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Review Article

MICROWAVE ASSISTED SYNTHESIS AND ANTIMICROBIAL ACTIVITYOF SOME NOVEL HYDRAZONES

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ABSTRACT
Hydrazone and acylhydrazone derivatives have been considerable interest in the development of novel compounds with anticonvulsant, antiinflammatory, antidepressant, analgesic, antiplatelet, antimalarial, antimicrobial, antimycobacterial, anticancer activities. Hydrazones containing an azometine –NHN=CH- proton are synthesized by heating the appropriate substituted hydrazines/hydrazides with aldehydes and ketones in solvents like ethanol, methanol, tetrahydrofuran, butanol, glacial acetic acid, ethanol-glacial acetic acid. Hydrazide-hydrazones compounds are not only intermediates but also very effective organic compounds in their own right. When they are used as intermediates, coupling products can be synthesized by using the active hydrogen component of –CONHN=CH- azometine group.

Keywords: Hydrazones, arachidonic acid, Nabumetone, Naproxen, prostaglandins.

INTRODUCTION

Hydrazones and acylhydrazones possessing an azometine – NHN=CH- and O=C-NH-N=CH proton constitute an important class of compounds for new drug development. The biological profiles of compounds presenting this subunit are related to its relative acidity and its capacity to stabilize free radicals, mimicking bisallyl fragment of certain unsaturated fatty acids, for example arachidonic acid,contributing to inhibit the active site of oxidative catabolic enzymes cyclooxygenase (COX) and/or 5-lypoxygenase (5-LOX) which are responsible for the biosynthesis of prostaglandins, thromboxanes and leukotrienes (LTs) [1]

Several aspects of utilizing the hydrazine building blocks for the design of potential drug candidates can be outlined [2]. Despite hydrazines themselves are rarely thought as promising drug candidates, some successful examples of drugs possessing hydrazine moiety can be found,

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In view of the potentiality of hydrazone analogs which contain hydrazone pharmacophore, it has been planned to synthesize various substituted hydrazones containing other interesting structural features such as naphthyl methyl moieties associated with anti-inflammatory activity. These compounds are also structurally similar to Naproxen, Nabumetone [3].

METHODOLOGY

Synthesis of hydrazones

In a 20ml microwave vial, equimolar quantities of hydrazide and substitutes benzaldehyde were heated to 40°C via microwave irradiation for 5 minutes.
The mixture was then allowed to cool to room temperature and then an ice cold water was added.The precipitate was then collected and purified by recrystallization from ethanol affording the pure product as a white crystal compound. The physical and spectral data were presented [4]

Physiochemical data of Compounds

Compound A

Name	:	Benzaldehyde
Formula	:	C_7H_6O
TLC	:	R_f (9:1

Chloroform:Methanol) : 0.425
IR (KBr) : 3176 cm^{-1} (N-H), 2950 cm^{-1} (ArC-H), 1668 cm^{-1}

Compound B
Name : Salicylaldehyde
Formula : C7H6O2
TLC : Rf (9:1 Chloroform:Methanol): 0.4
IR (KBr) : 3047 cm^{-1} (N-H), 2941 cm^{-1} (ArC-H), 1621 cm^{-1}

Compound C
Name : Vanilin
Formula : C8H8O3
TLC : Rf (9:1 Chloroform:Methanol):0.595
IR (KBr) : 3062 cm^{-1} (N-H), 2851cm^{-1} (Ar-H), 1629 cm^{-1} (C=O),

Compound D
Name : paradimethyl amino benzaldehyde
Formula : C9H11NO
TLC : R_f (9:1 Chloroform:Methanol): 0.5
IR (KBr) : 3280 cm^{-1} (N-H), 3111 cm^{-1} (Ar-H), 1628 cm^{-1}

ANTIBACTERIAL ACTIVITY

It is evident that acyl hydrazones exhibit a pronounced anti bacterial activity. Therefore, it has been worthwhile to screen the synthesized substituted acyl hydrazones for antibacterial activities [6]

MATERIALS AND METHODS

Four bacterial test organisms, two Gram-positive bacteria: Bacillus subtilis, Staphylococcus aureus and two Gram-negative bacteria: Escherichia coli, and Klebsiella pneumoniae were selected and obtained from the Institute of Microbial technology. Cultures of test organisms were maintained on nutrient agar slants and were sub cultured in Petri dishes prior to testing. The media used was nutrient agar and nutrient broth procured from Himedia Laboratories, Mumbai [7]

Various substituted acyl hydrazones wre synthesized as described earlier. Stock solutions of the synthesized compounds were prepared inconcentrations of 100micrograms/ml using dimethyl formamide (DMF) as solvent for anti bacterial [8]

Procedure:

The antibacterial activity of title compounds has been assayed against four different strains of bacteria by agar diffusion method.

Two Gram-positive bacteria: Bacillus subtilis, Staphylococcus aureus and two Gram-negative bacteria: Escherichia coli, and Klebsiella pneumoniae.[9]

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial inhibition can be measured by two methods: one is serial dilution method and the other is diffusion method. The serial dilution method is very much useful for the determination of the antibacterial activity. The agar diffusion method is of three types.[10]

- Cup-plate method
- Filter-paper method
- Gradient plate method

The method adopted in this investigated was Cup-plate method.

Antibacterial activity was performed by cup-plate method by measuring zone of inhibition. All the test compounds were screened for antibacterial activity against bacterial strains *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* at a concentration of 10mg/10ml. Streptomycin was used as standard drug at a concentration of 1mg/ml.[11]

Culture medium:

Nutrient broth was used for preparation of inoculums of the bacteria and nutrient agar was used for the screening method.

Composition of nutrient agar medium

Peptone	2.0gm
Sodium chloride	5.0gm
Beef extract	3.0gm
Agar	20.0gm
Distilled water	upto 1000ml
pH	7.0

The nutrient agar medium was sterilized by autoclaving at 121ºC (15lb/sq inches) for 15min. The petriplates and other required glassware was sterilized in hot air oven at 160ºC, for an hour. Into each sterilized petriplate (10cm diameter), about 20ml of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6ml of inoculum to 300mlof nutrient agar medium) was transferred aspetically.[12]

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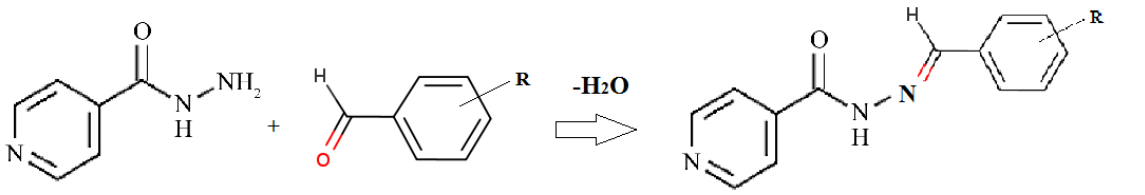
DMSO was used as a solvent control. Laminar airflow bench was swapped with 70% alcohol and UV lamp was switched on. After 30min, the UV lamp was switched off. All the reagents, media, inoculums and glassware were placed in laminar airflow bench observing all aseptic conditions. The plates were incubated within minutes of preparation of suspension, so that density does not change.[16] A sterile cotton swab over was dipped into the suspension and the medium was inoculated by even streaking of the swab over the entire surface of the plate in three directions. After inoculums had dried, cups of diameter 6mm were made in the agar plate with a sterile cork borer. The test samples (1mg/ml)were added to these cups with a micro pipette and plates were then incubated at 37ºC for 24hrs. The zone of inhibition was measured using mm scale (Deepthy Kohli et al.,2009) [17].

Scheme



Isoniazid (1) **Substituted aldehyde (2)** **Isoniazid substituted aldehyde hydrazone 3(a-d)**

Table 1. Substituted aldehyde

Compound	Substituted aldehyde	R
A	Benzaldehyde	-H
B	Salicyaldehyde	2-OH
C	Vanillin	4-OH,3-OCH_3
D	4-Dimethylamino benzaldehyde	4-$N(CH_3)_2$

Table 2. Batch and microwave conditions for hydrazones

Comp.no	Batch yield%	Batch time/h.	Microwave yield %	Microwave time/min.
3a	61	3	93	5
3b	57	3	91	5
3c	63	3	94	5
3d	69	3	93	5

Table 3. Anti bacterial activity of hydrazones

Compound	R	Zone of inhibition in mm			
		S.a	B.s	E.c	K.p
3a	-H	10	8	8	10
3b	**2-OH**	**18**	**19**	**19**	18
3c	**4-OH,3-OCH_3**	15	18	17	**19**
3d	4-$N(CH_3)_2$	15	18	17	16
Standard	Streptomycin	**21**	**20**	**22**	**23**

S.a – Staphylococcus auereus
B.s Bacillus Subtilis
E.c – Eischerichia coli
K.p – Klebsiellia Pneumonia

3a- Isoniazid benzaldehyde hydrazone
3b- Isoniazid salicylaldehyde hydrazone
3c- Isoniazid vanilin hydrazone

RESULTS AND DISCUSSION

Synthesis of hydrazones

The synthesis of hydrazones were obtained with in three hours at room temperature in good percentage yield using absolute ethanol as a solvent.continuation of our interest in the development of green organic synthesis, a microwave protocol was used as a simple and efficient protocol to achieve this new series of hydrazones in shorter reaction time and excellent percentage yield.[18]

The reaction was irradiated for five minutes at 40 C under solvent free conditions. The progress of the reaction was followed by TLC check.the product was obtained in an excellent yield within only 5 minutes.[19]

It is obvious from the results the performing the reaction under batch conditions using ethanol as a solvent provide 57-69% of the pure products while under solvent free conditions 90-95% of the pure products were obtained when the reaction accomplished using microwave irradiation.only five minutes was sufficient to obtain the products in high yield under microwave irradiation which was much better than the conventional batch conditions.[20].

CONCLUSION

In this, we reported solvent free ecofriendly synthesis of hydrazones under microwave condition.The advantage of this protocol is to avoid using of solvent and increase the percentage yield of the product. It is clear that synthesizing this series of hydrazones using microwave irradiation under solvent free conditions was found to be the optimal or most suitable reaction method which produces a higher product yield with shorter reaction time compared with the conventional batch method in the presence of ethanol as a reaction media.

The Antibacterial activity showed that the compound **3b** is more potent against *S.auerus, B.subtilis and E.coli* whereas compound **3c** showed highest activity aginst *K.pnemonia* organisms when compared to the standard Streptomycin indicating these novel hydrazones as antibacterial compounds.

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

No Interest

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