TRIMETHYLSILYL CHLORIDE CATALYZED SYNTHESIS OF SUBSTITUTED BENZIMIDAZOLES USING TWO PHASE SYSTEM UNDER MICROWAVE CONDITIONS, AND THEIR ANTIMICROBIAL STUDIES

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ABSTRACT

A convenient method using TMSCl (20 mol %) and microwave-induced technique for the synthesis of various benzimidazole is described. This has reduced the reaction time drastically as well as improved the yield when compared to conventional heating. The synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activities against four strains each. Preliminary results indicated that, compounds **3e, 3f, 3g, 3k, 3m, 3n** and **3o** demonstrated very good antimicrobial activity, comparable to the first line standard drugs. The most effective compounds have exhibited activity at MIC of 6.25 µg/mL.

Keywords: Microwave, TMSCl, Benzimidazole, Antibacterial activity, Antifungal activity.

INTRODUCTION

Benzimidazole moiety is of great importance to chemist as well as biologist as it found in a large variety of naturally occurring compounds and also chemically useful molecules having diverse biological activities. Compounds that exhibit the functionality of benzimidazoles have been used in the area of pharmaceuticals[1-5]. These high profile applications of compounds with benzimidazoles structures have prompted extensive studies for their synthesis. Methods of benzimidazole synthesis include the condensation of 1,2-phenylenediamine with carboxylic acids or their derivatives in the presence of strong acids such as poly phosphoric acids[6]. In another method, benzimidazole has been prepared by classical cyclocondensation of 1,2-phenylenediamine with the corresponding aldehydes under oxidative conditions[7-8]. Recent publications have shown synthesis of substituted benzimidazole via tosylation of N-aryl amidoxime[9], synthesis of benzimidazole using air as an oxidant[10] and using microwave irradiation[11]. However, most of the process suffers limitations such as low yields, tedious workup procedure and co-occurrences of several side reactions. As a consequence, the introduction of novel method and further work on technical improvements to overcome the limitation is still an important experimental challenge.

In continuation of our research work on the development of useful synthetic methodologies, we have observed that benzimidazole can be synthesized efficiently by treatment of o-phenylenediamine with carboxylic acid using Trimethylsilyl chloride (TMSCl) in good yields. In this study we wished to report the use of Trimethyl silylchloride for the synthesis of various benzimidazoles. During the research, we found that TMSCl could catalyze the synthesis of benzimidazole in a selected pair of solvents.

Pharmacology

All the title compounds were subjected to *in vitro* antibacterial and antifungal screening against pathogenic strains using ampicillin and itraconozole as standards, respectively. Their MIC values were determined.

3. CONCLUSION

In conclusion, an easier approach for the synthesis of substituted benzimidazoles has been explored by using Trimethylsilyl Chloride (TMSCl) in catalytic amount. The salient features of this method include: comparatively simple procedure, easy purification and high percentage of conversion. The research study reports the successful synthesis and antimicrobial activity of substituted benzimidazole carrying biologically active group. Their antimicrobial study revealed that presence of substituted phenyl ring at position **2** of benzimidazole might be responsible for increased antimicrobial activity in synthesized title compounds. In this synthesis we made use of microwave initiator, which not only made reaction time less but also gave very good yields. Work aimed at investigating further scope of the reaction is currently being pursued.

Biological results and discussion

The investigation of antibacterial screening data revealed that compounds **3g, 3k, 3n** and **3o** showed comparatively very good activity against all the four pathogenic strains (MIC-6.25µg/mL) in DMSO. The investigation of antifungal screening data revealed that compound **3f** showed a very good activity against all the four pathogenic strains (MIC-6.25 µg/mL) in DMSO and **3e, 3m** and **3o** showed moderate activity (12.5 µg/mL) in DMSO. Compound **3o** shows antibacterial as well as antifungal activity enhanced activity is due to the presence of substituted phenyl ring at position **2** of Benzimidazole derivatives.

Early preparations of substituted benzimidazoles from phenylenediamine and carboxylic acids or carboxylic acid derivatives were carried out under vigorous dehydrating conditions[12]. Long reaction times for this reaction have been reduced by the use of microwave heating. During the course of our studies, for the preparation of different benzimidazoles we have tried with different organic solvents in conventional method, but we have not been observed much improvement in the yield and rate of reaction. Finally we have tried a biphasic system by mixing the organic solvent along with water in a microwave and we observed little change in the yield and for further increasing the yield and rate of the reaction by adding TMSCl. We have got satisfactory yield and reduced the reaction time by using microwave conditions. With the initial success of this reaction, to test the general scope and versatility of this procedure in the synthesis of a variety of substituted benzimidazoles, we examined a number of differently substituted phenylenediamines and different carboxylic acids. We used a standard set of conditions to test this new method. We optimized a simple, microwave-based procedure for the preparation of benzimidazoles by cyclization of o-phenylenediamine and carboxylic acids, (scheme 1) this we have achieved by reducing the reaction time required and enhancing the yield of the process by using TMSCl as catalyst. In order to establish the optimum condition for this reaction, the effect of solvent was studied, (table 1) DMF was best among the solvents tested. The best yield was obtained with water / DMF (table 1). Clearly, DMF (entry 4) stands out as the solvent of choice, with its fast conversion and high yield. Next various ratios of TMSCl were examined (table 2). TMSCl was added in different ratios in different pair of solvents (aqueous phase along with an organic phase at 120°C in microwave as shown in the table 1). Very little of the desired product was obtained in the absence of TMSCl and the best yield were obtained with 20% TMSCl. The study revealed that even 20 mol% of the catalyst was sufficient to carry forward the reaction with minimal time period. The above conditions show that the diamine and acid bearing both electron donating and electron withdrawing substituent gave the desired benzimidazole in good yields. Thus equal moles of phenylenediamines and carboxylic acids were heated to 120°C in Microwave in DMF/water in presence of TMSCl (20 mol %) as catalyst.

The products were confirmed by the characterization studies like ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry. All the products exhibited physical and spectral properties in accord with the structures.

Table 3 shows both phenylenediamine and carboxylic acid bearing electron donating (entry 3,6&8) and electron withdrawing substituent (entry

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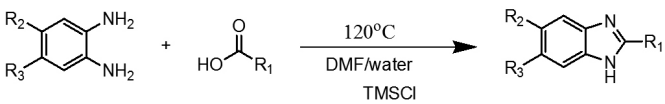
2,4,12&15) substituents gave desired benzimidazole in excellent yield.

In conclusion, an easier approach for the synthesis of substituted benzimidazoles has been explored by using TMSCl in catalytic amount. The salient features of this method include: comparatively simple procedure, easy purification and high percentage of conversion. Work aimed at investigating further the scope of the reaction is currently being pursued.



Scheme 1.
Synthesis of benzimidazole.

Table 1: Effect of solvent in cyclization of o-phenylenediamine and carboxylic acid.

Entry	Solvent[a]	Avg: time(min)*	yield[b]
1	CCl_4	35	35
2	THF	28	42
3	Xylene	40	45
4	**DMF**	**10**	**89**
5	DMSO	25	65
6	Dichloroethane	28	64
7	Benzene	25	70
8	water[d]	90	40
9	None[e]	20	15

[a] *Organic solvent / water;* [b] *Isolated yield after silica gel chromatography;* [d] *Only in aqueous phase;* [e] *Solvent free condition; *All reactions carried out with 20% TMSCl at 120°C.*

Table 2: Various ratios of TMSCl for the synthesis of benzimidazoles.

Entry	TMSCl (mol %)	Avg: time(min)	yield[a]
1	0	15	55
2	5	15	70
3	10	15	85
4	**20**	**15**	**89**
5	40	15	89
6	50	15	89
7	100	15	89

[a] All yields refer to isolated product.

Table 3: Synthesis of benzimidazoles catalyzed by TMSCl [a].

Entry	R1	R2	R3	Reaction time (min)	Yields [b] (%)
1	Br	H	H	10	86
2	H	NO_2	H	12	90
3	H	NH_2	H	10	81
4	H	Cl	H	15	89
5	$ClCH_2$	H	H	12	92
6	$CH_3(CH)CH_3$	H	H	15	75
7	CH_3	COOH	H	20	78
8	H	CH_3	CH_3	10	93
9	$(CH_2)_3OH$	H	H	15	80
10	Cl	H	H	17	85
11	C_6H_5	H	H	18	90
12	NO_2PhOCH_2	Br	H	20	92
13	$C_6H_4NH_2$	H	H	20	81
14	C_6H_5	SO_3H	H	17	76
15	$OCH_3C_6H_4$	NO_2	H	23	80
16	H	H	H	12	92

[a]The reaction was carried out with 1.0 eq of phenylenediamine, 1.0 eq of carboxylic acid and 20mol% TMSCl in 10 mL of DMF and 10 mL of water; [b] Isolated yield after silica gel chromatography.

EXPERIMENTAL

2.1. General.

^{1}H NMR spectra were recorded on a Bruker 300-MHz spectrometer in the solvent indicated with TMS as the internal standard. All chemical shifts are given in ppm and the coupling constants are given in Hz. For column chromatography Kieselgel 60 (ROCC, 0.040-0.060 mm) was used. Except TMSCl (Spectrochem) and DMF (Sonia industries), all phenylene- diamines and all carboxylic acids were obtained from Aldrich and used as such. Microwave-assisted syntheses were performed in single mode Biotage Initiator. The microwave reaction was carried out in a vial type. The absorption level was kept high with a pre-stirring of 30 s and stirring rate at 600 rpm. Initially the power was kept zero, followed by a gradual increase to 150W, the reaction proceeded at constant power of 30W with a temperature of 120°C and being measured using infrared sensor and 2-bar pressure the microwave irradiation was continued at 30W for the time indicated in table3.

2.2. General experimental procedure.

1,2-phenylenediamine (1 eq), carboxylic acid (1 eq) and TMSCl (0.2 eq) were taken in a microwave Pyrex tube which was introduced into a Biotage Initiators 60 microwave reactor fitted with a rotational system. DMF (10 mL) and water (10 mL) were added to it and heated in the microwave at 120°C for the time indicated (table 3). Reaction was monitored by TLC. After the completion of the reaction, reaction mixture was cooled to room temperature and ammonia solution was added to the reaction mixture to make alkaline (pH 9-10). It was then extracted with ethyl acetate (25 mL × 2) and separated organic layer was dried over Na_2SO_4. Organic layer was filtered and concentrated under vacuum, and the residue was purified by flash column chromatography on silica gel.

4.3 Antibacterial studies

The synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (recultured) bacterial stains by serial plate dilution method [13]. Serial dilutions of the drug in Mueller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37°C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

A number of antimicrobial discs are placed on the agar for the sole purpose of producing zone of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for an hour. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentration of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Antibacterial activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with ampicillin as standard. Zone of inhibition was determined for **3(a-o)** and the results are summarized in Table 4.

4.4 Antifungal studies

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flovus* (NCIM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method[14]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal stain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37°C for 1 h. Using a punch, wells were made on these seeded agar plates minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with Itraconozole as standard. Zone of inhibition was determined for **3(a-o)** and the results are summarized in Table 5.

Table 4: Antibacterial activity of the title compounds 3(a-o).

Compound	MIC in µg/mL and zone of inhibition in mm			
	S.aureus **(ATTC-25923)**	***E. coli*** **(ATTC-25922)**	***P.aeruginosa*** **(ATTC-27853)**	***K.pneumoniae*** **(recultured)**
3a	--	--	--	--
3b	--	--	--	--
3c	--	--	--	--
3d	--	--	--	--
3e	--	--	--	--
3f	--	--	--	--
3g	6.25(6-12)	6.25 (6-12)	6.25(6-12)	6.25(6-12)
3h	--	--	--	--
3i	--	--	--	--
3j	--	--	--	--
3k	6.25(6-12)	6.25 (12-16)	6.25(6-12)	6.25(6-12)
3l	--	--	--	--
3m	--	--	--	--
3n	6.25(6-12)	6.25 (6-12)	6.25(6-12)	6.25(6-12)
3o	6.25(6-12)	6.25 (6-12)	6.25(6-12)	6.25(6-12)
Ampicillin	10.0(16-22)	6.25(8-16)	6.25(16-22)	10.0(16-20)

Note: The MIC values were evaluated at concentration range, 6.25-12.5 µg/mL. The figures in the table show the MIC values in µg/mL and the corresponding zone of inhibition in mm; (--), Resistant (lack of inhibitory activity).

Table 5: Antifungal activity of the title compounds 3(a-o).

Compound	MIC in µg/mL and zone of inhibition in mm			
	P.marneffei **(recultured)**	***T.mentagrophytes*** **(recultured)**	***A.flavus*** **(NCIM No. 524)**	***A.fumigatus*** **(NCIM No. 902)**
3a	--	--	--	--
3b	--	--	--	--
3c	--	--	--	--
3d	--	--	--	--
3e	12.5(6-12)	12.5(6-12)	12.5(6-12)	12.5(6-12)
3f	6.25(8-16)	6.25(8-16)	6.25(8-16)	6.25(8-16)
3g	--	--	--	--
3h	--	--	--	--
3i	--	--	--	--
3j	--	--	--	--
3k	--	--	--	--
3l	--	--	--	--
3m	6.25(8-16)	6.25(8-16)	6.25(8-16)	6.25(8-16)
3n	--	--	--	--
3o	12.5(6-12)	12.5(6-12)	12.5(6-12)	12.5(6-12)
Itraconozole	10.0(16-20)	12.5(8-16)	6.25(16-20)	10.0(16-20)

Note: The MIC values were evaluated at concentration range, 6.25-12.5 µg/mL The figures in the table show the MIC values in µg/mL and the corresponding zone of inhibition in mm.; (--), Resistant (lack of inhibitory activity).

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