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SYNTHESIS AND BIOEVALUATION OF ALKYL DERIVATIVES AND PRODRUG OF PARACETAMOL TO REDUCE HEPATOTOXICITY

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ABSTRACT

Paracetamol, the most commonly used analgesic – antipyretic drug, damage multiple organs. The half life of Paracetamol is 1- 4 h which is very less and thus given in repeated high dose (Daily dose : 1000 mg per single dose and up to 4000 mg per day for adults). This lead to higher toxicity especially in liver and kidney. The toxicity of paracetamol is due to its metabolites, *N*-acetyl-*p*-benzoquinoneimine. Paracetamol overdose leads to the accumulation of NAPQI, which undergoes conjugation with glutathione. Conjugation depletes glutathione, a natural antioxidant. This directed cellular injury by NAPQI, leads to cell damage cause hepatotoxicity and even death. The formation of NAPQI can be minimized by reducing metabolism by CYP-450 which can be achieved by derivatization of paracetamol and also supplying a precursor of glutathione with drug reduced depletion of glutathione.

KEYWORDS- Paracetamol, Alkyl Derivatives, Prodrug, N-acetyl-p-benzoquinoneimine, glutathione.

INTRODUCTION

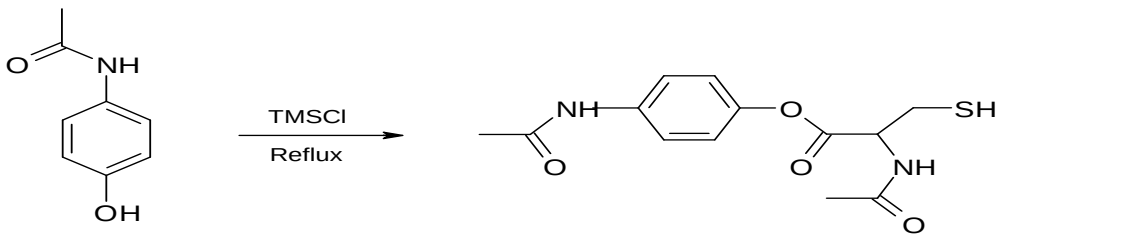
Drug discovery is a continuous process which aims at finding a drug better than currently available medications. A new drug must be able to improve current therapy and be able to fulfill many more and stricter requirements than does the lead compound. N-acetyl-p-benzoquinoneimine leads to cell damage in liver cause hepatotoxicity. Basis of these specific points in mind the present research work was undertaken on synthesize paracetamol derivative by substitution of alkyl group on N-position and preparation of prodrugs with amino acid those are precursors of glutathione like N-acetylcysteine, cysteine and methionine.

Synthesis of N-(4-methoxyphenyl)-N-methylacetamide

Into a dry three-necked round-bottomed flask, equipped with stirrer, reflux condenser and addition funnel introduced 0.2 mole of sodium hydride and 30 ml of xylene. Then added 0.1mole of paracetamol in 100 ml of boiling xylene and the mixture was refluxed for eight hours, under nitrogen atmosphere. After reflux reaction mixture was allowed to cool on the crushed ice and than 0.2 mole of methyl iodide was added and the reaction mixture was further refluxed for 8-10 h. The hot mixture was filtered, residue washed with 50 ml. of benzene and recrystallized with petroleum ether.

Synthesis of 4-(Acetylamino) phenyl 2-(Acetylamino)-3-sulfanyl ropanoate

N-Acetylcysteine (0.1 mol) was taken in a round bottom flask. Chlorotrimethyl silane (0.2 mol) was added slowly and stirred. Then paracetamol (0.1mole) was added and the resulting suspension was stirred at room temperature the progresses of reaction were monitored by TLC. The reaction mixture was concentrated on rotary evaporator to give amino acid ester hydrochloride.



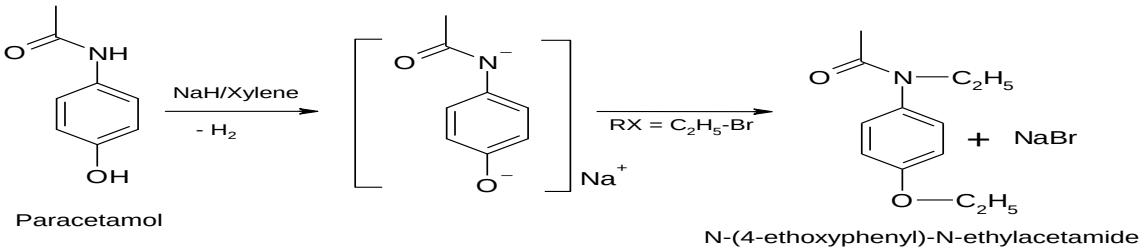
Paracetamol

4-(acetylamino)phenyl-2-(acetyl amino)-3-sulfanylpropanoate

Synthesis of N-(4-ethoxyphenyl)-N-ethylacetamide

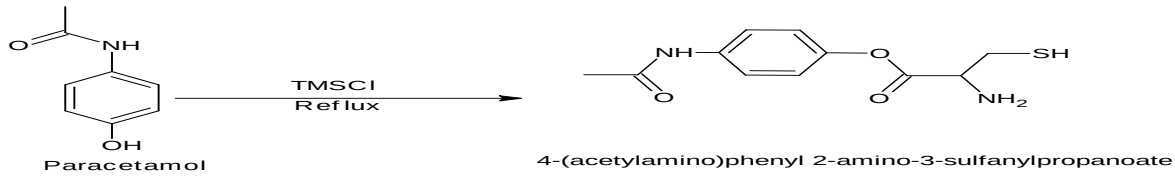
Into a dry three-necked round-bottomed flask, equipped with stirrer, reflux condenser and addition funnel sodium hydride (0.2 mol) and 30 ml. of xylene was introduced. Then added 0.1mole of paracetamol (0.1 mol) in 100 ml. of boiling xylene and the mixture was refluxed for eight hours, under nitrogen atmosphere.

After reflux reaction mixture was allowed to cool, ethyl bromide (0.2 mol) was added and the reaction mixture was refluxed for 8-10 hours. The hot mixture was filtered, residue washed with 50 ml. of benzene and recrystallized with petroleum ether.



Synthesis of 4-(acetylamino) phenyl 2-amino-3-sulfanyl propanoate

Cysteine amino acid (0.1mol) was taken in a round bottom flask. Chlorotrimethyl silane (0.2mol) was added slowly and stirred. Then paracetamol (0.1mol) was added and the resulting suspension was stirred at room temperature. The progresses of reaction were monitored by TLC. The reaction mixture was concentrated on rotary evaporator to give amino acid ester hydrochloride.



Synthesis of 4-(Acetylamino)phenyl 2-amino-4-(methylsulfanyl) butanoate

Methionine amino acid (0.1mol) was taken in a round bottom flask. Chlorotrimethyl silane (0.2mol) was added slowly and stirred with magnetic stirrers. Then paracetamol (0.1mol) was added and the resulting solution was stirred at room temperature. The progresses of reaction were monitored by TLC. The reaction mixture was concentrated on rotary evaporator to give amino acid ester hydrochloride.

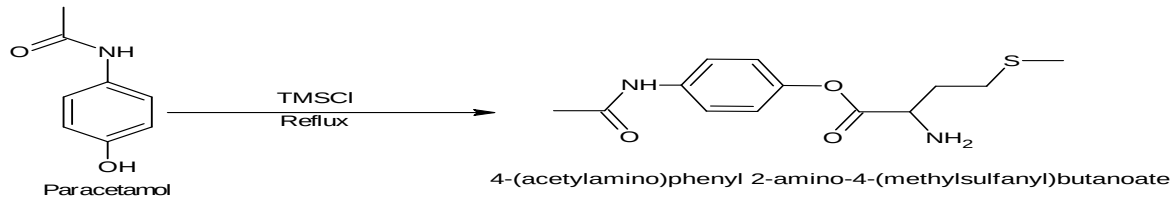


Table 1: Solubility data of synthesized compounds.

Comp.	10% HCl	10% NaOH	Ethanol	$CHCl_3$	Water	Benzene
PD-1	+	+++	+	++	--	--
PD-2	+	++	+	++	--	+
PD-3	++	+++	+++	+	+++	+
PD-4	++	+++	+++	--	+++	+
PD-5	++	+++	++	--	+++	++

Where

-- Insoluble

+ Slightly soluble

++ Soluble

+++ Freely soluble

Pharmacological Evaluation

From antipyretic study of synthesized compounds it was found that all compounds showed antipyretic activity. Antipyretic effect of synthesized compounds in comparison to paracetamol was found as follows:

Paracetamol > PD-2 > PD-3 > PD-5 > PD-4 > PD-1

Compound PD-3, PD-4 and PD-5 showed more efficient activity at 200 mg/kg dose. It reveals that higher concentration is required for causing antipyresis by these compounds as compared to paracetamol. Concurrently hepatotoxic potential study of synthesized compounds is performed on animal model by considering biochemical parameters (SGPT, SGOT, SALP), morphological parameter (Liver weight) and histopathological parameters. The observed hepatotoxic potential as compared to paracetamol was found as follows:

Paracetamol > PD-1 > PD-2 > PD-5 > PD-4 > PD-3

It show that alkyl (CH_3, C_2H_5) derivatives of paracetamol have antipyretic activity but also have higher hepatotoxic potential. Ester prodrugs of paracetamol with sulphar containing amino acid (N-Acetylcysteine, cysteine, methionine) have antipyretic activity and very less hepatotoxic potential than paracetamol.

Compounds PD-3, PD-4 and PD-5 were found to exhibit significant antipyretic activity and least hepatotoxicity.

Drug discovery is a continuous process which aims at finding a drug better than currently available medications. A new drug must be able to improve current therapy and be able to fulfill many more and stricter requirements than does the lead compound. Generally such requirements include

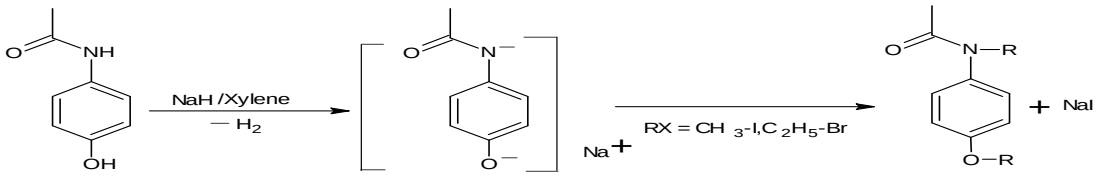
- A sufficiently high potency in order to keep dosage within reasonable limits
- A reasonable duration of effect
- Effectively on oral administration if oral dosing is required

- A reasonable selectivity in order to avoid unacceptable and undesirable side effects, and
- A reasonably low toxicity

It is long standing experience of medicinal chemist that all these properties can be improved *via* structural modification of the lead compound. The extensive literature review shows Paracetamol, the most commonly used analgesic – antipyretic drug, which used repeated high dose so its metabolites, *N*-acetyl-*p*-benzoquinoneimine leads to cell damage in liver cause hepatotoxicity. Basis of these specific points in mind the present research work was undertaken on synthesize paracetamol derivative by substitution of alkyl group on N-position and preparation of prodrugs with amino acid those are precursors of glutathione like N-acetylcysteine, cysteine and methionine.

The synthesis of the title compounds consist of the following schemes.

7.1 SCHEME I: SYNTHESIS OF PD-1 AND PD-2



SCHEME II: SYNTHESIS OF PD-3, PD-4 AND PD-5

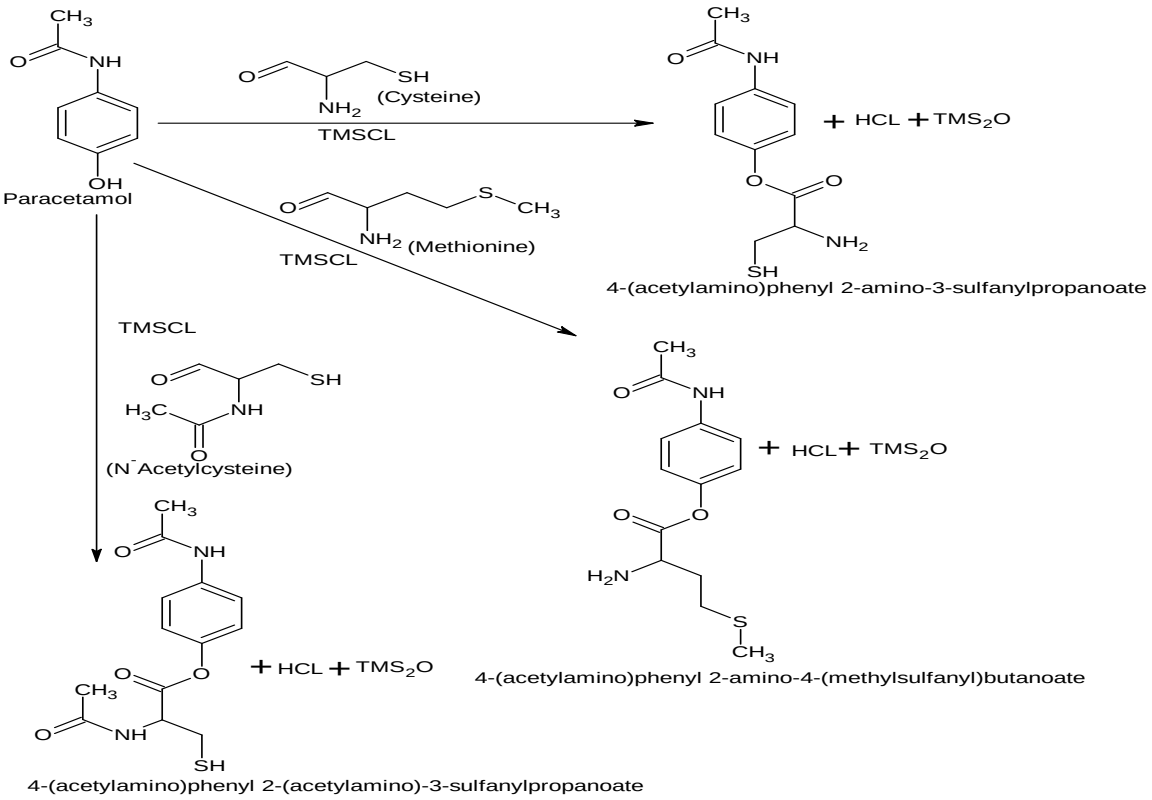


Table 2: List of Synthesized compounds.

S. No.	Code No.	Chemical name	%Yield	MP(^{0}C)
1	PD-1	N-(4-methoxyphenyl)-N-methyl acetamide	71	105-108ºC
2	PD-2	N-(4-ethoxyphenyl)-N-ethyl acetamide	78	93-95ºC
3	PD-3	4-(acetylamino) phenyl 2-(acetyl amino)-3-sulfanyl propanoate.	81	168-170ºC
4	PD-4	4-(acetylamino) phenyl 2-amino-3-sulfanyl propanoate	78	180-182ºC
5	PD-5	4-(acetylamino) phenyl 2-amino-4-(methylsulfanyl) butanoate	75	172-174ºC

The purity of compounds was ascertained by TLC and melting. The structures of synthesized compounds were confirmed on the basis of physical properties, IR and ^{1}HNMR.

Antipyretic Activity Evaluation

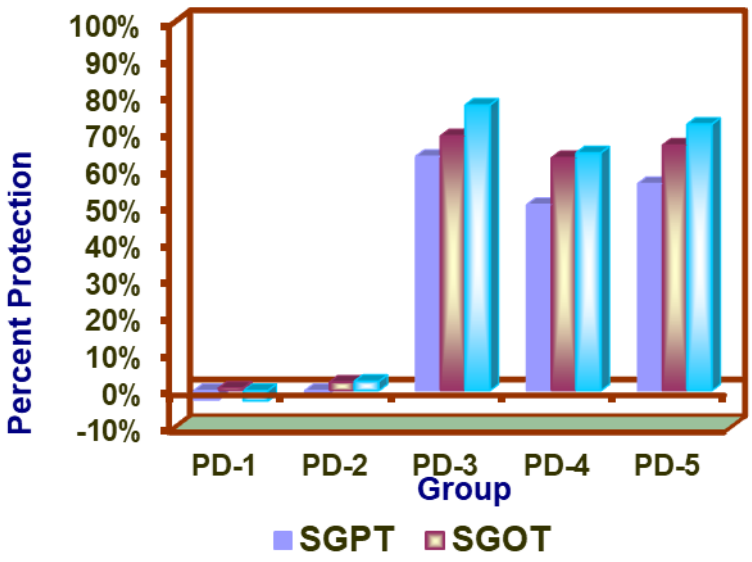
The antipyretic activity of synthesized compounds was evaluated using Brewer's yeast induced pyrexia in Wister rat's method. The results clearly demonstrated that all synthesized compounds have antipyretic activity but effectiveness was different, PD-1 have very less activity as compered to paracetamol.PD-2, PD-3, PD-4 and PD-5 showed significant antipyretic activity at 100 mg/kg and 200 mg/kg doses. PD-2 and PD-3 have the most potent activity than all other synthesized compounds.

Hepatotoxicity Evaluation

Hepatotoxic potential of synthesized compounds was determined by observing morphological parameters (Liver weight), biochemical parameters (SGOT, SGPT, SALP enzyme level in blood), histopathological parameters in albino rats and compared with paracetamol. The results obtained showed that PD-1 and PD-2 have higher hepatotoxic potential. PD-3, PD-4 and PD-5 have very less hepatotoxic potential in compression to paracetamol.

Table 2: effect of various Prodrugs in SGOT, SGPT, SALP enzyme level in blood.

	SGPT	SGOT	SALP
PD-1	-2.50%	0.65%	-2.80%
PD-2	-0.60%	2.30%	2.50%
PD-3	63.50%	69%	77.40%
PD-4	50.40%	63.10%	64.43%
PD-5	56.10%	66.50%	72.20%



CONCLUSION

At last it may be concluded that alkyl (CH_3, C_2H_5) derivatives of paracetamol have antipyretic activity but also have higher hepatotoxic potential. Ester prodrugs of paracetamol with sulphar containing amino acid (N-Acetylcysteine, cysteine, methionine) have antipyretic activity and very less hepatotoxic potential than paracetamol.

Compounds PD-3, PD-4 and PD-5 were found to exhibit significant antipyretic activity and least hepatotoxicity.

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