

Note

Synthesis and antimicrobial screening of *N*-substituted -3-chloro-4-dithiocarbamato azetidin-2-ones

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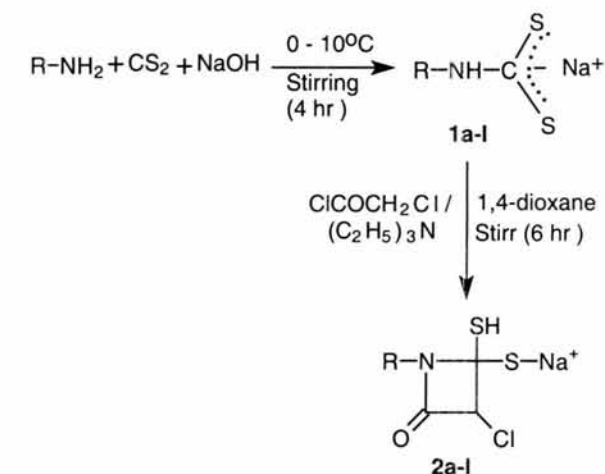
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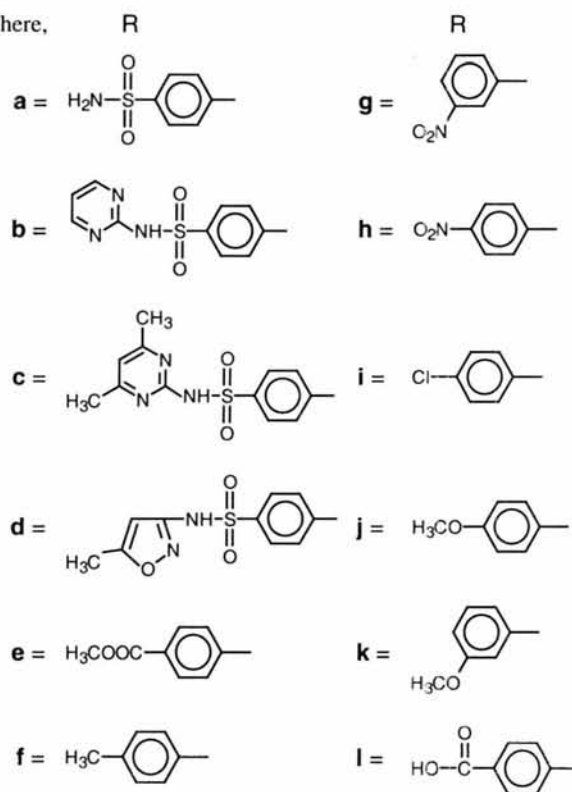
Some new *N*-substituted-3-chloro-4-dithiocarbamato azetidin-2-ones have been synthesized and their structures are established on the basis of their elemental, IR and ¹H NMR data. All the synthesized compounds have been evaluated for their *in-vitro* growth inhibitory activity against *P. diminuta*, *B. subtilis*, *E. coli*, *S. Aureus*, *R. rhodochrous*. All the compounds show significant antibacterial activity. *N*-[4'-(*N*'-4,6-Dimethyl pyrimidinyl) sulphonamoyl amino phenyl]-3-chloro-4-dithiocarbamato azetidin-2-one **2_c** has been found to be more potent antimicrobial agent against *B. subtilis*. A perusal of data reveals that molecular refractive index (*M_R*) and vander Waal's volume (*V_w*) are correlated linearly to the drug activity with correlation coefficient of 0.91 and 0.98, respectively. The synthesized compounds have been subjected to acute toxicity studies to find out LD₅₀ values. The compounds do not show any toxicity up to the dose of 8.5-mg/kg body weights in rats.

Azetidines and their derivatives have been extensively explored for their applications in the field of medicines^{1,2}. Likewise, azetidin-2-ones are of great importance because of the use of β-lactam derivatives as an antimicrobial agents³. Penicillin i.e., a β-lactam antibiotic, still remains the most widely prescribed compound for the treatment of infectious diseases^{4,5}. A large number of antibiotics contain β-lactam moiety^{6,7} in them. A recently developed β-lactam antibiotic fosfomycin⁸ has high antibacterial activity against urinary tract infection caused by *E. coli*. Similarly, certain other 2-azetidinones^{9,10} were found to exhibit a wide range of biological activities e.g., fungicidal, bacteriocidal etc. Keeping this in view, we report herein a new synthesis of *N*-substituted -3-chloro-4-dithiocarbamato azetidin-2-ones **2_{a-l}**. A number of substituents viz., sulphanilamido, 2-pyrimidinyl, 4,5-dimethyl pyrimidinyl, 5-methyl-3'-isoxazoyl and 4-COCH₃, 4-CH₃, 3-NO₂, 4-NO₂, 4-Cl, 4-OCH₃, 3-OCH₃, and 4-COOH, derived from various

sulphonamides and anilines have been incorporated in the title compound **2**. The dithiocarbamates **1_{a-l}** on vigorous stirring for 6 hr with triethylamine and chloroacetyl chloride in the presence of 1,4-dioxane gave substituted azetidinones **2_{a-l}** (Scheme I). All the synthesized compounds have been characterized by



Where,



Scheme I

elemental, IR and ^1H NMR data. An attempt has been made to understand the antimicrobial behaviour of these compounds *in vitro*.

Experimental Section

All the chemicals used were of AR grade. IR spectra were recorded on Shimadzu 460 IR spectrophotometer and ^1H NMR spectra on a Varian 270 MHz instrument. The melting point was determined on an electric melting point apparatus in open capillaries and is uncorrected.

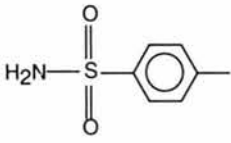
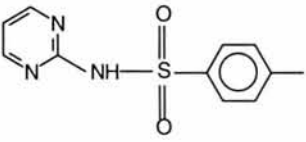
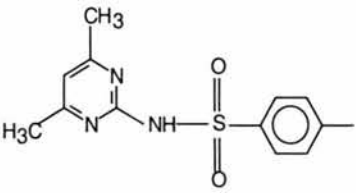
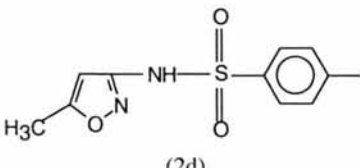
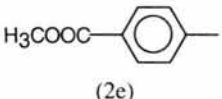
N-Substituted-3-chloro-4-dithiocarbamate - azetidin-2-ones 2_{a-i} . Sodium salts of the substituted dithiocarbamates 1_{a-i} were prepared by treating equimolar quantity of aniline/sulphonamide with carbon disulphide and sodium hydroxide by the procedure reported in literature.¹¹

Compound 1_{a-i} (0.02 M) was added to a constantly stirred solution of 1,4-dioxane (50 mL) and triethyl amine (2.26 mL, 0.02 M). After the reaction completed (6 hr), the reaction mixture was kept at room temperature for 30 min and then kept overnight in freeze. The precipitates so obtained was filtered, washed with water and dried to obtain compounds 2_{a-i} . The product was recrystallized from a mixture of ethanol and DMF (1:1, v/v). A synthetic pathway is depicted in **Scheme I**.

2f; m.p. 227-229°C, yield 64% (Found; C, 43.10; H, 3.25; N, 5.20; Calc. For $\text{C}_{10}\text{H}_9\text{NS}_2\text{ClONa}$ C, 43.02; H, 3.19; N, 4.97%).

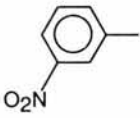
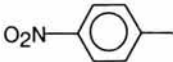
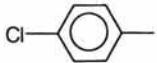
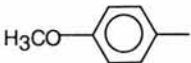
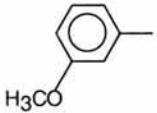
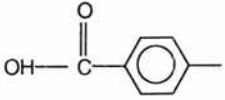
Spectral data of all the compounds 2_{a-i} is depicted in **Table I**.

Table I—Spectral data

Sl. No.	R	IR (ν_{KBr} , cm^{-1})	^1H -NMR (δ ppm)	^{13}C NMR (δ ppm)
1.	 (2a)	3440 (NH, sym), 3350 (NH, asym), 3070 (C-H, sp^2), 2850 (C-H, sp^3), 2550 (SH), 1750 (C=O), 1590 (C=C), 1425 (C-N), 1351 [$\text{S}(\text{=O})_2$], 980 (C-S), 690 (C-Cl)	2.2 (s, CH, azetidine ring), 5.1 (bs, 2H, NH_2), 7.1-7.3 (d, 2'CH, 6'CH), 7.6-7.8 (d, 2H, 3'CH-CH), 14.1 (s, SH)	207.9 (C-2); 129.8 (C-3); 120.3 (C-4), 150.1 (C-1); 136.8 (C-2', 6'), 134.2 (C-4'), 128.4 (C-3', 5')
2.	 (2b)	3300 (NH), 3050 (=C-H), 2850 (-C-H), 1740 (C=O), 1600, 1590 (C=C / C=N), 1350 [$\text{S}(\text{=O})_2$], 790, 810 (aromatic), 700 (C-Cl)	2.1 (s, CH), 7.2-7.3 (d, 2H, 2'CH-6'CH), 7.5-7.8 (d, 2H, 3'CH-5'CH), 7.9 (5''CH), 8.1 (dd, 2H, 4''CH-6''CH), 11.1 (s, NH), 12.1 (s, SH),	189.5 (C-2), 129.6 (C-3), 131.5 (C-4), (C-1'), 124.8 (C-2', 6'), 138.2 (C-3', 5''), 143.1 (C-4'), 151.5 (C-2''), 136.6 (C-4'', 6''), 148.6 (C-5'')
3.	 (2c)	3250 (NH), 3090 (=C-H), 2932 (-C-H), 1738 (C=O), 1600, 1500, 1485 (C-N), 1355 [$\text{S}(\text{=O})_2$], 975 (C-S), 860, 740 (aromatic), 620 (C-Cl)	—	—
4.	 (2d)	3300 (NH), 3100 (=C-H), 2870 (C-H), 1760 (C=O), 1600, 1800 (aromatic), 1470 (C-N), 1352 [$\text{S}(\text{=O})_2$], 960 (C-S), 550 (C-Cl)	1.4 (s, 3H, CH_3), 2.0 (s, CH), 4.6 (s, CH, Isoxazole ring), 6.9-7.2 (d, 2H, 2CH-6'CH), 7.5-7.8 (d, 3'CH-5'CH), 9.7 (s, NH), 11.8 (s, SH)	—
5.	 (2e)	3050 (=C-H), 2850 (C-H), 2580 (SH), 1760 (C=O, ester), 1600, 1500, 840, 710 (aromatic), 660 (C-Cl)	2.4 (s, CH), 3.6 (s, 3H, CH_3), 6.9-7.2 (d, 2H, 2'CH-6'CH, $J=8$ Hz), 7.9-8.1 (d, 2H, 3'CH-5'CH, $J=8$ Hz), 12.1 (s, SH),	181.5 (C-2), 130 (C-3), 124.6 (C-H), 134.2 (C-1'), 121.2 (C-2', 6'), 137.1 (C'-4'), 129.8 (C3''-C5'), 167.0 (C=O), 51.5 (CH_3)

—Contd

Table I—Spectral data—Contd

Sl. No.	R	IR (ν_{KBr} , cm^{-1})	$^1\text{H-NMR}$ (δ ppm)	$^{13}\text{C NMR}$ (δ ppm)
7.	 (2g)	3050 (=C-H), 2870 (C-H), 2660 (S-H), 1755 (C=O), 1660 (C=C), 1600, 1500, 680, 750 (aro. sub.), 1360, 1500 (NO_2), 500 (C-Cl)	—	—
8.	 (2h)	3075(=C-H), 2890 (C-H), 2700 (S-H), 1745 (C=O), 1620 (C=C), 1590, 1500, 790 (aromatic), 1360, 1540 (NO_2), 610 (C-Cl),	2.0 (s, CH), 6.9-7.2 (d, 2H, 2'CH-6'CH), 7.4-7.9 (d, 2H, 3'CH - 5'CH), 10.3 (s, SH)	192.1 (C-2), 138.5 (C-3), 142.1 (C-4), 134.5 (C-1'), 127.6 (C-2', 6'), 138.3 (C-4'), 1260 (C-3', C-5')
9.	 (2i)	3080 (=C-H), 3000 (C-H), 2700 (S-H), 1750 (C=O), 1640 (C=C), 1590, 1490, 810 (sub. Phenyl), 710 (C-Cl)	2.5 (s, CH), 7.0-7.2 (d, 2H, 2'CH - 6'CH), 7.8-8.1 (d, 2H, 3'CH-5'CH), 11.5 (s, SH)	181.9 (C-2), 136(C-3), 141.4 (C-4), 132.6 (C-4'), 129.8 (C-3', C-5'), 124.6 (C-2',6')
10.	 (2j)	3100 (=C-H), 2870 (C-H), 2660 (S-H), 1745 (C=O), 1600, 1500 (aromatic ring stretch), 1100 (C-O) 795 (<i>para</i> sub. Phenyl) 610(C-Cl),	—	179.6(C-2), 128.6(C-3), 138(C-4) 143.1 (C-1') 127.5 (C-2',6'), 126.7 (C-3',5'), 155.1 (C-4'), 15.6 (OCH ₃)
11.	 (2k)	3050 (=C-H), 2880 (C-H), 2710 (S-H), 1760 (C=O), 1600, 1500 (aromatic), 1050 (C-O), 720, 810 (<i>meta</i> sub. Phenyl), 650 (C-Cl)	2.5 (s, CH), 3.3 (s, 3H, OCH ₃), 7.5(s, 2'CH), 7.6 - 7.8 (m, 4H, Ar - H), 11.2(s, SH)	—
12.	 (2l)	3070 (=C-H), 2870 (C-H), 2810 (S-H), 3000-2500 (very broad, COOH), 1740 (C=O), 1760 (C=O, azetidine ring), 1600, 1500 (aro. str.), 1050 (C-O), 910, 740 (sub. Phenyl), 600 (C-Cl)	2.6 (s,CH), 7.0-7.3 (d, 2H, 2'CH-6'CH, $J = 8$ Hz), 7.9-8.2 (d, 2H, 3'CH-5'CH, $J = 8$ Hz), 10.37(s,1H, COOH), 12.1 (s,SH)	185.6 (C-2), 131.3 (C-3), 129.1 (C-4), 128.3 (C-3',5'), 137.3 (C-4'), 205.8 (C=O), 127.4 (C-2',6')

Antimicrobial activity

Nutrient agar media of the requisite composition viz., peptone (5 g), and beef extract (1 g), agar-agar (20 g) and distilled water (1000 mL), was prepared and pH of the media was adjusted to 6.6. For the preparation of media all the above ingredients (except agar-agar) were weighed and dissolved in distilled water (500 mL) by application of gentle heat. After dissolving the ingredients completely, more distilled water and weighed quantity of agar-agar was added. Then it was filtered through cotton to obtain a clear solution. The mixture was autoclaved for 30 min at a pressure of 1.5 kg/cm². All the glass apparatus were cleaned with chromic acid and then sterilized by keeping in oven. Medium was cooled to 37°C and homogenous suspension was prepared by transferring aseptically a loopful of all the corresponding micro-

organism from fresh subculture into agar medium followed by vigorous shaking; 20 mL of this medium was poured into each sterilized petri dishes under aseptic conditions and allowed to set. After preparing the medium, the paper disc (6 mm) was immersed in seeded agar containing petri dishes. The solution (0.1 mL) was dropped into the filter paper disc. Petri dishes were incubated at 37°C for 24 hr. The inhibition zone for each test solution was measured in mm.

The synthesized compounds were tested for their antimicrobial activity against *P. diminuta*, *B. subtilis*, *E. coli*, *S. aureus* and *R. rhodochrous* using standard paper disc method of Rapper¹³. Inhibition (%) = $[(\alpha - \beta)/\alpha] \times 100$ was calculated¹⁴ and presented in Table II, where α and β stands for inhibition zone of

Table II—Antibacterial activity of **2a-i** (zone of inhibition)

Compd	M _R	V _w	LD ₅₀ (mg/kg)	<i>P. diminuta</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>R. rhodochrous</i>
2a	65.48	2.501	3.60	-27.40	-20.15	-19.12	-15.10	-10.10
2b	79.03	2.765	6.82	-30.45	-22.25	-20.42	-16.42	-11.25
2c	88.62	3.073	8.20	-35.42	-32.25	-30.48	-20.48	-12.75
2d	79.84	2.840	5.25	-31.42	-28.42	-27.00	-14.40	-11.75
2e	58.28	2.251	3.60	-16.48	-14.50	-13.50	-7.56	-6.54
2f	54.33	1.990	5.25	-16.00	-14.10	-10.25	-7.24	-5.80
2g	59.58	2.155	8.50	-20.62	-16.15	-15.95	-9.25	-7.80
2h	59.58	2.155	7.75	-18.00	-14.15	-12.55	-11.58	-8.80
2i	54.41	2.057	8.42	-11.65	-9.06	0.50	1.32	3.15
2j	55.98	2.134	6.42	-9.00	-4.78	-3.95	1.00	2.02
2k	55.98	2.134	8.20	-13.25	-10.08	-8.45	-3.90	1.05
2l	55.87	2.165	8.00	-4.75	-4.26	1.25	4.25	5.92

perusal of the data presented in table, reveals that compound *N*-[4'-(*N'*-4'', 6''-Dimethyl pyrimidinyl) sulphonamoyl amino phenyl]-3-chloro-4-dithiocarbamate-azetidin-2-one was found to be more active in the series. Most of the compounds of the series were found to be highly active against *B. subtilis*, while least against *P. diminuta*. The data exhibited in table reveals that the activity shown by synthesized azetidin-2-ones follows the pattern *B. subtilis* > *E. coli* > *S. aureus* > *R. rhodochrous* > *P. diminuta*.

To study quantitative structure-activity relationship (QSAR), molecular refractive index (M_R) by the method of Dreishach¹⁵ and vander Waal volume (V_w) by the suggested method of Marriguchi and Kanada¹⁶ have been calculated and reported in **Table II**. A perusal of the data reveals that the M_R and V_w are correlated linearly to the drug activity with correlation coefficient of 0.91±0.072 and 0.88±0.079, respectively.

Furthermore, an interesting correlation between M_R values and Hammett substituent constant (σ) has been drawn to deduce the linear relationship between biological activity of the compounds and substitution pattern. All the substituents tends to fit in the regression line, whereas, ortho derivatives exhibit deviation owing to the ortho effect.

Moreover, it has been concluded that alkylation of carboxyl group and introduction of NO₂ group increases the antimicrobial activity. The groups such as -OCH₃, COOH and -Cl at para position causes a marked decrease in the activity.

Determination of LD₅₀

Before screening for above activities, synthesized substituted azetidin-2-ones were subjected to acute toxicity studies to find out LD₅₀. It has been found that all the compounds did not show any toxicity up to the dose of 8.5 mg/kg body weights in rats. The results of screening are presented in **Table II**.

Acknowledgement

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