Available online on www.ijddt.com
International Journal of Drug Delivery Technology 2019; 9(2); 226-235
doi: 10.25258/ijddt.9.2.18

ISSN: 0975 4415

Research Article

Synthesis of Novel Ibuprofen-Tranexamic Acid Codrug: Estimation of The Clinical Activity Against HCT116 Colorectal Carcinoma Cell Line and The Determination of Toxicity Profile Against MDCK Normal Kidney Cell Line

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Received: 7th Mar, 19; Revised: 30th Apr, 19, Accepted: 18th May, 19; Available Online: 25th Jun, 2019

ABSTRACT

Objective: Both ibuprofen and tranexamic acid were tried to treat colorectal carcinoma, however, combined drugs were not. Accordingly, with the aid of SciFinder®, online absence of the mutual prodrug (codrug) was affirmed. This persuade the authors to conduct this research. Methods: The ibuprofen-tranexamic acid codrug was synthesized and characterized with 83% yield. The purified white powder codrug was tested against HCT116 colorectal cancerous cell line and MDCK normal non-cancerous cell line. Results: The newly synthesized and characterized ibuprofen-tranexamic acid codrug has significant parameters. One of which it absolutely obeyes the Lipinski rule of five. Moreover, like the Lipinski rule of five, the number of rotatable bonds (7 rotatable bonds) and the topological polar surface area tPSA (66.4 A^2) shows a very favourable oral absorption drug candidate. The IC50 of the mutual prodrug against HCT116 colorectal cells was 5.33 mg/ml, while the IC50 for the MDCK normal kidney cell line was 6.4 g/ml. Conclusion: The authors conclude that the newly synthesized ibuprofen-tranexamic acid codrug has fair anticancer activity against HCT116 colorectal cancer cell line with tolerated toxicity profile acquired with the MDCK normal kidney cell line.

Keywords: Ibuprofen, tranexamic acid, codrug, mutual prodrug, HCT116 colorectal cell line, MDCK kidney cell line, NMR, DSC, anticancer activity, toxicity profile.

INTRODUCTION

Ibuprofen is classified as one of the most well-known non-steroidal anti-inflammatory drugs (NSAIDs)[1]. Furthermore, ibuprofen was concluded to reduce the high risk of colorectal cancer[2]. In colorectal carcinoma, the incidence of bleeding is considered very common and its one of the diagnostic signs of this malignant disease[3]. This in turn indicates the prescription of tranexamic acid to counterfeit the bleeding tendency of this patient group[4]. In one preliminary evidence, scientists suggest the utilization of tranexamic acid for cancer treatment as it is correlated with the pathogenic attributes of human cancer via the capability of neoplastic cell growth mediation[5]. Nevertheless, the oral bioavailability of tranexamic acid is approximately 34%[6].

All the above encouraged the authors to perform this work, in which, the authors aim to synthesize an ibuprofen-tranexamic acid mutual prodrug (codrug) and to investigate its biological and toxicological profiles with the employment the appropriate cell lines.

MATERIALS AND METHODS

Chemicals and reagents

Tranexamic acid was purchased from Haihang Industry (China).

Ibuprofen acid chloride was prepared in our laboratory from ibuprofen, which was gifted from Samarra Drug Industry (SDI), Iraq.

THF and Potassium carbonate were purchased from SCR (China).

Dichloromethane HPLC-grade and n-Hexane were purchased from GCC (UK).

Sodium hydrogen carbonate was purchased from Himedia (India)

NYLON Syringe Filters Polypropylene housing diameter: 25mm pore size: 0.22µm non-sterilized purchased from Giorgio11185's store Jiangsu, Mainland, China.

Methanol (HPLC grade), was purchased from Biosolve Chimie SARL (France).

Dimethyl sulphoxide from CDH (India).

Cellulose acetate membrane filter pore size 0.2 µm diameter 25 mm was purchased from chm (Spain).

MTT from Roth (Germany).

Celltreat® 96 Well Cell Culture Plates from CELLTREAT Scientific Products (USA).

Instrumentation

4-digit balance Sartorius Lab (Germany).

DSC (Differential Scanning Calorimeter) Thermal Analyzer Shimadzu (Japan).

Hotplate stirrer LabTech (Korea).

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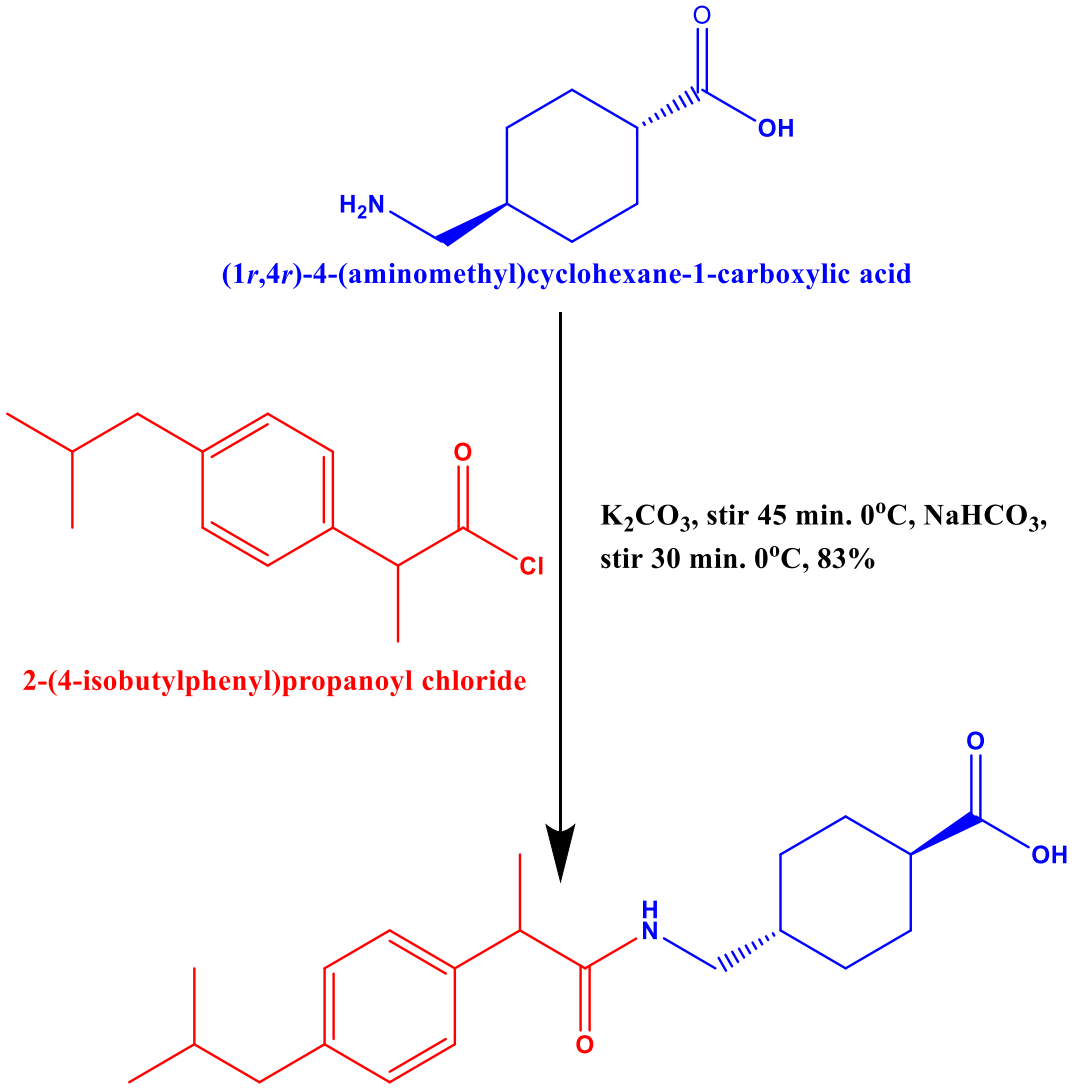

Scheme 1: Chemical synthesis and reaction conditions for the ibuprofen-tranexamic acid codrug (mutual prodrug).

1-stage vacuum pump 5 Pa ¼ HP Wenling Aitcool (China).
Microplate reader 800 TS BioTek (USA).
Inverted Microscope Zeiss (Germany).
Incubator UN 55 Memmert (Germany).
Clean Bench LabTech (Korea).

Methods

Synthesis of (1r,4r)-4-((2-(4-isobutylphenyl)propanamido)methyl)cyclohexane-1-carboxylic acid[7]

To a stirred solution of tranexamic acid (566 mg, 3.6 mmol) and potassium carbonate (2.48 g, 18 mmol) in THF (24 mL) at 0°C was added 2-(4-isobutylphenyl)propanoyl chloride (990 mg, 4.4 mmol) and the reaction mixture was stirred at 0°C for 45 min. The reaction mixture was added gradually to a cold, stirring solution of 10% $NaHCO_3$ (24 mL) and stirred at (0°C) for 30 min. A white solid precipitated out which was isolated by filtration. The solid was washed with cold water, hexane and dissolved in a mixture of methanol/dichloromethane (50:50, 20 mL) and concentrated under reduced pressure. The residue obtained was suspended in cold water (40 mL), Et3N was added to it and it was extracted with ethyl acetate (2 x 40 mL). The combined ethyl acetate extract was washed with water (20 mL), concentrated under reduced pressure in a desiccator to get the target product (1.034 g, 83%).

Cell lines preparation

Both the HCT116 and MDCK cell lines were grown in 1640 media (RPMI-1640, Gibco-BRL), with 10% heat-inactivated fetal bovine serum (FBS) (Gibco). Thereafter, the specified cell lines were cultured in Celltreat® 96-well plates and incubated to grew at 37°C. The 24 hours was the optimized time of cell culture.

Preparation of working concentrations from the synthesized codrug for the HCT116 cell-line

A 50 mg of the synthesized codrug was dissolved in 10 ml DMSO to get a stock solution with a concentration of 5mg/ml. The concentration was optimized for a 50µg/ml from witch a serial dilution was performed to obtain the

following concentrations (50, 25, 12.5, 6.25, 3.125, and 1.5625) µg/ml.

Application of the synthesized codrug on the HCT116 cell-lines

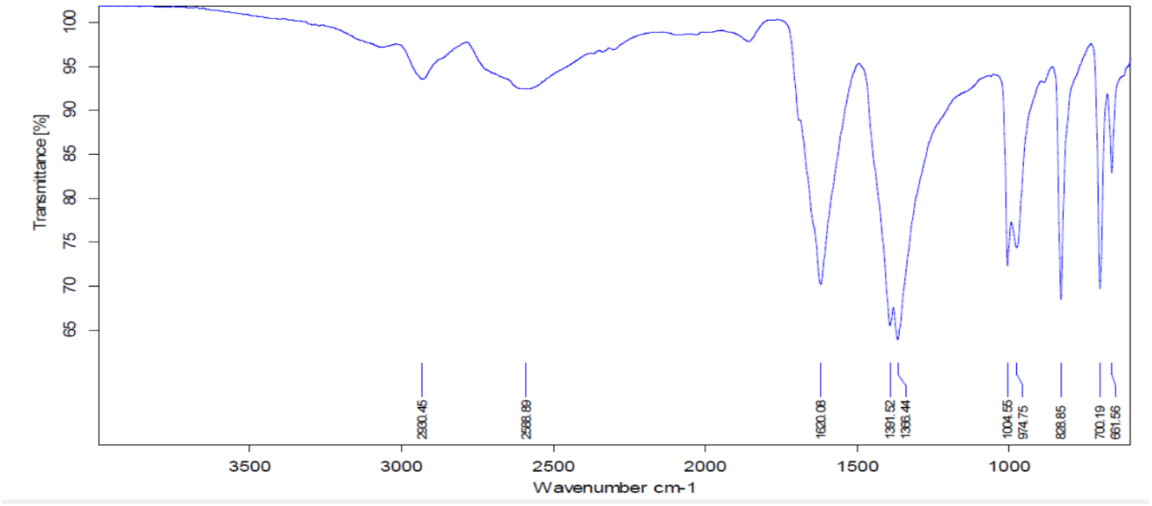

Figure 1: Reveals the IR peaks of the synthesized Ibuprofen-Tranexamic acid codrug.

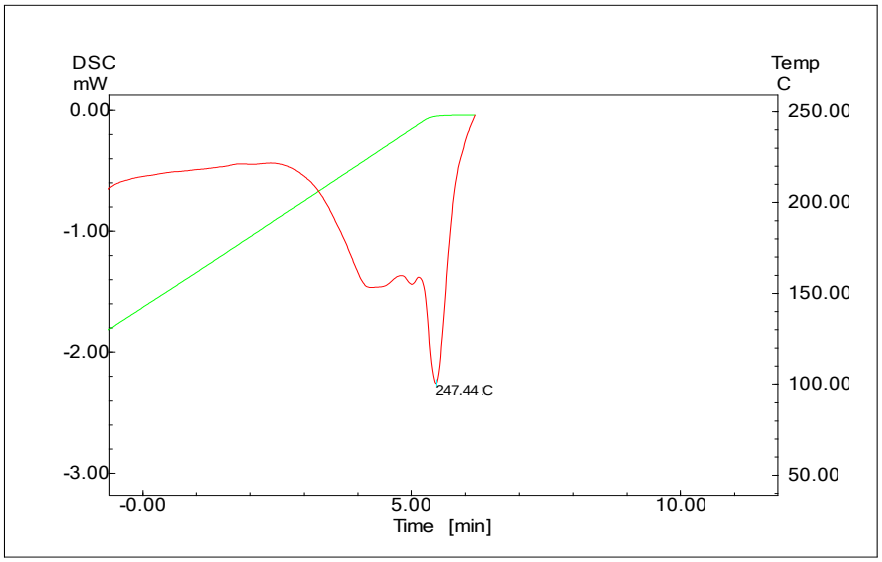

Figure 2: The melting point of the ibuprofen-tranexamic acid codrug acquired with the DSC Thermal Analyzer.

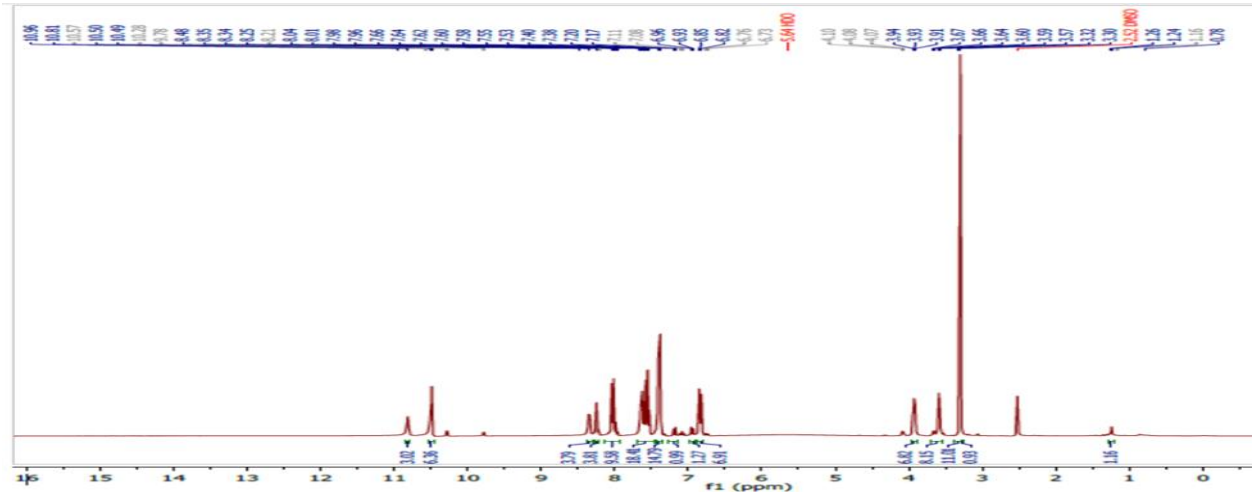

Figure 3: The ^{1}H NMR spectrum of the synthesized ibuprofen-tranexamic acid codrug shows the chemical shifts

(ppm), multiplicity, and the integrations of each peak. The DMSO was used as solvent.

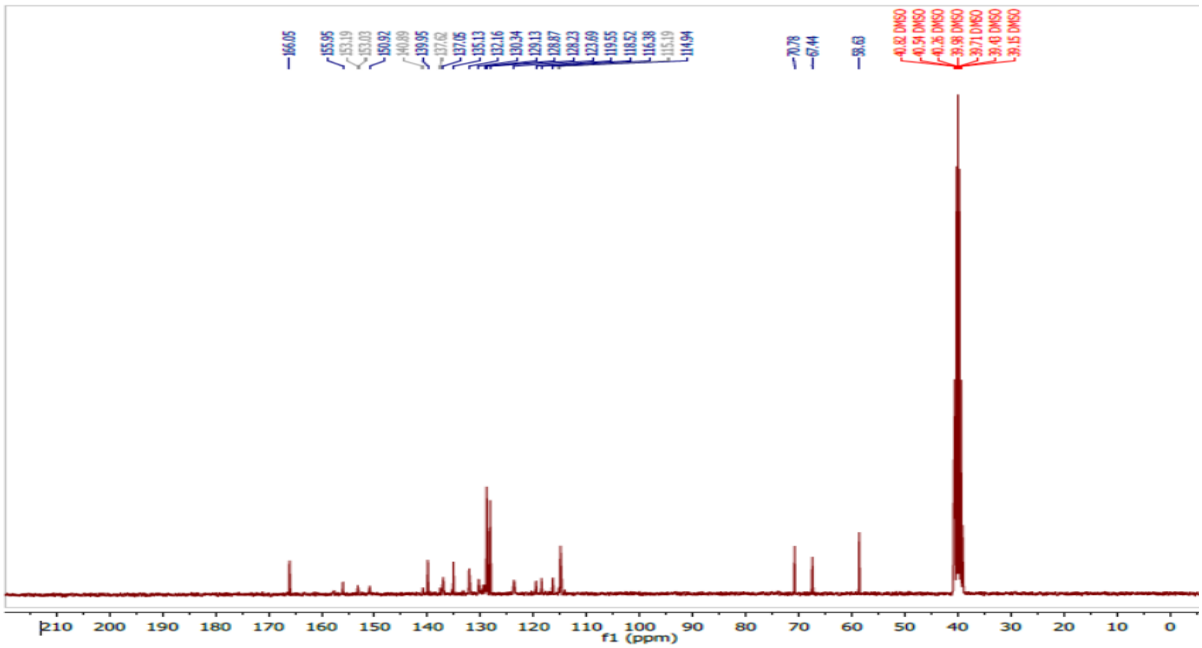

Figure 4: The ^{13}C NMR spectrum of the synthesized ibuprofen-tranexamic acid codrug reveals the chemical shifts (ppm) of each peak. The DMSO was used as solvent.

Table 1: Some of the physicochemical properties of the synthesized ibuprofen-tranexamic acid mutual prodrug (codrug).

No.	Parameter	Value
1	IUPAC name	(1r,4r)-4-((2-(4-isobutylphenyl)propanamido)methyl)cyclohexane-1-carboxylic acid
2	Formula	$C_{21}H_{31}NO_3$
3	Molecular mass	345.48
4	Yield %	83%
5	H Bond Donor Count	2
6	H Bond Acceptor Count	4
7	Rotatable bond count	7
8	tPSA*	66.4
9	Melting point**	247.44
10	Physical appearance	White crystalline powder
11	Log P	4.48

*tPSA = topological polar surface area.
** melting point was obtained with DSC.

The different concentrations solutions were poured in a 200-µl portions for the 96-weel plate. This was performed in triplicates and was incubated for 24hrs. Thereafter, the visualization of these plates were performed with the employment of the inverted microscope where the screenshots were captured for each well. The replacement of media with 10% MTT medium were performed. Then, the plate was incubated for another 3 hours. After that, the medium was sucked and the wells were washed with phosphate buffer saline. Lastly, DMSO was added into each well (a 200-µl aliquots) and left for 0.5 hour. Then, the plate was read at 630 nm with the aid of plate reader.

RESULTS

The IR spectrum:

The IR spectrum of the synthesized codrug is shown in figure (1):

Melting point measurement

The melting point was acquired with the employment of the DSC (Differential Scanning Calorimeter) Thermal Analyzer. The labelled melting point was recorded as shown in figure (2) below.

^{1}H NMR spectrum

The ^{1}H NMR spectrum of the synthesized ibuprofen-tranexamic acid codrug is revealed in figure (3).

^{13}C NMR spectrum

The ^{13}C NMR spectrum of the synthesized ibuprofen-tranexamic acid codrug is revealed in figure (4).

Physicochemical properties

Some of the physicochemical properties of the synthesized codrug are tabulated in table (1) below:

HCT116 colorectal cancer cell line

The effect of the application of the codrug on the HCT116 cell line is shown in figure (5) below.

MDCK normal kidney cell line

The effect of the application of the codrug on the MDCK cell line is shown in figure (5).

DISCUSSION

The ibuprofen-tranexamic acid codrug was successfully characterized utilizing the IR, ^{1}H NMR, ^{13}C NMR, and the DSC instruments. Infrared spectroscopy is considered as rapid, reliable, and non-destructive tool that is used

commonly in drug characterization[8]. One of the very characteristic bands utilized frequently in the IR spectroscopy is the carbonyl functional group[9]. In this work, the IR spectroscopy is basically used to identify and confirm the chemical structure of the synthesized ibuprofen-tranexamic acid codrug relying on the appearance of the amide carbonyl band, which is very characteristic of the produced compound.

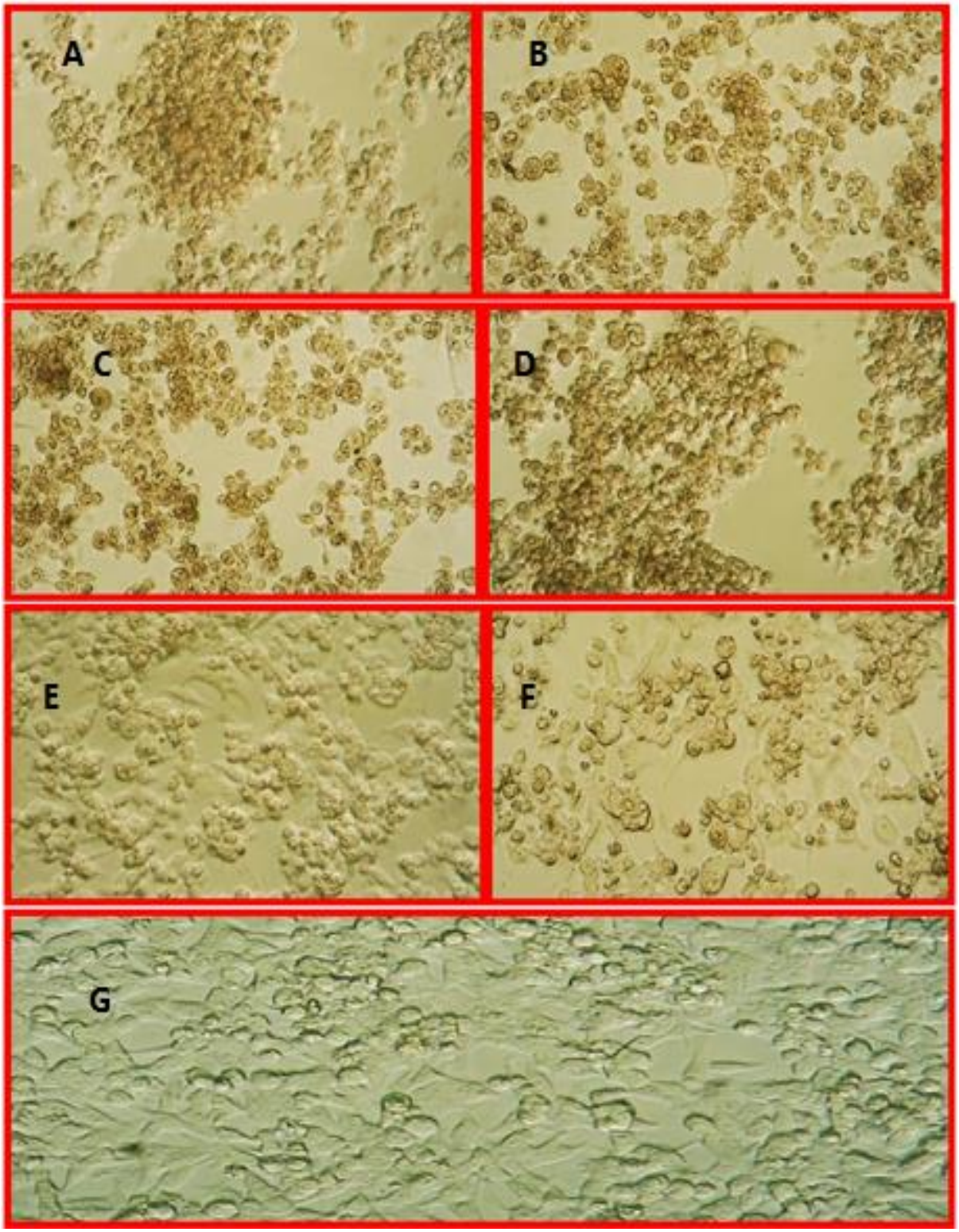

Figure 5: The effect of the application of the ibuprofen-tranexamic acid codrug on the HCT116 colorectal carcinoma cell line. The applied concentrations were (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.0) µg/ml assigned as A, B, C, D, E, F, and G, respectively.

Another issue is that the observed melting point, which was acquired with the DSC, which is considered as a cornerstone in the acquisition of the melting points. DSC is a very sensitive instrument from Shimadzu that provided the melting point up to two decimal places, which is obviously extremely hard for the classical capillary apparatus to perform. This is due to the ability of the DSC to record enthalpies and transition temperatures with appreciated sensitivity[10].

Moreover, the Lipinski rule of five was carefully obeyed. This is crystal clear when talking about the hydrogen bond donors, hydrogen bond acceptors, the estimated log P (Octanol-water partition coefficient), and the molecular

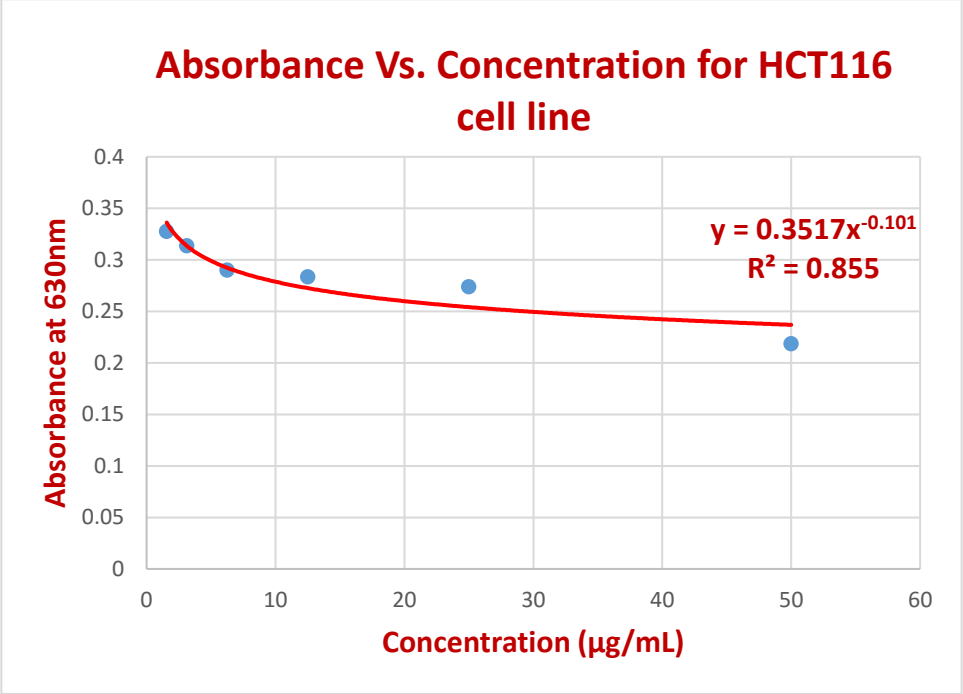

Figure 6: The absorbance of MTT versus concentration of the ibuprofen-tranexamic acid codrug after 24-hours' incubation of HCT116 colorectal cancer cell line.

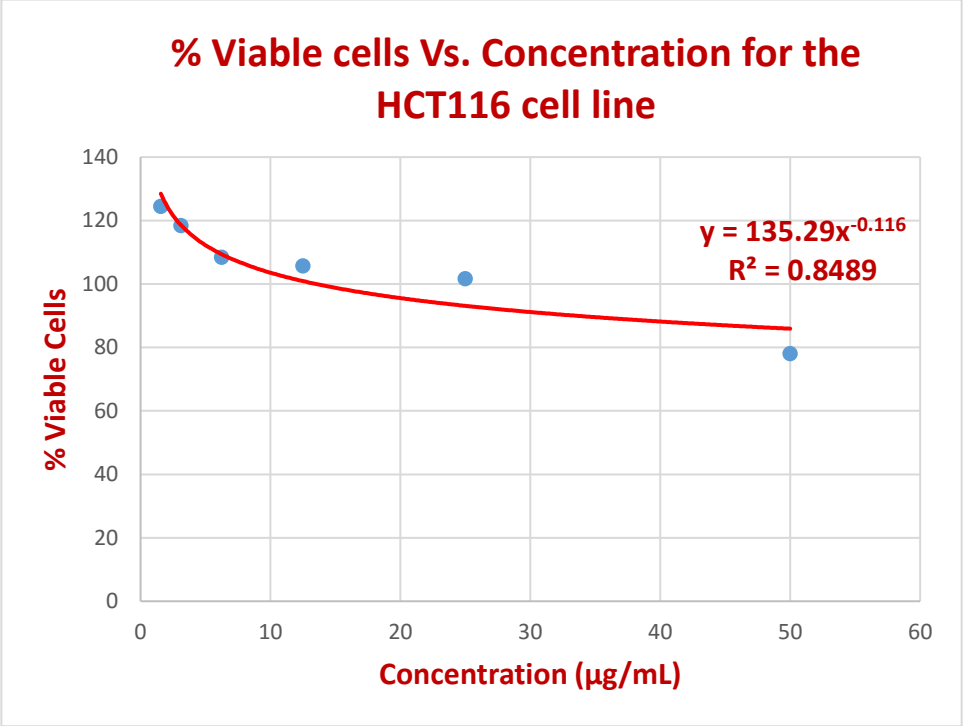

Figure 7: The percent viable cells versus concentration of the ibuprofen-tranexamic acid codrug after 24-hours' incubation of HCT116 colorectal cancer cell line. The IC50 was calculated to be 5.33 mg/ml.

mass. In 1997, Christopher A. Lipinski and his colleagues examined more than 2000 drug molecules and concluded

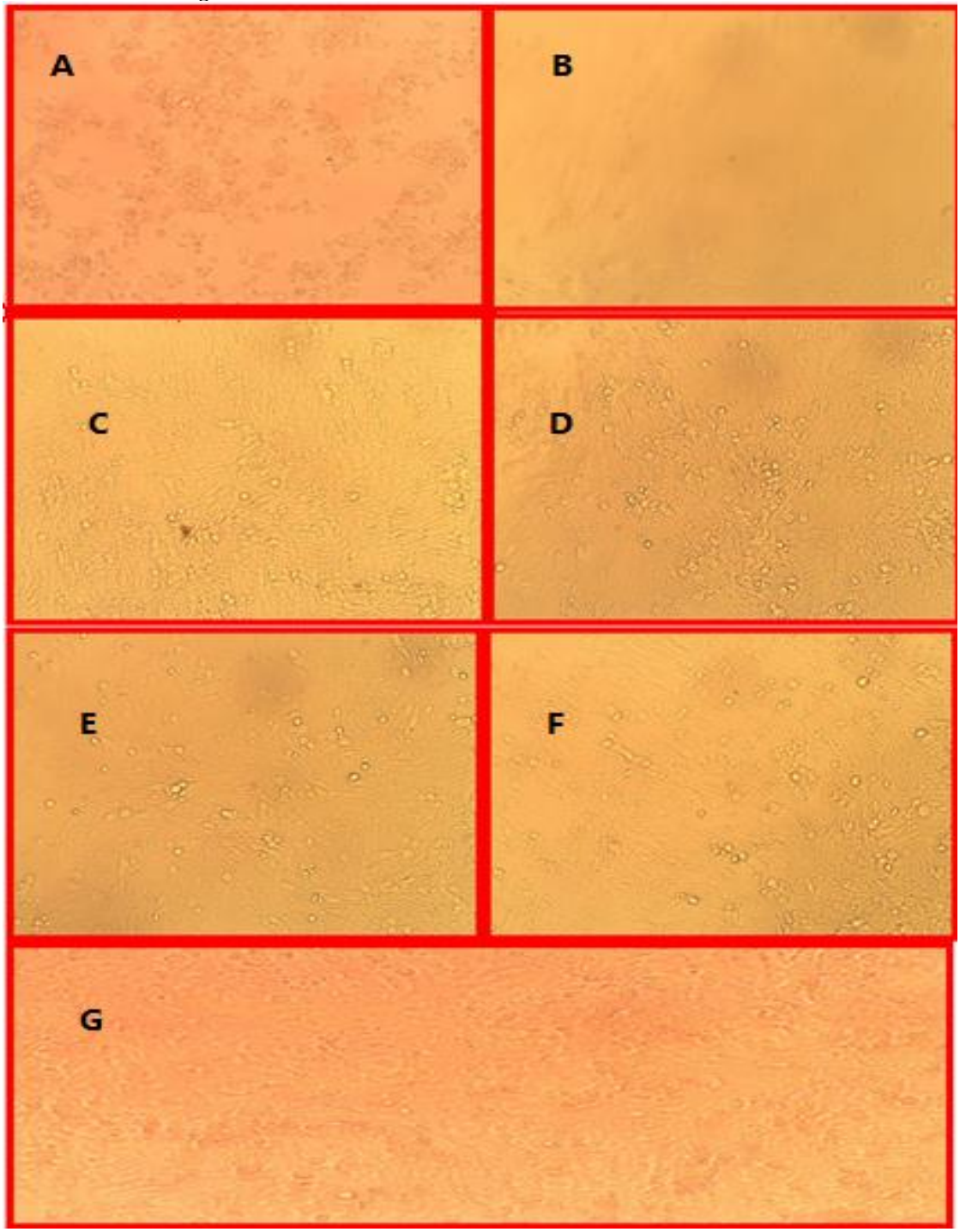

Figure 8: The effect of the application of the ibuprofen-tranexamic acid codrug on the MDCK normal kidney cell line. The applied concentrations were (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.0) µg/ml assigned as A, B, C, D, E, F, and G, respectively.

that a compound is more likely to be orally active if it has no more than one breach of the following rules[11-15]:
No more than five hydrogen bond donors.
No more than ten hydrogen bond acceptors.
No greater than five hindered daltons of the molecular mass.
No greater than five units for the log P.

On the other hand, the oral absorption is also correlated with the rotatable bond count. No more than 10 rotatable bonds alongside with no more than 140 A^2 of the topological polar surface area are crucial for the drug candidate to be orally bioavailable[16]. This feature is achieved with 7 and 66.4 A^2 for total rotatable bonds and for the topological polar surface area, respectively.
In chemistry, the reaction is efficiently measured with the employment of the per cent yield. The percent yield is

defined as the amount of the gained product divided by the calculated amount within a chemical reaction. The

therapeutic index of both combined drugs with good patient tolerance may encourage the use of this codrug.

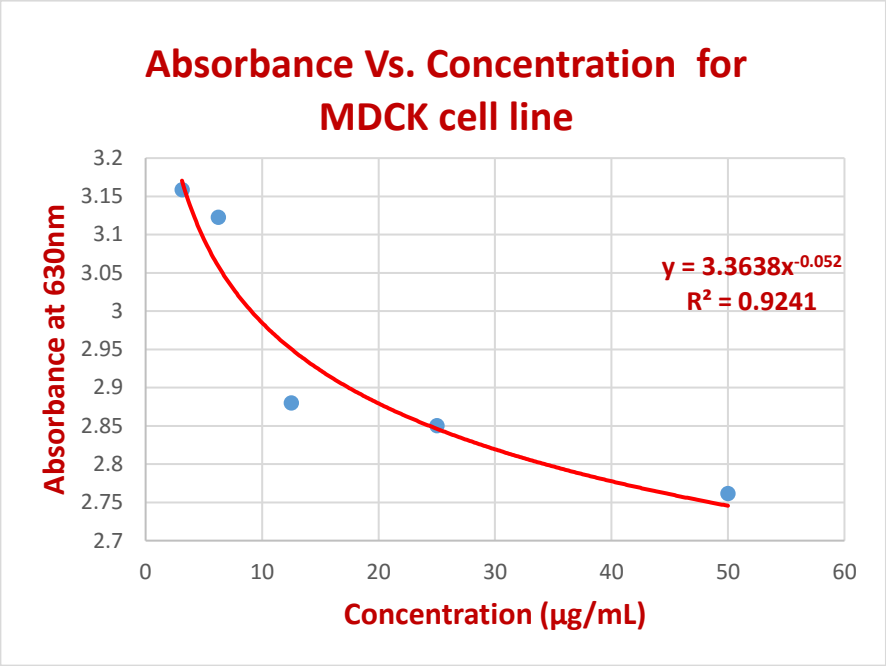

Figure 9: The absorbance of MTT versus concentration of the ibuprofen-tranexamic acid codrug after 24-hours' incubation of MDCK normal kidney cell line.

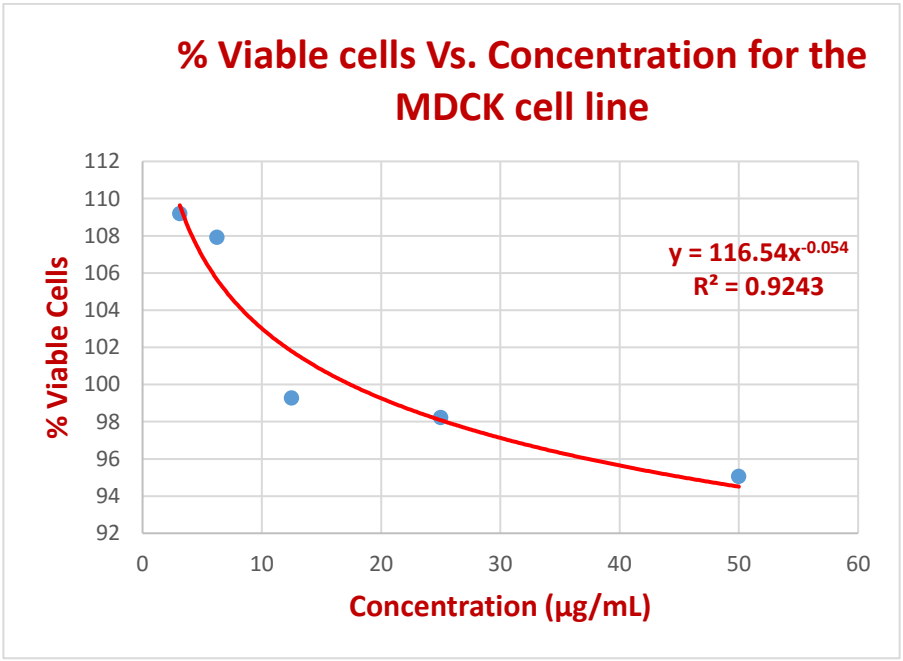

Figure 10: The percent viable cells versus concentration of the ibuprofen-tranexamic acid codrug after 24-hours' incubation of MDCK normal kidney cell line. The IC50 was calculated to be 6.4 g/ml.

percent yield that is greater than 80% is regarded as a very good yield[17,18]. Accordingly, the per cent yield of the ibuprofen-tranexamic acid codrug is considered very good.

Fairly anticancer activity of the newly synthesized codrug against HCT116 colorectal cancer cell line was obtained. The calculated IC50 was 5.33 mg/ml. Even though this concentration is considered high, however, the high

Concomitantly, to judge whether to consider the preclinical studies of this codrug, the toxicity profile is considered as a cutting edge. This is performed by considering the selectivity of the newly synthesized codrug against the cancer cells in respect to the normal (non-cancerous) cells[19-21]. A significant number of researchers employed the MDCK cell line to evaluate the toxicity profile of the candidate drugs[22-30]. With an IC50

of 6.4 g/ml, the ibuprofen-tranexamic acid codrug may be considered as extremely safe in this aspect.

CONCLUSION

In this study, the authors conclude that the newly synthesized ibuprofen-tranexamic acid codrug has fair antineoplastic activity against HCT116 colorectal cancer cell line with excellent safety profile acquired with the MDCK normal kidney cell line. The current finding encourages the utilization of further animal studies to affirm the present results and to investigate other important ADMET parameters.

ACKNOWLEDGEMENT

The authors would like to express their appreciation to the College of Pharmacy-University of Kerbala for offering their laboratories and all other facilities. The authors also would like to acknowledge the Samarra Drug Industry (SDI), in Iraq, for their collaboration and offering of standard.

CONFLICT OF INTEREST

The authors declare that, in this study, no conflict of interest is encountered.

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