Synthesis, antibacterial and antifungal possession of amino acids containing sulfonamide moieties

Muhammad Abdul Qadir[1], Mahmood Ahmed[1]* and Anum Khaleeq[2]
[1]Institute of chemistry, University of Punjab, Lahore-Pakistan
[2]Govt Kinnaird College, Lahore-Pakistan

Abstract: Sulfonamides were developed by the simple reaction of amino acid with p-toluenesulfonyl chloride and structures of the new products (2a, 2b and 2c) were confirmed by elemental and spectral analysis (FT-IR, ^{1}HNMR and^{13}CNMR). *In vitro*, developed compounds were screened for their antibacterial and antifungal activities against two sensitive bacteria belonging to both gram positive and gram-negative types and two fungi. The synthesized sulfonamides (2a, 2b, 2c) exhibited excellent antifungal activities against the tested fungi. Among the tested compounds 2a and 2b have marked activity against *E. coli* with zone of inhibition (mm) 22.3±0.11and 20.2±0.26 (MIC: 12.5µg/mL, 12.5µg/mL) and *S. aureus* with zone of inhibition (mm) 20.2±0.26 and 23.2±0.55 (MIC: 12.5µg/mL, 6.25µg/mL). Compound 2c is moderately efficient towards *E. coli* (zone of inhibition (mm) 14.2±0.64, MIC: 100µg/mL) and no activity against *S. aureus.*

Keywords: Inhibition zone; bacterial strains; sulfonamides.

INTRODUCTION

Sulfonamides group occurs in various biological active compounds and are therapeutic agents as antimicrobial drugs, carbonic anhydrase inhibitors, insulin-releasing sulfonamides, antithyroid agents, HIV protease inhibitors and antitumor drugs. Sulfonamides are among the most widely used antibacterial agents in the world, because of their low cost, low toxicity and excellent activity against common bacterial diseases (Jain *et al*., 2013; Özbek *et al*., 2007). Amino acids especially the beta class, have vital importance in synthesis of new biologically active substances and their role has been proven in synthesis of novel drug candidate. Some lipopeptides and toxins comprise of these beta amino acids, which exhibit antifungal and antibacterial activities (Okada, 2009; Bonmatin *et al*., 2003). Glycine, arginine and lysine are among the most important beta amino acids, which are important part of various natural derivatives that are characterized by potent pharmacological and toxicological activities. These amino acids are potential therapeutic candidates with enhanced bioavailability and resistance to metabolic degradation (Patočka, 2010a, 2010b, 2010c; Steer *et al*., 2002). The sulfonamides exhibit the amphoteric behavior as a result of the inductive properties of sulfoxide group. This amphoteric behavior of sulfonamides has been found to play an extremely important role in the antibacterial activity, found the relationship between acid dissociation constant of sulfonamides and bacteriostatic activity. Due to low solubility in water and high pKa value around 10, these are crystalize in kidney, but with advance research newer sulfonamide compounds have a pKa of around 5-6, which avoids the above problem of crystallization in the kidney (Bell and Romblin, 1942; Seydel *et al*., 1980). The rationale behind the research work is to synthesize some novel sulfonamides with better antimicrobial properties using classical approach with new reactant species. Glycine, arginine and lysine were selected as starting material and reacted with p-toluenesulfonyl chloride (Scheme 1).

MATERIALS AND METHODS

Chemistry

Chemicals used in present work, were of analytical grade obtained from E-Merck (Germany) and BDH (UK) without further purification to synthesize desired compounds, grade 1 quality water (0.01µS/cm) was prepared in our own laboratory (Jeffery *et al*. 1989). Alpha IR spectrometer (FTIR-ATR), Bruker and NMR spectrometer, Bruker were used to record the IR and ^{1}HNMR, ^{13}CNMR spectra respectively. PG-T80^{+} UV-Vis spectrophotometer, Flash HT Plus elemental analyzer, Thermo Scientific were used for λ_{max}, and concentration of carbon (C), hydrogen (H), sulfur (S) and nitrogen (N) of respective synthesized compounds respectively while the melting point was measured by Gallenkamp apparatus. The ^{1}HNMR, ^{13}CNMR spectra of all the synthesized compounds were measured using $CDCl_3$. Purification and progress of the synthesized compounds were confirmed on pre-coated TLC silica plate (Merck-Germany) and spots were located in UV light.

General procedure of synthesis

Amino acids were weighed accurately and dissolved completely by addition of distilled water by constant stirring using magnetic stirrer. The pH of the reaction contents was strictly monitored and maintained at 8-10 at

**Corresponding author:* e-mail: mahmoodresearchscholar@gmail.com

regular intervals during the experimental reaction using 1 M Na_2CO_3 solution (Qadir *et al.,* 2015a, 2015b, 2015c, 2015d). Then p-toluenesulfonyl chloride was accurately weighed and added carefully into the above solution. The reaction was carried in round bottom flask equipped with magnetic stirrer. Alkaline environment made the removal of hydrogen easier. During stirring p-toluenesulfonyl chloride initially floats on the surface and the completion of reaction was examined by the change in pH value due to formation of HCl by the consumption of p-toluenesulfonyl chloride during the reaction. On completion of the reaction pH was adjusted at 2-3 using HCl solution (2M). The precipitates formed were filtered through Whatmann filter paper No.42, washed several times with distilled water and re-crystallized using methanol and dried over anhydrous $MgSO_4$.

N-((4-methylphenyl)sulfonyl)glycine (2a)

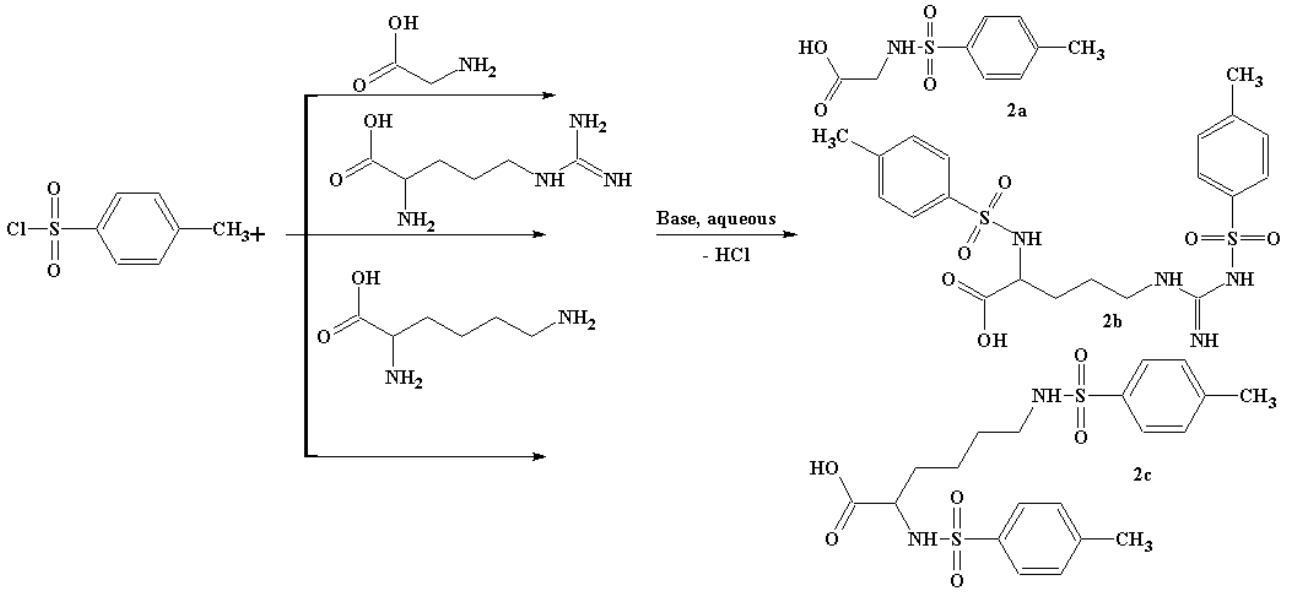
Compound (2a) was obtained as white solid by treating glycine (300.28mg, 4.0mM) with p-toluenesulfonyl chloride (762.6mg, 4.0mM); yield: 852.0mg (92.9%); m.p. (^{0}C): 101-103;λ_{max}: 285 nm;IR: 3250 (O-H $_{carboxylic}$), 3350 (N-H $_{amide, stretching}$), 1055 (C-N $_{amine}$), 1650 (N-H $_{amide, bending}$), 1030 (S=O$_{stretching}$), 1157, 1332 (-N-S=O $_{stretching}$),

Table 1: MIC, MBC and MFC (µg/mL) values of sulfonamides

Microorganism		Compounds				
		2a	2b	2c	Ciprofloxacin	Clotrimazole
E. coli	MIC	12.5	12.5	100.0	0.0625	-
	MBC	50.0	25.0	100.0	0.125	-
S. aureus	MIC	12.5	6.25	100.0	0.250	-
	MBC	50.0	25.0	200.0	0.50	-
A. flavus	MIC	6.25	6.25	6.25	-	0.50
	MFC	12.5	12.5	12.5	-	1.00
A. alternate	MIC	6.25	6.25	6.25	-	0.50
	MFC	12.5	12.5	6.25	-	1.00

Table 2: Diameter of zone of inhibition of sulfonamides

Compounds	Diameter of zone of inhibition (mm ± SD)			
	E. coli	*S. aureus*	*A. flavus*	*A. alternate*
2a	22.3±0.11	21.4±0.34	24.2±0.15	23.3±0.32
2b	20.2±0.26	23.2±0.55	24.1±0.12	23.4±0.34
2c	14.2±0.64	4.0±0.15	24.5±0.15	22.4±0.45
Ciprofloxacin	32	28	-	-
Clotrimazole	-	-	30	28



Scheme: Synthetic route for new sulfonamides

1620, 1580 (C=C $_{aromatic}$), 1150, 741 (C-H $_{aromatic}$) cm^{-1}; ^{1}HNMR ($CDCl_3$, δ/ppm): 2.23 (3H, J=2.69 Hz, CH_3), 6.98 (1H, d, J=7.97, CH), 7.59 (1H, d, J=7.96, CH), 5.61 (1H, NH), 3.28 (2H, d, J=11.48, CH_2), 5.63 (1H, OH); ^{13}CNMR ($CDCl_3$, δ/ppm): 142.8 (C-1), 129.1 (C-2), 127.28 (C-3), 21.1 (C-7), 42.9 (C-12), 169.75 (C-13); Anal. Calc. (%) for $C_9H_{11}NO_4S$ (229.25): C 47.15, H 4.84, N 6.11, S 13.99; Found (%): C 47.11, H 4.86, N 6.15, S 13.91.

N^5-(imino{((4-methylphenyl)sulfonyl)amino}methyl)-N^2-((4-methylphenyl)sulfonyl)ornithine(2b)

Slight yellow compound (2b) was obtained by the reaction of arginine (348.4mg, 2.0mM) with p-toluenesulfonyl chloride (760.0 mg, 4.0mM); yield: 760.0 mg (78.7%); m.p. (^{0}C): 168-170;λ_{max}: 380 nm; IR: 3252 (O-H $_{carboxylic}$), 3352 (N-H $_{amide,\ stretching}$), 1057 (C-N $_{amine}$), 1650 (N-H $_{amide,\ bending}$), 1035 (S=O$_{stretching}$), 1157, 1334 (-N-S=O $_{stretching}$), 1620, 1580 (C=C $_{aromatic}$), 1150, 743 (C-H $_{aromatic}$) cm^{-1}; ^{1}HNMR ($CDCl_3$, δ/ppm): 2.31 (6H, J=2.79 Hz, 2xCH_3), 3.78 (1H, t, J=10.17, CH), 7.59 (1H, d, J=7.99, CH), 2.19 (2H, d, J=11.96, CH_2), 7.31 (4H, 4xNH), 1.38 (2H, t, J=6.48, CH_2), 3.31 (2H, t, J=13.18, CH_2), 7.33 (1H, OH); ^{13}CNMR ($CDCl_3$, δ/ppm): 143.5 (C-1), 129.7 (C-2), 126.8 (C-3),136.5 (C-4), 21.5 (C-7), 51.9 (C-12), 29.75 (C-13), 25.7 (C-14),43.3 (C-15), 158.1 (C-17), 177.8 (C-19), 137.4 (C-26); Anal. Calc. (%) for $C_{20}H_{26}N_4O_6S_2$ (482.58): C 49.78, H 5.43, N 11.61, S 13.29; Found (%): C 49.71, H 5.46, N 11.15, S 13.31.

N^2, N^6-bis((4-methylphenyl)sulfonyl)lysine (2c)

Lysine (913.3mg, 5.0mM) reacts with p-toluenesulfonyl chloride (1190.0 mg, 10.0mM) and gave yellow compound (2c); yield 1300.0 mg (59.1%); m.p. (^{0}C): 224-226; λ_{max}: 325 nm; IR: 3256 (O-H $_{carboxylic}$), 3348 (N-H $_{amide,\ stretching}$), 1056 (C-N $_{amine}$), 1648 (N-H $_{amide,\ bending}$), 1036 (S=O $_{stretching}$), 1157, 1332 (-N-S=O $_{stretching}$), 1620, 1580 (C=C $_{aromatic}$), 1150, 736 (C-H $_{aromatic}$) cm^{-1}; ^{1}HNMR ($CDCl_3$, δ/ppm): 2.38 (6H, J=2.79 Hz, 2xCH_3), 4.1 (1H, t, J= 10.13, CH), 7.59 (1H, d, J=7.96, CH), 2.1 (2H, d, J= 18.8, CH_2), 6.61 (2H, 2xNH), 1.33 (2H, t, J=6.38, CH_2), 1.91 (2H, t, J=7.18, CH_2), 2.93 (2H, t, J=14.1, CH_2), 6.56 (1H, OH); ^{13}CNMR ($CDCl_3$, δ/ppm): 143.4 (C-1), 129.8 (C-2), 126.9 (C-3),136.1 (C-4), 21.4 (C-7), 54.2 (C-12), 31.7 (C-13), 21.7 (C-14),29.1 (C-15),44.43 (C-16), 176.1 (C-20), 137.8 (C-23), 137.4 (C-26); Anal. Calc. (%) for $C_{20}H_{26}N_2O_6S_2$ (454.56): C 52.85, H 5.77, N 6.16, S 14.11; Found (%): C 52.81, H 5.76, N 6.15, S 14.12.

Bacterial strains and fungus

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Aspergillus flavus* ATCC 9643 *and Alternaria alternate* ATCC 46582 were collected from Mycology Department, University of Punjab, Lahore-Pakistan were maintained in tryptic soy agar (TSA) and malt extract agar (MEA) medium respectively slants at 5 ^{0}C until use.

Solutions preparation

A series of ten2-fold dilutions were made by dissolving 10-30 mg of each sulfonamide (2a, 2b, 2c) separately in 1 mL dimethyl sulphoxide (DMSO). All the dilutions were made sterile in an autoclave at 121°C for 30 min with 15psi pressure after filtration through 0.22μm membrane filter.

Antimicrobial assay

The lowest concentration or dilution of the synthesized compounds in a serial dilution sequence that results in the absence of observable growth was reported as the minimal inhibitory concentration (MIC) and the lowest concentration or dilution that kills 99.9% of the test organism (bacteria and fungi) was reported as the bactericidal and minimal fungicidal concentration (MBC& MFC). Ten screw-capped test tubes (13 mm x 100 mm) were sterilized and numbered individually. Tube 1 was filled with 2mL of culture media (tryptic soy broth for bacterial strains while Sabouraud culture media for fungi) including the stock solution of synthesized compounds. 1.0mL of this solution was introduced into tubes 2 and diluted with 1.0mL culture media and repeated the procedure up to tube 10. Compounds (2a, 2b, 2c) concentration used for MIC value was 2-0.0039mg/mL obtained by two fold serial dilution technique. The tubes were incubated at 25^{o}C for 72 hrs. The tube with the lowest concentration at which no growth or turbidity was observed was reported as the MIC against the organism. 100μL contents from the tubes containing no turbidity were cultured on the medium and incubated at 37^{o}C for 24 hrs determine the minimum bactericidal and fungicidal concentrations. Ciprofloxacin was used as reference for bacterial strains while the clotrimazole as reference antifungal agent. The optical density of the bacterial inoculum at 0.3 to 0.4 for gram positive like *S. aureus* and 0.2 to 0.3 for gram negative such as *E. coli* was obtained at a wavelength of 620nm, equivalent to 1 to 3 x10^8 cfu/mL while fungal suspension (*A. flavus and A. alternate*) with cell density of 10^5 cfu/mL was studied in present work. All the compounds (2a, 2b, 2c) and reference solutions were applied (50μL) onto a 6mm sterile filter paper disc separately and inoculated plates were incubated at 37^{o}C for 72 hrs. Antibacterial and antifungal activity was evaluated by measuring the zone of inhibition in mm. Studies were performed in triplicates and zone of inhibition was calculated with the mean ± SD values.

RESULTS

Three new sulfonamides were prepared using classical approach by the reaction of glycine, arginine and lysine with p-toluenesulfonyl chloride in basic media with continuous stirring and after completion of reaction pH were adjusted at 2-3. The products were recrystallized after washing with methanol and dried over magnesium

sulphate. The highest yield 92.9% was obtained of 2a, while 78.7% and 59.1% yield were of 2b and 2c respectively.

DISCUSSION

Elemental analysis was performed for the conformation of all the compounds and measurement of absorption maximum (λ_{max}) provided the justification. The products have additional value of λ_{max} resulting in bathochromic shift to longer wavelengths; details are given in experimental section. The structures of all the compounds were confirmed by ^{1}HNMR and^{13}CNMR by dissolving in $CDCl_3$.^{1}HNMR spectra of 2a, 2b and 2c showed a signal at δ 6.98-7.59ppm for CH of benzene ring and 2.23-2.38ppm of CH_3 attached to ring. The signal at 5.61-7.31ppm corresponds to NH group of sulfonamide. The characteristics C-NH-SO signal δ 42.9 ppm (C-12) for 2a and δ 51.9ppm, 158.1ppm (C-12, C-17) for 2b δ 54.2ppm, 44.43ppm (C-12, C-16) was showed by ^{13}CNMR, which identified the structures correctly. The synthesized compounds were also characterized by FT-IR, the characteristics band at 3252-3256 cm^{-1} of (O-H $_{carboxylic}$), N-H amine stretching at 3348-3352$cm^{-1}$and 1332-1338 and 1152-1159cm^{-1} for (-N-S=O) of 2a, 2b, 2c. Synthesized compounds were also screened for their antibacterial and antifungal activity against bacterial and fungal stains respectively by following the guidelines of CLSI (CLSI, 2006a, 2006b). The antimicrobial and antifungal activities of amino acid conjugates of sulfonamides (2a, 2b, 2c) along with reference (ciprofloxacin and clotrimazole as antibacterial and antifungal respectively) against *E. coli, S. aureus, A. flavus* and *A. Alternate* respectively presented in table 1 and 2. The effectiveness of antimicrobial and antifungal sensitivity testing is based on the size of the zone of inhibition. The zone of inhibition however, varies with the infusibility of the agent, the size of the inoculums and the type of medium. *E. coli,* and *S. aureus* were found to be sensitive to 2a and 2b, while the 2c is resistant to *E. coli* and has no effect on *S. aureus,* the zone of inhibitions are presented in table 2. It was also noted that greater antifungal potential possessed by 2a, 2b and 2c for all fungi and lowest MIC values. The maximum zone of inhibition (23.2±0.55mm) was of compound 2b against gram-positive *S. aureus* among all other micro-organisms which found to be the most sensitive bacterial strain while 2cshowed almost no antibacterial activity (zone of inhibition: 4.0±0.15mm) against *S. aureus*.

CONCLUSION

In conclusion, three amino acid conjugate sulfonamides were synthesized, progress of reaction was monitored by TLC and their structures were confirmed by spectral and elemental analysis. The antibacterial and antifungal activities of new novel sulfonamides products were evaluated against gram negative, gram-positive bacteria and fungus. Compounds 2a, 2b and 2c have excellent antifungal activities while 2c has weak antibacterial activity against *E. coli*. Best antibacterial activities were possessed by 2a and 2b.

REFERENCES

Bell PH and Romblin RO Jr (1942). Sulfonamides amphoteric behavior. *J. Amer. Chem. Soc.,* **64**: 2509.

Bonmatin JM, Laprévote JM and Peypoux F (2003). Diversity among microbial cyclic lipopeptides: Iturins and surfactins. Activity structure relationships to design new bioactive agents. *Comb. Chem. High. Throughput Screen,* **6**: 541-556.

Clinical and Laboratory Standard Institute (2006). Performance Standard for Anti-microbial Disk Susceptibility Tests: Approved Standard M2-A-9, Clinical and Laboratory Standard Institute, Wayne, Pa, USA.

Clinical and Laboratory Standard Institute (2006). Method for dilution Anti-microbial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M7-A-9, Clinical and Laboratory Standard Institute, Wayne, Pa, USA.

Jain P, Saravanan C and Singh SK (2013). Sulphonamides: Deserving class as MMP inhibitors? *Eur. J. Med. Chem.,* **60**: 89-100.

Jeffery GH, Bassett J, Mendham J and Denney RC (1989). Vogel's textbook of quantitative chemical analysis. John Wiley and Sons, New York. 90-91

Okada Y (2009). Overview in 45 years of studies on peptide chemistry. *Yakugaku. Zasshi.,* **129**: 1141-54.

Özbek N, Katırcıoğlu H, Karacan N and Baykal T (2007). Synthesis, characterization and antimicrobial activity of new aliphatic sulfonamide. *Bioorg. Med. Chem.,* **15**: 5105-5109.

Patočka J (2010). Beta-natural amino acids and their biologically active derivatives. I. Derivatives of beta-alanine and beta-leucine. *Mil. Med. Sci. Lett.,* **79**: 15-22.

Patočka J (2010). Beta-natural amino acids and their biologically active derivatives. II. Derivatives of the beta-beta-lysine and arginine. *Mil. Med. Sci. Lett.,* **79**: 50-53.

Patočka J (2010). Beta-natural amino acids and their biologically active derivatives. III. Derivatives of the beta-beta-phenylalanine and tyrosine. *Mil. Med. Sci. Lett.,* **79**: 114-18.

Qadir MA, Ahmed M, and Iqbal M (2015). Synthesis, characterization, and antibacterial activities of novel sulfonamides derived through condensation of amino group containing drugs, amino acids, and their analogs. *BioMed Res. Inter.,* **2015**: Article ID 938486, 7 pages.

Qadir MA, Ahmed M, and Khaleeq A (2015). Synthesis and biological evaluation of amino terminalmodified

new sulfonamides of contemporary drugs. *Lat. Am. J. Phar.* **34**: 719-724.

Qadir MA, Ahmed M, Aslam H, Waseem S and Shafiq MI (2015). Amidine sulfonamides and benzene sulfonamides: Synthesis and their biological evaluation. *J. Chem.* **2015**: Article ID 524056, 8 pages.

Qadir MA, Ahmed M, Shafiq MI, Mahmood N, Anwar S, Habib T and Nosheen S (2015). Synthesis, spectral characterization and biological activities of methyl substituted amino acid conjugated sulfonamides. *Lat. Am. J. Phar.* **34**: 1511-1515.

Seydel JK, Trettin D and Cordes HP (1980). Quantitative structure pharmacokinetic relationships derived on antibacterial sulfonamides in rats and its comparison to quantitative structure-activity relationships. *J. Med. Chem.,* **23**: 607-613.

Steer DL, Lew RA, Perimutter P, Smith A and Aguilar MI (2002). β-Amino acids: Versatile peptidomimetics. *Curr. Med. Chem.,* **9**: 811-22.