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MICROWAVE-ASSISTED SYNTHESIS AND EVALUATION OF SOME THIAZOLE ANALOGUES AS ANTITUBERCULAR AGENT

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ABSTRACT

Thiazoles and their derivatives have long been used as precursors for the synthesis of biologically active molecules because of the wide spectrum of activity shown by the thiazole moiety. Numerous thiazole compounds substituted with a different group of various positions have been prepared, despite this importance of 2-aminothiazoles have not been previously reviewed. In recent years, several new methods for the preparation of 2-aminothiazole derivatives and reactions have been reported, including waste free techniques. Various substituted phenyl thiazoles were synthesized by the reaction of substituted acetophenone and thiourea in the presence of iodine, where cyclization takes place and these thiazoles were further treated with isatin, those gave respective Schiff bases. The newly synthesized compounds were characterized by IR spectral studies and screened for antitubercular activity against *Mycobacterium tuberculosis* whereas antimicrobial activity was performed against *B.subtilis*, *E.coli,* and *C.albicans*. The findings of the present investigation highlights, the synthesized thiazoles possessing good anti-tubercular, anti-microbial activities due to the incorporation of secondary amines with the substituted thiazoles.

Key Words: Isatin, Substituted acetophenones, Antitubercular activity, Antimicrobial activity.

INTRODUCTION

Thiazole derivatives show a wide range of biological activities such as cardiotonic, fungicidal, sedative, anaesthetic, bactericidal, and anti-inflammatory. The synthesis of thiazole derivatives is important of their wide range of pharmaceutical and biological properties. The most straight forward procedure reported by Hantzch in 1887 involved condensation of halo ketones and thiourea or thioamides in refluxing alcohol. This method is long reaction and time consuming (24-25 hr) and Hantzch reaction [1-3]. Thiazole derivatives are known to exhibit biological activities such as bacteriostatic, fungistatic, antithrombotic, anaesthetic, antihypertensive and sedative. Some thiazole derivatives particularly 2-amino-4-aryl thiazoles have been showed to possess antitubercular activity, antimicrobial, anti-inflammatory [4-7]. Acetophenones prepared by known methods were condensed with thiourea in the presence of iodine to give 2-amino-4-aryl thiazoles. Isatins (1H-indole-2,3-dione) are synthetically versatile substrates, where they can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, as raw materials for drug substances [8,9]. Isatin has also been found in mammalian tissue & their function as a modulator of biochemical processes. Isatin was obtained as a product from the of oxidation of indigo by nitric and chromic acids. A Schiff's base named after Hugo Schiff is a compound with a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, not hydrogen. Schiff's bases in a broad sense have the general formula $R^1R^2C=NR^3$, where R^1 is an organic side chain. In this definition, Schiff's base is synonyms with azomethine. Some restrict the term to secondary aldimines (azomethine, where carbon is connected to a hydrogen atom), thus with the general formula $RCH=NR^1$. The chain on the nitrogen makes the Schiff base stable imines. Amine reacts with aldehyde or ketones to form imines (Schiff's base) [10-14].

MATERIALS & METHODS

All the solvents and chemicals were purchased from MERCK, NICE chemicals, and SD Fine Chemicals. Melting points were determined by using melting point apparatus MP-DS TID 2000v and the values were uncorrected. Reactions were monitored by Thin layer chromatography (TLC) on pre-coated silica gel G plates using iodine vapour as a visualising agent. IR spectra were recorded on SHIMADZU FT-IR 01504 spectrophotometer by using KBr pellets technique [15-17].

Step 1

Microwave assisted synthesis of 4-phenyl thiazol-2-amine

A mixture of Iodine (0.01mol) and thiourea (0.02mol) was triturated and this mixture was poured into a beaker containing acetophenone (0.01mol). To this mixture 25ml of ethanol was added and irradiated at 350W in the microwave for 1-5 min. The solid was washed with diethyl ether and saturated sodium thiosulphate solution. Finally, it was washed with distilled water and then dried. Purification was done by recrystallisation from ethanol to give S_1A. Compounds S_2H, S_3M, S_4C and S_5N were prepared by similar method [18].

Step 2

Microwave assisted synthesis of Schiff's base of isatin

Equimolar quantities of Isatin (0.01mol) and Para-substituted 4-phenylthiazole-2-amine (0.01mol) were added into 20ml of absolute ethanol containing a few drops of glacial acetic acid and irradiated at 200W in the microwave for 2-6 min. The solid was washed with distilled water and then dried. Purification was done by recrystallisation from ethanol [19-21].

Compound S_1A

IR (KBr,cm-1): 1463.97(ArC=C Str) 1612.49 (N=C Str) 3172.9(ArC-H Str) 754.17 (C-S Str) 1728.22 (C=O Str)3396.64 (N-H Str)

Compound S_2H

IR (KBr,cm-1): 1462.04 (ArC=C Str)1612.49(N=C Str)3176.76 (ArC-H Str)771.53 (C-S Str)1724.36 (C=O Str)3224.34(N-H Str)3037.89 (Ar-OH Str)

Compound S_3M

IR (KBr,cm-1):1463.97(ArC=C Str)1612.49 (N=C Str)3170.97(ArC-H Str)754.17(C-S Str)1728.22(C=O Str)3394.72(N-H Str)3282.84(Ar-OCH_3 Str)

Compound S_4C

IR (KBr,cm-1):1460.11(ArC=C Str)1616.35(N=C Str)3186.4(ArC-H Str)771.53(C-S Str)1728.22(C=O Str)3374.60(N-H Str)734.88(Ar-Cl Str)

Compound S_5N

IR (KBr,cm-1):1460.11(ArC=C Str)1614.42(N=C Str)3188.33(ArC-H Str)771.53(C-S Str)1201.65(C=O Str)3296.62(N-H Str)1460.11(Ar-NO_2 Str)

Screening for antitubercular activity

The Anti-mycobacterial activity of compounds was assessed against M. tuberculosis using Microplate Alamar Blue assay (MABA).This methodology shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on the plate. The final drug concentrations tested were 100 - 0.2 µg/ml. Plates were covered and sealed with paraffin and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue Reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink [6,13].

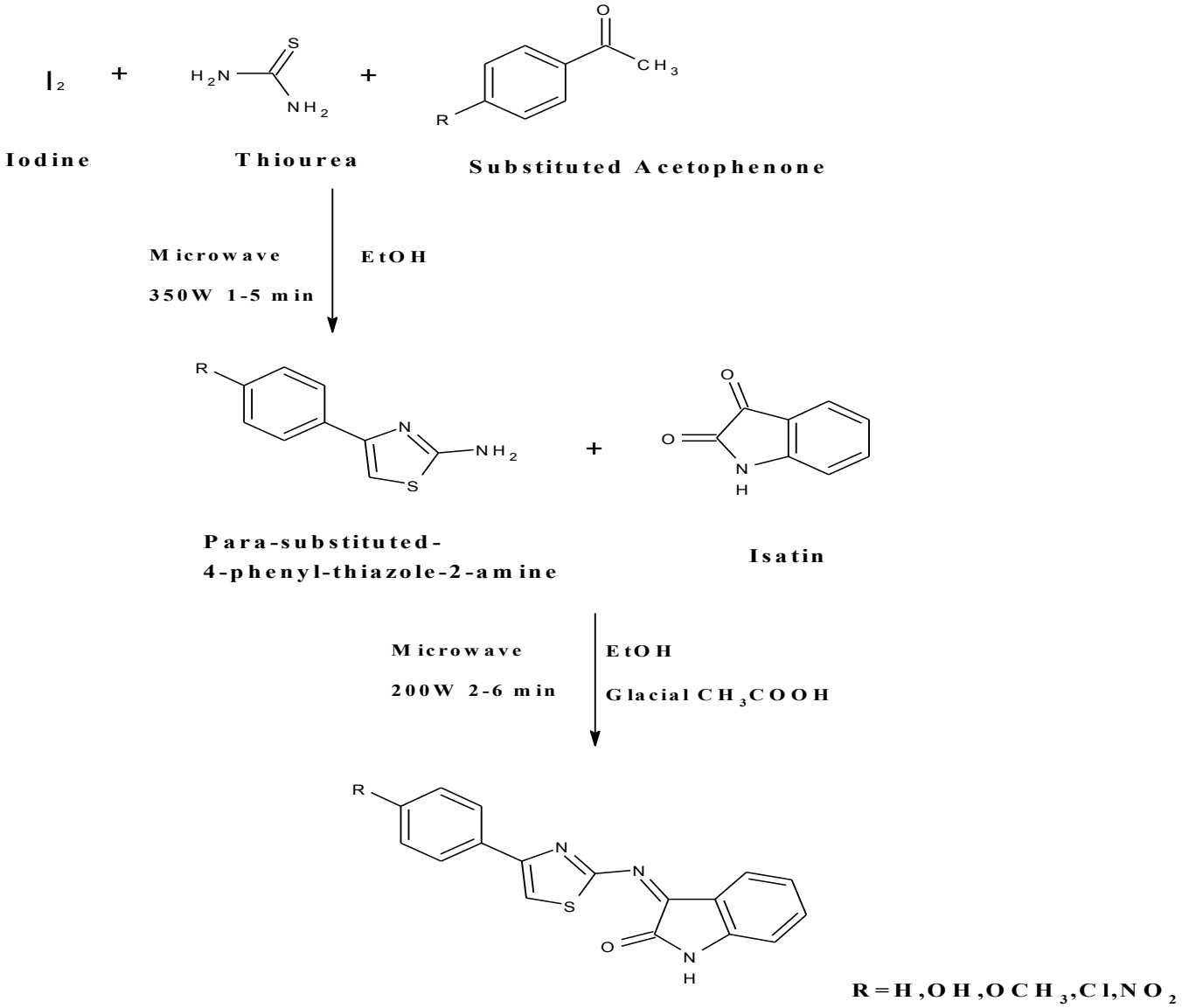
Antibacterial activity

The sterilized (autoclaved at 120^{o}C for 30 minutes) nutrient agar medium (40-50^{o}C) was inoculated with the suspension of microorganisms and mixture was transferred to sterile Petri dishes and allowed to solidify. In each plate or cups of 6mm diameter bores were made of equal distances using sterile steel borer. All the cups were filled with 0.1ml standard drug i.e. Gentamicin, 0.1ml phosphate buffer and 0.1ml synthesised compounds solution in DMSO respectively. Then plates were kept in the refrigerator for 30min and incubated at 37±2^{o}C for 24 hours. The diameters of zone of inhibition were measured and compared [8,10].

Antifungal activity

The sterilized (autoclaved at 120^0c for 30 minutes) Sabouraud dextrose agar (45-50^0c) was inoculated with the suspension of microorganisms and mixture was transferred to sterile Petri dishes and allowed to solidify. In each plate cups of 6mm diameter bores were made at equal distances using sterile steel borer. All the cups were filled with 0.1ml of standard drug i.e. Ketoconazole lotion, 0.1ml of phosphate buffer and 0.1ml of synthesised compounds solution in sterile DMSO respectively. Then plates were kept in the refrigerator for 30minutes and incubated at 37± 2^0C for 24 hours. The diameters were measured compared [11,13].

General Scheme



Synthetic route of titled compounds S_1A-S_5N

Table 1. Physiochemical properties of synthesized compounds

Compound Code	Chemical Name	Molecular Formula	Molecular Weight	Percentage Yield (Microwave)	Melting Point	R_f value
S_1A	(3*Z*)-3-[(4-phenyl-1,3-thiazol-2-yl)imino]-1,3-dihydro-2*H*-indol-2-one	$C_{17}H_{11}N_3OS$	305.35g	74.7	240-242	0.42
S_2H	(3*Z*)-3-{[4-(4-hydroxyphenyl)-1,3-thiazol-2-yl]imino}-1,3-dihydro-2*H*-indol-2-one	$C_7H_{11}N_3O_2S$	321.35g	72.3	230-231	0.36
S_3M	(3*Z*)-3-{[4-(4-methoxyphenyl)-1,3-thiazol-2-yl]imino}-1,3-dihydro-2*H*-indol-2-one	$C_{18}H_{13}N_3O_2S$	335.37g	73.5	254-256	0.38
S_4C	(3*Z*)-3-{[4-(4-chlorophenyl)-1,3-thiazol-2-yl]imino}-1,3-dihydro-2*H*-indol-2-one	$C_{17}H_{10}ClN_3OS$	339.79g	76.8	166-170	0.72
S_5N	(3*Z*)-3-{[4-(4-nitrophenyl)-1,3-thiazol-2-yl]imino}-1,3-dihydro-2*H*-indol-2-one	$C_{17}H_{10}N_4O_3S$	350.35g	78.9	178-180	0.36

Solvent System- Chloroform:Methanol (9:1)

Table 2. Screening of compounds for antitubercular activity

Sl. No	Compound code	Concentration in µg/ml							
		100	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.6**	**0.8**
1	**S_3M**	S	S	S	S	S	S	R	R
2	**S_1A**	S	S	S	S	S	S	S	R
3	**S_5N**	S	S	S	S	S	S	S	S
4	**S_2H**	S	S	S	S	S	R	R	R
5	**S_4C**	S	S	S	S	S	S	R	R
Standard	**Pyrazinamide**	S	S	S	S	S	S	R	R
	Streptomycin	S	S	S	S	S	R	R	R

S-Sensitive R- Resistant

Table 3. Screening of test compounds for antimicrobial study

Compound Code	Zone of Inhibition(mm)	
	Escherichia coli **(100µg)**	***Bacillus subtilis*** **(100µg)**
S_1A	24	28
S_2H	22	27
S_3M	26	30
S_4C	22	28
S_5N	27	35
Gentamicin	34	40
Control- PB P^H 7.4	-	-

Table 4: Screening of test compounds for antifungal activity

Compound Code	Zone of Inhibition (mm)
	***Candida albicans* (100µg)**
S_1A	27
S_2H	20
S_3M	21
S_4C	19
S_5N	22
Ketoconazole	30
Control -PB P^H 7.4	-

RESULT AND DISCUSSION

The compounds were synthesised in two steps process; in the first step, various substituted 2-amino phenyl thiazole was synthesised and in the second step, the Schiff's bases of Isatin was prepared. Infrared spectroscopy was taken for all the synthesised compounds. The characteristic absorption peaks were observed for all relevant groups. The absorption peaks around 1400 -1500 cm^{-1} indicates the formation of C=N Schiff's bases.

The synthesised compounds melting point and its reactants melting point were recorded by open capillary tube method are uncorrected. The techniques were performed for all synthesized compound and it gave a single spot whose R_F values are different from reactants. This ultimately shows that the compounds purity and completion of the reaction.

The synthesised compounds were evaluated for in-vitro antitubercular activity by Microplate Alamar Blue Assay Method. The result indicates the synthesised compound S_5N showed excellent activity in comparison with standard (pyrazinamide 3.125 microgram per ml/ streptomycin 6.25 microgram per ml). Antimicrobial activity of the synthesised compounds was evaluated by cup and plate method against *Bacillus subtilis, Escherichia coli* and *Candida Albicans*.

The maximum degree of activity was observed against *Escherichia coli, Bacillus subtilis* for the compounds S_5N and S_3M. The maximum degree of activity was observed against fungi *Candida albicans* for the compounds S_1A.In conclusion, the study concludes that among the synthesised compounds, S_5N and S_3M showed good activity against *E.coli* and *B.subtilis* respectively and S_1A showed remarkable activity against *C.albicans*. Hence, we can use these compounds as antimicrobial and antifungal agents in the future. Further studies are needed to make these compounds safe for the human consumption.

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