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SHORT COMMUNICATION

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME HETEROCYCLIC DERIVATIVES OF SULFANILAMIDE

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ABSTRACT. Considering the promising antimicrobial potential of carbonic anhydrase inhibitors and heterocyclic compounds some heterocyclic derivatives of sulfanilamide (**2a-e**) were synthesized. The diazotisation of sulfanilamide followed by substitution with ethylacetoacetate and further condensation yielded compounds **2a-c**. Schiff base of sulfanilamide with salicylaldehyde on reaction with thioglycollic acid and chloroacetyl chloride resulted in compound **2d-e**. The susceptibility of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* to the title compounds (300 µg/disc) was investigated and compared to that of nitrofurantoin (300 µg/disc) and ciprofloxacin (25 µg/disc). The title compounds showed good antimicrobial activity.

KEY WORDS: Carbonic anhydrase, Sulfanilamide, Heterocyclic compounds, Antimicrobial activity

INTRODUCTION

The field of research on development of carbonic anhydrase inhibitor based antimicrobials has shown promising results due to presence of carbonic anhydrases in a multitude of bacteria and protozoa [1-3]. Sulfanilamide, a prototype of carbonic anhydrase inhibitor has good potential for antibacterial action [4-5]. Conjugations of heterocyclic groups reportedly enhance antibacterial action of the original compound [6]. Besides derivatives of azetidinone, thiazolidinone [7], oxazoles [8] and imidazoles [9] are widely reported with antibacterial action. It is thus envisageable that conjugation of heterocyclic compounds with sulfanilamide will be able to enhance the antibacterial action of sulfanilamide leading to novel types of pharmacological agents useful in the fight against infections. Keeping this in view, we have attempted synthesis of heterocyclic derivatives of sulfanilamide, and investigated the *in vitro* susceptibility of two gram-positive bacteria (*S. aureus*, *E. faecalis*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*) to them.

EXPERIMENTAL

The synthesized compounds **2a-e** (Scheme 1) were purified with repeated washing and recrystallisation from different solvents. Purity of compounds was checked by TLC using chloroform: methanol: DMF (100+10+05 v/v) as developing solvents and iodine as visualizing agent. Melting points were determined in open capillary tubes (Sisco) and were uncorrected. Infrared spectra were recorded on Shimadzu-8400S spectrophotometer using KBr powder. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-300 NMR spectrophotometer (300 MHz) using TMS as internal standard. The CHN elemental analysis was carried out using EURO-EA elemental analyzer.

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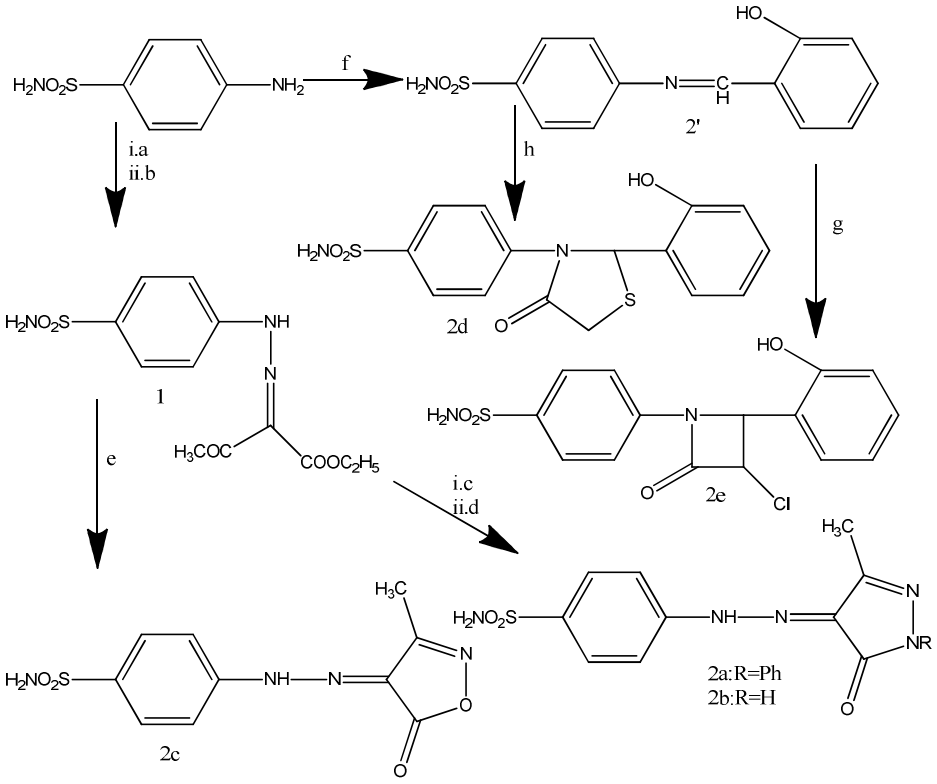

a. sodium nitrite, b. ethylacetoacetate, c. phenyl hydrazine, d. hydrazine hydrate, e. hydroxylamine hydrochloride, f. Salycylaldehye, g. chloroacetyl chloride, h. thioglycollic acid.

Scheme 1. Synthesis of heterocyclic derivatives of sulfanilamide.

Synthesis of compound **1** and **2a-e** were done as per the reported method [7-8] with little modification.

*Synthesis of ethyl-3-oxo-2-(2-(4-sulfamoylphenyl)hydrazono)butanoate (**1**)*. Sulfanilamide (0.01 mol) was taken as the starting material in a mixture of HCl (8 mL) and water (6 mL). It was cooled to 0 ^{o}C in an ice bath and a cold aqueous solution of sodium nitrite (0.03 mol) was added. The diazonium salt solution was filtered directly into a cold solution of ethylacetoacetate (0.01 mol) and sodium acetate (0.122 mol) in ethanol (50 mL). The resulting solid was washed with water and recrystallised from alcohol to yield ethyl-3-oxo-2-(2-(4-sulfamoylphenyl) hydrazono) butanoate (**1**).

*Synthesis of 4-(2-(3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidine) hydrazinyl) benzene sulfonamide (**2a**)*. Compound **1** (0.002 mol) was dissolved in glacial acetic acid (20 mL) and phenyl hydrazine (0.002 mol). The mixture was refluxed for 4 h, cooled and allowed to stand overnight. The product so formed was filtered, dried and recrystallised from aqueous ethanol to produce 4-(2-(3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidine) hydrazinyl) benzene sulfonamide. The compound 4-(2-(3-methyl-5-oxo-1H-pyrazol-phenyl ylidine) hydrazinyl) benzene sulfonamide (**2b**) was prepared using hydrazine hydrate.

*Synthesis of 4-(2-(3-methyl-5-oxoisoxazol-4(5H)-ylidine) hydrazinyl)benzene sulfonamide (**2c**)*. To the compound **1** (0.001 mol) in ethanol a solution of sodium acetate (1 g) and hydroxylamine hydrochloride (0.001 mol) in water was added and the solution was refluxed for 4 h. On cooling the solid obtained was recrystallised from ethanol to yield 4-(2-(3-methyl-5-oxoisoxazol-4(5H)-ylidine) hydrazinyl) benzene sulfonamide (**2c**).

*Synthesis of 4-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzene sulfonamide (**2d**)*. Schiff base of sulfanilamide with salicylaldehyde (**2**) was prepared by laboratory method. It (0.01 mol) was refluxed with thioglycollic acid (0.01 mol) in presence of anhydrous aluminium chloride (0.05 g) at 120 ºC for 12 h. The reaction mixture was then cooled and triturated with an excess of 10% sodium bicarbonate solution. The product formed was filtered, washed repeatedly with water, dried and recrystallised from aqueous ethanol to give 4-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzene sulfonamide (**2d**).

*Synthesis of 4-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl) benzene sulfonamide (**2e**)*. The Schiff base (0.01 mol) was dissolved in DMF (40 mL) and triethylamine (2.8 mL) was added to it. Chloroacetylchloride (0.01 mol) was added drop wise over a period of 30 min and then refluxed for 5 h. The reaction mixture was concentrated to half of its initial volume and then poured on to crushed ice. The product obtained was filtered, washed, dried and recrystallised from ethanol to yield 4-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl) benzene sulfonamide.

*Ethyl-3-oxo-2-(2-(4-sulfamoylphenyl)hydrazono)butanoate (**1**)*. Yellow color solid. Yield 54%. m.p. 145 ºC, IR (cm^{-1}, KBr): 3201.12 (N-H str.), 3011.42 (Ar-H str.), 1712.12 (C=O str), 1631.2 (C=N str), 1341.49 [$(S=O)_2$ asymmetric str], 1148.56 [$(S=O)_2$ symmetric str]. Mol. wt. anal. found = 312.4. Cacld. for $C_{12}H_{15}O_5N_3S$ = 313.

*4-(2 –Hydroxy pheyl)imno benzenesulfonamide (**2**)*. Yellow color solid. Yield 75%. m.p. 212 ºC, IR (cm^{-1}, KBr): 3412.21 (O-H str.), 3223.65(N-H str.), 3035.97(Ar-H str.), 1652.21(C=N str), 1344.53 [$(S=O)_2$ asymmetric str], 1155.96 [$(S=O)_2$ symmetric str], 603.89 (C-S str). ^{1}H NMR (δppm, $CDCl_3$): 6.9-7.89 (m, Ar-H), 2 (s, 2H, -NH_2), 5.1 (s, 1H, - OH). Mol. wt. anal. found = 275.7. Cacld. for $C_{13}H_{12}O_3N_2S$ = 276.

*4-(2-(3-Methyl-5-oxo-1H-pyrazol-4(5H)-ylidine) hydrazinyl) benzene sulfonamide (**2a**)*. Red color solid. Yield 58 %. m.p. 238 ºC. IR (cm^{-1}, KBr): 3210.54 (N-H str.), 3015.23 (Ar-H str.), 1703.3 (C=O str), 1638.21(C=N str), 1462.12 (C=C str), 1344.23 [$(S=O)_2$ asymmetric str], 1159.26 [$(S=O)_2$ symmetric str], 607.22 (C-S str). ^{1}H NMR (δppm, $CDCl_3$): 6.8-7.6 (m, 4H, Ar-H), 2 (s, 2H, -NH_2), 2.33 (s, 3H, -CH_3). Anal. found: C, 53.5;H, 4.87; N, 18.98%. Cacld. for $C_{16}H_{15}O_3N_5S$: C, 53.78; H, 4.2; N, 19.6%.. Mol. wt. anal. found = 356.7. Cacld. for $C_{16}H_{15}O_3N_5S$ = 357.

*4-(2-(3-Methyl-5-oxo-1H-pyrazol-phenyl-ylidine) hydrazinyl)benzene sulfonamide (**2b**)*. Yellow color solid. Yield 56 %. m.p. 232 ºC. IR (cm^{-1}, KBr): 3210.39 (N-H str.), 3023.52 (Ar-H str.), 1703.89(C=O str), 1623.72(C=N str), 1456.62 (C=C str), 1341.43 [$(S=O)_2$ asymmetric str], 1155.96 [$(S=O)_2$ symmetric str], 604.32 (C-S str). ^{1}H NMR (δppm, $CDCl_3$): 6.8-7.9 (m, 4H, Ar-H), 2 (s, 2H, -NH_2), 2.31 (s, 3H, -CH_3). Mol. wt. anal. found = 280.29. Cacld. for $C_{10}H_{11}O_3N_5S$ = 281.

*4-(2-(3-Methyl-5-oxoisoxazol-4(5H)-ylidine) hydrazinyl) benzene sulfonamide (**2c**)*. Cream color solid. Yield 62 %. m.p. 201 ºC. IR (cm^{-1}, KBr): 3215.45 (N-H str.), 3023.33 (Ar-H str.), 1730.21 (C=O str), 1651.21(C=N str), 1462.09 (C=C str), 1344.43 [$(S=O)_2$ asymmetric str],

1159.26 [(S=O) $_2$ symmetric str], 603.74 (C-S str). ^{1}H NMR (δppm, $CDCl_3$): 6.9-7.6 (m, 4H, Ar-H), 2.2 (s, 2H, -NH_2), 3.1 (s, 3H, - CH_3). Anal. found: C,43.1;H, 3.4; N, 19.3%. Cacld. for $C_{10}H_{10}O_4N_4S$: C, 42.55; H, 3.54; N, 19.85%. Mol. wt. anal. found = 282.4. Cacld. for $C_{10}H_{10}O_4N_4S$ = 282.

*4-(2-(2-Hydroxyphenyl)-4-oxothiazolidin-3-yl) benzene sulfonamide (**2d**)*. Brown color solid. Yield 54 %. m.p. 320 ºC. IR (cm^{-1}, KBr): 3439.86(O-H str.), 3221.39 (N-H str.), 3022.43 (Ar-H str.), 1712.87(C=O str), 1633.54(C=N str), 1344.43 [(S=O) $_2$ asymmetric str], 1155.32 [(S=O) $_2$ symmetric str], 607.82 (C-S str). ^{1}H NMR (δppm, $CDCl_3$): 6.7-7.9 (m, Ar-H), 6.3(s, 1H-OH),5.1 (s,1H-CH-), 4.1 2 (s, 2H, -CH_2-), 2. 1(s, 2H, -NH_2). Anal. found: C, 51.2;H, 4.7; N, 8.11%. Cacld. for $C_{15}H_{14}O_4N_2S_2$: C, 51.42; H, 4; N, 8.2%. Mol.wt. anal. found = 349.61. Cacld. for $C_{15}H_{14}O_4N_2S_2$ = 350.

*4-(3-Chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl) benzene sulfonamide (**2e**)*. Brown color solid. Yield 48 %. m.p. 264 ºC. IR (cm^{-1}, KBr): 3431.55(O-H str.), 3232.11 (N-H str.), 3041.62 (Ar-H str.), 1694.11(C=O str), 1334.74 [(S=O) $_2$ asymmetric str], 1163.52 [(S=O) $_2$ symmetric str], 608.78 (C-S str). ^{1}H NMR (δppm, $CDCl_3$): 6.9-7.9 (m, Ar-H), 5.8 (s, 1H-OH), 5.16 (s,1H-CH-), 5.42 (s, 1H, -CH-), 2 (s, 2H, -NH_2). Mol. wt. anal. found = 352.94. Cacld. for $C_{15}H_{13}ClN_2O_4S$ = 352.5.

RESULTS AND DISCUSSION

The physico-chemical data were used to characterize the compounds. The synthesized compounds exhibited characteristic IR (KBr, ν cm^{-1}) peaks in the region of 3319.60 (N-H str), 1680.05 (C=O str), 1344.43 [(S=O) $_2$ asymmetric str], 1159.26 [(S=O) $_2$ symmetric str], 603.74 (C-S str) 3057.27 [C-H str (aromatic)], 1663 (C=N str) and 1433.16 (C=C str). The title compounds exhibited characteristic ^{1}H NMR peaks. The molecular weights determined by Rast's procedure and the elemental proportions of compounds were close to the theoretical values.

For the *in vitro* screening pure strains were obtained from Post Graduate Department of Microbiology, Orissa University of Agricultural Technology, Bhubaneswar, India. The organisms were identified [7] and screened using disc diffusion method [11-12]. The compounds were dissolved in dimethyl formamide (6%), which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. A solution of concentration 30 mg/mL was made for each test compounds and finally sterilized by filtration using 0.45 μm millipore filters. The sterile discs (Hi-media, 6mm) were impregnated with 10 μL of the test solutions (300 μg/disc) and placed in inoculated agar. The density of the bacterial suspension was standardized by using McFarland standard method [8-9]. Nitrofurantoin (300 μg/disc) and ciprofloxacin (25 μg/disc) were used as standard drugs. The control was prepared using dimethyl fomamide. The inoculated plates were incubated at 37 ^{o}C for 24 h. The antibacterial activity of test compounds against the bacterial strains is given in Table 1 as zone of inhibition (mm).

The control did not show any zone of inhibition. Compounds **2a-e** exhibited significant ($p < 0.001$) antimicrobial action compared to control. Compound **2d** showed highest zones of inhibition against *E. coli* and *P. aureginosa*. Activity was better for **2b**, **2c** and **2d**, against *S. aureus*. Compared to nitrofurantoin, most of the compounds exhibited comparable or better antimicrobial activity against all the strains (Table 1). The zone of inhibition against *E. faecalis* was highest for compound **2c**.All the compounds exhibited better zones of inhibition than that of sulfanilamide against all microbial strains. Analysis of structural features reveals that substitution with heterocyclic group has increased the antimicrobial potential of sulfanilamide and the increment was more pronounced for the thiazolidinone derivative.

Table 1. Antimicrobial activity of test compounds.

Compound	μg/disc	Zones of inhibition (mm)			
		E. coli	*P. aeruginosa*	*S. aureus*	*E. faecalis*
Sulfanilamide	300	13.4±0.55	12.3±1.21	11±2.1	10.5±1.4
2a	300	17.2±1.41	16.45±0.32	14.4±2.14	17±0.47
2b	300	17.6±0.34	18.2±0.43	17.1±1.52	19±1.45
2c	300	18.4±1.06	17.7±2.07	18.4±1.34	23.4±2.16
2d	300	21.7±0.84	22.4±1.41	17.6±1.55	16.3±1.21
2e	300	14.7±2.15	16.1±0.74	14.8±1.53	19.7±0.87
Control	-	-	-	-	-
Ciprofloxacin	25	28±0.15	26.4±0.45	25.3±0.15	26.4±0.45
Nitofurantoin	300	18.4±0.45	12.6±0.65	11.8±0.75	15.3±0.37

CONCLUSIONS

The successful syntheses of some heterocyclic derivatives of sulfanilamide were reported. It is noticeable that derivatisation of sulfanilamide, a carbonic ahydrase inhibitor potentiates its antimicrobial action. From the results of the antimicrobial screening, it can be concluded that the new synthesized compounds (**2b, 2c** and **2d**) have good potential for antimicrobial property and hence can be used as leads for further development.

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REFERENCES

1. Chirica, L.C.; Elleby, B.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1997**, 244, 755.
2. Nafi, B.M.; Miles, R. J.; Butler, L.O.; Carter, N.D.; Kelly, C.; Jeffery, S. *J. Med. Microbiol*. **1990**, 32, 1.
3. Smith, K.S.; Ferry, J.G. *Microbiol. Rev*. **2000**, 24, 335.
4. De Benedetti, P.G.; Rastelli, A.; Melegari, M.; Albasini.A. *J. Med. Chem.* **1978**, 21, 1325.
5. Shankaman, S.; Makineni, S.; Gold, V. *Archives Biochem. Biophys.* **1963**, 100, 431.
6. Suresha, G.P; Prakasha, K.C.; Shivkumara, K.N; Kapfeo W.; Gowda,D.C. *Int. J. Peptide Res. Therapeutics* **2008**, 15, 25.
7. Havaldar, F.H.; Mishra, S.K. *Indian J. Heterocyclic Chem*. **2004**, 13, 197.
8. Gupta, U.; Sareen, V.; Khatri, V.; Chugh, S. *Indian J. Heterocyclic Chem*. **2004**, 13, 351.
9. Martin, A.R. *Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry*, 10th ed., Lippincoptt-Raven: Philadelphia; **1998**; pp 185-204.
10. Collins, C.H.; Lyne, P.M.; Grange, J.M. *Microbiological Methods*, 6th ed., Butterworths: London; **1989**; pp 127-129.
11. Jorgensen, J.H.; Turnidge, J.D.; Washington, J.A. *Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods*, Murray, P.R.; Pfaller, M.A.; Tenover, F.C.; Baron, E.J.; Yolken, R.H. (Eds.), ASM Press: Washington, D.C.; **1999**, pp 1526-1543.
12. Ringertz, S.; Rylander, M.; Kronvall, G. *J. Clin. Microbiol*. **1991**, 29, 1604.