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Research Article

Synthesis of NSAID with Sulfonamide Conjugates as Possible Mutual Prodrugs using Amino Acid Spacer

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ABSTRACT

The purpose of this research work is to synthesize conjugates of NSAIDs (ibuprofen, and naproxen) with sulfadiazine as possible mutual prodrugs to overcome the local gastric irritation of NSAIDs with free carboxyl group by formation of ester linkage that supposed to remain intact in stomach and may hydrolyze in intestine chemically or enzymatically; in addition to that attempting to target the synthesized derivative to the colon by formation of azo bond that undergo reduction only by colonic bacterial azoreductaze enzyme to liberate the parent compound to act locally (treatment of inflammation and infections in colon).

Key words: mutual prodrug, ester linkage, azo bond, colon targeting.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are most commonly prescribed drugs for the treatment of pain, inflammation and fever[1]. However gastric irritation caused by most of the NSAIDs used today restricts their use.The pharmacological activity of NSAIDs is related to the blocking of prostaglandin H_2 (PGH_2) biosynthesis from arachidonic acid by inhibiting theactivity of cyclooxygenases (COXs). The COX enzyme exists in two isoforms: a constitutive isoform, COX-1, found in most tissues including stomach, kidney, and platelets, and an inducible isoform, COX-2, expressed at the site of inflammation[2]. Classical NSAIDs, such as ibuprofen, flufenamic acid, diclofenac, and aspirin, preferentially inhibit COX-1, thus suppressing the biosynthesis of prostaglandins that maintain gastric mucosal integrity and leading to gastrointestinal (GI) side effects, including ulceration and hemorrhage (systemic effect)[3]. Moreover the local effect on GI Tract involves a local action comprising of a direct contact effect and an indirect effect on the GI mucosa. The direct effect can be attributed to the local inhibition of prostaglandin (PG) synthesis in the GI tract[4]. The indirect effect can be attributed to a combination of an ion-trapping mechanism of NSAIDs in mucosal cells and back diffusion of H^+ ions from the lumen into the mucosa[5]. Topical irritation by the free carboxylic group of the NSAIDs is considered an important factor in establishing superficial stomach erosion[6].Since the introduction of specific COX-2 inhibitors, which are less harmful to the GI tract, the use of conventional NSAIDs has declined. However, the safety profile of COX-2 inhibitors has been questioned due to the risk of ulcer complications in high-risk individuals and to cardiovascular adverseeffects[7]. Thus, the need for NSAIDs with improved GI tolerabilitystill exists. Figure (1) shows prostanoid synthesis and its inhibition by NSAIDs [8]

One approach that has been used to decrease NSAID induced GI toxicity without adversely affecting their anti-inflammatory activity is to mask the carboxylic acid group by synthesizing the corresponding ester prodrugs[9]. A Prodrug is a chemically modified inert drug precursor which upon biotransformation liberates the pharmacologically active parent compound[10]. A major requisite for these prodrugs is that they must be readily hydrolyzed, enzymatically or chemically, after oral absorption to quantitatively release the parent drug[11]. In addition, the pro-moiety should be non-toxic and readily excreted. Since they offer enormous potential in terms of low toxicity, amino acids have been considered the ideal carriers for the development of prodrugs; using amino acids like L-tryptophan, L-histidine, L-glycine, and tyrosine as carriers since they have marked anti-inflammatory activity of their own[12].Mutual prodrug, where the carrier used is

another biologically active drug instead of some inert molecule. A mutual prodrug consists of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and viceversa. The carrier selected may have the same biological action as that of the parent drug and thus might give synergistic action, or the carrier may have some additional biological action that is lacking in the parent drug, thus ensuring some additional benefit. The carrier may also be a drug that might help to target the parent drug to a specific site or organ or cells or may improve site specificity of a drug. The carrier drug may be used to overcome some side effects of the parent drugs as well[13].

Site specific drug delivery

A drug, after its absorption into systemic circulation, gets distributed to target site as well as non-targeted tissues. The distribution of drug to non-targeted tissues may lead to undesirable toxic effects in those tissues and insufficient concentration in the target site to evoke any therapeutic response. If the target site has a longer distribution time, the drug may get eliminated without reaching such a site; and even if the drug reaches the targeted area in sufficient concentrations, it may have such a low penetration power that it may not penetrate the target cells at all[14]. Targeting the drug to its site of action through prodrug concept has been utilized to overcome these problems. While designing the prodrug, utilization of the enzymes that are specifically present in that organ or tissue or specific constant pH of that area which is different from body pH should be made so that the prodrug releases the drug only in the targeted organ[15].

Colon targeted drug delivery

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeuticpeptides. The colon has some unique features, which make this organ attractive for site-specific drug delivery. There is a considerable interest in the colon specific drug delivery in order to treat diseases of the large intestine, such as colitis, colon cancer, constipation, irritable bowel syndrome, and infectious diseases[16].

To achieve successful colonic delivery, a drug needs to be protected from absorption and /or the environment of the upper gastrointestinal tract (GIT)and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs[17].

The colonic micro flora produces a variety of enzymes, including azoreductase, various glycosidases and amidases, which were not present in the stomach or small intestine. Therefore, enzyme dependent drug release, which relies on the existence of enzyme-producing microorganisms in the colon, could be used to deliver drug to the colon.After enzymatic cleavage of degradable carrier bonds, drug release occurs[18].

The presence of azoreductaze enzymes in colon, from colonic microflora, play important role in the release of drug from azo bond prodrugs since these enzymes cause reduction, and thus cleavage, of the azo bonds[19].

Sulfonamides are one of the least expensive drugs and this factor largely accounts for their greater extent of use in developing countries. These drugs are considered useful for gastrointestinal (GI) tract infections. An infection always leads to inflammation therefore sulfa drugs can be coupled with NSAIDs so that these mutual prodrugs can be used for infections as well as for inflammation[20]. In this work sulfadiazine is coupled with ibuprofen and naproxen using Boc-tyrosine as spacer.

MATERIALS AND METHODS

Ibuprofen, naproxen, and sulfadiazine were purchased from SDI (Iraq); Boc-tyrosine was purchased from Fluka (Switzerland). All chemicals were reagent grade and obtained from commercial sources. Elemental microanalysis was performed using CHNS analyzer (Jordan); melting points were measured on Barnstead Electrothermal melting point apparatus (USA) and are uncorrected; infra red spectra were recorded as KBr disks on FTIR spectrophotometer (College of Pharmacy, University of Al-Mustanseriya); UV spectra were done using UV spectrophotometer (College of pharmacy, University of Baghdad).

Chemical synthesis

Synthesis of compound 1A (diazonium salt formation)[21]

Sulfadiazine (2.5g, 10 mmole) was dissolved in a mixture of equal quantities (12.5ml) of each of conc. HCl and water in a suitable beaker; the resulting solution was stirred and cooled by immersing in a bath of crushed ice; throughout the reaction the temperature was kept below 5ºC. A cold solution of (0.75g,11 mmole) sodium nitrite in(5ml) water was placed in a dropping funnel which was cooled using crushed ice, then it was added dropwise into the first solution in the ice bath with continuous stirring ; the temperature should not be allowed to rise above 10ºC.The last quantity of the sodium nitrite solution was added more slowly and after stirring for 3-4 minutes, the solution was tested for excess sodium nitrite using potassium iodide-starch paper.A solution of sulfamic acid (1.5ml) of 2% was added and stirring was continued for 20 minutes. The diazonium salt formed was used immediately in the following step.

Synthesis of compound1B (Azo bond formation)[21]

Boc- tyrosine (2.8 g, 10 mmole) was dissolved in (8 ml) of (10 %) NaOH in a suitable beaker immersed in an ice bath. The solution was stirred vigorously and the temperaturewas kept below 5ºC by the addition of crushed ice. The cold diazonium salt solution from the previous step (compound 1A) was placed in a dropping funnel, then it was added drop by drop to the cooled, stirred Boc-tyrosine solution; an orange color was developed and orange crystals soon separated. At the end of the addition the mixture was stirred for 3hours in the ice bath. Then the solution was filtered through a Buchner funnel with gentle suction, washed well with water, and recrystallized from ethanol: water mixture (1:5) to obtain (34 %) of compound 1B.

Synthesis of compound 1C[22]

To a suspension of compound 1B (2.71 g, 5 mmole) in methanol;dimethylaminopyridine (0.06g, 0.5 mmole) was added.The mixture was stirred and cooled to -10ºC followed by dropwise addition ofthionyl chloride (0.7 ml, 10 mmole) with continuous stirring during which the temperature of the reaction mixture was kept below 0ºC. After completion of the addition, the temperature of the mixture was allowed to rise and was kept at 40ºC for 3 hours followed by refluxing for further 3 hours, then left at room temperature overnight. The solvent was evaporated to dryness in vacuum. Red powder appeared which was re-dissolved in methanol and evaporated several times to ensure the complete removal of excess thionylchloride. The residue was then collected and recrystallized from methanol: diethylether mixture (1:5) to obtain (95 %) of compound 1C as darkred residue.

Synthesis of compounds 1D, 2D[23]

Ibuprofen 1g, 5mmole (or naproxen 1.17g, 5mmole) was dissolved in chloroform (20ml) in a round bottom flask; to it few drops of DMF were added, the mixture was stirred inside an ice bath where the temperature should be below 0ºC. A slight excess of thionyl chloride (0.7 ml, 10 mmole) was added drop wise over a period of 15-20 minutes with continuous stirring. After complete addition of thionyl chloride the temperature was allowed to rise gradually then refluxing for 8 hours. The solvent and the excess thionyl chloride were evaporated under vacuum followed by re-dissolving in chloroform and re-evaporation several times. The acid chloride was obtained as a yellow oily residue and used immediately in the following step.

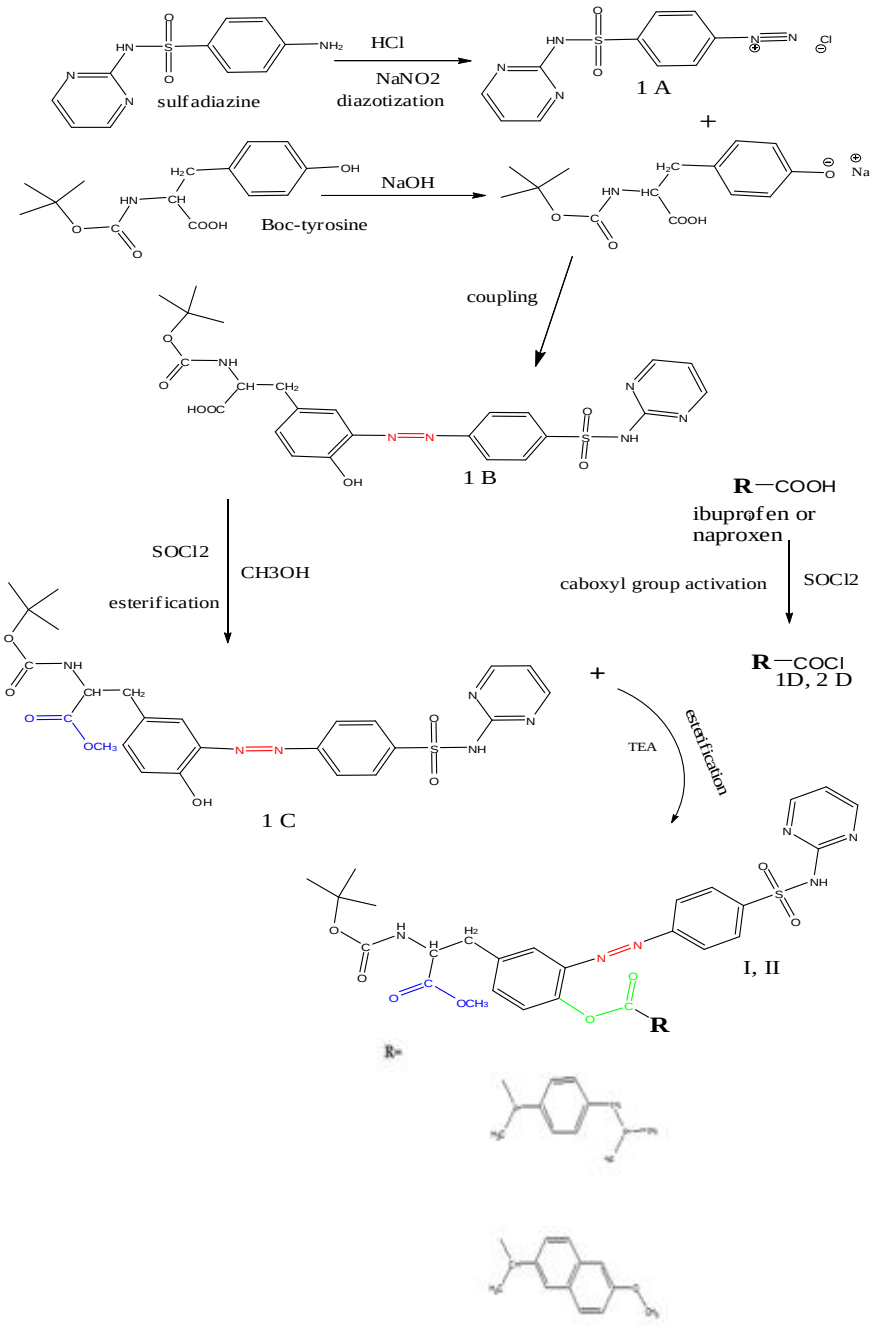
Synthesis of compound I, II[24]

A suspension of compound 1C (2.78 g, 5 mmole) and TEA (1.4ml, 10 mmole) in dry THF (100ml) was stirred in an ice bath. Followed by a dropwise addition of a solution of compound 1D or 2D (in dry acetone) (5 mmole) over a period of 1 hour, the temperature of the mixture was kept below -5ºC during the addition. After that the mixture was stirred for 72 hours at room temperature. The solvent was evaporated then the residue was dissolved in chloroform followed by filteration to remove solids. The chloroform layer was shaken with 1M sodium carbonate solution for 15 minutes (3× 25ml),D.W. (3× 25ml) ,0.05N HCl (3× 25ml) ,D.W. (3× 25ml) ,and finally with (25ml) brine solution (saturated NaCl solution).The chloroform extract was dried over anhydrous magnesium sulfate. The residue, after evaporation of solvent, was collected and recrystallized from chloroform: petroleum ether (40-60) mixture (1:5) to obtain (26%) yield red residue compound I; (25%) yellow residue compound II.

RESULTS AND DISCUSSION

Primary aromatic amines, on reaction with nitrous acid in the presence of HCl (or other mineral acid) at about 0ºC, yield diazonium salts. Coupling reaction is an electrophilic aromatic substitution with the diazonium ion acting as the electrophile which reacts at the position of greatest electron availability (the position para or ortho to the electron releasing group)[25].Conversion of acid chloride into ester:on treatment with the appropriate nucleophile, an acid chloride can be converted to an ester by nucleophilic acyl substitution mechanisms.Nucleophilic acyl substitution reactions take place in to steps:

1. Addition of the nucleophile.
2. Elimination of a leaving group.[26]



Scheme of synthesis of compound I, II

Determination ofλ max

Scanning the solutions of compounds I, II (25µg /ml) in chloroform by UV/visible spectrophotometer at 200-800 nm gave different peaks with λ max at 332nm; see figures(2), (3).

The molar extinction coefficient for compounds I, II were determined atλ max = 332 nmand presented in table (1).

Preparation of calibration curve

Calibration curve of compound I was constructed in chloroform using different concentration solutions (25, 50, 100, 200 µg/ml) at λ max (332 nm) and presented as straight line; see figure (5).

Determination of partition coefficient[27]

A drug partition coefficient is a measure of its distribution in a lipophilic/hydrophilic phase

system, and is indicative of its ability to penetrate biological multiphase system. The partition coefficient of compound I was determined in two systems: n-octanol/HCl buffer (PH=1.2) where the value was 30.211 and n-octanol/ phosphate buffer (PH=7.4) where the value was 30.64. This indicates that the compound is highly lipophilic.

CONCLUSION

The synthesis of the designed compounds has been successfully achieved and the structural formula for these compounds was characterized using IR spectroscopy, elemental microanalysis, melting points, UV spectra, and $R_f$values. From the results compound I is highly lipophilic.

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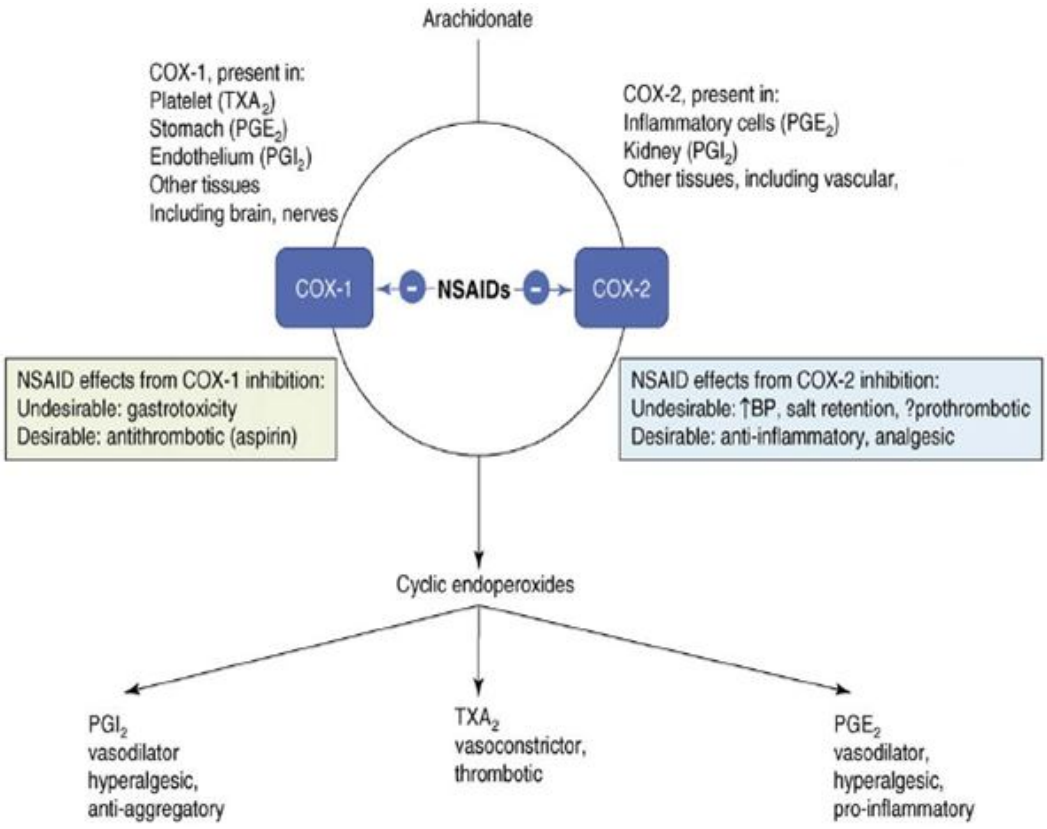

Fig. 1: Prostanoid synthesis and its inhibition by NSAIDs

Table 1: Physical appearance, percentage of yield, melting points, R_f values, and molar extinction coefficient of intermediates and final compounds

compound	Physical appearance	Yield%	Melting Point (°C)	R_f * value A	B	Є at 332 nm
1B	Orange powder	34%	143-145	0.3	0.1	-
1C	Dark red powder	95%	90-92	0.7	0.2	-
I	Red powder	26%	152-155	0.9	0.46	1173
II	Yellow powder	25%	157-160	0.96	0.37	3413.6

* A) Chloroform: ethanol (8:2) B) Toluene: ethanol (8:2)

Table 2: Elemental microanalysis results of compounds I and II

Compound	Molecular formula	Molecular weight	Elemental microanalysis%		
			Element	Calculated	Observed
I	$C_{38}H_{44}N_6O_8S$	744.86	C H N S	61.27 5.95 11.28 4.30	61.319 6.341 12.064 4.444
II	$C_{39}H_{40}N_6O_9S$	768.83	C H N S	60.93 5.24 10.93 4.17	60.998 5.197 11.534 4.623

Table 3: FT IR characteristic bands of the synthesized compounds

Compound	Band (cm^{-1})	Interpretation
Compound 1B	3385 1707 1622 1581 1276 1498 1348, 1166	O-H stretching and N-H stretching of amide C=O stretching of carboxyl group C=O stretching of amide N-H bending (amide II band) C-O stretching of carboxyl group N=N stretching O=S=O sulfonamide two bands
Compound1C	3417 1737 1618 1585 1344, 1170 1280	O-H stretching of phenol and N-H stretching of amide C=O stretching of ester C=O stretching of amide N-H bending (amide II band) O=S=O sulfonamide two bands C-O stretching of ester
Compound I	3416 1743 1658 1585 1357,1132	N-H stretching of amide C=O stretching of ester C=O stretching of amide N-H bending (amide II band) O=S=O sulfonamide two bands
Compound II	3288 1737 1651 1581 1354,1166 1269	N-H stretching of amide C=O stretching of ester C=O stretching of amide N-H bending (amide II band) O=S=O sulfonamide two bands C-O stretching of ester

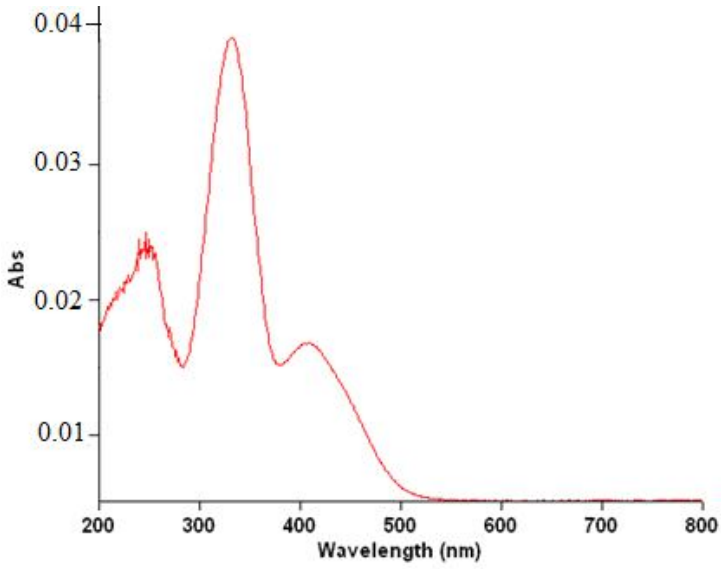

Fig. 2: UV spectrum of compound I shows 3 peaks; 246, 332, and 409 nm; λ max is 332 nm

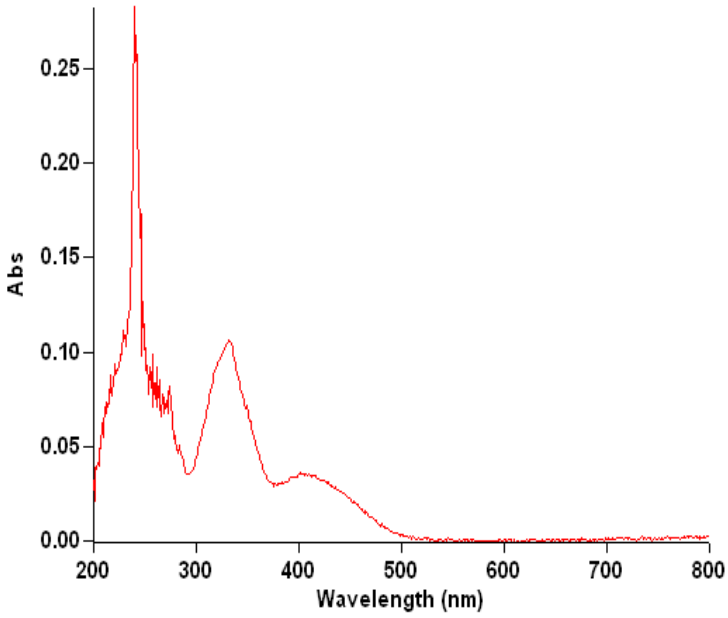

Fig. 3: UV spectrum of compound II shows 3 peaks; 241, 332, and 409 nm; λ max is 332 nm

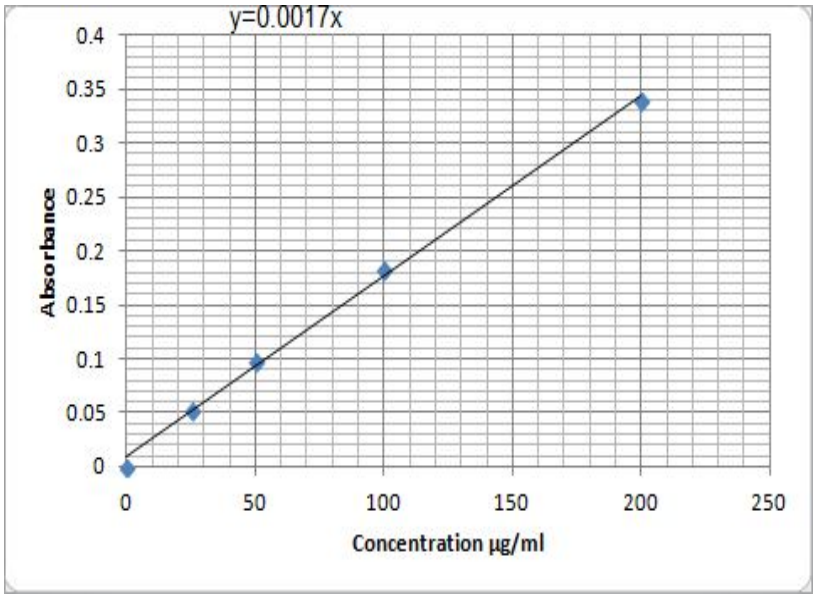

Fig. 5: The calibration curve of compound I

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