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Synthesis, spectral, and anti-microbial studies of thioiminium iodides and amine hydrochlorides

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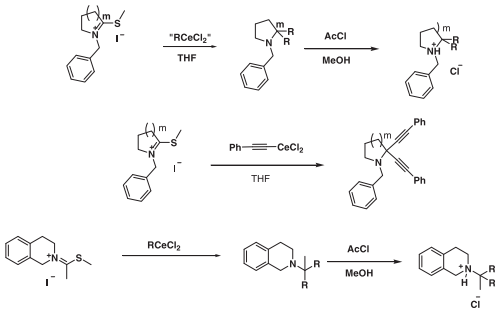
HIGHLIGHTS

- The use of simple nucleophiles results in bis and mono addition.
- The ultrasound sonication is used to prepare the organocerium reagents.
- The arrangement of protons in the amine hydrochlorides is investigated by COSY.
- The amine hydrochlorides are found to be active against some microorganisms.
- The functionalities in the molecules that cause for such activity are rationalized.

GRAPHICAL ABSTRACT

A series of germinal bis-alkyl cyclic amines and their hydrochlorides have been prepared by simple nucleophilic addition on appropriate thioiminium iodides using organocerium reagents. The amine hydrochlorides thus prepared display considerable antimicrobial activity.



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ABSTRACT

To avoid the undesired deprotonation during the addition of organolithium and organomagnesium reagents to ketones, the thioiminium salts, easily prepared from lactams and amides are converted into 2,2-disubstituted and 2-monosubstituted amines by reaction with simple nucleophiles such as organocerium and organocopper reagents. The reaction of thioiminium iodides with organocerium reagents derived by transmetalation of corresponding lithium reagents with anhydrous cerium(III) chloride has been investigated. These thioiminium iodides act as good electrophiles and accept alkylceriums towards bisaddition. The newly synthesized amines have been characterized by ^{1}H and ^{13}C NMR, IR and mass spectra. The amines have been converted into their hydrochlorides and characterized by COSY. These hydrochlorides have been subjected to antimicrobial screening with clinically isolated microorganisms, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Candida albicans*. The hydrochlorides show quite good activity against these bacteria and fungus.

Introduction

Bis addition products of thioiminium ions have been extensively studied and are reported to be done by organomagnesium and organocerium reagents [1]. Heterocycles having methylsulfonyl group and particularly cyclic amines with N-benzyl substituent possess considerable biological activity [2,3]. Thioiminium ions are prepared from lactams converted into thiolactams and reaction of them with methyl iodide. Bis alkylation is mainly dependent on nucleophilicity of organometallic reagents. For instance, we test alkylmagnesium reagents in bis-alkylation and find they are not quite nucleophilic enough to give addition products on

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thioiminium ions. Now we report that organocerium reagents are very effective for addition reactions of these salts. The preparation of thioiminium ions are shown in the scheme 1.

Experimental

All reactions were performed under nitrogen atmosphere in oven-dried flasks (120 °C) unless otherwise stated. Dry solvents for reactions were filtered through a column of dry alumina under positive pressure of argon. Solvents for flash chromatography were of technical grade and used without

purification. Other chemicals were obtained from commercial sources and used without further purifications. The reactions were monitored by TLC (analytical plates, Merck silica gel 60 F254) and visualized under UV light and/or stained with a solution of $KMnO_4$ or phosphomolibdic acid followed by heating. Flash chromatography (FC) was performed using *Baker* silica gel (0.065–0.200 mm). Melting points (m.p.) determined are not corrected. ^1H and ^{13}C NMR spectra were recorded on *Bruker Avance*-300 (^1H: 300 MHz, ^{13}C: 75.5 MHz) or *Bruker DRX*-400 (^1H: 400 MHz, ^{13}C: 100 MHz) or *Bruker Avance-III* (^1H: 400 MHz, ^{13}C: 100 MHz) spectrometers. The ^1H spectra were referred to an internal standard (TMS, 0 ppm) or to the residual ^1H of $CDCl_3$ (7.26 ppm). ^{13}C spectra were referred to residual signal of $CDCl_3$ (77.0 ppm). IR spectra were recorded on *Jasco FT-IR* 460 *plus*. Mass spectroscopy (MS) and high resolution mass spectroscopy (HRMS) analyses were performed on *Waters Micromass Autospec Q/Qstar Pulsar*.

Five bacterial cultures one gram-positive *Staphylococcus aureus*, three gram-negative namely, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi* and one fungal strain namely *Candida albicans*, were used for the bioassay. All the organisms were isolated from clinical patients and obtained from K.A.P. Viswanatham Government Medical College, Tiruchirappalli, India.

The organisms were maintained on agar slopes at 4 °C and sub cultured for 24 h before use. While the bacteria strains were incubated into nutrient broth throughout 24 h, the fungal strains were incubated into Saubouraud's dextrose agar throughout 48 h.

General procedure A: synthesis of thioiminium ions in Scheme 1

Lawesson's reagent (2.02 g, 5.0 mmol) was added to a solution of the lactam (10 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred at room temperature for 1–4 h until the starting material was consumed (TLC monitoring). The solvent was evaporated and the crude mixture was filtered on short column of silica gel (cyclohexane/EtOAc). The thiolactam (10 mmol) was suspended in dry THF (100 mL) and MeI (1.0 mL, 16 mmol) was added. The mixture was stirred at room temperature overnight. The thioiminium ion was isolated by filtration and washed with cold THF to obtain yellowish solid.

General procedure B: synthesis of gem-dialkylated amines in Scheme 2

A suspension of $CeCl_3$ (0.296 g. 1.2 mmol) in dry THF (4 mL) was soniccated at room temperature for about 30 min. The suspension was cooled down to −78 °C and the alkyllithium reagent solution (1.2 mmol) was added dropwise. The solution became pale yellow and stirring was continued for about 30 min. The thioiminium salt was added as a solid (0.3 mmol), the cooling bath was removed and the mixture was stirred for about 6 h. The dark brown suspension was treated with saturated NH_4Cl and the aqueous phases were extracted with dichloromethane (3×). The collected organic phases were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by FC (cyclohexane/*tert*-BuOMe) to afford the *gem*-dialkylated amine.

General procedure C: preparation of amine hydrochlorides in Scheme 2

The free amine was treated with HCl (generated from the addition of acetyl chloride to MeOH at 0 °C) to give the amine hydrochlorides.

Results and discussion

Synthesis of thioiminium iodides

The thioiminium iodides (**7–9**, **12** and **15**) were prepared by the action of methyl iodide on thiolactams. These thiolactams were obtained from the commercially available and easily prepared lactams (**1–3**, **10** and **13**) (Scheme 1). The lactam **1** is commercially available, whereas, **2** and **3** [4] are prepared by N-benzylation of δ-valerolactam and 6-caprolactam respectively. Conversion of lactams (**1–3**) into thiolactams enables to prepare the thioiminium ions (**7–9**) [5]. The reaction of thiolactams with methyl iodide took about 8 h to give the salts with good yield. The reaction between thiolactams and other alkyl halides like ethyl, propyl and butyl iodides was found to be slow. The lactams 1-(pyrrolidin-1-yl)ethanone (**13**) and 1-(3,4-dihydroisoquinolin-2(1H)-yl)ethanone (**10**) are prepared simply by acetylation of pyrrolidine and 1,2,3,4-tetrahydroisoquinoline respectively. They are converted into thioiminium ions (**15** and **12**). Biological activities of some of thioiminium iodides were also screened (Table 1).

Among the above thioiminium iodides, **7**, **8** and **12** are stable at room temperature for a long time in a closed container. The salt **9** is unstable at room temperature and **15** is hygroscopic in nature.

FT-IR spectra of **7–9**, **12** and **15** showed the characteristic C═N, C—S stretching frequencies that could be seen in 1600–1495 and 700–693 cm^{-1} respectively. C—C stretching appeared between 1299–1243 cm^{-1}. C—H (aromatic) stretch and C═C ring stretch (aromatic) appeared at 3085–3000 and 1590–1466 cm^{-1} respectively.

^1H NMR spectra of **7–9** showed a sharp singlet that was observed at the range of δ 5.23–4.93 ppm. This is assigned to two benzylic protons. The signal for CH_2 protons adjacent to N atom in **9** is shifted to the downfield at δ 4.22 ppm. This is the typical triplet with unexpected intensity ratio and this downfield shift is because of the adjacent positively charged nitrogen atom, which may deshield the methylene protons by the electron withdrawing nature. The CH_2 protons adjacent to positively charged nitrogen atom showed a triplet around at δ 4.17 ppm (**7**) and δ 3.75 ppm

Table 1
Antimicrobial screening of some selected compounds.

Name of the organism	Standard antibiotic	Standard zone of inhibition for selectivity (mm)	Diameter of zone of inhibition obtained (mm)												
			+ve control*	7	8	12	30	31	32	34	35	36	40	46	48
Staphylococcus aureus	Ampicillin	17	–	6	6	5	1	2	–	1	1	–	1	1	–
Salmonella typhi	Chloramphenicol	18	3	9	7	6	–	2	–	–	2	–	–	1	–
Pseudomonas aeruginosa	Gentamicin	15	9	9	8	3	1	1	1	1	4	2	1	2	3
Candida albicans	Ketoconazole	18	–	10	–	4	–	–	–	1	–	–	–	2	3
Klebsiella pneumoniae	Streptomycin	15	–	–	–	–	–	–	–	1	1	–	–	1	–

* zone of inhibition obtained (mm) for the standard antibiotics used.

(J = 6 Hz) (**8**). The C3 protons in **7** and **8** were observed as a triplet around δ 3.79 and 3.23 ppm (J = 6 Hz) respectively. The C4 protons of **7** showed a quintet in the upfield at δ 2.41 ppm. The multiplets appeared like singlets for C3, C4, C5 and C6 protons in **9** may be due to the relaxation effect. The same kind of effect could be seen for all the ring protons in **15**. The merged multiplet appeared for C4 and C5 protons of **8**. The aromatic protons of **7–9** also appeared as merged multiplets. The salt **12** is obtained as a mixture of rotamers, therefore, all the eight aromatic protons appeared as a merged mutiplet. Two singlets for C1 protons adjacent to the positively charged nitrogen atom and two triplets for C3 protons were appeared. One triplet for C4 protons of two rotamers appeared, this may be due to the same environment possessed by all the four protons. For the two methyl groups, four singlets appeared like two doublets. The appearance of a signal around at δ 193.69–187.52 ppm in ^{13}C NMR and mass peaks in HRMS confirmed the presence of iminium moiety in all the thioiminium ions.

Synthesis of bis-alkylated and alkynylated amines

We report that thioiminium salts thus prepared from lactams and amides, can be converted into 2,2-disubstitued amines by reaction with simple nucleophile i.e. organocerium reagents. Treatment of the thioiminium salt **7** with allylmagnesium bromide afforded the desired *gem*-diallylated pyrrolidine [1]. The best results are obtained with 3 equiv of allylmagnesium bromide in THF at room temperature.

In contrast to Klaver et al. [6] neither the solvent nor the temperature influences the product distribution. For instance, when the reaction of **7** was performed with only one equivalent of allylmagnesium bromide in dichloromethane at −78 °C, no monoallylation was observed. Instead, the bisallylated amine was obtained in 40% yield together with lactam **7** arising from aqueous workup.

Introduction of geminal dialkyl group proved not to be feasible by using either alkylmagnesium halides or alkyllithium derivatives, despite the isolated example reported by Klaver et al. [6]. These reagents are presumably too basic and deprotonate the thioiminium salts rather than undergoing the desired addition. Organocerium derivatives are often used to avoid undesired deprotonation during addition of organolithium and organomagnesium reagents to ketones [7]. Alkylcerium dichlorides, easily prepared from the commercially available organolithium derivatives are tested with thioiminium iodides (**7–9**, **12** and **15**). These reagents reacted cleanly with the thioiminium iodides **7–9**, **12** and afforded the *gem*-dialkyl derivatives **16–19**, **20–23**, **24–26** and **41–44** and *gem*-dialkynyl amines **27–29** (Scheme 2) in good yields over the three steps from the parent lactams. The progress of the reaction was followed by TLC analysis using cyclohexane : TBME as eluent. Interestingly, the reaction in the presence of 1 equiv of *n*-butylcerium with **7** affords **18** as single isolated product in 20% yield. According to the results of the organomagnesium allylation, no product resulting from the monoaddition was detected [8].

The reaction was also tested with acetamide **15**, but was not successful. Then, we prepared *N-tert*-butyltetrahydroisoquinoline **41** from acetamide **10** via the thioiminium ion **12**. The acetylation-*gem*-dimethylation process represents a useful method for the conversion of secondary amines into *tert*-butyl tertiary amines.

Secondary and tertiary alkylcerium reagents do not add to the thioiminium salts and after aqueous work up, the parent lactams are recovered [1]. It is unclear whether this result is due to a lack of reactivity or a competitive deprotonation. Amines in Schemes 2, 3 and 5 are not stable for a long time at room temperature. Hence, they are immediately converted to their hydrochlorides.

The compounds **16–19** and **20–26** showed their characteristic C—H (aromatic), C=C ring stretch (aromatic) and C-N stretch in the range of 3000–2996, 1605–1466 and 1258–1026 cm^{-1} respectively. The amines **27–29** showed C≡C stretch in the range of 1598–1597 cm^{-1}.

The merged multiplet was observed for aromatic protons of **16–29** in the range of δ 7.45–7.11 ppm. A sharp singlet appeared in the range of δ 3.73–3.42 ppm for benzylic protons of **16–26** and the same was shifted to δ 3.99 ppm for **27–29**. This peak appeared as broad singlet for **28**. The ring protons of these amines appeared in the range of δ 1.69–1.23 ppm and the same was shifted to δ 2.42–2.21 ppm for **25** and **27**. This is due to the electron withdrawing effect of phenyl rings as well as the triple bonds. The characteristic triplet for CH_2 protons adjacent to the ring nitrogen appeared in the range of δ 2.68–2.26 ppm for **16–20**, **28** and **29**. These triplets are looking like singlets due to the relaxation effect in **21–23**. In all the amines, methylene protons in the alkyl groups showed merged peaks with ring protons.

The ipso carbon of the compounds **16–19** and **20–23** was observed in the range δ 141.72–141.55 ppm, for **27** and **28**, in the range δ 140.02–138.62 ppm, and for 7 membered amines **24–26**, in the range δ 143.25–143.08. This may be due to the substituent effect. ^{13}C NMR spectra of **16**, **25–29** showed a signal for the quaternary carbon in the region of δ 60.26–60.02 ppm. The same was observed in the region of δ 64.82–64.51 ppm for **17–19** and δ 57.65–57.25 ppm for **21**, **22**, and **27**. The six membered amines **20** and **28** showed the quaternary carbon at δ 53.24 ppm and δ 58.23 ppm respectively. The seven membered amine **24** showed the same carbon at δ 55.98 ppm. The signal for alkyne carbons in **27** were showed at δ 87.82 and 83.69 ppm. The same in **28** were observed at δ 88.31 and 84.40 ppm. These carbons in **29** presented at δ 90.72 and 82.05 ppm. The difference in the chemical shifts may be due to the different ring size.

The multiplets for aromatic protons in the region of δ 7.43–7.03 ppm were observed for **41–44**. The C2 protons of these amines showed a sharp singlet in the region of δ 3.80–3.69 ppm and C3 and C4 protons appeared as multiplets in the region of δ 2.83–2.70 ppm.

Synthesis of amine hydrochlorides

The preparation of hydrochloride salts of above amines is shown in Scheme 4 and 6. The characteristic N-H stretch of **30–32**, **36** and **38–40** could be seen between 2597–2534 cm^{-1}, whereas, **33** and **37** showed the same at 2857 cm^{-1}. The compounds **34** and **35** showed the N-H stretch between 2489–2477 cm^{-1}. This variation may be due to the difference in ring size.

A broad singlet was observed in the range δ 12.10–11.03 and 10.96–10.38 ppm for **30–40**. This difference may due to the variation in ring size. The aromatic protons peaks are well separated.

They show the coupling of protons clearly and explain the splitting pattern of benzylic as well as ring protons satisfactorily. The spin coupling of N—H protons and geminal coupling of benzylic protons resulted in the doublet of doublets appeared for benzylic protons. In **Fig. 1**, the splitting of all the signals in ^{1}H NMR of the free amine **16** by N—H proton in the corresponding amine hydrochloride **30** has been shown. The COSY of 30 proves this spatial arrangement of N—H very well (**Fig. 2**). The interaction of N—H protons with the ring protons made the peaks for the ring protons to be multiplets. This interaction is found to be weak in the cases of **34**, **35** and **38**.

The spectra of **30** and **35** showed the quaternary carbon in the range of δ 69.03–69.02 ppm, whereas, **31–33** show in the range δ 75.87–75.17 ppm. The hydrochloride **34** showed the same at δ 62.74 ppm, whereas, **36** and **37** showed in the range of δ 68.39–68.31 ppm. The seven membered amine hydrochloride **38** showed the quaternary carbon at δ 67.31 ppm, whereas, others **39** and **40** show in the range of δ 73.78–73.10 ppm. This variation may be due to the increase in number of α, ß, γ and δ carbons.

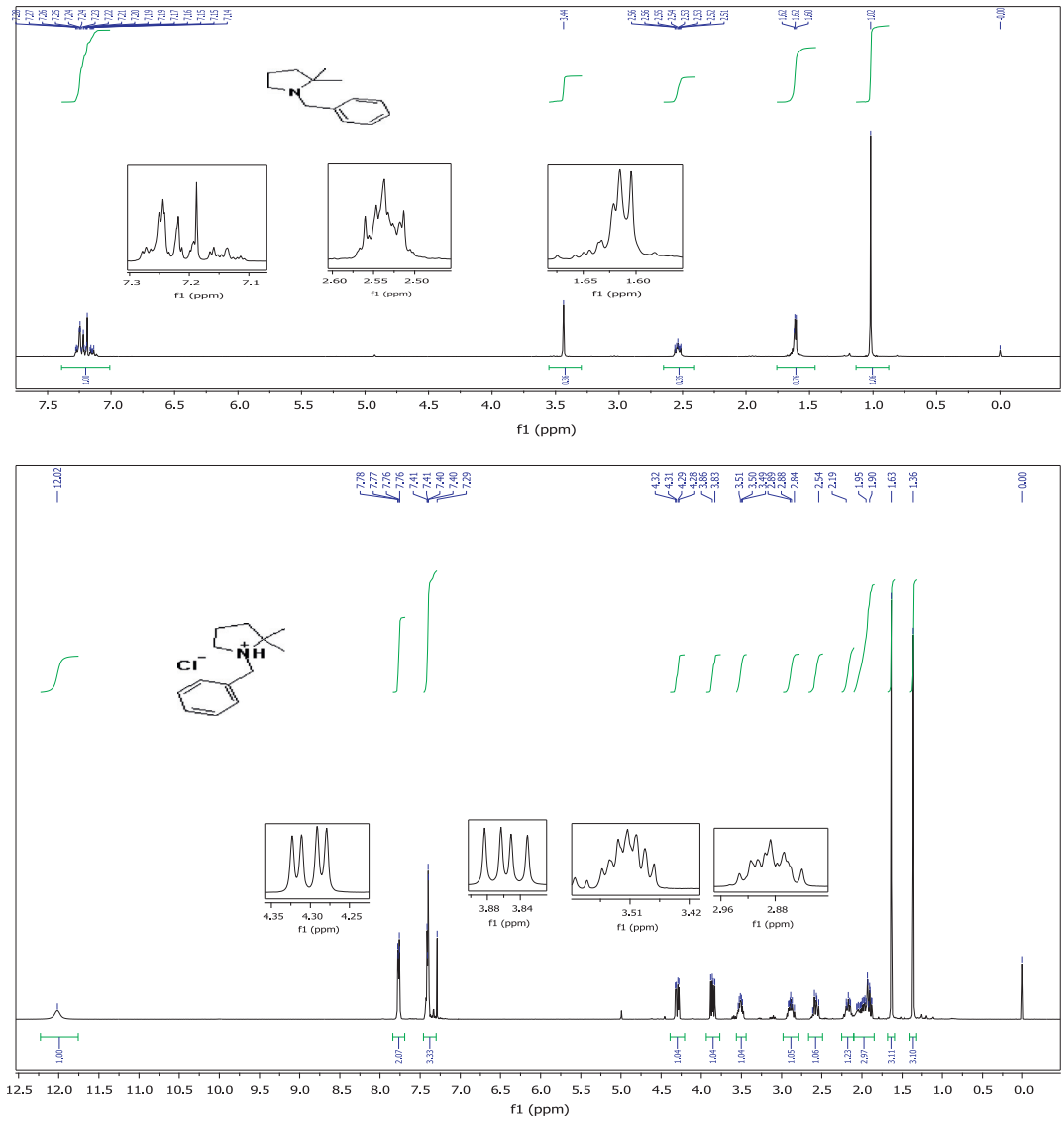

Fig. 1. Comparison of ^{1}H NMR spectra of amine **16** and its hydrochloride **30**.

Coupling of N—H protons with ring protons

The coupling pattern of protons especially the interaction of N—H protons with the ring protons was very much clear by their spectra. The hydrochlorides (**96–99**) of amines **92–95** were prepared and characterized by their IR, ^{1}H, ^{13}C and mass spectral data.

They showed the characteristic quaternary carbon in the range of δ 70.55–69.48 ppm whereas **96** shows at δ 63.55 ppm. This may be due to the increase in the number of α, ß, γ and δ carbons.

Antimicrobial screening

For the investigation of the antibacterial and antifungal activity, filter paper disc- agar diffusion method (Kirby-Bauer method) was employed [9]. Thioiminium iodides **7**, **8** and **12** amine hydrochlorides **30–32**, **34–36**, **40**, **46**, and **48** were weighed and dissolved in DMSO to obtain 100 mg/ml concentration. These solutions were impregnated with Whatman No. 3 filter paper discs, dried under laminar air flow and placed on the Muller–Hinton agar plate for bacteria and Saubouraud's dextrose agar plate for fungi, which had been inoculated with a lawn of 0.1 ml of a 24 h broth culture of test bacteria and fungi. The inoculated plates were incubated at 37 °C, for a period of 18–24 h for bacteria and at 25 °C, for 24–48 h for fungi. Discs treated with 10 µl of DMSO was used as negative controls and commercial discs of gentamicin (10 µg), chloramphenicol (30 µg), streptomycin (100 µg), ampicillin (10 µg) and ketoconazole (10 µg) which were purchased at Himedia lab. Pvt. Ltd. were used as positive control. All determinations were done duplicate. The compounds produced distinct, clear, circular zones of inhibition around the discs and the diameters of clear zones were measured with calipers (Attaie et al., 1987) and used as an indication of antibacterial and antifungal activity [10].

All the tested organisms (*S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi* and *C. albicans*) were found to be multidrug resistance to various commercial antibiotics available (results not shown).

The effect of bis-quaternary ammonium salts on the growth of various bacteria and the yeast depends on substituents at the quaternary nitrogen atom [3]. We prepared the thioiminium salts as well as the hydrochlorides of cyclic amines with N-benzyl substituent. So this study will be useful to understand the efficiency of benzyl group against the microorganisms tested.

The compounds **7** and **8** were found to be moderately active against *Staphylococcus* and highly active against *Salmonello* and *Pseudomonas*. But the ion **57** showed poor response against

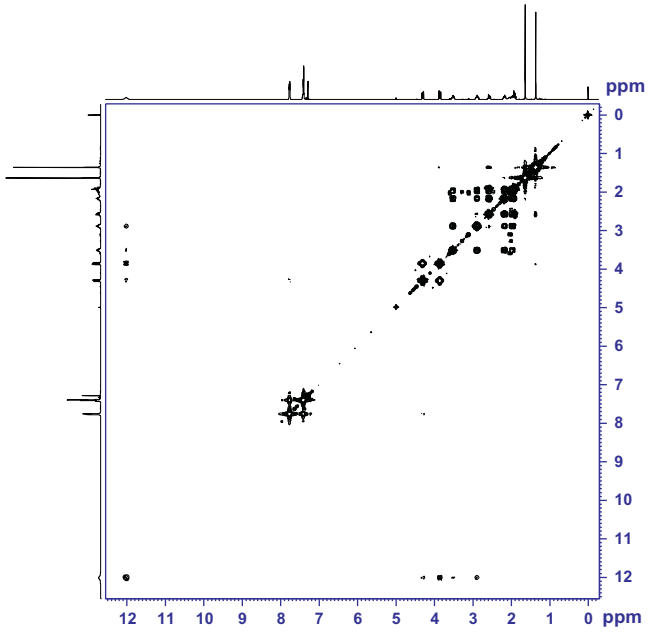

Fig. 2. COSY of amine hydrochloride **30** showing the coupling of N–H proton with other protons.

Candida. Both the salts showed the poor response to *Klebsiella*. The compound **12** responded moderately against all the above organisms except *Klebsiella*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.09.108.

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