Efficient and Green Procedure for the Synthesis of Novel Sydnonimines as Antimicrobial Agents

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ABSTRACT

A series of substituted sydnonimine hydrochlorides has been synthesized by nitrosation of 3-aryl substituted glycinonitriles under microwave irradiation in presence of dry hydrogen chloride gas. The present ecofriendly procedure represents an alternative to the existing methods for synthesis of some novel sydnonimines. The structures of the investigated sydnonimines have been elucidated using infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy and their biological effects have been evaluated, in particular, their antimicrobial activities against gram positive bacteria, gram negative bacteria and fungi in the present paper.

KEYWORDS

Green chemistry, Microwave irradiation, Sydnonimines, antimicrobial activity

INTRODUCTION

Sydnonimines are members of a class of compounds known as mesoionic, and are specifically exo-imino analogs of sydnones [1-3]. These molecules have been studied extensively in terms of their chemical, physical, and biological properties and applications. Sydnonimines, like sydnones, do not occur in nature and have no known analogs among the natural and synthetic biologically active compounds presently known. Studies involving their biological activity have been the focus of considerable attention due to their high and diverse reactivity and the possibility that they might be converted under mild conditions into bioactive N-nitroso compounds or amino acids. Data on the antibacterial, antitumor, pharmacological, and biological activities of a number of sydnonimine derivatives have been collected [4-5]. Daeniker and Druey [6] and other workers [7-8] described biological activities like antibacterial, antifungal, insecticidal, analgesic, antipyretic, anti-inflammatory, antiallergenic, antiblastic, anti-tumor, and anti-hypertensive effects of sydnonimines, with the 3-benzylsydnonimine salt being particularly active. Along with those properties, some 3-arylsydnonimines display anti-inflammatory action [9]. 3-Aminosydnonimine salts containing 4-chloro, 4-bromo, or 4-alkylsubstituents) have a dilating effect upon the coronary artery [10-14]. Spasmolytic activity also has been demonstrated in 3,4-diaryl and 3-amino sydnonimines [15-16].

Literature survey revealed that more research work has been carried out so far on the conventional methods of synthesis of sydnones and sydnonimines but less attention was found to be paid for their eco-friendly synthetic procedures [17-18] and evaluation of their antimicrobial activity. Bearing all the above aspects, the present paper describes the synthesis of some novel sydnonimines using different efficient and environmentally benign techniques and also to their antimicrobial activity.

EXPERIMENTAL

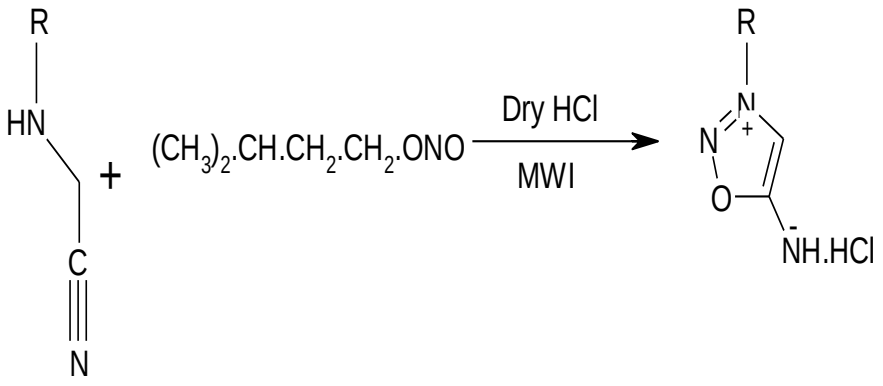
Materials and Methods

All the chemicals and solvents used for the synthesis were of analytical grade. The solvents were purified by standard methods. The infrared spectra of the ligands and metal complexes were run as KBr discs in the range 4000- 400 cm-1 on a Shimadzu Infrared Spectrophotometer. Electronic spectra in the solid state as well as in solution were recorded on a Shimadzu UV-160, UV-visible spectrophotometer. Melting points were measured on an Electro-thermal 9100 apparatus and are uncorrected. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using NMR Varian-Mercury 300 MHz spectrometer with Tetramethylsilane (TMS) as an internal standard. The chemical shift was measured in ppm on the δ scale and coupling constant was measured in Hertz.

Elemental analyses were recorded on a Carlo-Erba EA1110CNNO-S analyzer. Microwave assisted synthesis were carried out in open glass vessel on a modified microwave oven model 2001 ETB with rotating tray and a power source 230 V, at output energy of 800W and 2450 MHz frequency. A thermocouple device was used to monitor the temperature inside the vessel of the microwave. The microwave reactions were performed using on/off cycling to control the temperature. The completion of reaction and purity of the products were monitored by performing TLC and melting points. Thin layer chromatography (TLC) was carried out on silica gel plates (Fluka-Kieselgel, 0.2 mm thickness) and the plates were scanned under 254 nm ultraviolet light.

Green approach of Synthesis of Sydnonimines

A molar solution of the respective glycinonitrile (7.5 mmol) in PEG-300 was added two molar isoamyl nitrite (15 mmol) at 310K in microwave oven and stirred. After the completion of the reaction (8-12 minutes, TLC, solvent: PEG-300), the mixture was cooled in an ice bath and hydrogen chloride was bubbled through gently until the N-nitroso intermediate had completely reacted (usually 1-3 minutes, TLC) till coloured solid separated. Usually the crude coloured sydnonimine products crystallized out almost immediately following the addition of HCl gas. The hydrochloride salt were collected by vacuum filtration, washed with ethanol and recrystallized from absolute ethanol and their purity was checked by TLC on precoated silica gel plate (scheme-1).



I **II**

i. R= Ph, ii. R= *p*-tolyl, iii. R= *p*-methoxyphenyl, iv. R= *m*-chlorophenyl & v. R= *m*-bromophenyl

Scheme-1: *Synthesis of 3-Aryl Sydnonimine Hydrochlorides*

Antimicrobial activity: Zone of inhibition (Cup-plate method)

All the target compounds were tested for their antimicrobial activity by agar cup plate method [19]. The organisms used for antibacterial activity were gram positive bacteria: *S. aureus*, *B. subtilis* and *S. epidermis*, gram negative bacteria: *E. coli*, *S. typhi* and *P. aeruginosa* and the media were nutrient agar broth. The antifungal activity was performed against *A. niger* and *C. albicans* and the medium was potato dextrose agar. Amoxycilin and fluconazole were used as standards for antibacterial and antifungal activity respectively.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) against the above mentioned organisms was determined by the broth dilution method [20].

RESULTS AND DISCUSSION

The process used in this work to prepare the sydnonimine hydrochlorides **II** from the corresponding N-substituted glycinonitriles **I** was that already developed by Turnbull and Beal [21] under conventional methods. Their one-pot procedure was most efficient and the toxic N-nitroso species did not have to be isolated. **Table-1** gives the yields and physical characteristics of the sydnonimine starting materials **1-5** prepared from the corresponding aminoacetonitriles. The reaction times for nitrosation did not vary for the 3-aryl substituted glycinonitriles used and, in each case, after five to ten minutes at about 310K temperature under MWI all of the starting material was converted to the corresponding N-nitroso species by evidence of TLC. After the corresponding N-nitroso species had been made *in situ*, dry HCl gas was bubbled into the reaction using a small glass sparking tube to affect cyclisation to the corresponding sydnonimine hydrochloride. This process was environmentally benign process due to the one-pot procedure which accordingly proceed in a single reaction solvent (PEG-300), work-up, and purification step. Therefore, it is often associated with cost savings in terms of solvents, catalysts, and reagents in addition to time and effort.

In the infrared spectra of the sydnonimines, intense bands for the exocyclic C=N (1671-1700 cm^{-1}) and the medium bands at 3110 to 3205cm^{-1} due to the C(4)-H were used to confirm the identities of the sydnonimine salts. Further, confirmation was provided by the ^{1}H-NMR spectra of **i-v**, wherein a signal in the range 7.4-8.1 δ established the presence of the sydnonimine proton at the 4-position.

Table-1

Characteristic data of synthesized 3-arylsydnonimines (by green chemical method)

Compound	Colour	Molecular Formula	Melting Point	Reaction Time (in minute)		Overall Yield (%)	
				Conventional Method	Green chemical approach	Conventional Method	Green chemical approach
i	Light brown		177-179	25	6	78	95
ii	Brown		176-178	30	7	77	93
iii	Light brown		190-191	20	5	86	98
iv	Light gold		176-177	30	7	72	89
v	Light brown		187-189	54	10	12	52

Table-2

Analytical and Spectroscopic data of investigated compounds

Compound	Mol.Formula (Mol.Wt.)	Analytical results (%) Calcd (Found)			IR (KBr, cm^{-1})	^{1}H NMR (DMSO-d_6)(δ, ppm)
		C	H	N		
i	$C_8N_3H_7O.HCl$ (197.53)	48.60 (48.75)	4.05 (4.12)	21.26 (21.28)	1687cm^{-1} (exocyclic C=N), 1594cm^{-1} (immonium group) and 3134cm^{-1} (aromatic CH)	10.101 (s, 2H), 8.635 (s, sydnonimine C-H), 7.965 (s, 1H), 8.123-8.003 (t, 1H), 7.852-7.769 (m, 2H)
ii	$C_9N_3H_9O.HCl$ (211.53	51.05 (51.12)	4.72 (4.80)	19.85 (19.88)	1683cm^{-1} (exocyclic C=N), 1590cm^{-1} (immonium group) and 3156cm^{-1} (aromatic CH)	10.115 (s, 2H), 8.765 (s, sydnonimine C-H), 8.421(s, 1H), 8.075-8.013 (t, 1H), 7.815-7.658 (m, 2H) 2.38(s,3H, CH_3)
iii	$C_9N_3H_9O_2.HCl$ (279)	38.71 (38.80)	3.85 (3.88)	15.05 (15.12)	1684cm^{-1} (exocyclic C=N), 1590cm^{-1} (immonium group) and 3166cm^{-1} (aromatic CH)	10.103 (s, 2H), 8.758 (s, sydnonimine C-H), 8.313 (s, 1H), 8.033-8.010 (t, 1H), 7.641-7.586 (m, 2H) 4.36 (s, 3H, OCH_3)
iv	$C_8N_3H_6OCl.HCl$ (235)	40.85 (40.90)	2.97 (3.04)	17.87 (17.92)	1638cm^{-1} (exocyclic C=N), 1597cm^{-1} (immonium group) and 3166cm^{-1} (aromatic CH)	10.027 (s, 2H), 8.452 (s, sydnonimine C-H), 8.215 (s, 1H), 8.102-8.005 (t, 1H), 7.985-7.576 (m, 2H)
v	$C_8N_3H_6OBr.HCl$ (276.5)	34.71 (34.80)	2.53 (2.58)	15.18 (15.20)	1679cm^{-1} (exocyclic C=N), 1585cm^{-1} (immonium group) and 3168cm^{-1} (aromatic CH)	10.133 (s, 2H), 8.752 (s, sydnonimine C-H), 8.355 (s, 1H), 8.088-8.028 (t, 1H), 7.751-7.697 (m, 2H)

The observations related to antimicrobial activity of investigated compounds are presented in **Table-3 & 4** for bacteria and fungi respectively.

Table-3

Zone of inhibition against bacteria

Compound	Concentration µg /10µl	Zone of inhibition in mm (SEM)					
		Gram positive bacteria			Gram negative bacteria		
		S. aureus	*S. epidermis*	*B. subtilis*	*E. coli*	*S. typhi*	*P. aeruginosa*
Amoxycilin	30	26	27	29	27	25	28
i	50 100 150	16 17 13	17 15 18	15 19 14	19 14 15	19 13 15	18 15 17
ii	50 100 150	10 14 27	9 13 25	11 15 27	10 15 28	12 17 24	11 16 253
iii	50 100 150	8 12 23	9 13 19	10 11 20	11 14 22	7 15 21	8 13 20
iv	50 100 150	8 12 17	7 15 16	9 13 17	8 14 18	9 12 18	8 16 20
v	50 100 150	8 12 17	7 13 19	6 11 16	8 12 18	9 13 18	7 13 16

Table-4

Zone of inhibition against fungi

Compound	Concentration µg /10µl	Zone of inhibition in mm (SEM)	
		A. niger	*C. albicans*
Fluconazole	30	27	29
i	50 100 150	10 15 20	13 14 22
ii	50 100 150	12 18 25	15 20 30
iii	50 100 150	11 15 18	14 18 20
iv	50 100 150	15 12 15	20 15 18
v	50 100 150	19 15 18	21 18 21

The results related to minimum inhibitory concentration (MIC) against the above mentioned organisms are given in **Table-5**.

Table-5

Minimum inhibitory concentration for bacteria

Compound	**MIC (in µg per mL)**					
	Gram positive bacteria			**Gram negative bacteria**		
	S. aureus	***S. epidermis***	***B. subtilis***	***E. coli***	***S. typhi***	***P. aeruginosa***
i	23	25	26	29	31	34
ii	15	20	23	25	21	18
iii	12	12	12	12	12	12
iv	27	30	25	26	32	25
v	12	15	25	23	27	20

The data indicate that antimicrobial activity is affected by the nature of R. The results showed that all the compounds were moderately active against different strains of bacteria and fungi as compared to standards and compound **iii** exhibiting highest zone of inhibition and least MIC (12 µg/10µl)) against all the organisms.

CONCLUSIONS

A series of substituted sydnonimine hydrochlorides has been synthesized by nitrosation of 3-aryl substituted glycinonitriles under microwave irradiation in presence of dry hydrogen chloride gas. This procedure offers several advantages including mild reaction conditions, high yields, ease of workup, which makes it a useful and attractive protocol for the synthesis of these compounds. The present ecofriendly procedure represents an alternative to the existing methods for synthesis of some novel sydnonimines. The structures of the investigated sydnonimines have been elucidated using infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy and their biological effects have been evaluated, in particular, their antimicrobial activities against gram positive bacteria, gram negative bacteria and fungi in the present research project.

Conflicts of interest

The authors declare no conflict of interest.

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