds were tested *in vitro* against HepG2 cells, a cell line derived from human hepatocellular carcinoma and MCF-7 cells, a cell line derived from adenocarcinoma of mammary gland epithelial cells. This is the first stage in developing newer drugs.

MATERIALS AND METHODS

Study area: This study was conducted in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia from 2018 to 2019.

Chemicals and plating of cells: As 6-mercaptopurine, 6-hydroxy-2-mercaptopurine and 2-amino-9-butyl-6-mercaptopurine (Sigma Chemicals, US) (Fig. 1(a-b) were dissolved in 0.1% Dimethyl Sulfoxide (DMSO; Sigma Chemicals, US) at 100 mM stock solution. A serial dilution with Dulbecco's modified Eagle's medium (DMEM; Life Technologies, US) to give a final concentration of 200 M. The HepG2 cells, a cell line derived from human hepatocellular carcinoma and MCF-7 cells, a cell line derived from adenocarcinoma of mammary gland epithelial cells, were purchased from American Type Culture Collection (ATCC: Rockville, Maryland, US). Both cell lines were cultured in the following growth medium as recommended by ATCC. All media were supplemented with 10% fetal calf serum (Sigma Chemicals, US) and flasks with 90 to 100% confluency were harvested and detached from the flask by trypsinization. The concentration of cells was done by Trypan blue (Sigma Chemicals, US) exclusion[10]. While 1×10^5 cells was pipetted into each well of the 96-well microtiter plate, incubated overnight at 37°C with 95% O_2/5% CO_2 prior to treatment.

Dosing and 3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide assay: Approximately 24 hrs of incubation, serial dilution with final concentration of 100, 50,

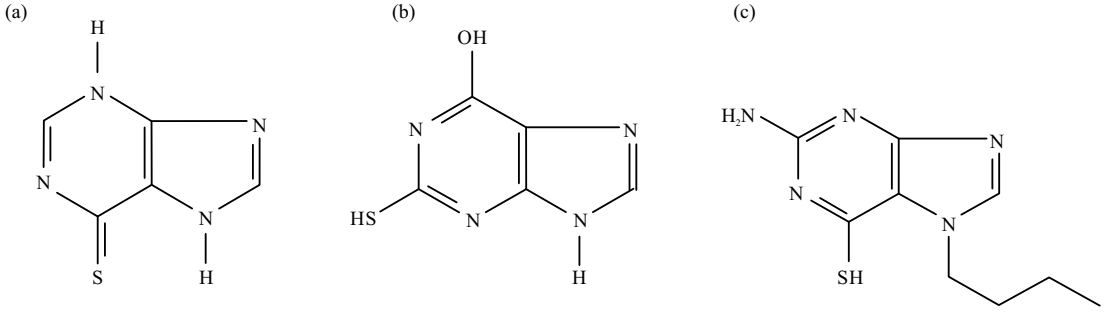

Fig. 1(a-c): Chemical structures of 6-mercaptopurine and derivatives, (a) 6-mercaptopurine, (b) 6-hydroxy-2-mercaptopurine and (c) 2-amino-9-butyl-6-mercaptopurine
(a) MW=152.177, (b) MW=168.178 and (c) MW=223.301

25, 12.5, 6.25, 3.125 and 1.5625 M of compounds were added to the wells. Detailed procedures have been published by Somchit *et al.*[11]. The plates were then further incubated for 72 hrs. Cell viability was determined using 3-(4,5-Dimethylthiazole- 2-yl)-2, 5-Diphenyltetrazolium bromide (MTT) assay[11,12].

Statistical analysis: Data are expressed as Mean±SD of 4 separate experiments using SPSS Ver. 19 software. Statistical significance was defined at $p<0.05$ using analysis of variance or student's t-test. Significant treatment means were further subjected to the Tukey's post-test. The calculations of IC_{50} were performed using GraphPad Prism 5.2 (USA).

RESULTS

Table 1 demonstrated that 6-MP incubations induced a dose-dependent reduction in cell viability of both HepG2 and MCF-7 cells. However, HepG2 cells were statistically more susceptible to 6-MP when compared to MCF-7 cells. The viability of HepG2 cells was only at 37.20 and 19.50% at 50 and 100 M concentrations of 6-MP, respectively. The MCF-7 cells were more resistant to 6-MP where viability 60.31 and 55.41% at the same 6-MP concentration, respectively (Table 1).

Incubation of cells with 6H2-MP was shown in Table 2. Again, a dose-dependent effect was observed for both cell lines. Similar to 6-MP, HepG2 cells were more sensitive to 6H2-MP when compared to MCF-7 cells. Viability of HepG2cells were at 67.51 and 53.20% at the 2 highest concentration of 6H2-MP. The MCF-7 showed better viability at 82.71 and 79.95% at 50 and 100 M concentration of 6H2-MP, respectively. Both cell viability was statistically higher than the same concentration of 6-MP (Fig. 2).

Table 3 shows the viability of cells incubated with 2A9B6-MP. The 2A9B6-MP was more cytotoxic to HepG2 cells than MCF-7 cells like the other 2 related compounds. The viability of HepG2 cells was at 57.20 and 38.71% at the 2 highest concentrations of the compound. The viability was statistically higher than the same concentration of 6-MP but lower than the same concentration of 6H2-MP (Fig. 2). Like the other 2 compounds, MCF-7 was more resistant to 2A9B6-MP where the viability of MCF-7 cells was approximately double to the viability of HepG2 at 100 M 2A9B6-MP concentration (Table 3). The IC_{50} was calculated and revealed that HepG2 cell 6-MP and 2A9B6-MP of 32.25 M and 64.51 M, respectively. Other IC_{50}s (for MCF-7 and 6H2-MP are all above 100 M.

Table 1: Viability of hepatocytes treated with various concentration of 6-mercaptopurine

	Viability of cells (%)	
6-Mercaptopurine (μM)	HepG2	MCF-7
0	112.3 ± 7.14^{abx}	105.84 ± 3.71^{ax}
1.5625	118.75 ± 10.1^{ax}	102.27 ± 2.42^{ax}
3.125	94.06 ± 9.27^{bx}	97.78 ± 2.79^{ax}
6.25	80.10 ± 7.40^{cx}	91.20 ± 2.38^{by}
12.5	69.70 ± 15.47^{cx}	80.13 ± 5.23^{cy}
25	55.57 ± 12.59^{dx}	69.82 ± 2.02^{dy}
50	37.20 ± 9.15^{ex}	60.31 ± 4.80^{dy}
100	19.50 ± 14.12^{fx}	55.41 ± 4.76^{ey}

[a-f]Means with different superscripts differ significantly ($p<0.05$) in the same column, [x-y]Means with different superscripts differ significantly ($p<0.05$) in the same row and n=4/group from four separate experiments

Table 2: Viability of hepatocytes treated with various concentration 6-hydroxy-2-mercaptopurine

	Viability of cells (%)	
6-Hydroxy-2-mercaptopurine (μM)	HepG2	MCF-7
0	114.95 ± 14.23^{ax}	107.22 ± 8.12^{ax}
1.5625	106.07 ± 10.27^{ax}	100.25 ± 2.42^{ax}
3.125	104.20 ± 9.17^{abx}	98.02 ± 2.55^{ax}
6.25	92.25 ± 5.24^{bx}	92.15 ± 4.12^{abx}
12.5	81.45 ± 7.71^{bcx}	94.53 ± 2.75^{by}
25	79.27 ± 4.45^{cdx}	89.28 ± 3.38^{bcy}
50	67.51 ± 9.75^{dx}	82.71 ± 5.22^{cdy}
100	53.20 ± 4.98^{dx}	79.95 ± 4.26^{dy}

[a-e]Means with different superscripts differ significantly ($p<0.05$) in the same column, [x-y]Means with different superscripts differ significantly ($p<0.05$) in the same row and n=4/group from four separate experiments

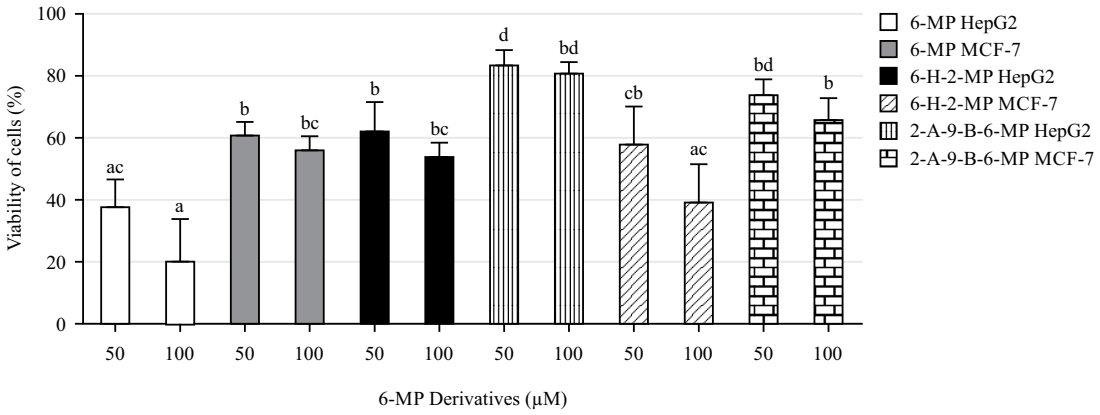

Fig. 2: Percentage cell viability of liver cells incubated with 50 and 100 M 6-mercaptopurine derivatives

[a-d]Means with different superscripts differ significantly (p<0.05) 6-Mercaptopurine (6-MP), 6-Hydroxy-2-Mercaptopurine (6H2MP) and 2-amino-9-butyl-6-mercaptopurine (2A9B6-MP) and n=4/group from four separate experiments

Table 3: Viability of hepatocytes treated with various concentration of 2-amino-9-butyl-6-mercaptopurine.

	Viability of cells (%)	
2-Amino-9-butyl-6-mercaptopurine (μM)	HepG2	MCF-7
0	114.2±9.24^{ax}	108.28±4.54^{ax}
1.5625	110.25±7.31^{abx}	103.25±3.67^{ax}
3.125	92.15±3.82^{bcx}	101.29±5.52^{abx}
6.25	85.68±5.42^{cdx}	94.65±3.78^{bx}
12.5	72.40±10.65^{dex}	82.41±2.10^{cx}
25	65.43±10.19^{ex}	80.25±4.12^{cy}
50	57.20±8.10^{ex}	73.15±5.28^{dy}
100	38.71±12.54^{fx}	65.14±7.22^{dy}

[a-e]Means with different superscripts differ significantly (p<0.05) in the same column, [x-z]Means with different superscripts differ significantly (p<0.05) in the same row and n=4/group from four separate experiments

DISCUSSION

This present study demonstrated that all three compounds 6-Mercaptopurine (6-MP), 6-Hydroxy-2-Mercaptopurine (6H2MP) and 2-amino-9-butyl-6-mercaptopurine (2A9B6-MP) induced dose-dependent cytotoxicity to both HepG2 and MCF-7 cells. However, 6-MP was the most cytotoxic and 6H2-MP was the least toxic. Interestingly, all three compounds were effective towards HepG2 cells and were moderately toxic to M CF-7 cells. The IC_{50} values for 6-MP for HepG2 and MCF7 cells were 32.25 M and >100 M, respectively. Both cells had >100 M for 6H2-MP and 2A96-MP had 64.51 M and >100 M IC_{50}, respectively. Collectively, 6-MP had the most cytotoxic potency towards HepG2 cells.

The major problem for cancer chemotherapy is the resistance of cancer cells towards the drug[13]. Therefore, combination anti-cancer agents are popular and currently 6-MP combinations are useful for not only various types of leukemia but other cancers such as lungs, liver and breast[3]. Indeed, Johnston *et al.*[14] had reported cancer cell lines that were resistant to 6-MP and also several other 6-MP analogs such as 6-mercaptopurine-9-beta-D-ribofuranoside 5'-monophosphate, bis(6-mercaptopurine-9-beta-D-ribofuranoside)-5', 5'''-monophosphate, bis(O2',O3'-dibutyryl-6-mercaptopurine-9-beta-D-ribofuranoside)-5', 5'''-monophosphate and O2',O3'-dibutyryl-6-mercaptopurine-9-beta-D-ribofuranoside 5'-monophosphate. Therefore, this study evaluated the effectiveness of 6-MP and 2 other analogs against 2 cancer cell lines.

The 6-MP and its pro-drug Azathioprine interfere with the process of DNA synthesis and inhibit the proliferation of rapidly dividing cells, especially cells of the immune system. It is used as an immunosuppressive agent in patients undergoing organ transplantation and in the treatment of autoimmune diseases and acute lymphoblastic leukemia[6]. The metabolism of 6-MP by Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT) to cytotoxic 6-thioguanine nucleotide metabolites causes tumor cell death. Interestingly, Johnston *et al.*[14] described the inactivation of 6-MP by Thiopurine Methyltransferase (TPMT). In patients with reduced TPMT activity, 6-MP may accumulate

in the body and can be converted to 6-thioguanine metabolite. As 6-thioguanine is cytotoxic and may lead to bone marrow toxicity such as myelosuppression[9]. In this current investigation, 6-MP was the most potent cytotoxic agent against HepG2. Therefore, indeed this supports the usefulness of 6-MP towards other cancers, especially liver cancer[3].

Another strategy for reducing the ADRs of any drug is nano-delivery[15]. With an improved delivery system, better efficacy can be achieved with lower dosage making ADRs of another anti-cancer drug tamoxifen can be reduced to minimum[16]. The ADRs are commonly reported any all prescribed drugs in medicine[17] and are especially very common in anti-cancer drugs[9]. Liver is the most common site of ADRs as it is the main organ for drug metabolism[18]. Future studies of nano-encapsulated 6-MP must be done to further investigate the reduction of ADRs in patients. Collectively, results from the present study demonstrated the potential use of 6-MP in liver cancers but not breast cancers. The mechanism of action studies also need to be performed to understand the action thus reducing its ADRs.

CONCLUSION

This present study revealed that 6-MP and derivatives (6H2-MP and 2A9B6-MP) have the potential to be investigated as agents for other cancers. Interestingly, 6-MP appeared to be more effective in killing cancer cells when compared to analogs of 6-MP. As this is a preliminary study, further studies must be performed to elucidate their mechanism of action.

SIGNIFICANCE STATEMENT

The 6-Mercaptopurine (6-MP) is used to treat autoimmune diseases and inflammation such as rheumatoid arthritis, Crohn's disease and ulcerative colitis. It is also useful for acute leukemia. This study evaluates the potential use of 6-MP and its derivatives towards Liver (HepG2) and Breast (MCF-7) cancer cells. The HepG2 cells were more susceptible to all derivatives when compared to MCF-7 cells. The 6-MP appeared to be more effective in killing cancer cells when compared to analogues of 6-MP.

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REFERENCES

1. Lennard, L., 1992. The clinical pharmacology of 6-mercaptopurine. Eur. J. Clin. Pharmacol., 43: 329-339.
2. Barrera, P., A.M.T. Boerbooms, L.B.A. van de Putte and J.W.M. van der Meer, 1996. Effects of antirheumatic agents on cytokines. Semin. Arthritis Rheumatism, 25: 234-253.
3. Kaur, G., H. Behrsing, R.E. Parchment, M.D. Millin and B.A. Teicher, 2013. Analyses of the combination of 6-MP and dasatinib in cell culture. Int. J. Oncol., 43: 13-22.
4. Siegel, R.L., K.D. Miller and A. Jemal, 2016. Cancer statistics, 2016. CA: Cancer J. Clinicians, 66: 7-30.
5. Zhao, Y., K. Yuan, Q. Hu, D. Li and M. Liu*et al*., 2024. Preparation of GSH-responsive nanoparticles for combined chemo-photothermal therapy codelivering 6-MP and Ce6. J. Drug Delivery Sci. Technol., Vol. 93. 10.1016/j.jddst. 2024.105405
6. Nielsen, O.H., B. Vainer and J. Rask-Madsen, 2001. The treatment of inflammatory bowel disease with 6-mercaptopurine or azathioprine. Aliment. Pharmacol. Ther., 15: 1699-1708.
7. Kantarjian, H.M., T.M. Kadia, C.D. DiNardo, M.A. Welch and F. Ravandi, 2021. Acute myeloid leukemia: Treatment and research outlook for 2021 and the MD Anderson approach. Cancer, 127: 1186-1207.
8. Al Hadithy, A.F.Y., N.K.H. de Boer, L.J.J. Derijks, J.C. Escher, C.J.J. Mulder and J.R.B.J. Brouwers, 2005. Thiopurines in inflammatory bowel disease: Pharmacogenetics, therapeutic drug monitoring and clinical recommendations. Dig. Liver Dis., 37: 282-297.
9. Farrella, R.J., Y. Anga, P. Kileena, D.S. O'Briainb, D. Kellehera, P.W.N. Keelinga and D.G. Weira, 2000. Increased incidence of non-Hodgkin's lymphoma in inflammatory bowel disease patients on immunosuppressive therapy but overall risk is low. Gut, 47: 514-519.
10. Zuraini, A., A.H. Hasiah, H.M. Khairi, M.N. Somchit, S. Faizah and Z.A. Zakaria, 2009. Selective *in vitro* cytotoxic effects of piroxicam and mefenamic acid on several cancer cells lines. Res. J. Pharmacol., 3: 15-18.
11. Somchit, N., S.M. Hassim and S.H. Samsudin, 2002. Itraconazole-and fluconazole-induced toxicity in rat hepatocytes: A comparative *in vitro* study. Hum. Exp. Toxicol., 21: 43-48.
12. Hussein, M.Z., S.H. Al Ali, Z. Zainal and M. Nazrul Hakim, 2011. Development of antiproliferative nanohybrid compound with controlled release property using ellagic acid as the active agent. Int. J. Nanomed., 6: 1373-1383.
13. Cao, L., Y. Zhu, W. Wang, G. Wang, S. Zhang and H. Cheng, 2021. Emerging nano-based strategies against drug resistance in tumor chemotherapy. Front. Bioeng. Biotechnol., Vol. 9. 10.3389/fbioe.2021.798882.

14. Johnston, H.P., P. Hawley, S.E. White, I. Gibson and D.M. Tidd, 1985. The effects of 6-mercaptopurine nucleotide derivatives on the growth and survival of 6-mercaptopurine-sensitive and-resistant cell culture lines. Br. J. Cancer, 51: 505-514.
15. Al Ali, S.H.H., M. Al-Qubaisi, M.Z. Hussein, M. Ismail, Z. Zainal and M. Nazrul Hakim, 2012. Controlled-release formulation of antihistamine based on cetirizine zinc-layered hydroxide nanocomposites and its effect on histamine release from basophilic leukemia (RBL-2H3) cells. Int. J. Nanomed., 7: 3351-3363.
16. Al Ali, S.H.H., M. A l-Qubaisi, M.Z. Hussein, Z. Zainal and M. Nazrul Hakim, 2011. Preparation of hippurate-zinc layered hydroxide nanohybrid and its synergistic effect with tamoxifen on HepG2 cell lines. Int. J. Nanomed., 6: 3099-3111.
17. Somchit, N., F. Sanat, E.H. Gan, I.A.W. Shahrin and A. Zuraini, 2004. Liver injury induced by the non-steroidal anti-inflammatory drug mefenamic acid. Singapore Med. J. 45: 530-532.
18. Somchit, N., C.W. Wong, A. Zuraini, A.A. Bustamam and D.A. Israf *et al*., 2006. Involvement of phenobarbital and SKF 525A in the hepatotoxicity of antifungal drugs itraconazole and fluconazole in rats. Drug Chem. Toxicol., 29: 237-253.