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Microwave Assisted Synthesis And Screening Of Benzimidazoles And Other Heterocyclic Compounds For Various Biological Activities.

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Abstract:

Various heterocyclic compounds especially with Benzimidazole and thiazolinones moieties were synthesized and investigated for their biological activities[1]

The increasing global tuberculosis burden due to the curse of HIV, MDR and XRD TB has led to the search of newer therapeutic agents to tackle the menance. Substituted benzaldehydes react with acetic anhydride at MWI at 350 W for 60 min to form substituted Aryl cinnamic acid derivatives by Perkin method. Then this substituted Aryl Cinnamic acid reacted with O-phenylene diamine at 350 W for 30 min to get corresponding Benzimidazole substitutes and which further reacted with thioglycolic acid at 350 MWI for 15 min to obtain corresponding 4-thiazolidinone[2,3,20]

This methodology offers distinct advantages of reduced reaction time as well as enhanced purity and yield.It is an environmentally safe synthesis as like in green chemistry. Green Chemistry to meet the fundamental scientific challenges of protecting the human health and environment while maintaining the commercial viability.The emerging area of green chemistry envisages minimum hazards as the performance crieteria while designing new chemical processes. So Microwave technology is advatagious because this method minimized by-product or waste as well as eliminating the use of conventional organic solvents.

The biological activities {antimicrobial, antitubercular, antifungal} were compared with standard drug[4]

Keywords: Environ. Safe, Antitubercular, Invitro, Benzimidazole .

Introduction:

Microwave-accelerated chemical synthesis in solvent as well as under solvent free conditions have witnessed an explosive growth .The technique has found widespread application predominantly exploiting the inexpensive unmodified household microwave (MW) ovens although the use dedicated MW equipment has been rapidly gaining ground of special interest is the environmentally friendlier solvent free approach that often involves explosure of neat reactants to MW irradiation. The rapid one-pot preparation of heterocyclic compounds from in situ generated reactive intermediates and the genral application to multicomponent reactions,that are adaptable for building a library of compounds has been accomplished using this MW technique.More recently,the stragety has been extended to combinatorial chemistry and the synthesis of newer class of solvents ,ionic liquids that are essentially molten with barely measurable vapour pressure. The sailent features of these high yield protocols namely the selectivity.

The increasing global tuberculosis burden due to the curse of HIV, MDR and XRD TB has led to the search of newer therapeutic agents to tackle the menance[1-4]

A survey of literature has revealed that compounds having nitrogen and sulphur and thiazole nucleus possesses interesting biological activities[17-18]

Thiazolidinones and Benzimidazole derivatives have acquired a special place in heterocyclic chemistry, since they possesses various biological activities including antibacterial activity. Because of their excellent activities, benzimidazole and its derivatives have a long history as antimicrobial agents. Several thousands of benzimidazole analogs have been synthesized and screened for pharmacological activity. They are of wide interest because of their diverse biological activity and clinical applications. These heterocyclic systems have different activities as they can act as bacteriostats or bactericides, as well as fungicides and they are present in numerous antiparasitic, antiprotozoal and antiviral drugs [21-23]. Some of 2-substituted benzimidazole derivatives were confirmed to have a moderate in vitro anticancer activity, as well as some of them exhibited antitumor activity against human hepatocellular carcinoma (HEPG2), human breast adenocarcinoma (MCF7) and human colon carcinoma (HCT 116) cell lines [24]. In continuation of studies on the inhibitory activities of benzimidazole derivatives [25-27]

Objective:

Various heterocyclic compounds especially with Benzimidazole and thiazolinones moieties were synthesized and investigated for their biological activities[1] Substituted benzaldehydes react with acetic anhydride at MWI at 350 W for 60 min to form substituted Aryl cinnamic acid derivatives by Perkin method. Then this substituted Aryl Cinnamic acid reacted with O-phenylene diamine at 350 W for 30 min to get corresponding Benzimidazole substitutes and

which further reacted with thioglycolic acid at 350 MWI for 15 min to obtain corresponding 4-thiazolidinone[2,3,20]

Purpose:

Despite the availability of highly potential antitubercular agents, tuberculosis remains primary cause of comparatively high mortality worldwide. The statistics shows that around three million people through out the world die annually from tuberculosis and today more people die from tuberculosis than ever before. Therefore, the development of new drugs with activity against multi drug-resistant (MDR) TB, extensively drug –resistant (XDR) TB, and latent TB is a priority task. Although new agents that will shorten the duration of current chemotherapy are also needed[1] A new dimension was added in the year 1980 due to the spread of HIV with high prevalence of tuberculosis and mycobact avium complex infection among the patients[2].

The present first line drugs like INH, pyrazinamide, ethambutol, and rifampicin are potent antitubercular agent. They act by inhibition of mycolic acid and RNA / DNA synthesis. They possess numerous adverse reactions[3]. To avoid these effects it seemed promising to look for more selective compounds, at other targets to suppress the activity[4]. Various derivatives of benzimidazole [5-7]exhibit interesting biological properties like antiviral ,antiprotozoal,antifungal,anthelmintic,antifilarial activities, hence attempt was made to synthesized benzimidazole and other heterocyclic derivatives for promising antitubercular activity. Microwave Methodology offers distinct advantages of reduced reaction time as well as enhanced purity and yield.It is an environmentally safe synthesis as like in green chemistry. Green Chemistry to meet the fundamental scientific challenges of protecting the human health and environment while maintaining the commercial viability.The emerging area of green chemistry envisages minimum hazards as the performance crieteria while designing new chemical processes. So Microwave technology is advatagious because this method minimized by-product or waste as well as eliminating the use of conventional organic solvents.

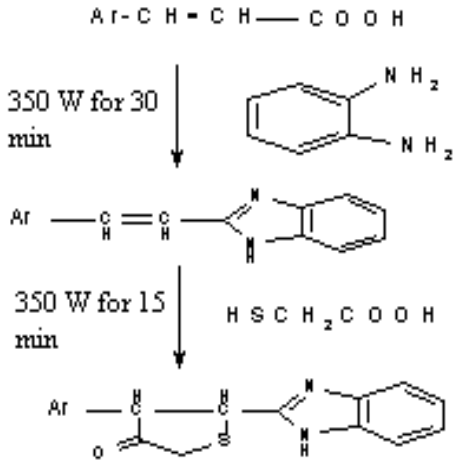
The biological activities {antimicrobial, antitubercular, antifungal} were compared with standard drug.

Material and Methods:

Experimental

Melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Thermo Nicolet IR 200 spectrophotometer using KBr disc method. The ^{1}H-NMR spectra were recorded on sophisticated multinuclear FT-NMR Spectrometer model Avance-III (Bruker), using dimethylsulfoxide-d_6 as solvent and tetramethylsilane as internal standard.

Proposed Scheme:



[Ar = phenyl, Chlorophenyl, Flurophenyl, Methylphenyl,Methoxyphenyl,Hydroxyphenyl,Furaldehyde**]**

Substituted benzaldehyde reacts with acetic anhydride at MWI at 350 W for 60 min to form Aryl cinnamic acid derivatives by Perkin method. Then this substituted Cinnamic acid reacted with O-phenylene diamine at 350 W for 30 min to get corresponding Benzimidazole substitutes and which further reacted with thioglycolic acid at 350 MWI for 15 min to obtain corresponding 4-thiazolidinone **[2,3,20].**

General method for preparation of cinnamic acids.(Perkins Method).

A reaction mixture of Aromatic aldehyde (0.09 mole),Potassium Carbonate (0.065 mole) and Acetic anhydride (0.075 mole) was subjected to MWI at 350 W for 60 min.Completion of reaction was monitored by TLC.Workup the reaction mixture involving pouring on ice cold water and its basification with 10% Ammonia and extraction with solvent ether to remove untreated aromatic aldehyde.Aqueous layer was treated with dilute hydrochloric acid .The precipitated solid obtained was washed with water dried and recrystalised to obtained (CA_1) **[2]**Yield -70.1%, m.p. -133-134^{0}C.

Synthesis of 2-(2-Phenyl ethenyl) – 1 H- benzimidazole:

A reaction mixture of aryl cinnamic acid (0.05 mol) and o-Phenylenediamine (0.05 mol) was treated with 4 N Hydrochloric acid and stirred at room temperature for 1hour. Until it goes into a solution then the reaction mixture was subjected to MWI for 350 W for 30 min then cooled and neutralized with dilute ammonia. The precipitate that separated was filtered and washed with water and crystallized from methanol to get solid crystal of pure (BZ_1) **[10]**yield – 82.5%, m.p. –173-174^{0}C.

Synthesis of 5-(1-H benzimidazole - 2 – yl)–4-Phenyl dihdrothiophene–3 (2H) –one

A reaction mixture of 2 (2-Phenyl ethenyl) 1-H- benzimidazole (0.01 mol) and thioglycollic acid (0.01mol) in 30 ml of dry benzene was subjected to MWI for 350 W for 15 min to obtain corresponding 4-thiazolidinone [2,3,20] The residue was washed with Sodium bicarbonate solution and the product was washed with water thoroughly and crystallized from alcohol to get solid crystals of pure (BT_1) [11-12]Yield- 86.1%, m.p. –98-100 ^{0} C.

Analytical data of synthesized compounds.

BT_1 : FT-IR(KBr) cm^{-1} : 3436 (-O-H free), 2918 (-Ar-C-H Str.), 1672 (-C=O str.), 1348 (-C=N Str.), 749 (-Ar-), 693 (C-S str).

^{1}H NMR ($CDCl_3$) : 7.22-7.59 (9H of Ar), 3.61 (2H of CH_2).

BT_2 :FT- IR(KBr) cm^{-1} : 3438 (-O-H free), 2919 (-Ar-C-H Str.), 1673 (-C=O str.), 1349 (-C=N Str.), 749 (-Ar-), 693 (C-S str).
^{1}H NMR ($CDCl_3$) : 7.22-7.59 (9H of Ar), 3.61 (2H of CH_2).

BT_3 : FT-IR(KBr) cm^{-1} : 3452 (-O-H str), 1552 (-C-NO_2 str.), 1348 (-C=N Str.),

BT_4 : FT-IR(KBr) cm^{-1} : 3229 (-O-H str), 1608 (-C=O str.), 1347 (-C=N Str.), 1225 (-CH_2-), 829 (-C-S str).

BT_5 : FT-IR(KBr) cm^{-1} : 2927 (-O-H free), 1684 (-C=O str.), 1430(-C-N Str.), 1252(-CH_2)
^{1}H NMR ($CDCl_3$): 6.95-7.59 (9H of Ar), 4.84 (2H of CH_2), 3.81 (3H of OCH_3) .

BT_6 : FT-IR(KBr) cm^{-1} : 2920(-O-H free), 1681 (-C=O str.), 1348 (-C=N Str.), 682 (-C-S Str.),
^{1}H NMR ($CDCl_3$): 7.19-7.60 (9H of Ar), 4.83(2H of CH_2), 2.34 (3H of CH_3).

BT_7 : FT-IR(KBr) cm^{-1} : 3356(-O-H free), 2832 (-Ar-C-H Str.), 1682 (-C=O str.), 1348 (-C=N Str.), 749(-C-S Str)

Antimicrobial activity

The antimicrobial activity of the synthesized compounds was determined by cup-plate method. The antibacterial activity was determined against gram-positive organism

Staphylococcus aureus and gram-negative organism *Escherichia coli* at 50-mcg/ml and 75mcg/ml concentration of sample compounds. Dimethyl Formanide was used as control. The bacteria were subcultured on nutrient agar broth and incubated at 37^0 C for 18-24 hrs. Standard antibacterial drug Ciprofloxacin was also screened under similar conditions at 50-mcg/ml and 75mcg/ml concentration for comparison. [30,31]

The antifungal activity was carried out against the fungi *Candida albicans* and A.fumigatus at 50 mcg/ml and 75mcg/ml concentration of sample compounds. The fungi were subcultured in Sabourod's dextrose agar medium. The fungal susceptibility testing was done by cup-plate method using Fluconazole (50 mcg/ml and 75mcg/ml concentration) as standard. The petridishes were incubated a 37^0 C for18-24 hrs.[Table-I]

Antitubercular evaluation

The antitubercular screening of synthesized compounds was carried out by middle brook 7H9 broth base (M198) medium against

H_{37}Rv strain at100 mcg/ml, 125 mcg/ml and 250mcg/ml. middle brook 7H9 broth base (M198) medium was inoculated with mycobacterium tuberculosis of H_{37}Rv strain. The inoculated medium was incubated for 37^0 C for 6 weeks. At the end of 6 weeks the growth of mycobacterium tuberculosis was read. [Table-II].

Streptomycin (100 mcg/ml, 125 mcg/ml and 250mcg/ml) was used as a standard drug[30,31]

Table-I Antibacterial and antifungal activity of synthesized compounds.

Compounds Code.	Zone of inhibition at 75 & 50 μg/ml (in mm.)							
	E.Coli		S.aureus		A. Fumigatus		C. albicans	
	75	50	75	50	75	50	75	50
BT_1	8	6	R	R	R	R	10	8
BT_2	11	6	10	9	R	R	15	10
BT_3	R	R	R	R	R	R	10	5
BT_4	7	6	R	R	R	R	12	10
BT_5	18	16	10	8	R	R	14	13
BT_6	10	6	11	9	R	R	16	12
BT_7	8	6	R	R	R	R	18	15
Ciprofloxacin	21	21	22	20	-	-	-	-
Fluconazole	-	-	-	-	23	24	23	23

Table-II Anti tubercular activity of the synthesized compounds.

Compound Code	100 mcg/ml	125 mcg/ml	250 mcg/ml
BT_1	**S**	**S**	**S**
BT_2	R	R	S
BT_3	**S**	**S**	**S**
BT_4	R	R	S
BT_5	R	S	S
BT_6	**S**	**S**	**S**
BT_7	R	R	R
Streptomycin	**S**	**S**	**S**

S denotes Significant and **R** denotes Resistant

Table-III Reaction time and % yield of Products by Conventional and Microwave Methods.

Sr No	Compound Name	Conventional Reported method			Microwave Method			TLC Mobile Phase
		Reaction time (min)	% yield	Melting point	Reaction time (min)	% yield	Melting point	
1	Cinnamic acid (CA_1)	240	62.3	133-134	60	70.1	133-134	Benzene: EA (8:2)
2	Benzimidazole (BZ_1)	120	63.2	171-172	25	82.5	173-174	EA: methanol (8:2)
3	Thiophenone (BT_1)	230	70.0	182-183	15	86.1	98-100	Hexane: Chloroform:benzene (1:2:1)

Table-IV Characterization of data of compounds.

Sr.No.	Compounds	Melting point	Yield %
1	BT_1	98-100	86.1
2	BT_2	99-100	79
3	BT_3	102-103	82

4	BT_4	97-99	85
5	BT_5	98-100	84.2
6	BT_6	98-99	74
7	BT_7	101-103	87.9

Result And Discussion

The title compounds (BT_1-BT_7) were prepared from 2(2-substituted phenyl ethenyl) 1H benzimidazole by Microwave technology. [Table-IV] The structures of the compounds were confirmed by spectra and analytical studies. All the compounds synthesized matched with spectral data.

The compounds synthesized were screened mainly for the antitubercular activity by using middle brookMedia method using $H_{37}Rv$ strain. Compounds BT_1, BT_3, and BT_6 have shown very significant antitubercular activity when compared with the standard drug Streptomycin. Remaining compounds have also shown moderate antitubercular activity. (Table-II).

The compounds synthesized were also screened for antibacterial and antifungal activities by cup-plate method. Compounds BT_5, have shown significant antibacterial activity against gram-negative organism *Escherichia coli* standard drug Ciprofloxacin was used and BT_6 and BT_7 have shown significant antifungal activity against *Candida albicans* standard drug Fluconazole was used. (Table-I).

All compounds found to be very good antitubercular agents & present synthesized compound can definitely as lead compound for future molecular manipulation studies[8-10]

Comparitive study of Microwave and Conventional technology done and microwave technology is advantageous over conventional method.(Table-IV). And it is safe environmentaly safe as Green Chemistry to meet the fundamental scientific challenges of protecting the human health and environment while maintaining the commercial viability.The emerging area of green chemistry envisages minimum hazards as the performance crieteria while designing new chemical processes. So Microwave technology is advatagious because this method minimized by-product or waste as well as eliminating the use of conventional organic solvents. Microwave Methodology offers distinct advantages of reduced reaction time as well as enhanced purity and yield. It is an environmentally safe synthesis as like in green chemistry.[12-15]

Conclusion:

The present work was intended to study on the Microwave assisted synthesis and evaluation of certain Benzimidazole and Thiophen derivatives for antitubercular, antifungal and antibacterial activities[33-35]

Microwave heating, being specific and instantaneous, is unique and has found a place in expeditious chemical synthesis domain as a valuable tool.

This methodology offers distinct advantages of reduced reaction time as well as enhanced purity and % yield. It is an environmentally safe synthesis as like in green chemistry.

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