



Original Article

IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS  
Copyright © 2017 by Iran University of Medical Sciences

Iranian J Pharmacol Ther. 2017 (December);15:1-7.



## Indomethacin hybrids as novel cytotoxic compounds

Farzaneh Yazdi<sup>1</sup>, Maryam Abbasi<sup>2</sup>, Hojjat Sadeghi-Aliabadi<sup>1\*</sup>

<sup>1</sup> Department of Medicinal Chemistry, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>2</sup> Department of Medicinal Chemistry, Faculty of Pharmacy, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

### Please cite this article as:

Yazdi F, Abbasi M, Sadeghi-Aliabadi H. Indomethacin hybrids as novel cytotoxic compounds. Iranian J Pharmacol Ther. 2017 (December);15:1-7.

### ABSTRACT

Cancer is still one of the most invasive health problems around the world although many researches have been done in this field. Different kinds of drugs are developed and used to improve cancer therapy. Some evidence has shown that nonsteroidal anti-inflammatory drugs (NSAIDs) have anticancer activity in addition to anti-inflammatory effects. To improve the safety profile of NSAIDs and enhance anticancer potency, different strategies such as hybridization are used in several studies. Indomethacin is a lead compound in NSAIDs classes' demonstrated inhibitory activity for some malignancies including breast and ovarian cancer. Two kinds of hybrids were proposed and synthesized using diamine linkers via amid bonds: indomethacin-indomethacin (hybrid A) and indomethacin-methotrexate (MTX; hybrid B). To confirm the structures of newly synthesized hybrids, melting points, IR, H NMR were applied. The cytotoxic effects of synthesized hybrids against Hela and MCF-7 cancer cells were evaluated by MTT assay. The results showed that both hybrids were more cytotoxic than indomethacin and MTX alone.

Conflicts of Interest: Declared None

Funding: None

### Keywords

NSAIDs,  
Indomethacin,  
Hybrid,  
MTT,  
Methotrexate

### Corresponding to:

Hojjat Sadeghi-Aliabadi,  
Department of Medicinal  
Chemistry, Faculty of Pharmacy,  
Isfahan University of Medical  
Sciences, 81746-73461 Isfahan,  
Iran

Email:

sadeghi@pharm.mui.ac.ir

Received: 12 June 2017

Revised: 17 July 2017,

Accepted: 18 Sep 2017

### INTRODUCTION

Cancer is still one of the most invasive health problems around the world although many studies have done and some breakthroughs in the cancer treatment were developed. Inflammation is a recognized sign of cancer that is contributed to the development of malignancies. Systemic inflammation and local immune response have important role in the development of tumors and survival of patients with cancer. Therefore, it provides a chance to target these inflammatory responses to improve cancer treatments [1,2].

Tumor response to use nonsteroidal anti-inflammatory drugs (NSAIDs) especially indomethacin is observed in different experimental, clinical, epidemiologic studies that show NSAIDs could be used as anticancer drugs [3]. These studies have been experimentally shown that NSAIDs have two mechanisms that help to repress malignant alteration and

tumor growth such as inhibiting angiogenesis and stimulating apoptosis. Endothelial cells are affected by both selective and nonselective NSAIDs so the angiogenesis will be inhibited [4].

On the other hand, NSAIDs apply their activity by inhibition of prostaglandin synthesis catalyzed by cyclooxygenase (COX) enzyme. The COX enzyme has two isoforms, including: COX<sub>1</sub> with a cytoprotective role and COX<sub>2</sub> with a role in the inflammation and pain [5,6].

Some NSAIDs including indomethacin, aspirin and ibuprofen not only used as effective painkillers but they used for other diseases such as arthritis and cardiovascular diseases and recently, for the prevention of colon and breast cancers [4,7,8].

Relationship between NSAIDs consumption and

decreasing the rate of breast cancer is revealed in some studies back to 1980 [9].

The mechanism of suppressing breast cancer by indomethacin had been developed by other researchers. Results were represented that protein kinases, protein phosphatases or signaling peptides are indirect signaling ways that may influence choline phospholipid metabolism and as a result indomethacin may have inhibitory effects on the gene expression of breast cancer cells [10].

Animal experiments provided evidence that colorectal cancer has been controlled by some NSAIDs such as sulindac and selective COX-2 inhibitors [8]. In addition ibuprofen and indomethacin showed antitumor effect on K562 cells [11]. Although these two NSAIDs were effective agents against chronic myeloid leukemia but their antitumor properties would warrant further studies on other clinical applications of these drugs.

Animal models of colon and breast cancers have shown a causal connection between COX<sub>2</sub> consumption and epithelial tumor genesis. COX<sub>2</sub> is an enzyme that over express at several epithelial cancers including breast cancer and controls prostaglandin synthesis [11-15].

To improve the safety profile of NSAIDs and enhance drugs potency and anticancer activity of them, different strategies including hybridization and targeted drug delivery like biovehicles are proposed. Drug hybridization strategies are valuable tools in the development of new drugs with either improved affinity for one bioreceptor or dual action on more than one. Hybridization methods have been applied, for example, to optimize therapy with available drugs and evaluate drug-resistance reversers (chemo sensitizers) as well as new chemotherapeutic targets [16].

Some hybrids are composed of two anti-tumor moieties with various mechanism of action. Up to now, many studies have been done according to hybrid drugs idea (e.g. conjugate of indomethacin and 5-fluorouracil) for the treatment of different diseases like cancer, malaria, inflammation and blood pressure [17-20]. Indomethacin-naphthalimid hybrid is another effective example of anticancer hybrids that are synthesized by Wu et al in 2010. Both indomethacin and naphthalimide are known as anticancer drugs and the biological assay of this hybrid showed higher cytotoxic activity than indomethacin against cancer cell lines such as HeLa, HL-60, HCT-8, and A375 [21].

The other purpose of hybridization could be synthesizing targeted drug. Conjugation of methotrexate (MTX) with an analog of luteinizing hormone (LH) is an example of targeted drug that is synthesized by Zhu et al in 2016. MTX and LH analog alone are effective chemotherapeutic drugs against some cancers; but the new synthesized conjugated compound inhibited the growth of prostate tumor more effectively [22].

The main aim of our investigation was synthesize and characterize a new hybrid drug consist of MTX and indomethacin. It could be more effective against cancer cell lines than indomethacin alone. Also in cancer treatment we

prospect that hybridization of indomethacin with MTX could be a targeted drug with dual action because of this fact that tumor tissues are usually inflamed and indomethacin has also antitumor properties.

## MATERIALS AND METHODS

All reagents were purchased from Sigma-Aldrich or Merck companies via local vendors. Chloroform was dried by distillation before using. H-NMR was obtained in chloroform using a Bruker AV-400 spectrometer (Germany); chemical shifts are given as ppm in  $\delta$  scale (in CDCl<sub>3</sub> and TMS as internal standard). IR spectra were recorded as KBr pellets by a Perkin-Elmer 2000 Fourier-transform IR (FTIR) instrument (Japan). Melting points were determined in capillaries using electro thermal 9200 melting point apparatus. Column chromatography was performed on 200–300 mesh silica gels.

Indomethacin and MTX free bases were kindly provided by Jalinous and Osveh pharmaceutical companies (Tehran, Iran), respectively.

### Chemistry

#### *Conjugation of indomethacin-1,12diaminododecan-indomethacin (hybrid A)*

Indomethacin free base (1.43g, 4mmol) was dissolved in dry chloroform (50ml). To this solution, dicyclohexylcarbodiimid (4.1g, 20mmol) was added with stirring and the reaction mixture was stirred under nitrogen for 2 hours. 1, 12-diaminododecan (0.8g, 4mmol) was dissolved in chloroform (10ml) and added to the reaction mixture of activated indomethacin by dropping funnel. The reaction mixture was then stirred over night at refluxed temperature. Reaction progress was monitored by TLC until completion. Then the chloroform was distilled off under vacuum and the conjugated product was separated by column chromatography. The white crystalline product was obtained by 60% yield [23].

#### *Conjugation of indomethacin-1,6diaminohexan*

Indomethacin powder as free base (1.43g, 4mmol) was activated with DCC (4.1g, 20mmol) as mentioned above. This solution was then added drop wise to a solution of 1,6 diaminohexane (1.39g, 12mmol) dissolved in chloroform (25ml). The reaction mixture was stirred for 3 hours at room temperature. After completion (monitored by TLC), the reaction was quenched with water. To remove the water-soluble by products, the reaction mixture was washed 3 times by distilled water (3×100 ml). The organic layer was separated and dried under reduced pressure. The obtained residue was purified using column chromatography to give indomethacin-1,6diaminohexan as a white product (mp: 110°C, 57% yield) [24].

#### *Conjugation of indomethacin-1,6diaminohexane-Methotrexate (hybrid B)*

MTX (0.54g, 1.2mmol) was dissolved in dry chloroform (10ml) and dicyclohexylcarbodiimid (1.23g, 6mmol) was added and then the reaction mixture was stirred under

nitrogen for 6 hours. After completion of the reaction (monitored by TLC), activated MTX was added drop wise to a solution of indomethacin-1,6 diaminoxane conjugate in chloroform (10ml) over 10 min and the reaction mixture was stirred under nitrogen for 72 hours. Then chloroform was distilled off under vacuum and the residue was dissolved in dichloromethane (20ml) and extracted with hexane (10 ml) until the title compound (indomethacin-1,6diaminoxane-MTX) was precipitated as yellow crystals (mp: 150°C, 50% yield) [25,26].

### Cell lines

MCF-7 (breast cancer) and Hela (cervix cancer) cells were obtained from national cell bank of Iran, pasteur institute, Tehran, Iran. Cell were maintained in RPMI 1640 containing 100 µg/ml streptomycin, 100 units/ml penicillin supplemented with 10% heat-inactivated fetal bovine serum (FBS).

### Biological evaluation of synthesized hybrids

Cytotoxic effects of novel synthesized compounds were tested against MCF-7 and Hela cells, using MTT assay as reported previously [26].

Briefly cells were plated in 96-well plate at a concentration of  $5 \times 10^4$  cells/ml and incubated for 24 h. Different concentrations of the novel synthesized hybrid were added to each wells so that the final concentrations of

compounds were 0.1, 1, 10, 50, 100 µM and incubated for further 48 h, in a humidified environment at 37°C with 5% CO<sub>2</sub>.

After incubation, each well was treated with 20 µL of MTT dye (5 mg/ml) and re-incubated for 3 h at the same condition. Then, the culture media replaced with 150 µl of DMSO to dissolve formazan crystals and the absorbance was recorded at 570 nm using an ELISA plate reader (BioTek, USA). 20 µl of DMSO (1%) and doxorubicin (7.7µM) were added to the wells and used as negative and positive controls, respectively.

Each experiment was repeated three times and cell survival was calculated using following formula:

$$\text{Cell survival (\%)} = \frac{\text{absorbance of treated well} - \text{absorbance of blank}}{\text{absorbance of control well} - \text{absorbance of blank}} \times 100$$

IC<sub>50</sub> values were determined by plotting the cell viability against compound concentrations, at 50% cell survival. All statistical analysis was performed with the Microsoft Office Excel 2010 and SPSS by one-way ANOVA method [26].

## RESULTS

To confirm the structures of newly synthesized hybrids, melting points, IR, H-NMR were used (Fig. 1).

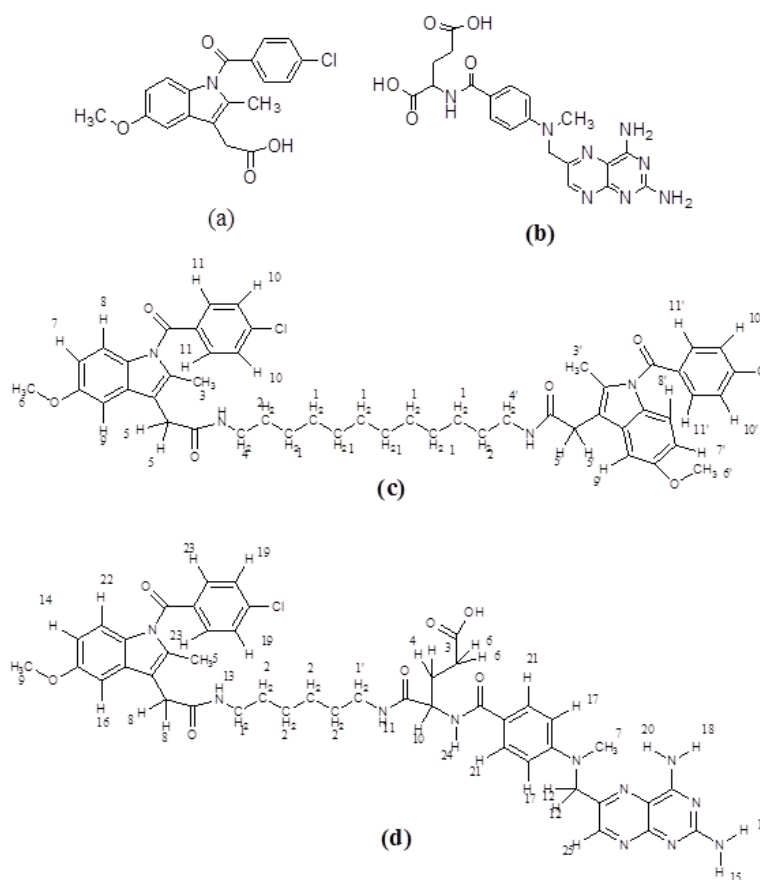


Figure 1. Structures of the tested compounds; Indomethacin (a); Methotrexate (b); Hybrid A (c); Hybrid B(d)

### Chemical results

#### Hybrid A

Melting point: 165°C; IR (KBr,  $\nu$ ): 3309(N-H), 2927.4(C-H), 1679(C=O), 1638.23(C=O)  $\text{cm}^{-1}$ ; H NMR ( $\text{CDCl}_3$ , 400 MHz),  $\delta\text{H}$ : (ppm): 1.09(16H, brs, 8×CH-1), 1.31(4H, q, 2×CH-2), 2.3(6H, s, 2×CH-3,3'), 3.1(4H, q, 2×CH-4,4'), 3.56(4H, s, 2×CH-5,5'), 3.7(6H, s, 2×CH-6,6'), 5.5(2H, t, 2×NH), 6.6(2H, d, 2×CH-7,7'), 6.7(2H, d, 2×CH-8,8'), 6.8(2H, s, 2×CH-9,9'), 7.4(4H, d, 4×CH-10,10'), 7.6(4H, d, 4×CH-11,11')

#### Hybrid B

Melting point: 150°C; IR (KBr,  $\nu$ ): 3319.86(N-H), 2930.31(C-H), 1649.8(C=O), 1613.16 (C=O)  $\text{cm}^{-1}$ ; H NMR ( $\text{CDCl}_3$ , 400 MHz),  $\delta\text{H}$ : (ppm): 1.2(4H, s, 2×CH-1,1'), 1.3(8H, s, 4×CH-2), 1.9(1H, d, CH-3), 2.05(1H, d, CH-4), 2.3(5H, s, 2×CH-5,6), 3.1(3H, s, CH-7), 3.56(3H, s, CH-8), 3.7(4H, s, CH-9,10), 4.48(1H, t, NH-11), 4.68(2H, s, CH-12), 5.79(1H, t, NH-13), 6.61(1H, d, CH-14), 6.68(2H, d,

NH-15), 6.79(1H, s, CH-16), 6.834(2H, d, CH-17), 7.26 (1H, d, CH-18), 7.4(2H, d, CH-19), 7.46(1H, d, CH-20), 7.58(3H, d, CH-21,22), 7.65(2H, d, CH-23), 7.9(1H, s, CH-24), 8.5(1H, s, CH-25).

### Biological results

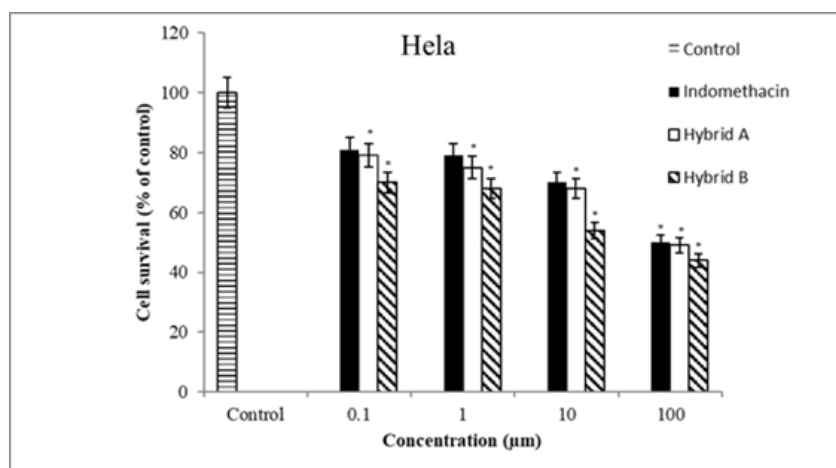
The cytotoxic effects of synthesized hybrids were examined by MTT assay. Indomethacin was used as control. In both cell lines, the cell viability reduced gradually in a concentration dependent manner. Results of inhibitory activity were represented by  $\text{IC}_{50}$ . As shown in Table 1, hybrid B was much more cytotoxic than indomethacin alone against both cell lines.

#### Effect of Indomethacin against HeLa cells

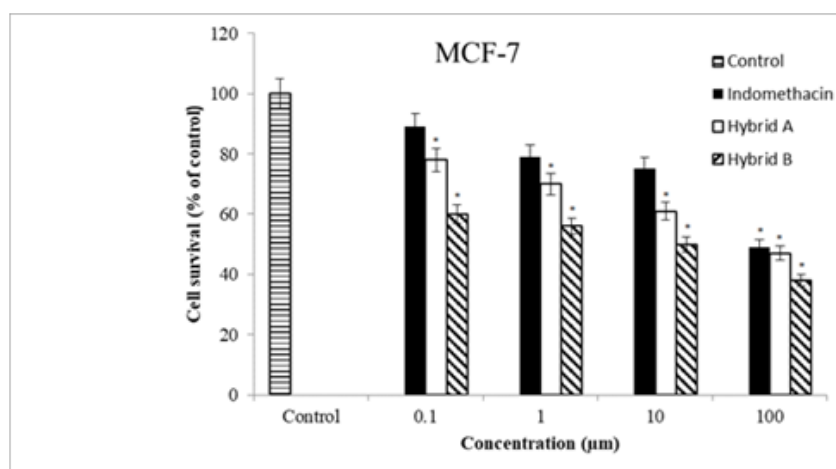
Logarithmic concentrations of 0.1, 1, 10 and 100  $\mu\text{m}$  of Indomethacin against HeLa cells reduced cell viability to 81, 79, 70 and 50%, respectively (Fig. 2).

#### Effect of Indomethacin against MCF-7 cells

MCF-7 cells were treated with indomethacin at concentrations of 0.1, 1, 10 and 100  $\mu\text{m}$  and cell survival



**Figure 2.** Percent cell survival of HeLa cells exposed to different concentrations (0.1, 1, 10, 100  $\mu\text{m}$ ) of indomethacin, hybrid A and hybrid B. The cytotoxicity was determined by MTT assay. Data are presented as percent of inhibition compared to negative control. Significant results were shown with an asterisk (\*) on histograms (Anova,  $p < 0.05$ ). The error bars represent Mean  $\pm$  SD.



**Figure 3.** Percent cell survival of MCF-7 cells exposed to different concentrations (0.1, 1, 10, 100  $\mu\text{m}$ ) of indomethacin, hybrid A, hybrid B. The cytotoxicity was determined by MTT assay. Data are presented as percent of inhibition compared to negative control. \* shows significant effects using one way ANOVA ( $p < 0.05$ ). The error bars represent Mean  $\pm$  SD.

was obtained 89%, 79%, 75% and 49%, respectively. The cell viability results were shown in a concentration dependent manner (Fig. 3).

#### *Effect of hybrid A against MCF-7 cells*

Cell survival reduced in a concentration dependent manner to 78, 70, 61 and 47% after 48h exposure to hybrid A at concentrations of 0.1, 1, 10 and 100  $\mu\text{m}$ , respectively. As seen in Figure 3, hybrid A at concentrations of 10 and 100  $\mu\text{m}$  were decreased cell viability significantly ( $p < 0.05$ ).

#### *Effect of hybrid A against Hela cells*

The effect of hybrid A against Hela cells (Fig. 2) was concentration dependent and at tested concentrations (0.1, 1, 10, 100  $\mu\text{m}$ ) cell viability was reduced significantly to 79, 75, 68 and 49%, respectively ( $p < 0.05$ ).

#### *Effect of hybrid B against MCF-7 cells*

A reduction in cell survival was seen in MCF-7 cell lines after treated with Hybrid B. Cell viability reduced to 60, 55, 50 and 38% at tested concentrations (0.1, 1, 10, 100  $\mu\text{m}$ ) respectively. This reduction in cell survival was statistically significant at concentrations of 10 and 100  $\mu\text{m}$  ( $p < 0.05$ ).

#### *Effect of hybrid B against Hela cells*

Logarithmic concentrations of hybrid B, (0.1, 1, 10 and 100  $\mu\text{m}$ ) were tested against HeLa cell line for 48 h. The results showed, as concentrations increased the percent of cell survival reduced to 70, 68, 54 and 44%, respectively which was statistically significant at concentrations in comparison with negative control ( $p < 0.05$ ) (Fig. 2).

## DISCUSSION

Cancer and inflammation are closely linked to each other, so that many anti-cancer agents are also used to treat inflammatory diseases, such as rheumatoid arthritis and vice versa. Moreover, chronic inflammation increases the risk for various cancers, indicating that eliminating inflammation may represent a valid strategy for cancer prevention and therapy [6, 8, 27-29].

Indomethacin as a lead of NSAIDs compounds has been studied as anticancer agent and the link between using indomethacin and increasing tumor inhibitory activity have been shown in several studies in the cancer fields [11, 20, 21]. To increase indomethacin anticancer effect, new hybrids of indomethacin have been synthesized. Biological evaluation of these synthesized hybrids showed better cytotoxic effects against breast and ovarian cancer cells. For example, combination of naphthalimide as a known anticancer drug when hybridized with indomethacin showed the synergic effects against some cancer cell lines such as Hela [21]. In the same regards Singh et al in 2009 synthesized new anticancer hybrid drug conjugated of indomethacin and 5-fluorouracil. In vitro evaluation of this

hybrid showed higher antiproliferative activity against some cell lines including breast, ovarian, colon and renal cancers [20]. According to the results obtained from indomethacin hybrids, we first proposed to synthesize a new hybrid (hybrid A) which is consist of two molecules of indomethacin conjugated via 1,12 diaminododecan as linker.

MTX is one of the effective chemotherapeutic drugs that is used clinically in different cancer therapy including breast, cervix, lung and prostate malignancies [22]. MTX has also potential to be used in clinic for the treatment of some chronic inflammatory diseases such as rheumatoid arthritis, dermatoses, ocular inflammation etc [30-32]. The anti-inflammatory effect of MTX is possibly a combination of different mechanisms including inhibition of pyrimidine and purine synthesis, reduction of antigen-dependent T-cell proliferation, suppression of transmethylation, reactions with accumulation of polyamines and promotion of adenosine release with adenosine-mediated suppression of inflammation. The latter mechanism of MTX has been supported by the in vitro, in vivo and clinical data. Generally, these mechanisms make MTX a good choice for hybridization drugs to treatment of inflammatory diseases and cancers [33-34]. Elmorsi and co-workers showed that NSAIDs such as indomethacin combined with MTX could increase MTX absorption and subsequently increase its cytotoxic effects [35]. Since most of cancers are contributed with inflammation [1, 14], in this study, we proposed to synthesize a new hybrid (hybrid B), that is a conjugation of indomethacin and MTX.

As shown in Table 1, hybrid A had better inhibitory activity than indomethacin against MCF-7 and HeLa cell lines which may relate to the increased molar ratio of indomethacin in the new hybrid. Consistent results from others showed that novel hybrid compounds including inorganic salts such as indomethacin-ZrO<sub>2</sub>-caprolacton [36], biotin-Pt-indomethacin hybrid [37] and indomethacin-macrolide hybrid [38] have been designed and synthesized according to this strategy. These novel hybrids increased the anti-inflammatory effects of indomethacin significantly.

Since MTX is widely used in the treatment of breast and ovarian cancers [22], this newly synthesized hybrid of indomethacin-linker-MTX was tested against MCF-7 and HeLa cell lines which were derived respectively from those organs.

Different linkers were used for drugs conjugation including: 1) Amide or peptide linkers such as amino acids or diamines which are protease sensitive and rapidly cleaved by lysosomal or extracellular enzymes while they have high plasma stability; 2) Esters linkers which are poorly stable in blood circulation [39]; 3) Phosphodiester linkers [40]; 4)

**Table 1.** IC<sub>50</sub> values of synthesized hybrids and indomethacin against Hela and MCF-7 cell lines

Compounds	IC <sub>50</sub> ( $\mu\text{m}$ )	
	HeLa	MCF-7
Indomethacin	100	94
Hybrid A	92	80
Hybrid B	16	10

Disulfide linkers [41]. In addition, it is reported that linkers with different structures have various biological activity. When the number of methylene group in the structure of diamine linkers reduced the cytotoxic activity would be increased [21]. Thus to improve the cytotoxic activity of proposed hybrid B, we decided to use 1, 6-diaminohexane as a cleavable linker instead of 1, 12-diaminododecane.

In both hybrids were formed drug-liker amid bond and molecular weight hybrids were more than 500, the same peptide compounds (42). Thus, we will expect that these compounds get inside the cells, like peptides.

As it has been shown in Table 1, hybrid B had higher activity than indomethacin and MTX in both cell lines in a concentration dependent manner; although it's inhibitory activity was more significant against MCF-7 than HeLa cell line.

### CONCLUSION

In conclusion, two novel hybrids of indomethacin with two different linkers were synthesized. The biological activity was measured against two different cell lines. The results showed that both hybrids were more cytotoxic than indomethacin and MTX alone. This study suggests novel hybrid drugs with improved bioactivity which could be lead compounds for further optimizations in future studies.

### CONFLICT OF INTEREST

The authors declare that this research does not have any conflict of interest with anyone or any institute.

### REFERENCES

- Greene ER, Huang S, Serhan CN, Panigrahy D. Regulation of inflammation in cancer by eicosanoids. *Prostag Oth Lipid M* 2011;96(1-4): 27-36.
- Taniguchi K, Karin M. NF- $\kappa$ B, inflammation, immunity and cancer: coming of age. *Nat Rev Immunol* 2018;18(5):309-324.
- Mathew B, Hobrath JV, Lu W, Li Y, Reynolds RC. Synthesis and preliminary assessment of the anticancer and Wnt/ $\beta$ -catenin inhibitory activity of small amide libraries of fenamates and profens. *Med Chem Res* 2017;26(11):3038-3045.
- Ackerstaff E, Gimi B, Artemov D, Bhujwala ZM. Anti-inflammatory agent indomethacin reduces invasion and alters metabolism in a human breast cancer cell line. *Neoplasia* 2007;9(3):222-235.
- Gallego GA, Prado SD, Fonseca PJ, Campelo RG, Espinosa JC, Aparicio LA. Cyclooxygenase-2 (COX-2): a molecular target in prostate cancer. *Clin Transl Oncol* 2007;9(11):694-702.
- Sinicrope FA, Gill S. Role of cyclooxygenase-2 in colorectal cancer. *Cancer Metastasis Rev* 2004;23(1-2):63-75.
- Wey SJ, Augustyniak ME, Cochran ED, Ellis JL, Fang X, Garvey DS, et al. Structure-based design, synthesis, and biological evaluation of indomethacin derivatives as cyclooxygenase-2 inhibiting nitric oxide donors. *J Med Chem* 2007;50(25):6367-6382.
- Liou JY, Ghelani D, Yeh S, Wu KK. Nonsteroidal anti-inflammatory drugs induce colorectal cancer cell apoptosis by suppressing 14-3-3 $\epsilon$ . *Cancer Res* 2007;67(7):3185-3191.
- Friedman G, Ury H. Initial Screening for Carcinogenicity of Commonly Used Drugs. *J Natl Cancer Inst.* 1980; 65(4): 723-733.
- Glunde K, Jie C, Bhujwala ZM. Mechanisms of indomethacin-induced alterations in the choline phospholipid metabolism of breast cancer cells. *Neoplasia* 2006;8(9):758-771.
- Bapiri A, Mohammadzadeh M. The Effects of Ibuprofen and Indomethacin on Human Chronic Myelocytic Leukemia K562 Cell Line: An In-Vitro Study. *J Isfahan Med Sch* 2015;33(328):389-399.
- Sarkar FH, Adsule S, Li Y, Padhye S. Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy. *Mini Rev Med Chem* 2007;7(6):599-608.
- Karmali RA. Prostaglandins and cancer. *Prostaglandins Med* 1980; 5(1):11-28.
- Bennett A, Gaffen JD, Melhuish PB, Stamford IF. Studies on the mechanism by which indomethacin increases the anticancer effect of methotrexate. *Br J Pharmacol* 1987;91(1):229-235.
- Rolland PH, Martin PM, Jacquemier J, Rolland AM, Toga M. Prostaglandin in human breast cancer: evidence suggesting that an elevated prostaglandin production is a marker of high metastatic potential for neoplastic cells. *J Natl Cancer Inst* 1980;64(5):1061-1070.
- Fortin S, Bérubé G. Advances in the development of hybrid anticancer drugs. *Expert Opin Drug Discov* 2013;8(8):1029-1047.
- Muregi FW, Ishih A. Next-generation antimalarial drugs: hybrid molecules as a new strategy in drug design. *Drug Dev Res* 2010;71(1):20-32.
- Charlier C, Michaux C. Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. *Eur J Med Chem* 2003;38(7-8): 645-659.
- García G, Serrano I, Sánchez-Alonso P, Rodríguez-Puyol M, Alajarín R, Griera M, et al. New losartan-hydrocaffeic acid hybrids as antihypertensive-antioxidant dual drugs: Ester, amide and amine linkers. *Eur J Med Chem.* 2012; 50: 90-101.
- Singh P, Kaur M, Verma P. Design, synthesis and anticancer activities of hybrids of indole and barbituric acids—Identification of highly promising leads. *Bioorg Med Chem Lett* 2009;19(11):3054-3058.
- Wu A, Xu Y, Qian X. Novel naphthalimide-indomethacin hybrids as potential antitumor agents: effects of linkers on hypoxic/oxic cytotoxicity and apoptosis-inducing activity. *Monatsh Chem* 2010;141(8): 893-899.
- Zhu S, Wang Q, Jiang J, Luo Y, Sun Z. A conjugate of methotrexate and an analog of luteinizing hormone releasing hormone shows increased efficacy against prostate cancer. *Sci Rep* 2016;6:33894.
- Kuka V, Bunchan R, Kong Thoo Lin P. Synthesis of Polyamines, Their Derivatives, Analogues and Conjugates. *Synthesis.* 2000;2000 (09):1189-1207.
- Liu C, Guan Y, Su Y, Zhao L, Meng F, Yao Y, et al. Surface charge switchable and core cross-linked polyurethane micelles as a reduction-triggered drug delivery system for cancer therapy. *RSC Adv* 2017; 7(18):11021-11029.
- Chen T, Xiao Y, Lu W, Liu S, Gan L, Yu J, et al. Facile preparation of core cross-linked nanomicelles based on graft copolymers with pH responsivity and reduction sensitivity for doxorubicin delivery. *Colloids Surf B Biointerfaces* 2018;161:606-613.
- Fattahi A, Asgarshamsi M, Hasanzadeh F, Varshosaz J, Rostami M, Mirian M, et al. Methotrexate-grafted-oligochitosan micelles as drug carriers: synthesis and biological evaluations. *J Mater Sci Mater Med* 2015;26(2):119.
- Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann Intern Med* 1994;121(4):241-246.
- Baandrup L, Faber MT, Christensen J, Jensen A, Andersen KK, Friis S, et al. Nonsteroidal anti-inflammatory drugs and risk of ovarian cancer: systematic review and meta-analysis of observational studies. *Acta Obstet Gynecol Scand* 2013;92(3):245-255.
- Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic and clinical issues. *J Natl Cancer Inst* 2002;94(4):252-266.
- Montesinos MC, Yap JS, Desai A, Posadas I, McCrary CT, Cronstein BN. Reversal of the anti-inflammatory effects of methotrexate by the nonselective adenosine receptor antagonist's theophylline and caffeine: evidence that the anti-inflammatory effects of methotrexate are mediated via multiple adenosine receptors in rat adjuvant arthritis. *Arthritis Rheumatol* 2000;43(3):656-663.

31. Jolivet J, Cowan KH, Curt GA, Clendeninn NJ, Chabner BA. The pharmacology and clinical use of methotrexate. *N Engl J Med* 1983; 309(18):1094-10104.
32. Smith JR, Levinson RD, Holland GN, Jabs DA, Robinson MR, Whitcup SM, et al. Differential efficacy of tumor necrosis factor inhibition in the management of inflammatory eye disease and associated rheumatic disease. *Arthritis Care Res* 2001;45(3):252-257.
33. Tian H, Cronstein BN. Understanding the mechanisms of action of methotrexate. *Bull NYU Hosp Jt Dis* 2007;65(3):168-173.
34. Chan ES, Cronstein BN. Mechanisms of action of methotrexate. *Bull Hosp Jt Dis* 2013;71(Suppl 1):S5-8.
35. Elmorsi YM, El-Haggag SM, Ibrahim OM, Mabrouk MM. Effect of ketoprofen and indomethacin on methotrexate pharmacokinetics in mice plasma and tumor tissues. *Eur J Drug Metab Pharmacokinet* 2013;38(1):27-32.
36. Catauro M, Bollino F, Papale F, Pacifico S. Modulation of indomethacin release from ZrO<sub>2</sub>/PCL hybrid multilayers synthesized via sol-gel dip coating. *J Drug Deliv Sci Technol* 2015;26:10-6.
37. Hu W, Fang L, Hua W, Gou S. Biotin-Pt (IV)-indomethacin hybrid: A targeting anticancer prodrug providing enhanced cancer cellular uptake and reversing cisplatin resistance. *J Inorg Biochem* 2017;175:47-57.
38. Cipic Paljetak H, Tomaskovic L, Matijasic M, Bukvic M, Fajdetic A, Verbanac D, et al. Macrolide Hybrid Compounds: Drug Discovery Opportunities in Anti-Infective and Anti-inflammatory Area. *Curr Top Med Chem* 2017;17(8):919-940.
39. Li J, Wang F, Sun D, Wang R. A review of the ligands and related targeting strategies for active targeting of paclitaxel to tumours. *J Drug Target* 2016;24(7):590-602.
40. Wang X, Huang Y, Liu Y, Chen Y, Jin H, Zheng Y, et al. Synthesis and biological evaluation of peptide-siRNA conjugates with phosphodiester unit as linker. *Sci China Chem* 2013;56(11):1542-1549.
41. Ducry L, Stump B. Antibody- drug conjugates: linking cytotoxic payloads to monoclonal antibodies. *Bioconjug Chem* 2009;21(1):5-13.
42. Wu K., Liu J., Johnson R. N., Yang J. and Kopecek J. Drug-Free Macromolecular Therapeutics: Induction of Apoptosis by Coiled-Coil-Mediated Cross-Linking of Antigens on the Cell Surface. *Angewan Chem* 2010;122:1493-1407.