SYNTHESIS OF MUTUAL PRODRUG OF P-AMINO SALICYLIC ACID (PAS) AND ISONIAZID(INH), WITH POSSIBLE ENHANCEMENT PHYSICOCHEMICAL PROPERTIES

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Abstract

Tuberculosis is one of the most widespread disease in the world, and can be deadly in patients with AIDS worldwide. The course of treatment is long (3-9) months, often need combination therapy to decrease microbial resistance, some of these drugs have serious side effects and undesirable physicochemical properties like, gastric irritation, short T1/2 (pas), hepatic toxicity and extensive metabolism by the liver (INH), through N-acetyl transferase enzyme.

So by using this approach we hope reduction of gastrointestinal toxicity of PAS and reduction of intestinal acetylation of Isoniazid. This drug was synthesized by amidation of free amine group of INH with free carboxyl group of glycine from one side and amidation of carboxyl group of **PAS** after protection of it, and amino group of glycine from other side, here amidation was done by using coupling agent (DCC) using conventional solution method and it was identified by the following methods: melting point, thin layer chromatography (TLC), infrared spectroscopy (IR) and the elemental analysis(CHN). The synthesized compound showed better partition coefficient compared with the original apromoiety (PAS,INH).

Keywords: Mutual prodrug, PAS, INH.

Introduction

Tuberculosis is one of the most disease widespread in the world, could be deadly in patients with AIDS worldwide (1). The treatment was done by using combination therapy because these drugs show better therapeutic results and the mycobacterium does not evolve resistance to drugs, during this long period, For PAS, the free acidic carboxylic group is responsible for gastrointestinal irritation and is extensively metabolized by acetylation of the amino group, T1/2 for the metabolism of the drug is one hour, therefore; a large dose given to maintain a minimum effective level of the PAS; INH is readily absorbed after oral administration, it is extensively metabolized to inactive metabolites (diacetyl hydrazide, acetylhydrazide, N-acetylizoniazide and hydrazine) (2) the major metabolite is N-acetyl Isoniazid. The enzyme N-acetyl transferase,is responsible for INH metabolism (3). PAS, when co administrated with INH, is found to reduce the acetylation of INH, itself being the substrate for acetylation, thus it increases the plasma level of INH.(4) Substitution at the 2-hydroxy group or removal of the amino group of PAS abolishes the antitubercular activity, therefore various prodrugs were synthesized in the past, including macromolecular prodrugs, (5-8)amides and ion pair complex.(9-11) However, formations of mutual prodrugs have never been tried before. As in the case of NSAIDs, Mutual prodrug, where the carrier used is another biologically active drug instead of some inert molecule. A mutual prodrug consists of two pharma-cologically active agents coupled together so that each acts as a promoiety for the other agent and *vice versa*. mutual prodrugs have been beneficial in reducing gastrointestinal irritation (12).

Materials and Methods

BOC-glycine amino acid, INH& PAS, DCC was supplied from Fluka AG/ Switzerland. All of the solvents and materials used were of annular type and used without further purification.The method used for synthesis of this analog was conventional

solution method in which we use N,N-Dicyclohexylcarbo-diimide (DCC) as coupling agent, tert-butyoxy carbonyl (BOC) amino acid was used as terminal amino protecting group, PAS was protected to prevent inter polymerization,BOC-glycine obtained fully protected from Fluka, final analog was purified by repeating recrystallisation using Petroleum ether 40-60: ethyl acetate mixture. The synthesis of analog includes the following general steps:

1. P-amino salicylic acid (PAS) protection: using acetic anhydride under reflux for protection hydroxyl and amino groups.
2. Coupling of INH & BOC-Glycine by DCC to form BOC-gly-INH.
3. Deprotection of BOC group of BOC-gly-INH by trifluoroacetic acid (TFA) to form Glycine-INH.
4. Coupling of glycine-INH. & protected PAS by DCC to form protected PAS-gly-INH.
5. Deprotection of protected group in PAS using ethanaloic HCL (15%) solution under reflux.
6. Product purification by repeating recrystallisation using Petroleum ether 40-60: ethyl acetate mixture several times
7. Partition coefficient estimation: using octanol water mixture

Synthesis of the Analog: PAS-GLY-INH:

1) Synthesis of 2-acetoxy-4-acetomidobenzoic acid

A solution of acetic anhydride (5ml) was added to a suspension of 2-hydroxy-4-aminobenzoic acid (1.53 gm, 10 mmole) in dry benzene (10ml) mixture which was then refluxed for 2hr. After cooling the solid was filtrated off and recrystallized from acetic acid containing 20% acetic anhydride.Percent yield was 85%. M.p and RF are value listed in Table (1). (13)

2) Synthesis of BOC-gly-INH

To a stirred solution of Boc-glycine (1.75 gm, 10 mmole) in (8 ml) N, Ndimethyl formamide (DMF), N-methyl morpholine (NMM) (1.10 ml, 10 mmole) was added followed by stirring for 10 minutes. A solution of compound INH (1.37 gm, 10 mmole) in 8 ml DMF was added to the reaction mixture. The mixture was then cooled to (–15 ^{o}C), DCC (2.1 gm, 10 mmole) was added with stirring,the stirrer was continued for (120 hrs.) at (0 ^{o}C) and for (72 hrs.) at ambient temperature (20 ^{o}C). Ethyl acetate (50 ml) was added to the reaction mixture and then filtered to get rid of the precipitated N,N-dicyclohexylurea (DCU). The filtrate was evaporated to dryness under vacuum, and the residue was re-dissolved in ethyl acetate (50 ml), the excess DCU which was still adhesive on the residue was precipitated out and filtered. The clear filtrate was washed twice with (10 ml) HCl (0.1 N) solution, once with (10 ml) D.W, and with (10 ml) saturated NaCl solution using the separatory funnel. The ethyl acetate layer was dried using anhydrous magnesium sulfate, and then evaporated to dryness. The analog was recrystallized from (ethyl acetate: petroleum ether 40-60) mixture. percent yield was 80%, Physical appearance and RF value are listed in Table (1).

3) Synthesis of Glycine-INH (compound 3)

Compound BOC-gly-INH (0.904 gm, 4 mmole) was dissolved in the minimum volume of DMF and TFA (trifluro acetic acid) 6 ml was added at 0 ^{o}C for 5 min with continuous stirring. and kept at 30 ^{o}C for 30 min. Then drying under vacuum to remove completely TFA, re-crystallization was carried out using ethyl acetate/ether mixture (1:10). The percent Yield was 59%, Physical appearance, melting point and, Rf value are listed in Table (1).

4) Synthesis of protected PAS- Glycine-INH:

The same procedure for synthesis of BOC-gly-INH was carried out with a percent yield of 54%. By using 2mmole of protected PAS, 2mmole of glycine-INH, Physical appearance, RF value are listed in Table (1).

5) Synthesis of PAS-Glycine-INH:

(Protected-PAS-Glycine-INH) (2mmole) was hydrolyzed with 10ml of 15% ethanolic hydrochloric acid (35.5%) under reflux for 30-45 min. The excess of acid was neutralized with 25% ammonium hydroxide solution, re-crystallisation was done from 50% aqueous ethanol and percent yield was 50%. Physical appearance & m.p& IR spectra & Rf are list in Table (1) C.H.N analysis list in Table (1).

Partition Coefficient Estimation

Estimated λmax for the compound and the preparation of standard curve were shown in Table (3) & Fig.(1).

Preparation of octanol / water mixture is as follows: 10ml octanol was mixed with 10 ml D.W and shacked for 24 hr. using magnetic stirrer and standing as long as possible to complete separation, to obtain octanol saturated with water and water saturated with octanol. 3mg of analog was dissolved in 10ml octanol saturated with water, 1ml was taken and completed to 10 ml with octanol saturated with water, this solution was mixed with 10ml of water saturated with octanol in sepertaory funnel and shacked for 30 min and left to achieve equilibrium, the octanol layer was separated and the absorbance measured at 311 λmax. According to the equation obtained from standard curve for straight line, the concentration was calculated in octanol layer. Then we can calculate then the conc. in water saturated with octanol layer by following equation:

The conc. in water = total conc. used – conc. in octanol layer

Partition Coefficient Estimation, According to the Following Equation:

P.C = conc. of analog in Octanol sat. Water layer / conc. of analog in water sat.octanol.

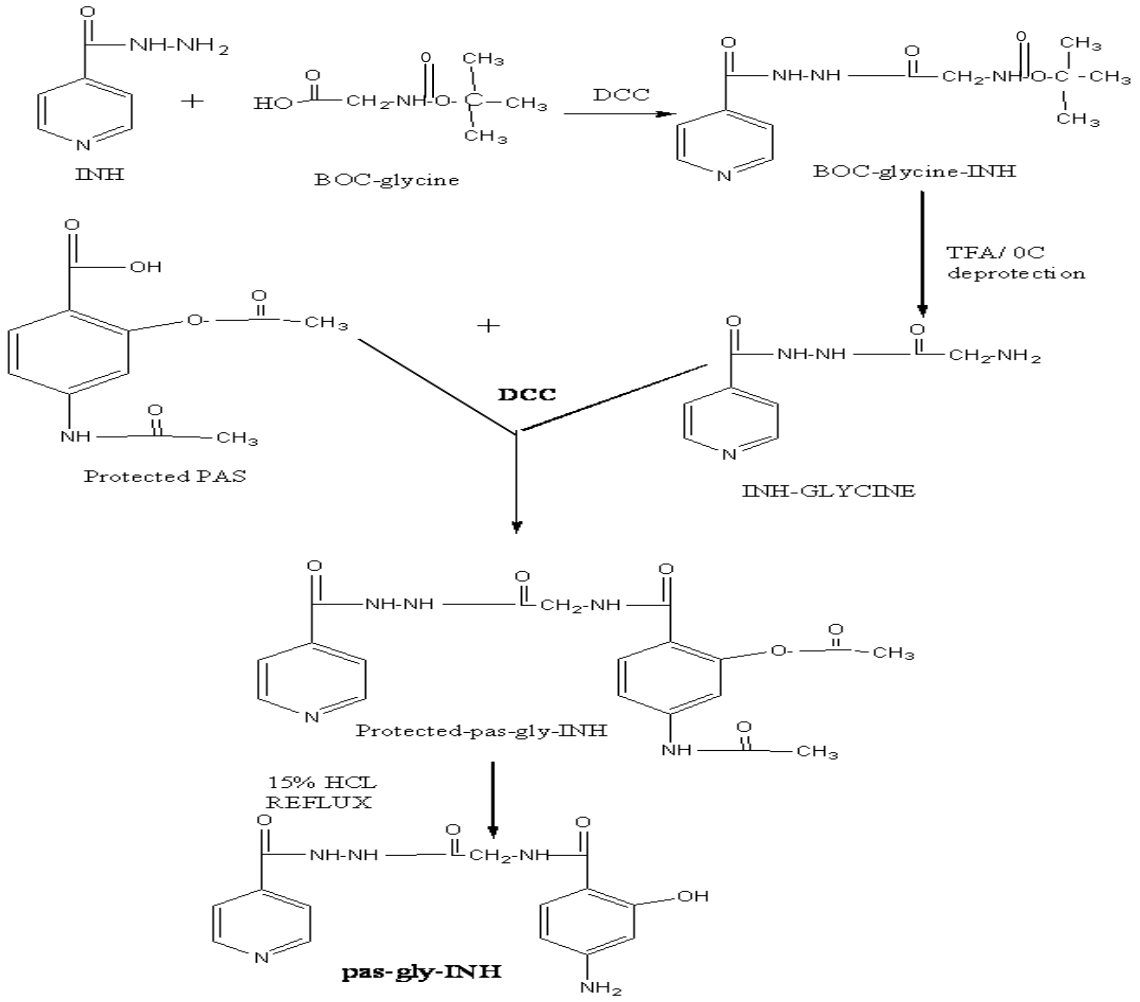
Log p values for the final analog and for the starting compounds (reported) are listed in Table (4)

Results and Discussion

The results of our work are shown in Scheme (1), Tables (1)-(3) and Fig. (1). The methodology that has been adapted in this work seems to be successful according to the result indicated previously, in addition partition coefficient estimation seems to be excepted to increase lipid solubility than original partition coefficient for pas & INH (+1.6, –0.8) respectively (14), in addition to that the synthesized prodrug may decrease gastric irritation for PAS (due to connect of free carboxyl group that responsible for gastric irritation with amine group of glycine also decrease extensive metabolism for INH (due to free amino group), the analog will hydrolyze by amidase enzyme.

Characteristic IR spectra of the prepared compound.

IR value	*Interpretation*
3487	Referred to O-H stretching of phenolic group.
3400	Referred to N-H stretching of 2nd amine.
3350&3331	Referred to N-H stretching of 1st amine.
3213	Referred to N-H stretching of 2nd amide.
2929&2853	Referred to C-H stretching of –CH2- group.
1668&1629	Referred to carbonyl stretching adjacent to –CH2- group, adjacent to benzene ring respectively, carbonyl group adjacent to benzene ring show less stretching vibration due to resonance and inductive effect of benzene ring.
1464&1367	Referred to C-H bending of –CH2- group
758&678&648	Referred to aromatic bending in and out of plane.



Scheme (1) : Synthesis of PAS-GLY-INH compound.

Table (1)
Physical properties of the intermediates & the analog Solvent systems:
A: Chloroform: Ethanol (3:7) C: Acetone: Ethyl acetate (1:2).
B: Chloroform: DMF (1:1) D: DMF: Methanol (1:2).

Compound name	*Physical appearance*	*Found m.p*	*Reported m.p*	*(A) RF value*	*(B) RF value*	*(C) RF value*	*(D) RF value*
2-acetoxy-4-acetomidobenzoic acid	White powder	194-196C	195C(13)	0.27	0.12	0.25	0.32
BOC-gly-INH	yellow powder	95-97C	–	–	–	0.06	0.37
INH-GLYCINE	yellow powder	191-193C	–	–	–	0.26	0.23
INH-GLY-protected pas	Clear oil	–	–	–	–	0.12	0.42
INH-GLY- pas	Yellow powder	137-138C	–	–	–	0.39	0.08

Table (2)
CHN elemental analysis of analog.

			Calculated/observed %		
Compound	*Molecular formula*	*Molecular weight*	*C*	*H*	*N*
INH-GLY- pas	(C15H15N5O4)	329	54.71 55.13	4.56 4.65	21.28 20.45

Table (3)
Show concentration and absorbance of prepared compound at 311nm.

CONC.(mg/ml)	*Absorbance at 311nm*
0.02831	0.624
0.0263	0.573
0.0222	0.498
0.0158	0.385
0.0126	0.301
0.0105	0.241

By application of the equation obtained for standard curve, we obtained the conc.from the absorbance obtained for octanol layer.

Absorbance = 0.456.

Total conc. Used = 0.03 mg / ml.

Conc in octanol layer = 0.0203 mg / ml.

Conc. in water layer = 0.0097 mg / ml

(Partition coefficient) p.c = Conc. in octanol layer/ Conc. in water layer

= 2.092.

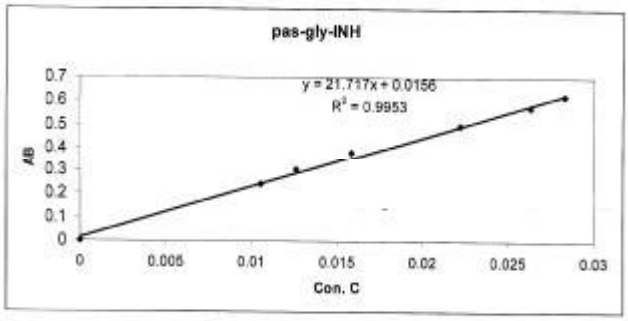
Log p = 0.32**.**

Table (4)
P.C & Log p for PAS and INH.

P.C & Log p for PAS	*P.C & Log p for INH*
P.c for PAS (reported) = 39. 8	p.c for INH (reported) = 0.158
Log p for PAS(reported) = +1.6(14)	Log p for INH (reported) = –0.8 (14)

Conclusion

The INH-GLY-PAS compound was synthesized successfully in pure form as confirmed by the sharp m.p & TLC results that showed only one spot for the product, in addition to I.R data and C.H.N micro analysis. Partition coefficient (p) estimation for analog was accepted according to Lipinski rule (15), to be less than 5 for good absorption. It is hoped that the analog may be acceptable since the carboxylic acid group of PAS & amino group of INH are not free, and this may give better absorption according to the estimated P.C.



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الخلاصة

أن مرض التدرن هو احد الأمراض المنتشرة عالميا و باستطاعته أن يفتك بالأشخاص المصابين بمرض العوز المناعي أن مدة العلاج غالبا ما تكون طويلة تتراوح من 3 اشهر إلى 9 أشهر و تحتاج إلى مزيج من العلاجات لتقليل مقاومة البكتريا.أن البعض من هذه العلاجات تحمل تأثيرات جانبية خطرة و خواص فيزوكمياوية غير مرغوب فيها مثل حامض الباراامينوسالسلك يعمل على تخدش جدار المعدة والمثال الأخر هو الايزونيازيد الذي يعمل على تسمم الكبد وتأثره السريع بعمليات الايض بواسطة أنزيم (إن–استيل ترانسفريس). لذلك باستخدام هذه الطريقة نتوقع التقليل من تأثير الباراامينوسالسلك أسد على جدار المعدة واختزال عمليات الايض السريع للايزونيازايد.

ان المركب المخلق صنع على شكل مركب امايد بواسطة ربط مجموعة ألامين الحرة للايزونيازايد مع مجموعة الكاربوكسيل من الطرف الأول للحمض الكلايسين وربط مجموعة الكاربوكسيل لحامض الباراامينوسالسلك مع مجموعة ألامين للطرف الثاني للكلايسين.ان عملية صنع مركب الامايد تمت بواسطة استخدام عامل الرابط (DCC) بواسطة طريقة الطور السائل وتم استخدام التقنيات التالية للتعرف على المركب الناتج مثل:قياس درجة الانصهار، كروماتوغلرافيا الطبقة الرقيقة، مطياف الأشعة تحت الحمراء، التحليل الدقيق للعناصر أن معامل الانتشار (P.C) للعقار المصنع هو أفضل أصلا من المواد المكونة له.