Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(10):251-259
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis of some anticancer agent conjugated to aminoacids through amide bond with expected biological activity

Abbas Abdulridha* and Kawkab Y. Saour

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq

---

ABSTRACT

purines are an important group of organic compounds where several compounds containing a purine residue are known to possess useful biological activity and used as antibacterial, antifungal and antitumor agents. These pharmacological properties of purines aroused our interest in synthesizing several new 6-mercaptopurine(6-MP) derivatives linked to amino acid via peptide bond with expected biological activity. 6-MP was S-alkylated by the methyl bromoacetate and then hydrolyzed with an alkaline sodium hydroxide solution to librate the free carboxylic group side chain. Also the alkylated 6-MP reacted with hydrazine hydrate to get hydrazide of 6-MP. Conventional solution method for peptide synthesis used as a coupling method between the free carboxyl and amine groups. The proposed analogues were successfully synthesized and their structural formulas were consistent with the proposed structures as they were characterized and proved by thin layer chromatography (TLC), melting point, infrared spectroscopy (IR) and elemental microanalysis. All tested analogues showed an improved antistaphylococcus activity in comparison with ofloxacin. Also the analogues showed cytotoxic activity on the HeLa cell line (cervical cancer cells) with inhibitory concentration percent of (IC %) range (16.79 % -39.28 %). It can be concluded from the results that the synthesized compounds are promising as new anticancer candidates in future.

Keywords: 6-mercaptopurine, amino acid, antibacterial and cytotoxic activity.

INTRODUCTION

Purine is the generic name of imidazo[4,5-*d*] pyrimidine . It is a bicyclic heterocycle consisting of a 6-membered pyrimidine ring and 5-membered imidazole ring. The fused ring system fulfills Hückels rule and is therefore aromatic giving it a flat geometry[1].

The first synthesis of purine was performed by Emil Fischer in the late 1800s and was part of the early endeavors in organic synthesis[2]. Purine is an aromatic compound with -deficient ring system; therefore, all its carbon atoms are susceptible towards nucleophilic attack and are deactivated towards electrophilic attack [3].

Purines are an important group of nitrogen-containing compounds that are present in all forms of plant and animal life and play a vital role in biological processes. The quantity of naturally occurring purines produced on earth is huge, fifty percent (50%) of the bases in nucleic acids, adenine and guanine which are purines [2]. They serve as the source of cellular energy in ATP and together with pyrimidine, are the building blocks of DNA and RNA, two of the four deoxyribonucleotides and two of the four ribonucleotide which are purines (the respective building blocks of DNA and RNA) [4,5]. Purines also participate in the structure of the co-enzymes (e.g. NAD+, NADP+ and FAD) and they are involved in membrane signal transduction, translation and protein synthesis (GTP, cAMP, cGMP, RNA) [5].

This class of fused [6:5] nitrogen containing heterocycles are the core structure of a wide range of natural compounds with pharmacological properties, including alkaloids (e.g. caffeine and theobromine), cytokines, and natural antibiotics, thus purines being considered privileged scaffolds for drug development [6].

Due to their similarity with cell components, purine analogues may act as substrates or inhibitors of enzymes of purine metabolism or as agonists/antagonists/inhibitors of adenosine receptors and protein kinases. This is particularly relevant in the development of antiparasitic chemotherapies, since most parasites rely heavily on purine salvage pathways as they cannot synthesize them de novo. As a result, a number of pharmacologically active purine analogues have been approved for their clinical application as chemotherapeutic agents such as 6-thioguanine (6-TG) , 6-MP and AZT. In addition, it can be found as a nucleobase in some synthetic nucleoside analogues which are structurally similar to natural nucleosides. Many of these nucleoside analogues have antiviral and antitumor properties [7,8].

MATERIALS AND METHODS

Materials

6-mercaptopurine, methyl bromoacetate and boc-tyrosine was purchased from Hangzhou hyper chemical lemited(China). Absolute ethanol, absolute methanol, n-hexane were purchased from BDH (England). Ethyl acetate purchased from Riedel-dehaen(Germany). L-methionine and N-methyl morpholine (NMM) purchased from Fluka AG(Switzerland). Hydrazine hydrate, Hydrochloric acid, Petroleum spirit and Thionyl chloride were purchased from BDH (U.K).

DCC (Dicyclohexyl carbodiimide) and 1-Hydroxy benzotriazole (HOBt) were purchased from Heifi JOYE (China). N,N Dimethyl formamide (DMF) were purchased from Fluka AG(Switzerland). All other reagents were of analytical grade

Methods of Identification

General methods were used for purification and identification of the synthesized analogues including:

- Thin Layer Chromatography: Ascending thin layer chromatography was run for monitoring the reaction progress as well as checking the purity of our products. The compounds were revealed by reactivity with iodine vapor.
- Melting Points: Electronic Melting Point Apparatus was used to determine all melting points reported in this work.
- Infrared Spectra: Determinations of infrared spectra were recorded by KBr film FTIR shimadzu (Japan).
- Elemental Microanalysis: Elemental microanalysis was done at the College of Science, Al-Mustansiriya University. It has been done using Euro-vector EA 3000A (Italy).

Synthesis

Esterification of amino acid

Synthesis of L-methionine methyl ester HCl (Met-O-Me HCl), Compound A

A suspension of L-methionine (7.46 g , 50 mmol) dissolved in (100 ml) of absolute methanol, was cooled down to -10 ^{0}C, then thionyl chloride (50mmol, 3.63 ml) was added drop wise (the temperature should be keep below –10 ^{0}C, the reaction mixture was left at 40 ^{0}C for 3h , then refluxed for 3h and left at room temperature overnight. The solvent was evaporated to dryness under vacuum, re-dissolved in methanol and evaporated, this process was repeated several times and re-crystallize the product from methanol-diethyl ether [9].

Percent yield, physical appearance, m.p and R_f values were listed in table 1, and IR characteristics absorption bands were listed in table 2.

Alkylationof 6-mercaptopurine, compound (B1)

To a suspension of 2-mercaptopurine (2 g , 13 mmol) in 75 ml distilled water in 150 ml round bottom flask add triethyl amine liquid (1.81 ml , 13 mmol) dropwise in 5 min with stirring to get clear solution. Then methyl bromoacetate (1.23 ml , 13 mmol) added dropwise in 30 min at room temperature and continue stirring at room temperature for additional 1h. White precipitate was formed which filtered off, washed with water and recrystallized by ethanol:water (60:40) [10].

Percent yield, physical appearance, m.p and R_f values were listed in table 1, and IR characteristics absorption bands were listed in table 2.

Synthesis (7H-Purin-6-ylsulfanyl)-acetic acid, compound (B2)
compound B1(2 gm , 8.919 mmole) dissolved in 20 ml of 20% sodium hydroxide solution in round bottom flask 100 ml with 2- 3 boiling chips and boil the solution under reflex for 1 h, then cool solution to room temperature, filter and acidify it to pH 5 with 5 M HCl solution and cool the mixture in ice bath, white precipitate formed which filtered, washed with water [11] .

Percent yield, physical appearance, m.p and R_f values were listed in table 1, and IR characteristics absorption bands were listed in table 2.

Synthesis of (9H-Purin-6-ylsulfanyl)-acetic acid hydrazide, compound (B3)
compound B1 (2.242 g ,10.0 mmol) dissolved in 60 mL absolute ethanol, then hydrazine hydrate (1.2 mL, 25 mmol) was added. The reaction mixture was refluxed for 4 hours .Reaction was monitored by TLC. After the completion of reaction, solution was concentrated and refrigerated overnight. White precipitates obtained on cooling, were dried and recrystallized in ethanol [12].

Percent yield, physical appearance, m.p and R_f values were listed in table 1, and IR characteristics absorption bands were listed in table 2.

Coupling method and reagents
Conventional solution method was used as a coupling method between the carboxy- protected methionine and carboxylic acid side chain of 6-mercaptopurine derivative and also coupling between compound B3 and free carboxyl group of boc-tyrosine. DCC (Dicyclohexyl carbodiimide) was used as a coupling reagent, while HOBt (1-Hydroxy benzotriazole) was used to decrease racemization and to increase the yields [13].

Synthesis of 4-Methylsulfanyl-2-[2-(9H-purin-6-ylsulfanyl)-acetylamino]-butyric acid methyl ester, compound (C1)
To a stirred solution of L-methionine Me ester HCl (comp. A) (0.998 g, 5 mmol) in 10 ml dry DMF, NMM (1.1 ml, 10 mmol) was added with stirring for 10 min. Compound B2 (1.05 g, 5 mmol) in 10 ml dry DMF was also added to the reaction mixture which was cooled down to -10 ^{0}C then HOBt (0.675 g, 5 mmol) and DCC (1.03 g, 5 mmol) were added with stirring, continue stirring for 2 days at 0 ^{0}C and then at room temperature for 5 days. At the end of reaction, DCU was filtered off, and washed with DMF. The filterate was evaporated to dryness and re-dissolved in cold DMF, the excess of DCU which that still adhesive on the peptide residue was precipitated out filtered off, this process was repeated several times then the clear filtrate concentrated and dissolved in chloroform (100 ml), transferred to seperatory funnel washed 3 times with 10 ml of 0.1N HCl solution, 3 times with 10 ml of 5% $NaHCO_3$ solution, once with water and once with 10 ml of saturated NaCl solution. The ethylacetate layer was dried using anhydrous magnesium sulfate powder and evaporated under vacuum; the resulted product was collected, recrystallized from chloroform and n-hexane, dried in vacuum oven at 60 ^{0}C for 4 h [13].

Percent yield, physical appearance, m.p and R_f values were listed in table 1, IR characteristics absorption bands were listed in table 2 and elemental microanalysis data are listed in table 3.

Synthesis of (2-(4-Hydroxy-phenyl)-1-{N'-[2-(9H-purin-6-ylsulfanyl)-acetyl]-hydrazinocarbonyl}-ethyl)-carbamic acid tert-butyl ester (compound C2)
To a stirred solution of compound B3 (0.876 g, 3.91 mmol) in 10 ml dry DMF, NMM (0.42 ml, 3.9 mmol) was added with stirring for 10 min. Boc-tyrosine (1.09 g, 3.9 mmol) in 10 ml dry DMF was also added to the reaction mixture which was cooled down to -10 ºC then HOBt (0.528 g, 3.91 mmol) and DCC (0.806 g, 3.9 mmol) were added with stirring, continue stirring for 2 days at 0 ^{0}C and then at room temperature for 5 days. The crude product was evaporated to exclude DMF and the filterate was evaporated to dryness and re-dissolved in cold DMF, the excess DCU which that still adhesive on the peptide residue was precipitated out filtered off, this process was repeated several times, then evaporate the filtrate to get clear oily residue that mixed with cold 5% $NaHCO_3$ forming a white precipitate which was filtered and washed with cold 0.2 N HCl solution and excess of water. The resulted product was collected and recrystallized by (ethanol:water)(60:40), dried in vacuum oven at 60 ^{0}C for 4 h [13].

Percent yield, physical appearance, m.p, R_f values were listed in table 1, IR characteristics absorption bands were listed in table 2 and elemental microanalysis data are listed in table 3.

Biological Activity

Antibacterial

A preliminary antibacterial activity has been carried out according to Well Diffusion Method. The prepared compounds have been studied for their antimicrobial activity in vitro against three tested bacteria (*Staphylococcus aureus.,* as gram positive bacteria and *Klebsiella pneumoniae*. and *E.Coli* as gram negative bacteria), were clinically activated and maintained on nutrient agar medium for testing antibacterial activity. Ofloxacin was used as a standard drug for antibacterial activity. The plates were incubated at 37 °C for 24 h and the antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the well [14], as shown in table 4.

Cytotoxic Assay

The in vitro cytotoxicity assays with cultured cells are widely used to evaluate chemicals including cancer chemotherapeutics, pharmaceuticals, biomaterials, natural toxins, antimicrobial agents and industrial chemicals because they are rapid and economical [15]. The cytotoxic effect of our analogues was evaluated by MTT assay; a non-radioactive, fast assay widely used to quantify cell viability and proliferation. MTT is a yellow water soluble tetrazolium salt, metabolic active cells are able to convert the dye to water insoluble dark blue formazan by reductive cleavage of tetrazolium ring that can be detected through UV light to give us a correlation between optical density and viable cells counts. A set of two fold in four concentrations (1, 5, 10,20 µg/ml) was made for each product and the exposure time of the assay was 24 h . HeLa Cell Line was used in this study. It is the first continuously cultured human malignant cell line, derived from the cervical carcinoma of Henrietta Lacks. These cells are used for virus cultivation and antitumor drug screening assays

RESULTS AND DISCUSION

The reaction pathways:

The aim of our research is to synthesize 6-mercaptopurine derivatives coupled to different amino acid or peptide (Scheme 1 and 2).

The overall synthesis strategy based on five major lines:

1. Amino acid derivatives

The amino acids were activated by thionyl chloride to get acyl chloride that attacks methanol to get methyl esters of the selected amino acid.

2. Alkylation of 6-mercaptopurine

6-mercaptopurine was alkylated by methyl bromoacetate in alkaline aqueous media (containing triethylamine as a base) to get ester of 6-mercaptopurine.

3. hydrolysis of ester group

Removal of methoxy group from the ester can be done by sodium hydroxide to form the conjugated base, then acidified with hydrochloric acid to get (7H-Purin-6-ylsulfanyl)-acetic acid.

4. synthesis of 6-mp hydrazide derivative

2-(9H-Purin-6-ylsulfanyl)acetohydrazide (B3) was synthesized by using conventional reaction between the ester (comp.B1) with the hydrazine hydrate.

5. Amide bond formation

Conventional solution method for amide bond formation used as a coupling method between the free carboxyl and amine groups . The DCC/HOBt coupling reagents used for peptide bond formation in an aprotic solvent (DMF) in the presence of hindered base such as NMM that enhance the step involving preactivation of carboxylic acid residue. The overall reaction pathway is shown in the following schemes (figure 1&2):

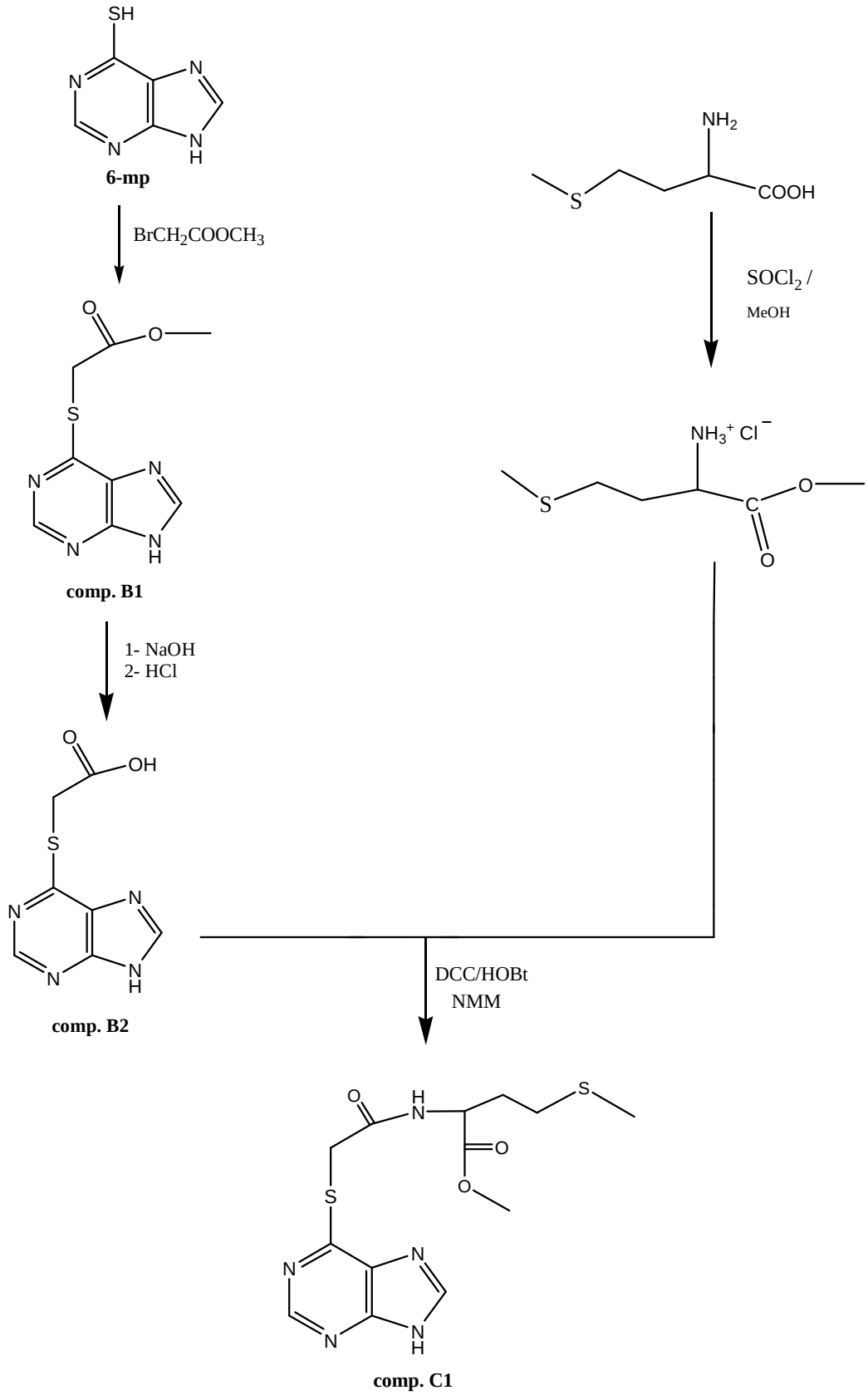

Fig.1: Scheme of overall pathway of synthesis of compounds C1

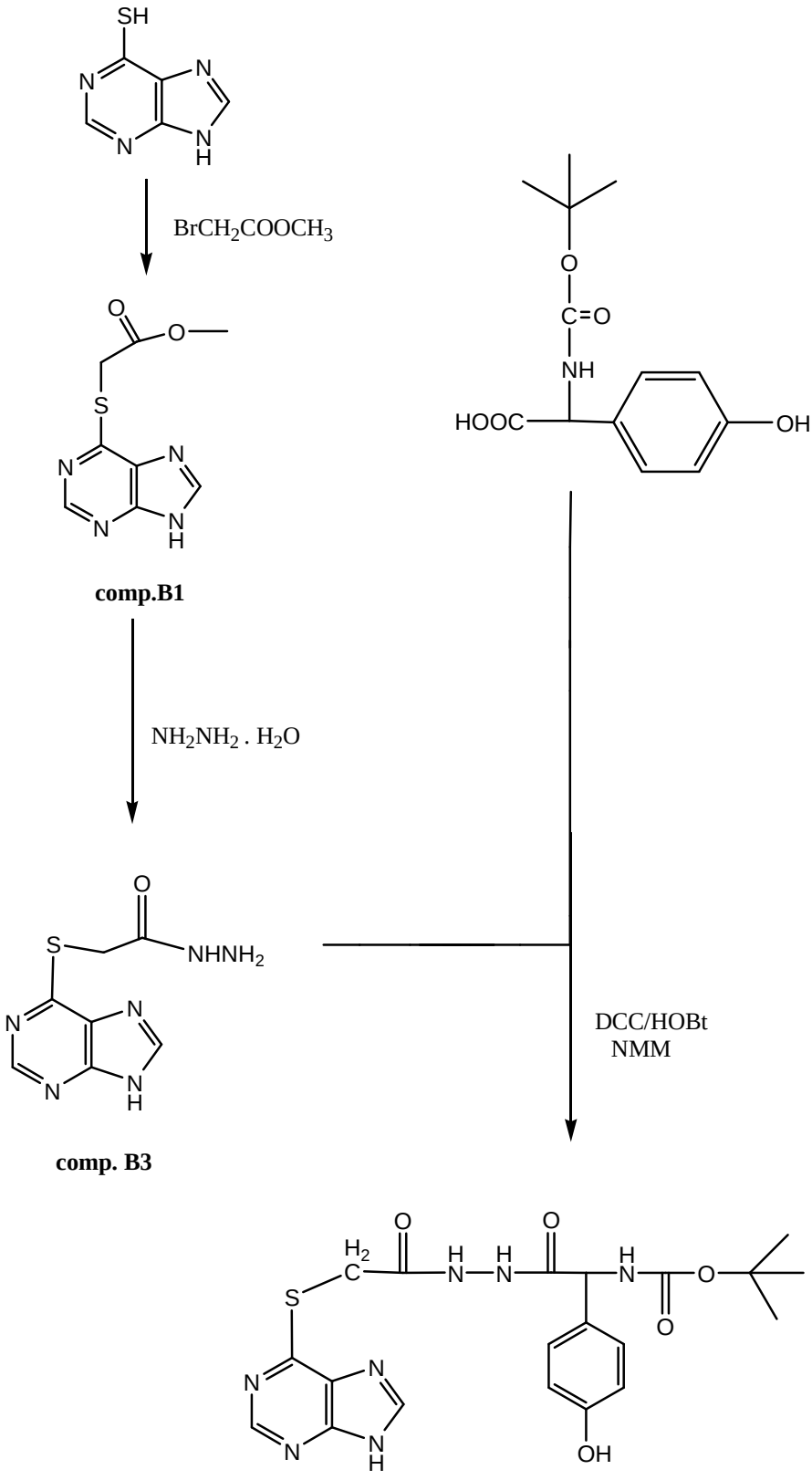

Fig.2: Scheme of overall pathway of synthesis of compounds C2

Table 1: The identification parameters of the synthesized compounds

Compound	Physical appearance	Melting Point observed (°C)	% yield	R_f value	Solvent system
A	white crystals	149-153	80	0.80	Chloroform 7 Methanol 3
B1	White fine powder	159-161	70	0.55	Ethylacetate 5 n-hexane 3 Methanol 2
B2	Faint yellow fine granules	250-252 With decomposed	85	0.14	Ethylacetate 5 n-hexane 3 Methanol 2
B3	White powder	232-234 With decomposed	81	0.22	Ethylacetate 5 n-hexane 3 Methanol 2
C5	Faint yellow powder	103-105	70	0.58	Ethylacetate 5 n-hexane 3 Methanol 2
C8	Beige powder	156-158	63	0.49	Ethylacetate 5 n-hexane 3 Methanol 2

Table 2: The characteristic IR bands of the synthesized compounds

(str. = stretching vibration , ar.= aromatic , alph.= aliphatic, asym.= asymmetric, sym.= symmetric, bend.= bendig vibration)

Com-pound	Compound name	IR Characteristic Absorption Bands (V cm-1)
A	Meth-O-Me HCl	(3250-2700 (NH_3^+ str. of amine salt), (2958,2920 asym. C-H str. of CH_3 and CH_2), (1743 C=O str. of ester), (1630 NH_3^+ bend), (1508 CH_2 bend), (1442,1394 CH_3 bend), (1230 CO-O str. of ester).).
B1	(9H-Purin-6-ylsulfanyl)-acetic acid methyl ester	(3599, 3562, 3392, 3195 N-H str.), (3059 ar. C-H str.), (2962 asym. str. of CH_3, CH_2), (2798 sym. str. of CH_3 ,CH_2), (1738 C=O ester), (1637,1564 C=N, C=C str.),(1196,1157 CO-O str. of ester), (640 C-S-C str.)
B2	(9H-Purin-6-ylsulfanyl)-acetic acid	(3199 N-H str.), (3168, 3105, 3006 ar. C-H str.), (2908 asym. str. of CH_2), (2837 sym. str. of CH_2), (1724 C=O str. of carboxylic acid), (1564 C=N and C=C str), (1196 CO-O str. of carboxylic acid), (636 C-S-C str.)
B3	(9H-Purin-6-ylsulfanyl)-acetic acid hydrazide	(3296-3138 N-H str), (3138, 3041 ar. C-H str.), (2916 asym. str. of CH_2), (2875,2777 sym. st. of CH_2),(1693 C=O str. of amide), (1637 C=N str.), (1595 amide II), (1564,1496,1442 ar. C=C str.),(1375 CH_2 bending), (627 C-S-C str.)
C1	4-Methylsulfanyl-2-[2-(9H-purin-6-ylsulfanyl)-acetylamino]-butyric acid methyl ester	(3278 N-H str.),(3057 ar. C-H str.),(2951,2916 asym. str. of CH_3, CH_2),(2794 sym. str. of CH_3,CH_2), (1739 C=O str. of ester),(1657 C=O str. of peptide bond, amide I),(1556 overlapped ar. C=C str. and amide II),(1238 CO-O str. of ester), (627 C-S-C str)
C2	(2-(4-Hydroxy-phenyl)-1-{N'-[2-(9H-purin-6-ylsulfanyl)-acetyl]-hydrazinocarbonyl}-ethyl)-carbamic acid tert-butyl ester	(3317,3199 N-H and O-H str.), (3041 ar. C-H str.),(2983,2931 asym. str. of CH_2, CH_3),(2848 sym. str. of CH_2, CH_3),(1693 C=O str. of peptide bond), (1622 C=N str.),(1576 amide II),(1161 CO-O str.), (692 C-S-C str.).

Table 3: The elemental microanalysis of the synthesized compounds

Compound.	Value type	C	H	N	S	Mol. wt.
C1	Calculated Observed	43.93 43.65	4.820 5.014	19.70 19.854	18.043 18.042	355.08
C2	Calculated Observed	51.73 54.76	5.170 5.156	20.11 19.645	6.58 6.250	487.16

Table 4: The antibacterial activity of the tested compounds

Compound No.		Zone of Inhibition in mm		
		S. aureus	***Klebsiella pneumoniae***	***E. Coli***
C1	1µg/ml	-	-	-
	5µg/ml	10	16	-
C2	1µg/ml	-	-	-
	5µg/ml	12	13	-
6-MP	1µg/ml	-	-	-
	5µg/ml	11	12	-
Ofloxacin	1µg/ml	-	12	14
	5µg/ml	8	21	16

Table 5: The cytotoxic activity of the tested compounds by comparing their optical density with the control

Comp	Mean Absorbance at 570 nm					Cell growth inhibition %			
	1 µg/ml	**5 µg/ml**	**10 µg/ml**	**20 µg/ml**	**Control 0 µg/ml**	**1 µg/ml**	**5 µg/ml**	**10 µg/ml**	**20 µg/ml**
C1	0.29862	0.3575	0.2747	0.2227	0.3596	16.79	0.608	23.61	38.07
C2	0.27812	0.3401	0.2437	0.2183	0.3596	22.67	5.43	32.32	39.28

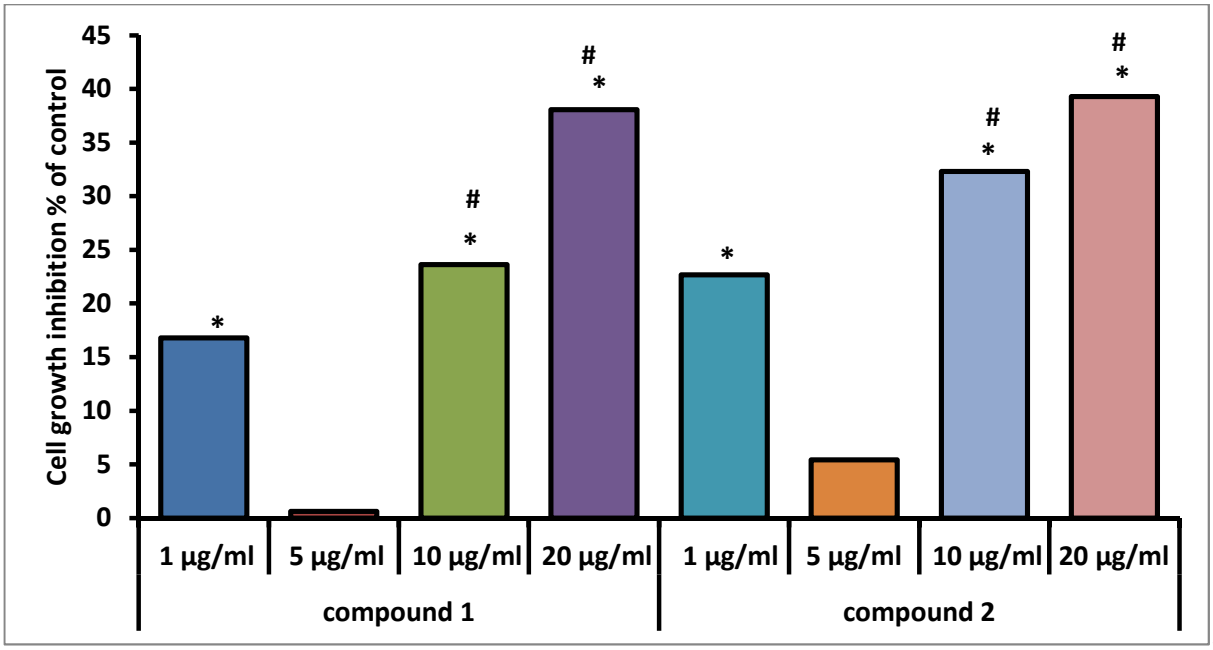

Fig. 3 : Cervical carcinoma cell line (HeLa) exposed to different concentration of compound C1 and compound C2 for 24 h at 37˚C, columns represent cell growth inhibition, * represent significant differences among the means of control untreated cells and cells treated with either compounds (p< 0.05), # represent significant difference between the highest concentration and lowest concentration (p<0.05)

Biological study

antibacterial activity

The antibacterial have been carried out according to Well Diffusion methodology. The prepared compounds have been studying for their antibacterial activity *in vitro* against three tested bacteria (*Staphylococcus aureus* as gram positive bacteria *and E.Coli and Klebsiella pneumoniae* as gram negative bacteria) using Ofloxacin as standard. All results are fixed in tables 4 , compound C1 and C2 show improved antibacterial activity against *S. aureus* , while both compounds had no or low activity against *E.Coli* and *Klebseila spp.* when compared with ofloxacin.

Cytotoxic activity

The cytotoxic study was done on HeLa cell line. Exposure time =24 h. Staining is MTT stain. Control number two. When the cancer cell line was treated with these products the result showed significant cytotoxic effect in all tested samples in comparison with the control. The toxic effect varied from one sample to another, all samples showed a significant toxicity ($P < 0.05$) at concentration of (1 , 10 and 20 µg/ml). The inhibitory concentration percent (IC %) was estimated according to the following equation:

IC% Inhibitory concentration = [(Control O.D. – Sample O.D)/(Control O.D.)] X 100 and the result was varied among samples as shown in table 5 and figure 3.

CONCLUSION

The proposed compounds were successfully synthesized by the conventional solution method as previously described and their structure formula were consistent with the proposed structures since conformity of their structures was achieved by using the following techniques: thin layer chromatography (TLC), melting point, infrared spectroscopy (IR) and elemental microanalysis (CHNS).

The derivatives with promising cytotoxic effect can be consider as a good anticancer drug candidates.

REFERENCES

[1] T. Bart et al; *Eur. J. Org. Chem*., **2009**, 1377-1383.
[2] Sachan Dinesh et al; *Journal of Pharmaceutical and Scientific Innovation* , March- April **2012**,29-34.
[3] Shaw, G.; In Comprehensive Heterocyclic Chemistry (Ed. K.T. Potts), **1984**,Vol. 5, PergamonPress, pp. 499-605.
[4] Di Iorio, P.; Ballerini, P.; Caciagli, F.; & Ciccarelli, R. (**1998**). *Pharmacol Res*, 37(3):169-178.
[5] Ralevic, V.; *CurrVasc Pharmacol*, **2009**, 7(1):3-14.

[6] Jeannette Calderón-Arancibia , Christian Espinosa-Bustos , Álvaro Cañete-Molina, Ricardo A. Tapia , Mario Faúndez , Maria Jose Torres , Adam Aguirre , Margot Paulino and Cristian O. Salas; *Molecules*, **2015**, 20, 6808-6826.
[7] J. Vesely et al; *Eur. J. Biochem*., **1994**,224,771-786.
[8] L. Meijer, E. Raymond, *Acc. Chem. Res*., **2003**,36, 417-425.
[9] AL-Ameri M. F.;Synthesis of new analgesic peptides analogues with expected biological activity. M. Sc. Thesis, College of Pharmacy, Baghdad University, Baghdad; **2000**.
[10] Azizi N, et al; *J. Iran. Chem. Soc*.; **2009**, 6 (4): pp. 749-753.
[11] Najim A. Al-Masoudi et al; *Acta Pharm*.; **2006**,56:175–188
[12] Ibrahim A. I. Ali; *ARKIVOC*; **2008**, xvii: 77-89.
[13] Henklein P, Rapp W.; *J. Peptide Chemistry*, **2008**; 14(8):10401-10421.
[14] Kuete. V., Tangmouo. J.G., Penlap. V., Mofo. F., Lontsi. D.*: Journal of Ethnopharmacology*, **2006**;104: pp. 5-11.
[15] Irena K.; *Curr. Med. Chem. - Anti-Cancer Agents*, Bentham Science Publishers Ltd., **2005**; 5: 29- 46.